“Federico II” University of Naples

PhD in Biology XXXV Cycle

(funded by Stazione Zoologica Anton Dohrn)

Ecological role and biotechnological applications of marine sponges and benthic diatoms and their natural products

Coordinator:

Prof. Sergio Esposito

Tutors:

Dr. Maria Costantini, Stazione Zoologica Anton Dohrn, Naples

Dr. Valerio Zupo, Stazione Zoologica Anton Dohrn, Naples

Dr. Stefania Crispi, Istituto di Bioscienze e Biorisorse, IBBR-CNR, Naples

Dr. Adele Cutignano, Istituto di Chimica Biomolecolare, ICB-CNR, Pozzuoli

Prof. Simona Carfagna, Università Federico II, Naples

Candidate:

Dr.ssa Roberta Esposito

Anno accademico 2021-2022

Journal of *Marine Science and Engineering*

MDPI

Review

Management and Sustainable Exploitation of Marine Environments through Smart Monitoring and Automation

Francesca Glaviano [1,2], Roberta Esposito [1,2], Anna Di Cosmo [2], Francesco Esposito [1], Luca Gerevini [3,4], Andrea Ria [3,5], Mario Molinara [4], Paolo Bruschi [5], Maria Costantini [1,*] and Valerio Zupo [1,*]

1 Stazione Zoologica Anton Dohrn, Department of Ecosustainable Marine Biotechnology, Villa Comunale, 80121 Naples, Italy; francesca.glaviano@szn.it (F.G.); roberta.esposito@szn.it (R.E.); francesco.esposito@szn.it (F.E.)
2 Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Naples, Italy; anna.dicosmo@unina.it
3 Sensichips Srl., Via Fanciulla d'Anzio 9, 00042 Anzio, Italy; luca.gerevini@unicas.it (L.G.); andrea.ria@sensichips.com (A.R.)
4 DIEI—Dipartimento di Ingegneria Elettrica e dell'Informazione, Università di Cassino e del Lazio Meridionale, Via G. Di Biasio 43, 03043 Cassino, Italy; m.molinara@unicas.it
5 Dipartimento di Ingegneria dell'Informazione, Università di Pisa, Via Caruso 16, 56122 Pisa, Italy; paolo.bruschi@unipi.it
* Correspondence: maria.costantini@szn.it (M.C.); vzupo@szn.it (V.Z.)

Abstract: Monitoring of aquatic ecosystems has been historically accomplished by intensive campaigns of direct measurements (by probes and other boat instruments) and indirect extensive methods such as aero-photogrammetry and satellite detection. These measurements characterized the research in the last century, with significant but limited improvements within those technological boundaries. The newest advances in the field of smart devices and increased networking capabilities provided by emerging tools, such as the Internet of Things (IoT), offer increasing opportunities to provide accurate and precise measurements over larger areas. These perspectives also correspond to an increasing need to promptly respond to frequent catastrophic impacts produced by drilling stations and intense transportation activities of dangerous materials over ocean routes. The shape of coastal ecosystems continuously varies due to increasing anthropic activities and climatic changes, aside from touristic activities, industrial impacts, and conservation practices. Smart buoy networks (SBNs), autonomous underwater vehicles (AUVs), and multi-sensor microsystems (MSMs) such as smart cable water (SCW) are able to learn specific patterns of ecological conditions, along with electronic "noses", permitting them to set innovative low-cost monitoring stations reacting in real time to the signals of marine environments by autonomously adapting their monitoring programs and eventually sending alarm messages to prompt human intervention. These opportunities, according to multimodal scenarios, are dramatically changing both the coastal monitoring operations and the investigations over large oceanic areas by yielding huge amounts of information and partially computing them in order to provide intelligent responses. However, the major effects of these tools on the management of marine environments are still to be realized, and they are likely to become evident in the next decade. In this review, we examined from an ecological perspective the most striking innovations applied by various research groups around the world and analyzed their advantages and limits to depict scenarios of monitoring activities made possible for the next decade.

Keywords: IoT; buoy; aquaculture; coastal; connectivity; transmission; real time; network

Citation: Glaviano, F.; Esposito, R.; Cosmo, A.D.; Esposito, F.; Gerevini, L.; Ria, A.; Molinara, M.; Bruschi, P.; Costantini, M.; Zupo, V. Management and Sustainable Exploitation of Marine Environments through Smart Monitoring and Automation. *J. Mar. Sci. Eng.* **2022**, *10*, 297. https://doi.org/10.3390/jmse10020297

Academic Editors: Gabriella Caruso, Francesco Tiralongo and Yannis N. Krestenitis

Received: 30 October 2021
Accepted: 10 February 2022
Published: 21 February 2022

1. Current Policies for Environmental Monitoring and Conservation

The monitoring of marine environments has attracted increasing attention due to the growing concerns about climate change, along with intensified transportation activities, possibly producing direct, indirect, and stochastic impacts. In fact, a key challenge in

contemporary ecology and conservation management is the accuracy of tracking of the spatial distribution of human impacts, including oil spills and chemical pollution, along with the evaluation of environmental quality and fishery activities [1]. Automation is an important part of the new generation of information technology, and it represents the ultimate achievement in the development of ocean monitoring programs. Various emerging technologies developed in the last decade include smart devices for the collection of information and their sharing over networks, as well as emerging technologies such as the Internet of Things (IoT), often foreseen as the future solution to an intelligent monitoring assembly [2].

The systems currently in use generally consist of observatories connected to a network system lying on the seafloor or connected to the surface by, for example, a buoy. In the first case, an example of a stable observatory is the Dense Ocean Floor Network System for Earthquakes and Tsunamis (DONET) by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). DONET is a submarine-cabled real-time seafloor observatory network intended for large-scale research and earthquake and tsunami monitoring. The program, which began in 2006, consists of several phases involving an increase in the number of observatories.

This system concept consists of a high-reliability backbone cable, which provides the power line and the communications channel, connecting several nodes with different measurement instruments [3].

Buoy systems are widely applied as well to monitor ocean environments, and meteorological and oceanographic instrumentation platforms able to share meteorological and environmental data in real time are critical to promptly respond to critical events. The development of newer buoys is able to improve early detection and real-time reporting of events in the open oceans, which is fundamental for the forecasting and reporting of tsunamis. For example, forecasting and reporting of tsunamis were made possible by the development of newer buoys able to improve early detection and real-time reporting of events in the open oceans [4,5]. Similarly, the realization of systems able to detect the presence of pollutants in the marine environment (including hydrocarbons, often requiring prompt reactions due to ship collisions and other disasters) has become extremely complex, involving various technologies and integrated know-how [6] further discussed below. Stations for deep-ocean assessment and reporting of tsunamis were developed ad hoc by NOAA (https://www.ndbc.noaa.gov/dart/dart.shtml; accessed on 30 November 2021) to acquire critical data for real-time forecasts in key regions [7]. The network is presently composed of 39 stations (Figure 1). This station system was named DART®, and it consists of bottom pressure recorders (BPRs) anchored to the seafloor coupled with a companion moored surface buoy for real-time communications [4]. An acoustic link transmits data from the BPR on the seafloor to the surface buoy. However, the main constraint for ocean monitoring systems is represented by communications, because it is almost impossible to deliver the measured data to remote monitoring sites without the aid of satellite communications [8]. To extend the communication coverage of a buoy network, a wireless mesh network (WMN) can be adopted (i.e., a communication network containing multiple radio nodes consisting of mesh routers and clients organized into a mesh topology). Since mesh routers can forward a message deriving from other nodes (even outside the transmission coverage of their destination), a multi-hop relay network (MHRN) may be arranged. An MHRN can extend the coverage of wireless communications, and it provides line-of-sight (LOS) links between couples of nodes. Mesh networks provide many advantages, including reliability, robustness, self-organization, and self-configuration [9].

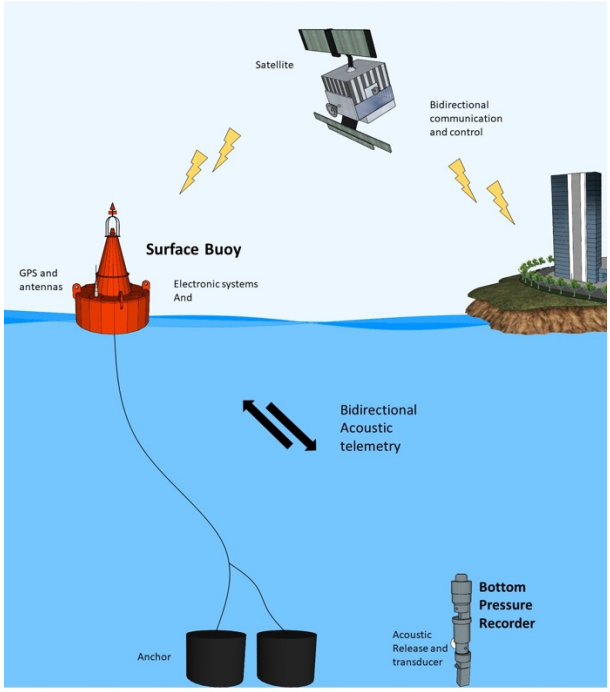

Figure 1. DART System set by NOAA (from www.noaa.gov, modified; accessed on 31 July 2021), containing 39 special stations transmitting GNSS data using bidirectional communications.

Thus far, it is evident that marine monitoring of natural environments is a tremendously wide field of study, taking advantage of various disciplines and comprising several aspects including the biology of species, the ecology of aquatic environments, the technology of new devices, and the chemistry of water as revealed by probes, with the inclusion of newer smart tools for detection and transmission. A complete analysis of all these aspects cannot be achieved and discussed within a single literature synthesis. For this reason, here we analyze the current literature to present several (but not necessarily all) recently developed methodologies and technologies to improve marine monitoring methods in order to highlight new trends and modern perspectives on the study of coastal and offshore environments, which are changing fast due the introduction of important innovations. In addition, we introduce some newly developed tools and experimental data collected at our laboratory in order to broaden the analysis of coastal tools with the introduction of smart sensors and autonomous monitoring buoys, facilitating video monitoring and immediate answers to critical events.

2. Sensing and e-Noses

The technological limits of probes and transmission devices must be taken into account when planning innovative monitoring stations and vessels. Some critical issues impose specific requirements for probes and monitoring stations, including simplicity, autonomy [10], adaptability, scalability, and robustness [11]. Some features should be assured due to the harsh characteristics of the marine environments [12]. Among these, we need to address the following specifically [13]:

- Self-standing devices: equipment should be designed against possible acts of vandalism, which are more frequent than commonly expected;
- Hardware robustness: all equipment needs strong resistance due to currents, waves, tides, typhoons, and other physical impacts producing frequent aggressions to weak structures;
- Salinity: sensor and actuator nodes need to have very high levels of robustness against corrosion and be adapted to a high electrical resistance to the medium;

- Stability of communications: specific techniques must be adapted to bad weather conditions (that can affect the stability of radio signals) and to the oscillation of the antennas due to waves and storms, which can cause unstable communications [14];
- Costs: energy storage and collection (eventually using energy accumulators) must be considered due to long communication distances and the need for probe functioning, data storage and transmission, and ultimately motion structures;
- Distance between receiving stations and buoy or mooring devices: sensor coverage needs to be carefully calculated because of the large areas often covered by a monitoring network [15];
- Stationary position: in the case of both fixed buoys and autonomous vehicles, the position of the sensor nodes should be assured, and its location should be assessed with high reliability because of the continuous movement in the fluid environment;
- The optical signal response is too low when compared with other targets and that one may have under certain circumstances of vegetation, soils, and also strong geometric effects (e.g., sun-view angle effects from optical data).

For these reasons, various monitoring systems have been developed in different areas, also according to the specific variables under analysis. Among them, the most powerful approaches employed to obtain sensitive data and rapidly compute them include synthetic aperture radar (SAR) [16], computer-aided imaging, and network analysis [17]. All these approaches account for some critical issues, including low detection capability (i.e., when wind speeds that are too low or too high influence the functioning of the SAR) or worse functioning during given times (e.g., at night, when sunlight is not available). In addition, both in oceanic environments and in coastal areas, hyperspectral and thermal imaging [18] and hydrodynamic mathematical modeling of stationary phenomena [19] may represent a possible solution.

Among the most modern and powerful systems, however, we must consider the chemical sensors for electronic nose-like systems [20–22]. Recently, a smart system based on electronic noses able to monitor the presence of pollutants (particularly hydrocarbons) on the sea surface was proposed [20]. The system was suggested to be employed, together with traditional methods, for a complete and exhaustive analysis of the marine pollution caused by hydrocarbons. It is composed of an array of sensors, a flow chamber, and electronics, and it was initially tested at the laboratory bench and then in the sea, demonstrating its efficiency and reliability in the detection of hydrocarbon pollutants present on the surface. It allows for an early intervention strategy from designated entities, as well as from the autonomous underwater vehicles (AUVs) themselves, when equipped for these circumstances. In addition, an e-nose-like technology may be integrated into an AUV in order to perform a dynamic check of the pollution status over a given area, and this possibility is increasingly stimulating various research groups because various noses are presently under study for implementation into smart vehicles able to independently monitor large coastal and oceanic areas [23–25]. This extension to the basic functions of AUVs was also performed by earlier prototypes [6], and it could embody an invaluable innovative contribution to the prevention strategies presently adopted throughout the world in this field, possibly establishing the basis for future multimodal marine monitoring implementations.

A number of different approaches have been employed to provide real-time acquisition of environmental data, especially to provide immediate reaction to incidents involving petroleum tanks or oil spills in coastal or oceanic areas, where continuous monitoring may be limited by economic or technical constraints [16]. Spills or leaks, as well as accidents [26,27], can induce dramatic consequences on the marine environments, and their immediate localization (followed by restoration activities) is critical to reduce long-term impacts over the marine biota. In these cases, various monitoring approaches have been widely applied in the past, such as hyperspectral and thermal imaging [18] and hydrodynamic mathematical modelling [19]. However, these large-scale approaches exhibit some limitations when the pollution sources are of a small size and the waves of pollution have

not yet been distributed over larger areas. In addition, weather conditions and light availability may drastically reduce their detection capabilities. To this end, newer intelligent technologies primed the development of AUVs (described in the next paragraph), independently sailing over large areas and able to ride out customized or pre-loaded explorations according to the needs of scientists and administrators [6]. This innovative approach is based on signals produced by electrochemical sensors reacting to the presence of possible pollutants [22], the signal of which is immediately sent to reference stations where the signal may be interpreted and eventually converted into an alert message, prompting the intervention of specialized personnel to assure marine environment preservation.

In parallel to atmospheric issues, as mentioned above, hydrocarbon pollution is one of the most serious concerns for the health of marine ecosystems, and the strategies for its timely monitoring have grown in complexity and number in the last decade. To this end, an AUV equipped with an *e*-nose-like system was proposed [20], employing sensors set both at the laboratory bench and at sea. The results confirmed the feasibility of the approach and the good reliability of the data acquired, confirming the possible employment of this system within an integrated marine monitoring tool.

The high costs of offshore mooring systems and traditional oceanographic cruises have suggested the use of innovative technologies, often based on intelligent devices and small monitoring platforms automatically collecting a wide range of environmental and meteorological data [28]. These approaches reached lower costs thanks to the new opportunities offered by emergent tools, representing cost-effective solutions to the need of modularity, flexibility, and real-time observing systems. Their affordability is guaranteed by the efforts dedicated to the design, development, and realization of new oceanographic devices, leading to rapid advances in the fields of probes and intelligent vehicles. In addition, innovative molecular technologies tremendously improved biodiversity studies, particularly in the case of microbes, rare species, "soft species" (or extremely small species), and cryptic species (to be studied combining molecular and morphological information [29,30], while new sensors and in situ technologies are being applied to the identification of life forms in remote deep-sea habitats [31,32].

In general terms, *e*-nose technologies are based on arrays of sensors connected to specific unit boards able to analyze the sensor's signals, compare their results, and compute an answer according to pollution thresholds set by the user. For some applications, photoionization detectors were employed, whose driving force relies on vacuum ultra-violet radiation capable of ionizing the volatile organic compounds (VOCs) contained in the air over the seawater [6]. In this case, the sensors do not analyze the chemical or physical properties of the seawater. They detect the VOCs present in the air immediately over the water surface, just like a "nose" exploring large areas along the coastline searching for the "smell" of petrol [20]. For these applications, a concentration of 100 ppm of each hydrocarbon among the ones most frequently present in polluted seas (e.g., gasoline, kerosene, diesel fuel, and crude oil) is considered sufficient [26,27]. The smart modules employed for these purposes are normally trained to evaluate the responses of various probes after the determination of the most relevant features among all the data collected by e-noses by means of principal component analysis (PCA). Using this system, the detected stimuli may be classified according to different levels of warning, depending on the intensity of the concentration of pollutants.

3. Autonomous Vehicles and Monitoring Platforms

Unmanned vehicles (UMVs) represent a significant innovation, improving the quality, affordability, and costs of environmental monitoring (Table 1). They are also used in the military field for the inspection of areas and targets of strategic interest [33], and they are divided into three kinds: AUVs, autonomous surface vehicles (ASVs), and remotely operated underwater vehicles (ROUVs). These vehicles can be also deployed in the air (unmanned aerial systems (UASs)), at the sea's surface (ASVs, also known as unmanned surface vessels (USVs)), or in the water column (AUVs). UMVs have various applications,

such as gathering oceanographic and meteorological data [34–39] and monitoring sea ice [40] and wildlife [41–44]. Most ROUVs are equipped with at least a video camera and lights. The main difference between these types is that an operator controls the ROUV, while AUVs and ASVs operate autonomously. Thus, some innovative vehicles are capable of sensing the environment and navigating on their own. UMVs include semi-submersibles and unmanned surface crafts.

Table 1. Features of unmanned vehicles (UMVs) classified according to the types (unmanned aerial system (UAS), autonomous surface vehicle or unmanned surface vessel (ASV/USV), autonomous underwater vessel (AUV), remotely operated underwater vessel (ROUV), and gliders). The main features are indicated in terms of environment explored, control, navigation system, and propulsion type.

	Operates			Controlled by		Navigation System		Propulsion	
UMV	**In Air**	**Water Surface**	**Under Water**	**Operator**	**Independent**	**GPS Navigation**	***e*-Compass**	**Propellers**	**Variable Buoyancy**
UAS	X			X	X	X		X	
ASV/USV		X			X	X		X	
AUV			X		X		X	X	
ROUV			X	X			X	X	
Glider	X	X	X		X	X	X		X

The advantage, with respect to aerial photogrammetry and other large-scale monitoring approaches, is that the measures are quite direct, punctual, and characterized by precision and accuracy, even if large territories may be explored for longer times by smart AUVs. Their employment in association with other classical monitoring systems can increase accuracy and efficiency, because the movements of autonomous vehicles can be semi-randomly influenced by alarms sent by satellites or other monitoring sources, modifying the programmed maps of cruises. Such systems may also find wide application in critical coastal zones, such as in marine protected areas (MPAs), because they are left free to iteratively explore transects and continuously transfer to reference centers (on land) signals of "all normal" conditions or, alternatively, warning messages prompting immediate inspection by coastal guards or other marine authorities [45–47]. Several MPAs have been set in Europe in the last decade after the evaluation of marine sites of ecological interest [48], where ship transits are totally or partially forbidden, and consequently, oil spills should be avoided. Since continuous and punctual environmental monitoring in these areas is critical, automation of smart monitoring activities may represent an obvious solution.

AUVs are widely used for monitoring survey and data collection. They can be equipped with various types of sensors, such as sonar, video cameras, and the means for measuring conductivity, temperature, pressure, and salinity, among other factors. AUVs collect information through sensors. Parameters such as the water temperature and speed are simply measured and easily interpreted. Other types of data are more complex to collect and analyze because they require further interpretation to convert the records into meaningful information. Therefore, the selection of sensors is important for successful detection. Equally important is the diagnosis of the problem, which requires the ability to analyze and interpret the data collected by eliminating sensor noise and therefore making the data reliable [49–52]. They have the advantage of huge spatial coverage, but they are limited by a small resolution [53]. The risk is that the collected data might not be representative from a temporal point of view. As part of the research, they can be involved in data collection for bathymetric and magnetic fields and conformation of the seabed [54]. They are also used for the evaluation of water parameters in specific locations, such as in the areas surrounding hydrothermal processes or coral reefs [55]. Currently, they find application in various fields ranging from scientific research to industrial purposes. In industrial applications, AUVs are used for the monitoring and maintenance management of oil, gas ducts, and electrical lines [56]. Evidently, AUVs and ASVs represent the most

recent advances in the field of smart tools compared with ROUVs, which were introduced several decades ago and have been improved in terms of efficiency and cost in the last few years. Additional equipment is commonly added to expand the vehicle's capabilities. These may include sonars, magnetometers, a still camera, a manipulator or cutting arm, water samplers, and instruments that measure the water clarity, water temperature, water density, sound velocity, light penetration, and temperature [57].

ASVs and AUVs suitable for marine monitoring can vary from relatively small vehicles lifted by one or two persons and deployed from a small inflatable boat to large diesel-powered surface vessels [58]. In particular, smaller vessels are able to operate with a high level of autonomy and are also capable of staying at sea for several months. In contrast, larger surface vehicles often tend to be more tightly controlled. Surface vehicles have the advantage of being able to continuously receive GPS position data while navigating, and their locations can be accurately recorded at all times. Subsurface vehicles do not receive GPS data while they are immersed and therefore must generally rely on depth measurements and dead reckoning using electronic compasses [59,60]. Moreover, ASVs can operate safely in hazardous locations and at night and can cover much larger areas, mitigating the risk of crew fatigue. In some cases, they can independently operate off large ships [61].

ASVs started to be developed at an academic level in 1993, when the MIT presented its first vessel, called ARTEMIS [62,63]. The newer ASV, called the Shallow Water Autonomous Multipurpose Platform (SWAMP), is a full-electric catamaran built with the purpose of being a modular multi-functional vehicle, having several applications for a range of missions, such as geomorphological analysis, water sampling, and physical and chemical data collection in harsh environments [64]. This vehicle has four thrusters, azimuth pump-jet thrusters that are flush with the hull, small-draft soft foam, an unsinkable hull structure with high modularity, and a flexible hardware and software architecture [64]. Generally, USVs are associated with unmanned surface vehicles (USVs) [65]. Usually, USVs are equipped with a central processing unit and different memories for saving and providing a preliminary management of the acquired data (e.g., compression and classification). In addition, batteries and photovoltaic panels are equipped to increase the electrical autonomy as much as possible, which generally turns out to be one of the major limiting factors [60]. ENDURUNS is an example of a system that integrates both an AUV and an USV system. The USV is equipped to support the power requests of both systems with photovoltaic panels and rechargeable battery packs. The peculiarity of this AUV is the ability to move using two different modes. The first, thruster mode, allows it to move in a precise and controlled way to perform transects parallel to the seabed and collect data with great accuracy. The second mode is called glider mode and allows it to cover larger areas for a longer time, as consumption is significantly reduced [66–68]. The USV autonomously follows the AUV, providing information for accurate geo-localization of the acquired data. Data transfers between the AUV and the USV are realized through acoustics communication or through a wireless connection [53]. It is also important to establish threshold values at the beginning of the mission for correct data processing. The last phase is represented by adaptation, in which the mission plan can be redesigned by changing the detection scheme and the trajectory of the vehicle [69]. The AUTOSUB Long Range 1500, which is being designed, built, and operated by the National Oceanography Center, is a highly capable AUV with the potential of providing measurements that would have been previously impossible to collect, therefore allowing key advances in marine ecology studies. This vehicle will be built to be able to reach a depth of 1500 m [70,71].

Finally, an underwater "glider" (Table 1) is a specific type of AUV which employs variable-buoyancy propulsion instead of traditional propellers or thrusters. It houses sensors capable of making multidisciplinary oceanographic observations with long-term deployments (months) and has the ability to cover large distances (hundreds to thousands of kilometers) because it has significantly greater endurance compared with traditional AUVs [72]. The typical up-and-down, sawtooth-like profile followed by a glider can

provide data on temporal and spatial scales unattainable by powered AUVs and which are much more costly to sample using traditional shipboard techniques. Four commercially available electric underwater gliders represent the main opportunities in this field: the Slocum electric [73], the Seaglider [34], the Coastal glider [74], and the Sea Explorer [75]. In addition, other gliders are under development, including Spray [76]. Coastal gliders are designed to be applied in the littoral zone (they are self-ballasting from essentially fresh to full ocean water) with a faster maximum speed (2 knots, according to Imlach and Mahr [74]). The Deep Glider, on the other hand, is designed to operate at depths of 6000 m [77]. These vehicles mostly extract energy from wave motion and convert it directly into forward motion. The vehicles also use solar or wind power to charge batteries used to power the navigation systems and the sensor payload.

4. Experimental Data

As mentioned above, the main advantage of coupling e-noses with smart autonomous vehicles relies on the possible customization of analytical procedures, as well as on the rapidity in intervention policies suddenly made possible after an accident or any type of pollution event. Attempts to quantify the ecological effects of special coastal areas, such as MPAs and MPA networks, are usually hampered by a lack of well-designed monitoring studies [78,79]. The management plans for an MPA network aim at protecting and conserving biodiversity and other natural values within protected areas. However, coastal monitoring in an MPA is not limited to the detection of oil pollution and the mapping of VOCs, because various ecological descriptors may be crucial to follow the chemical and physical state of key environments along the coastal waters in a timely manner [80,81], such as in seagrass meadows and recruitment areas. To this end, we designed and realized an innovative system for marine environmental monitoring whose main features are represented by the employment of an innovative probe carried aboard a smart ASV (Figure 2). Although the realization of the monitoring system is still in progress, it may be worth it to present the data obtained to date as a preliminary description of these innovative tools based on the newest technologies appearing on the market. In particular, we designed the prototype of a simple and inexpensive floating ASV able to independently move within an MPA located around the Isand of Ischia and send real-time data to a land-based station located at the local laboratory of Stazione Zoologica Anton Dohrn. The floating ASV was equipped with three electric propellers mounted under a floating plastic base, containing a glass bell that protected the main components. One of the main innovations was represented by the presence of pioneering probe technology.

The probe was a multi-metal detector produced by SensiChips [82], named "smart cable water" (SCW), based on the impedance generated by the presence of various pollutants. Such probes must be trained prior to be applied for ecological purposes, because their reactions to patterns of various substances are singular and not-linear. In this light, they represent a complex though interesting means to afford biomonitoring of coastal ecosystems. SCW is a multi-sensor microsystem (MSM) produced to monitor the presence of toxic chemicals (TICs), pollutants, hydrocarbons, and organics in water [83,84]. At the core of SCW there is SENSIPLUS, a microsensor platform which can interrogate on-chip and off-chip sensors with its versatile electrical impedance spectrometer (EIS) and potentiostat. Analyses performed with EIS allow for exploiting the RedOx dynamics of catalytic noble metals to aid the fine discrimination of chemicals along with the measurement of the conductivity and permittivity spectra. The on-chip potentiostat is used for a number of voltammetric or amperometric measurements and real-time discrimination of pollutants.

By cycling the electrodes with overvoltage, the device prevents or mitigates the formation of biofouling. Consequently, SCW may be considered a reliable multiparametric water analysis microsystem. Thanks to its analytical instruments and availability of catalytic interdigitated electrodes, SCW (Table 2) also represents an experimental microsystem for discriminative measurements (Figure 3).

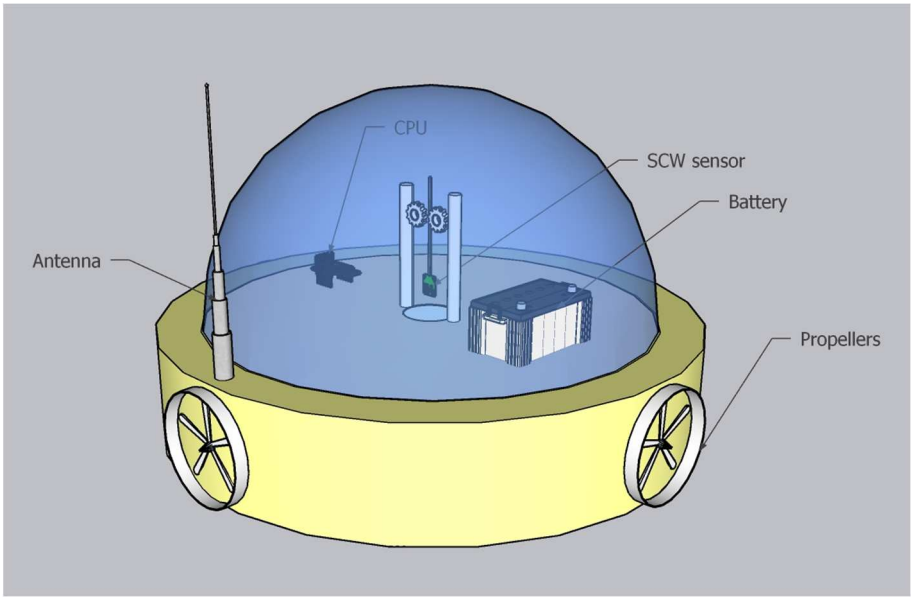

Figure 2. An experimental ASV equipped with an innovative probe under development in our laboratory. An SCW is located on board an ASV, and the CPU directs the movements of the instrument over a network of fixed points to transmit data sets and, eventually, alarms according to thresholds set by local administrators. The probe is automatically extracted from the marine water at given time intervals and mopped to avoid corrosion of the metal plates.

Table 2. Technical specifications of SCW used for our smart monitoring test.

ELECTRICAL	
Supply voltage	1.5–3.6 V
Max current	0.4 mA continuous when reading on-chip sensors with EIS
Size	12 × 15 mm, 3 mm thickness
Interface	I^2C or SENSIBUS, single data wire multidrop sensor array cable interface, 1.5–3.6 V
Unique identifier	OTP 48 bits unique device identifier, 16 bits user-defined
ELECTRICAL IMPEDANCE SPECTROSCOPY	
Frequency	From 3.1 mHz to 1.2 MHz
Vpp output sinewave	From 156 mV to 2.8 Vpp
Coherent demodulation	1st, 2nd, or 3rd harmonic
Output	Reciprocal of real or imagery component
Wide measurement range	From ohms to 100 MΩ
TEMPERATURE	
Range	−40–125 °C
Accuracy	±0.1 °C
Thermodynamics	Calorimetry, enthalpy, and exothermic or endothermic
ELECTROCHEMICAL METHODS	
pH	From 3 to 14, potential of platinum vs. clads-platinum
ORP	Total oxidation and reduction potentials
RedOx	Reduction or oxidation activity (free chlorine, hardness)
Voltammetry	Specific reduction or oxidation potentials
Anodic stripping voltammetry	Measures heavy metals
Electro-catalysis	Noble metal IDEs measure current specifically
IMPEDANCE METHODS	
Conductivity spectroscopy	Resistivity, salinity, EC, TDS, and absorption dynamics
Dielectric spectroscopy	Turbidity, SS, biomass total and active, and hydrocarbon detection

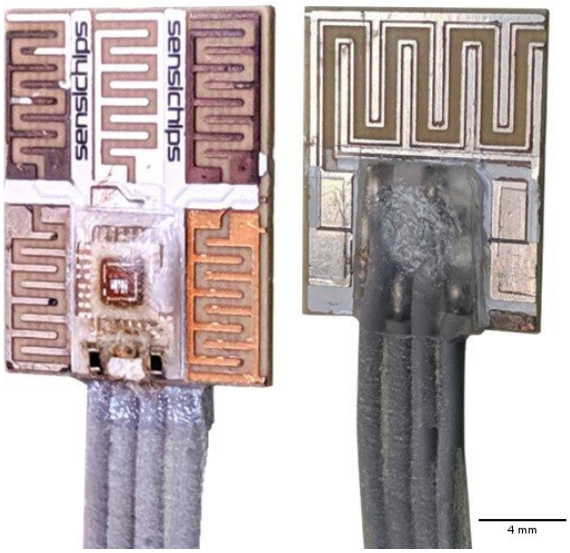

Figure 3. Front (left) and rear (right) sides of SCW adopted to produce a smart ASV for coastal monitoring (see Figure 2).

As mentioned above, an SCW needs to be trained to recognize pollutants and other substances of ecological interest. For this purpose, various amounts of key compounds (such as nitrogen and phosphorus compounds) were tested and used to calibrate the probe. Our results indicate that low amounts of important pollutants were detected by the instrument, but a full set of permutated measurements is needed to train the instrument to recognize compounds in any pattern of reciprocal concentrations.

Another constraint is represented by the oxidizing power of the seawater, because continuous immersion in water rich in NaCl produces fast deterioration of some of the metal plates, drastically reducing the performance, as demonstrated by our tests. For this reason, the SCW was mounted over the ASV by means of an immersion device able to move the SCW up and down at various time intervals, protecting it with frequent washes in distilled water followed by mopping and drying of its surface. However, this SCW-equipped ASV was demonstrated to be quite promising for coastal monitoring, because its performance may improve through auto-training and also because of the easy installation over small smart vehicles wirelessly connected to the control stations on land.

5. Autonomous Monitoring Networks

The increase in the exploitation of marine resources enforces the necessity to develop new methods of environmental monitoring which, with the integration of new technologies, make the reaching of new frontiers possible in the field of biological features, namely for environmental, physical, and chemical parameters and sampling surveys [85]. In fact, in recent years, several projects had the goal of identifying new tools for the optimization of monitoring and sampling techniques for the improved assessment of an environmental status, which is the basis of several international management policies [86,87]. The conception of new models of structures for data collection is necessary to cope with the different types of marine environments in which the survey is carried out to increase the operational range either in time or space [64]. While multiparametric cabled bases are a well-proven solution for the remote and continuous monitoring of marine environments [84], the implementation of more autonomous solutions is an important future prospect to ideally allow data collection at any depth and distance from the coast. In this light, network complementation with surface or aerial (radio frequency transmission) and underwater (acoustic) video monitoring may represent the smartest solution.

Video monitoring, in fact, can also be realized by taking advantage of a fixed-point cabled camera installed over a platform [88] or a mobile underwater television (UWTV) consisting of a towed camera sled. The sled is positioned on the seabed and dragged along

a transept. Care must be taken to try to keep the vessel speed stable, as it is affected by the surface conditions [89]. The advantages of the UWTV solution lay in the fact that if used properly, it allows for obtaining a relatively constant measurement while being more accurate and less invasive than trawling surveys. For example, the Scottish government and Joint Nature Conservation Committee [90] considered the use of UWTVs an excellent solution to identifying any new areas potentially eligible to become MPAs [91].

Upon the set-up of various autonomous monitoring vehicles (AUVs), a network composed of AUVs moving around a single buoy may produce timely maps of the marine areas under control (Figure 4). The network should contain a master buoy equipped with a wireless link receiving data from the AUVs and, eventually, satellite communication to an inshore station in order to raise warning signals to the station as well as to check the real-time evolution of pollution events.

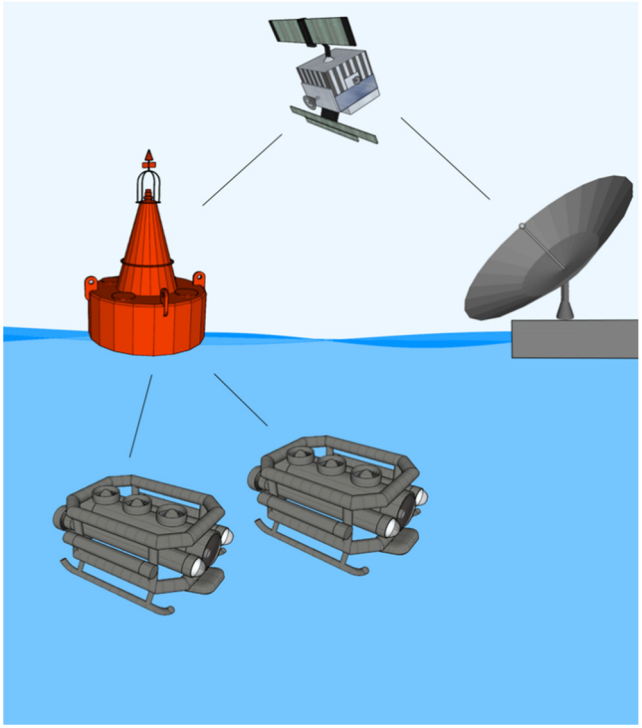

Figure 4. Real-time marine monitoring multimodal scenario (from [20], modified). A master buoy (red) receives pollution data from the AUVs (two gray vessels) and sends data to the land through satellite communications.

6. Marine Permanent Infrastructures

Currently, the largest existing networks of underwater observing stations are represented by permanent infrastructures specifically intended for multidisciplinary monitoring and research in the fields of geology, oceanography, and ecology. The advantage of permanent infrastructural networks is that they can be connected directly to the coast or through a succession of nodes [85,86]. Connection by a cable transmission line directly provides power and real-time data transmission to and from the marine observatory. However, networks of this magnitude are very expensive.

The operation costs for this kind of infrastructure are really high, considering the involvement of suitable ships and specialized equipment. Moreover, given the complexity and multidisciplinary nature of the projects, the use of specialized personnel is required in various areas, such as engineers, marine scientists, and data analysts [85,92]. Although the possibility of having a connection with the shore confers numerous advantages by finding an easy solution to the problems of energy supply and data transmission, at the same time, these prove to be a limit if the site of interest is not close enough to the coast [93]. Permanent

structures tend to also be limited due to their restricted spatial coverage and unpredictable bias in monitoring results that can be influenced by the infrastructure's presence [68]. To overcome these limitations, most of these infrastructures are integrated with mobile nodes that allow observations to be extended over a much larger area, taking into account different geographical gradients and different depths. A network designed by different nodes, including mobile ones, allows for collecting data in a more extensive and continuous way, making it possible to follow animal movement across different spatial gradients [94] and energy flux interchanges [95]. The data collected are transmitted through a cyber infrastructure, making it possible for anyone with an Internet connection to download the data in real time. Raw data are archived and read by a system code that separates them into data streams based on the content. According to the requirements, multiple levels of data products are processed with different algorithms to make them easier to consult at different levels of complexity. Each platform hosts several integrated scientific instruments, and they can contain multiple "nodes" to which the integrated instruments are attached, as well as a means for transmitting the data to the shore. Some examples of cabled observatories that integrate remote control systems and interactive sensors are the following.

The Ocean Networks of Canada (ONC) [96] is a research facility hosted and owned by the University of Victoria. This network operates with several ocean observatories in the deep ocean and coastal waters of Canada from the west and east coasts and the Arctic. It continuously collects data in real time, which are made available for scientific research, governments, and industry. Through the use of cabled observatories and remote-control systems, the ONC enables the development of several projects [97].

NEPTUNE is among the largest observatories. This observatory has several nodes, with various cabled instrument platforms and mobile crawlers that can cover around 15 km of linear distance with a depth oscillation of about 500 m [85]. This observatory is equipped with various instruments that can be used in different applications, such as a seismograph to monitor earthquake activity, bottom pressure recorders for real-time tsunami monitoring systems around the world, and specialized hydrophones to track marine mammals' activities [98,99] and investigate how they are influenced by human activities. Specialized sensors, cameras, and remotely controlled sampling devices make NEPTUNE's site easily adaptable for monitoring [98] commercially relevant fishery resources (such as the sablefish *Anoplopoma fimbria*) with life cycles that involve small-scale and large-scale geographic movements with both vertical and horizontal changes [100,101].

The American Ocean Observatory Initiative (OOI) funded by the National Science Foundation was designed as a long-term project to collect ocean data. The Ocean Observatories Initiative is made up of five major research components with several associated arrays located in the northern and southern Atlantic and Pacific according to the demand of the scientific community. Each array is composed of fixed and mobile platforms [102]. A platform can be stable, fixed, or mobile. Mobile components can move up and down in the water column or be a glider, which is able to move in three dimensions. Each platform hosts several integrated scientific instruments and can contain multiple "nodes" to which the integrated instruments are attached, as well as a means for transmitting the data to the shore. The OOI instrumentation is involved in the support of several research projects, including climate variability, ocean food webs, biogeochemical cycles, and coastal ocean dynamics and ecosystems.

The European Multidisciplinary Seafloor and water column Observatory (EMSO) [103] consists of a system of regional observatories located at key sites around Europe. Each platform is equipped with multiple sensors sited along the water column and on the seafloor. They constantly measure different parameters. Data are collected and available to different users, from scientists and industries to institutions and policy makers [104]. The EMSO infrastructure range runs at the European scale from the coastal area to the deep sea and open ocean, operating with both stand-alone observing systems and nodes connected to shore stations through fiber optic cable [105]. The data in both cases are transmitted in real time either through the cables or through acoustic networks featured by satellite-linked

buoys [106]. Data are collected from the surface of the ocean to the seafloor. In addition to generic sensors, specific modules with different instrument combinations are deployed to be able to respond to specific objectives [107]. Many physical and biological applications require observation of the physical and ecological parameters (such as concentrations of oxygen and chlorophyll) at high-resolution time series data over long periods. Other systems for marine ecological research require photo and video imaging, acoustic recording, and in situ collections [65–85].

KM3NeT is a research infrastructure located in the Mediterranean Sea which houses the next-generation neutrino telescopes. Still nearing completion, this structure aims to have a detector volume of several cubic kilometers of clear seawater [108]. The main purpose of this project is to allow an innovative framework for studying neutrinos from distant astrophysical sources. Nonetheless, given the arrays of thousands of sensor modules, this research infrastructure will also house instrumentation for other scientific investigations for long-term and online monitoring that may find application in such fields as marine biology, oceanography, and geophysics [108].

The Joint European Research Infrastructure of Coastal Observatories (JERICO) is a network of coastal observatories providing a European Research Infrastructure (RI) dedicated to the observation and monitoring of marine coastal seas to provide high-quality environmental data as tools for scientific researchers and societal and policy needs [109,110]. It comprises JERICO-S3. In parallel, JERICO-RI is an integrated pan-European multidisciplinary and multi-platform research infrastructure dedicated to the assessment of changes in the coastal marine system. JERICO-S3 officially started in 2020, entitled Marine coastal observatories, facilities, expertise, and data for Europe. Its aim is to be involved in the cooperation of coastal observatories in Europe by the implementation and improvement of the coastal structures of a European Ocean Observing System and to cooperate with other European initiatives. There are currently 10 structures between the different partner nations. These facilities provide wired observatories, AUVs, fixed and multi-platform structures, and calibration laboratories to allow the carrying out of different projects [111]. An example of some of these projects currently underway is the study focused on Algerian Basin (AB) circulation through the monitoring line ABACUS [112] through the AB between Palma de Mallorca and the southern part of the basin [113]. These projects involve partners both public (e.g., university and research institutes) and private (e.g., private non-profit research institutes) from European and non-European countries.

7. IoT Hardware Modules

The European Research Cluster of the Internet of Things defined the IoT as a technological revolution, consisting of a dynamic global network infrastructure with self-configuring capabilities. It is based on standard and interoperable communication protocols. In this system, physical and virtual "things" have identities, physical attributes, and virtual personalities, and they use intelligent interfaces natively integrated into an information network [114]. The IoT is characterized by the integration of various devices equipped with sensing, identification, processing, communication, actuation, and networking capabilities [115] (Figure 5).

The term Internet of Things was initially created in 1999 by Kevin Ashton, an expert in digital innovation [117]. IoTes (i.e., the "objects" taking part of the network) can be variously defined. Firstly, IoTes can be defined as intelligent objects, or "things having identities and virtual personalities" operating in smart spaces and using intelligent interfaces to connect and communicate within social, environmental, and user contexts [118]. IoTes are also considered an extension of the Internet with objects, devices, sensors, and items not ordinarily considered computers [119]. In addition, the IoT is understood as a global network infrastructure linking physical and virtual objects (IoTes) through the exploitation of data capture and communication capabilities [120]. Finally, the IoT can be regarded as a way to promote information interaction by linking people, things, and objects autonomously and intelligently without any temporal or spatial constraints [121,122].

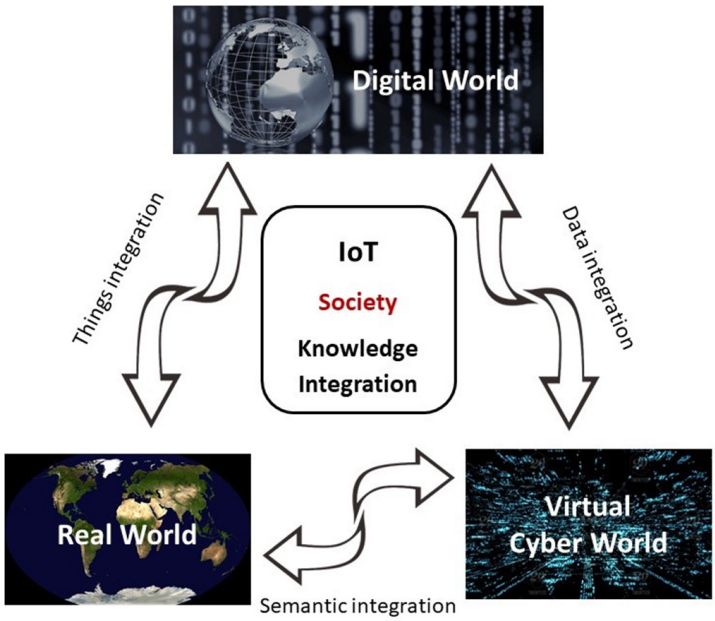

Figure 5. Knowledge integration for the logical relationships among the digital and cyber world as connected with the real world ([116], modified).

Even if the IoT and IoTes are still evolving, their effects are beginning to be seen and are making great strides, offering universal solution media for an interconnected scenario [123]. This is mainly due to the fact that the IoT guarantees high-speed and accurate data with secure processing and an improved client or user experience [124,125]. Its development depends on dynamic technical innovation in a number of important fields, from wireless sensors to nanotechnology [126]. In fact, the IoT can be applied to various fields of our daily life, such as eHealth (a relatively recent health care practice supported by electronic processes and communication) [127], security, entertainment, smart cities, defense, and many other fields [128]. The IoT can be used to manage soil moisture, irrigation and drainage systems, and crops in smart farming systems. Finally, the IoT is useful to monitor the conditions of marine environments, allowing scientists to monitoring such physical parameters as the water temperature, dissolved oxygen, salinity, pH, and turbidity [129]. Smart health sensors are used to collect human physiology information as well and use gateways and clouds to analyze and store the information and wirelessly send the analyzed data to caregivers for further analysis and review [130]. The IoT can also be operated in smart cities for (1) improving infrastructure, public transportation [125], and electrical conductivity thanks to smart grids that combine the information and communications technologies into an electricity network [131] and (2) helping predict natural disasters with the combination of sensors and their autonomous coordination and simulation [132]. However, the IoT is not limited to public uses. It can also be privately adopted for smart home and security systems, such as by natively connecting several household devices to the Internet [133].

Domingo [134] proposed the architecture of an IoT network in three layers: (1) perception, (2) network, and (3) application. The main function of the perception layer is to identify specific objects and gather information. It is formed mainly by sensors, actuators, monitoring stations (such as cell phones, tablet PCs, smart phones, and PDAs), nano-nodes, RFID tags, and readers and writers. Depending on the type of sensor, the information to be referred can be the location, temperature, orientation, motion, vibration, acceleration, humidity, or chemical changes, among other details. The collected information is then passed to the network layer for its secure transmission to the information processing system. This network layer consists of converging, privately owned, wired, or wireless networks where the transmission technology can be chosen (e.g., 3G, UMTS, Wi-fi, Bluetooth, infrared, or ZigBee) depending upon the features of sensor devices. Its main function is to transfer the

information obtained from the perception layer to the middleware layer. It receives the information from the network layer, stores it in the database, and autonomously makes some decisions based on the results and the agreed protocols. The application layer provides global management based on the object's information processed in the middleware layer. Finally, the business layer is responsible for the overall management of the IoT system, including applications and services. In particular, this layer eventually builds management models, graphs, and flowcharts, and it proposes the future actions and operative strategies based on the data received from the application layer [132].

Evidently, the IoT represents a future challenge in many technological applications, minimizing efforts, offering the use of efficient resources, and guaranteeing accurate quality data and a high speed of reaction. The reliability and validity of the data, performance, security, and privacy are additional advantages. However, various issues will need to be addressed in the future, such as privacy issues (hackers can break into the system and steal the data) and unemployment, because some activities performed by human operators will be replaced by machines [125,133].

8. The IoT Applied to Marine Environmental Monitoring

IoT-based technologies, as well as wireless sensor networks (WSNs), a subset of IoT, can be applied to the monitoring and protection of marine environments [13]. In particular, monitoring activities employing IoT technology can be used for ocean sensing and monitoring of water quality [134], coral reef protection, offshore and deep-sea fish farms, and wave and current watching [13].

The development of an adaptive, scalable WSN must foresee such critical properties as autonomy, scalability, adaptability, durability, and simplicity [135]. On the other hand, the design and deployment of a lasting and scalable WSN for marine environment monitoring should consider all of the following peculiar challenges mentioned in the second paragraph. Other issues can concern the devices and sensor nodes, which can be highly reliable because of the difficult deployment and maintenance. In addition, their coverage needs to be carefully evaluated, because their application over large areas far from direct control and with expensive, delicate equipment should be protected against possible acts of vandalism [13,135].

Overall, an online marine monitoring system needs (1) sensors adequate to measure seawater features, (2) a controller or processor unit to compute the data from sensors, and (3) communication equipment to send data from the processing unit to the cloud via ground stations. Sending large amounts of data (as large images or videos) to the cloud requires the combination of the IoT and cloud computing, because satellite communications may be expensive, in terms of both money and energy consumed [136,137] (Figure 6), and sensing stations should be relatively small and light.

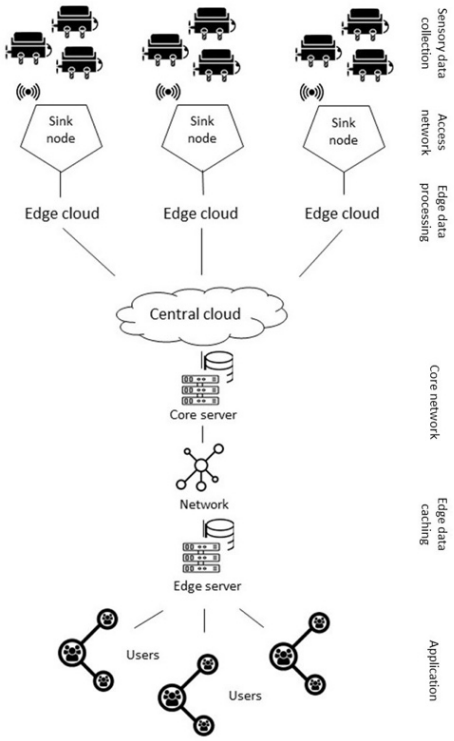

Figure 6. Physical architecture of smart monitoring systems with data collection networks.

9. Monitoring Applied to Aquaculture and Fishery Productions

Various technological applications of artificial intelligence (AI) were set for improving the sustainability of monitoring in coastal waters and oceans, as well as in aquaculture and smart fishery plants, and they are widespread nowadays [138]. In particular, the attention of scientists in AI-inspired fisheries focused mostly on monitoring the automation of fishery resources (mainly detection, identification, and classification). However, it is still unclear how fishers perceive AI needs and how governments exhibit a tangible strategy on the regulation of AI concerning smart fishery systems to promote the value and potential of the techniques of AI-inspired fisheries. AI has great influence on catch monitoring across fishery systems at sea [139], and several AI applications improved fishing activities, helping the economic evaluation of commercial fleets [140]. In addition, a fishery may be helped by electronic monitoring of the catch and bycatch [141], as well as the detection and forecasting of fishing grounds [142], eventually applying mathematical models to simulate fishing vessel behavior [143]. This also helps to reduce fishery wastes [144] by optimizing the sorting operations. Finally, automation of the monitoring of illegal fishery methods [145] is also possible for reducing the negative impacts of fisheries on coastal areas. The AI technologies of fish farming mainly focus on the means for optimizing the efficient use of resources in ecosystem management [146]. In several instances, fishery and ecological monitoring have been strictly interconnected. In fact, sustainable fisheries are related to environmental monitoring [147]. Various authors stressed the scope of smart fisheries because of the "epidemic of plastics entering the sea". This warrants urgent action if humanity is to stave off a collapse in fish stocks. Additionally, oil spills [148] and global changes [149], as mentioned above, are topics of great concern [150], prompting not only issues of environmental conservation but also large impacts on fisheries [151], requiring accurate and modern monitoring activities. The employment of smart systems able to autonomously tune their activities according to local perturbations and able to be trained for the detection of various compounds (both in the water and over the water, evolving into e-noses) is boosting improvements in various fields of environmental monitoring.

A special case of monitoring marine environments is the one applied to aquaculture activities [152,153], primarily because these activities may impact various coastal areas when practiced in cages, pens, floating tanks, and raceways deployed in open waters [154]. There is great concern about the potential environmental effects of marine finfish cages on the water quality [155] and a large interest in developing an ecologically responsible industry [156,157]. Several reviews [158] have broadly addressed this topic [159–161].

In addition, aquaculture ponds may be considered very special marine environments, and they need continuous monitoring and real-time reactions to negative changes impacting the organisms contained therein [162]. In addition, in this case, artificial intelligence and IoT devices may be applied to improve production efficiency and reduce impacts and risks. In fact, the connection between good environmental conditions and seafood health in aquaculture has been documented [163–165]. Sea cages can be more than 45 m in diameter and 30 m deep, and they need frequent inspections. Although a single cage can contain high value production [166,167], the level of surveillance of the product and of the closer environments is often low [168]. As for tank aquaculture, various remote sensor systems were proposed to have a conveyed gathering of sensor hubs organized together and be able to exchange the crude information up to a base station through an IoT network [169,170]. Using Arduino-like hardware and a few simple probes, automatic farming systems were developed based on IoT platforms (Figure 7).

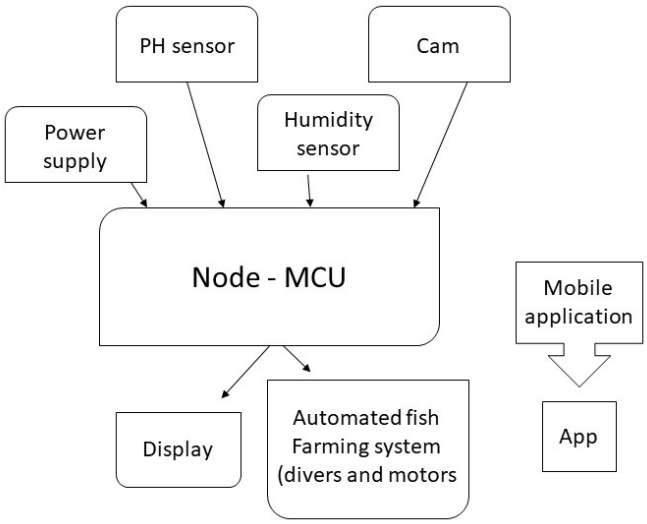

Figure 7. Block diagram of the system proposed in [169] for IoT control of aquaculture productions (modified).

All IoT systems for aquaculture are to be considered smart systems based on intelligent sensors, intelligent processing, and intelligent control. Their functions consist of data collection, real-time image acquisition, wireless transmission, intelligent processing, warning messages, and auxiliary decision making [171–173]. Any aquaculture IoT monitoring system fundamentally consists of water quality monitoring stations, including meteorological stations, water quality control stations, and on-site and remote monitoring centers. These structures are supported by a central cloud-processing platform [174]. The water monitoring stations are provided with monitoring sensors and take advantage of wireless data collection terminals. Local data are collected in ponds and transmitted to monitoring centers. In particular, such water quality parameters as dissolved oxygen, pH, and ammonia concentration are key elements to allow prompt answers to production issues. Generally, weather stations are also used to acquire in real time such meteorological data as wind speed, wind direction, and air humidity. The system analyzes the relationships between the water quality parameters and weather changes to predict water quality trends and ensure the optimal water quality in the culture tanks. The tank controllers include independent control terminals, an electrical control box, aerators, and other equipment,

with the control terminal receiving wireless instruction from the control equipment. On-site and remote monitoring centers based on wireless sensor networks and the Global System for Mobile Communications (GSM) central servers with central cloud processing platforms are included, favoring an intelligent control algorithm for water quality to achieve data acquisition, smart data processing, alarms, and their mailing to human managers [175]. Central cloud processing platforms provide the basis for decision making for farmers by providing a variety of models and algorithms of quality monitoring, feeding, and pond management [176]. These strategies reduce the risks of product losses, reduce the pollution of local environments by increasing the efficiency of culture procedures, and also reduce the need for using drugs, with obvious advantages for local environments.

10. Conclusions

Environmental monitoring solutions must be adapted to each individual situation, because communication systems and the rapidity of responses differently influence the monitoring activities in various environments. Evidently, pollution is concentrated off the coastal areas [177], where anthropized urban settlements are mainly located and maritime traffic is intense [178]. In the case of the Mediterranean, for example, which is almost completely surrounded by lands, ecosystems may be extremely fragile and vulnerable because their waters are slowly renewable, thus making them sensitive to all kinds of pollutants, especially when derived from commercial traffic, industrial pollution, or touristic activities [179]. In parallel, these areas are characterized by valuable and fragile environments such as seagrass meadows and coralligenous areas [180,181], and they deserve a higher degree of monitoring and conservation practices [182]. This task is partially accomplished by the institution of MPAs and sanctuaries, but again, they require a higher level of monitoring and immediate reaction to stresses produced by anthropic activities in order to conserve key reproductive areas and fragile environments [183,184]. In this case, communications are not the most important problem, since the presence of coasts closer to the monitoring areas guarantee a fast transfer of information to the computing centers [176]. In contrast, oil pollution has become a matter of serious environmental concern in all oceans [26], with petroleum hydrocarbons (gasoline, kerosene, fuel oil, etc.) penetrating shallow and deeper environments through spills or leaks, as well as after frequent accidents [27]. Here, the rapid delivery of signals becomes critical because coastal stations are quite far away, and satellite communications become indispensable.

An ocean-sensing and monitoring network is a monitoring system that has basically been applied since the last century because oceanographic and hydrographic research vessels were previously adopted for this purpose. A water quality monitoring system usually monitors water conditions and quality, including water temperature, pH, turbidity, conductivity, and dissolved oxygen (DO) in bays, lakes, rivers, and other water bodies. A coral reef monitoring system typically monitors coral reef habitats and the surrounding environments. A marine fish farm monitoring system checks water conditions and quality, including the temperature and pH. It measures the levels of waste and uneaten feed in a fish farm, as well as fish conditions and activities including the presence of dead fish. A wave and current monitoring system measures waves and currents for safe and secure waterway navigation [13]. The most common tools traditionally used to monitor marine environments are satellite imagery, underwater devices with various sensors, and buoys [184]. These devices transmit data by means of satellite communications or close-range base stations, which present several limitations and elevated infrastructure costs. Unmanned aerial vehicles (UAVs), as described above, are an alternative for remote environmental monitoring which provides new types of data and ease of use. These techniques are mainly used in video capture-related applications in its various light spectra and do not provide the same data as sensing buoys, nor can they be used for extended periods of time [184]. However, it is important to stress that monitoring the marine environment is quite challenging, because it requires waterproof robust technology to endure the high levels of humidity and salinity, wave collisions, and extreme weather conditions [135].

In this light, the development of newer "noses", coupled with the powerful features of various kinds of UMVs as classified above, may represent a tremendous innovation toward the collection of data in an efficient way, with minimum costs and fast delivery of strategic information. In this review, we have described, from an historical perspective, the main strategies of monitoring coastal and ocean areas, showing that several smart solutions are presently available, although most of them still need complete engineering to reach full applicability, perfect automation, and their best performance.

Author Contributions: Conceptualization, M.C. and V.Z.; methodology, F.G., F.E.; software, F.G., L.G., A.R., M.M., P.B. and R.E.; formal analysis, V.Z. and F.G.; resources, M.C., V.Z., F.E.; data curation, M.C.; writing—original draft preparation, F.G., M.C. and V.Z.; writing—review and editing, V.Z. and M.C.; supervision, A.D.C., M.C. and V.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval are not applicable for this review.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: SENSIPLUS is a microsensor platform developed by Sensichips.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chuaysi, B.; Kiattisin, S. Fishing Vessels Behavior Identification for Combating IUU Fishing: Enable Traceability at Sea. *Wirel. Pers. Commun.* **2020**, *115*, 2971–2993. [CrossRef]
2. Al-Absi, M.A.; Kamolov, A.; Al-Absi, A.A.; Sain, M.; Lee, H.J. IoT Technology with Marine Environment Protection and Monitoring. In *International Conference on Smart Computing and Cyber Security*; Springer: Singapore, 2021; pp. 81–89.
3. DONET System Concept. Available online: https://www.jamstec.go.jp/donet/e/ (accessed on 16 January 2022).
4. González, F.I.; Milburn, H.B.; Bernard, E.N.; Newman, J. Deep-Ocean Assessment and Reporting of Tsunamis (DART®): Brief Overview and Status Report. In Proceedings of the International Workshop on Tsunami Disaster Mitigation, Tokyo, Japan, 19 January 1998; pp. 19–22.
5. Meinig, C.; Stalin, S.; Stalin, S.E.; Nakamura, A.I.; Milburn, H.B. *Real-Time Deep-Ocean Tsunami Measuring, Monitoring, and Reporting System: The NOAA DART II Description and Disclosure*; NOAA: Washington, DC, USA, 2005.
6. Tonacci, A.; Lippa, M.A.; Pioggia, G.; Domenici, C.; Lacava, G.; Lupi, L.; Gualdesi, L.; Cocco, M. A Smart Multimodal Innovative Model For Marine Environmental Monitoring. In Proceedings of the 29th European Conference on Modelling and Simulation ECMS, Albena, Bulgaria, 26–29 May 2015; pp. 455–461.
7. Milburn, H.B.; Nakamura, A.I.; Gonzalez, F.I. Real-Time Tsunami Reporting from the Deep Ocean. In Proceedings of the Oceans Conference Record (IEEE), Lauderdale, FL, USA, 3–26 September 1996; Volume 1, pp. 390–394.
8. Kim, S.; Lee, W.; Kwon, H.; Kim, J. Design and Preliminary Implementation of an IoT-Based System for Ocean Observation Buoys. In Proceedings of the ITC-CSCC, Okinawa, Japan, 10 July 2016; pp. 865–867.
9. Kim, S.M.; Lee, U.H.; Kwon, H.J.; Kim, J.Y.; Kim, J. Development of an IoT Platform for Ocean Observation Buoys. *IEIE Trans. Smart Process. Comput.* **2017**, *6*, 109–116. [CrossRef]
10. Boonma, P.; Suzuki, J. An Adaptive, Scalable and Self-Healing Sensor Network Architecture for Autonomous Coastal Environmental Monitoring. In Proceedings of the IEEE Conference on Technologies for Homeland Security, Woburn, MA, USA, 16–17 May 2007; p. 18.
11. Albaladejo, C.; Sánchez, P.; Iborra, A.; Soto, F.; López, J.A.; Torres, R. Wireless Sensor Networks for Oceanographic Monitoring: A Systematic Review. *Sensors* **2010**, *10*, 6948–6968. [CrossRef] [PubMed]
12. Hadim, S.; Mohamed, N. Middleware: Middleware Challenges and Approaches for Wireless Sensor Networks. *IEEE Distrib. Syst. Online* **2006**, *7*, 1–23. [CrossRef]
13. Xu, G.; Shi, Y.; Sun, X.; Shen, W. Internet of Things in Marine Environment Monitoring: A Review. *Sensors* **2019**, *19*, 1711. [CrossRef]
14. Alippi, C.; Camplani, R.; Galperti, C.; Roveri, M. Effective Design of WSNs: From the Lab to the Real World. In Proceedings of the 3rd International Conference on Sensing Technology, ICST 2008, Taipei, Taiwan, 30 November–3 December 2008; pp. 1–9. [CrossRef]
15. Cardei, M.; Wu, J. Energy-Efficient Coverage Problems in Wireless Ad-Hoc Sensor Networks. *Comput. Commun.* **2006**, *29*, 413–420. [CrossRef]

16. Alpers, W.; Hühnerfuss, H. The Damping of Ocean Waves by Surface Films: A New Look at an Old Problem. *J. Geophys. Res. Ocean.* **1989**, *94*, 6251–6265. [CrossRef]
17. Topouzelis, K.; Karathanassi, V.; Pavlakis, P.; Rokos, D. Detection and Discrimination between Oil Spills and Look-Alike Phenomena through Neural Networks. *ISPRS J. Photogramm. Remote Sens.* **2007**, *62*, 264–270. [CrossRef]
18. Van der Meer, F.; Jong, S. De Imaging spectrometry: Basic principles and prospective applications. In *Basic Principles of Imaging Spectrometry*; Kluwer Academic: Alphen aan den Rijn, The Netherlands, 2001; pp. 21–23.
19. Martins, F.; Leitão, P.; Silva, A.; Neves, R. 3D Modelling in the Sado Estuary Using a New Generic Vertical Discretization Approach. *Oceanol. Acta* **2001**, *24*, 51–62. [CrossRef]
20. Tonacci, A.; Corda, D.; Tartarisco, G.; Pioggia, G.; Domenici, C. A Smart Sensor System for Detecting Hydrocarbon Volatile Organic Compounds in Sea Water. *CLEAN–Soil Air Water* **2015**, *43*, 147–152. [CrossRef]
21. Bourgeois, W.; Stuetz, R.M. Use of a Chemical Sensor Array for Detecting Pollutants in Domestic Wastewater. *Water Res.* **2002**, *36*, 4505–4512. [CrossRef]
22. Sobański, T.; Szczurek, A.; Nitsch, K.; Licznerski, B.W.; Radwan, W. Electronic Nose Applied to Automotive Fuel Qualification. *Sens. Actuators B Chem.* **2006**, *116*, 207–212. [CrossRef]
23. Pieri, G.; Cocco, M.; Salvetti, O. A Marine Information System for Environmental Monitoring: ARGO-MIS. *J. Mar. Sci. Eng.* **2018**, *6*, 15. [CrossRef]
24. Moroni, D.; Pieri, G.; Tampucci, M.; Salvetti, O. Environmental Monitoring Integrated with a Proactive Marine Information System. *Proceedings* **2018**, *2*, 98. [CrossRef]
25. Tonacci, A.; Lacava, G.; Lippa, M.A.; Lupi, L.; Cocco, M.; Domenici, C. Electronic Nose and AUV: A Novel Perspective in Marine Pollution Monitoring. *Mar. Technol. Soc. J.* **2015**, *49*, 18–24. [CrossRef]
26. Ines, Z.; Amina, B.; Mahmoud, R.; Dalila, S.-M. Aliphatic and Aromatic Biomarkers for Petroleum Hydrocarbon Monitoring in Khniss Tunisian-Coast, (Mediterranean Sea). *Procedia Environ. Sci.* **2013**, *18*, 211–220. [CrossRef]
27. Mille, G.; Asia, L.; Guiliano, M.; Malleret, L.; Doumenq, P. Hydrocarbons in Coastal Sediments from the Mediterranean Sea (Gulf of Fos Area, France). *Mar. Pollut. Bull.* **2007**, *54*, 566–575. [CrossRef]
28. Marcelli, M.; Piermattei, V.; Gerin, R.; Brunetti, F.; Pietrosemoli, E.; Addo, S.A.M.; Boudaya, L.; Coleman, R.; Nubi, O.A.; Jojannes, R. Toward the Widespread Application of Low-Cost Technologies in Coastal Ocean Observing (Internet of Things for the Ocean). *Mediterr. Mar. Sci.* **2021**, *22*, 255–269. [CrossRef]
29. Derycke, S.; Remerie, T.; Vierstraete, A.; Backeljau, T.; Vanfleteren, J.; Vincx, M.; Moens, T. Mitochondrial DNA Variation and Cryptic Speciation within the Free-Living Marine Nematode Pellioditis Marina. *Mar. Ecol. Prog. Ser.* **2005**, *300*, 91–103. [CrossRef]
30. Sogin, M.L.; Morrison, H.G.; Huber, J.A.; Welch, D.M.; Huse, S.M.; Neal, P.R.; Arrieta, J.M.; Herndl, G.J. Microbial Diversity in the Deep Sea and the Underexplored "Rare Biosphere". *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12115–12120. [CrossRef]
31. Danovaro, R.; Gambi, C.; Dell'Anno, A.; Corinaldesi, C.; Fraschetti, S.; Vanreusel, A.; Vincx, M.; Gooday, A.J. Exponential Decline of Deep-Sea Ecosystem Functioning Linked to Benthic Biodiversity Loss. *Curr. Biol.* **2008**, *18*, 1–8. [CrossRef] [PubMed]
32. Danovaro, R.; Snelgrove, P.V.R.; Tyler, P. Challenging the Paradigms of Deep-Sea Ecology. *Trends Ecol. Evol.* **2014**, *29*, 465–475. [CrossRef] [PubMed]
33. Aguado, E.; Milosevic, Z.; Hernández, C.; Sanz, R.; Garzon, M.; Bozhinoski, D.; Rossi, C. Functional Self-Awareness and Metacontrol for Underwater. Robot Autonomy. *Sensors* **2021**, *21*, 1210. [CrossRef] [PubMed]
34. Eriksen, C.C.; Osse, T.J.; Light, R.D.; Wen, T.; Lehman, T.W.; Sabin, P.L.; Ballard, J.W.; Chiodi, A.M. Seaglider: A Long-Range Autonomous Underwater Vehicle for Oceanographic Research. *IEEE J. Ocean. Eng.* **2001**, *26*, 424–436. [CrossRef]
35. Funaki, M.; Hirasawa, N. Outline of a Small Unmanned Aerial Vehicle (Ant-Plane) Designed for Antarctic Research. *Polar Sci.* **2008**, *2*, 129–142. [CrossRef]
36. Leong, S.C.Y.; Tkalich, P.; Patrikalakis, N.M. Monitoring Harmful Algal Blooms in Singapore: Developing a HABs Observing System. In Proceedings of the Program Book-OCEANS 2012 MTS/IEEE Yeosu: The Living Ocean and Coast-Diversity of Resources and Sustainable Activities, Yeosu, Korea, 21–24 May 2012. [CrossRef]
37. Meyer, D. Glider Technology for Ocean Observations: A Review. *Ocean. Sci. Discuss.* **2016**, *40*, 1–26. [CrossRef]
38. Williams, S.B.; Pizarro, O.; Webster, J.M.; Beaman, R.J.; Mahon, I.; Johnson-Roberson, M.; Bridge, T.C.L. Autonomous Underwater Vehicle–Assisted Surveying of Drowned Reefs on the Shelf Edge of the Great Barrier Reef, Australia. *J. Field Robot.* **2010**, *27*, 675–697. [CrossRef]
39. Wynn, R.B.; Huvenne, V.A.I.; Le Bas, T.P.; Murton, B.J.; Connelly, D.P.; Bett, B.J.; Ruhl, H.A.; Morris, K.J.; Peakall, J.; Parsons, D.R.; et al. Autonomous Underwater Vehicles (AUVs): Their Past, Present and Future Contributions to the Advancement of Marine Geoscience. *Mar. Geol.* **2014**, *352*, 451–468. [CrossRef]
40. Inoue, J.; Curry, J.A.; Maslanik, J.A. Application of Aerosondes to Melt-Pond Observations over Arctic Sea Ice. *J. Atmos. Ocean. Technol.* **2008**, *25*, 327–334. [CrossRef]
41. Forney, K.A.; Ferguson, M.C.; Becker, E.A.; Fiedler, P.C.; Redfern, J.V.; Barlow, J.; Vilchis, I.L.; Ballance, L.T. Habitat-Based Spatial Models of Cetacean Density in the Eastern Pacific Ocean. *Endanger. Species Res.* **2012**, *16*, 113–133. [CrossRef]
42. Hodgson, A.J.; Noad, M.; Marsh, H.; Lanyon, J.; Kniest, E. Using Unmanned Aerial Vehicles for Surveys of Marine Mammals in Australia: Test of Concept. Available online: https://espace.library.uq.edu.au/view/UQ:690328 (accessed on 31 July 2021).

43. Koski, W.R.; Allen, T.; Ireland, D.; Buck, G.; Smith, P.R.; Macrander, A.M.; Halick, M.A.; Rushing, C.; Sliwa, D.J.; Mcdonald, T.L. Evaluation of an Unmanned Airborne System for Monitoring Marine Mammals. *Aquat. Mamm.* **2009**, *35*, 347–357. [CrossRef]
44. Lyons, C.; Koski, W.R.; Ireland, D.S. Unmanned Aerial Surveys. In *Joint Monitoring Program in the Chukchi and Beaufort Seas, Open Water Seasons 2006*; LGL Alaska Research Associates: Anchorage, AK, USA, 2008; Chapter 8; 15p.
45. Llewellyn, L.E.; Bainbridge, S.J. Getting up Close and Personal: The Need to Immerse Autonomous Vehicles in Coral Reefs. In Proceedings of the OCEANS 2015-MTS/IEEE, Washington, DC, USA, 19–22 October 2016. [CrossRef]
46. Huvenne, V.A.I.; Bett, B.J.; Masson, D.G.; Le Bas, T.P.; Wheeler, A.J. Effectiveness of a Deep-Sea Cold-Water Coral Marine Protected Area, Following Eight Years of Fisheries Closure. *Biol. Conserv.* **2016**, *200*, 60–69. [CrossRef]
47. Benoist, N.M.A.; Morris, K.J.; Bett, B.J.; Durden, J.M.; Huvenne, V.A.I.; Le Bas, T.P.; Wynn, R.B.; Ware, S.J.; Ruhl, H.A. Monitoring Mosaic Biotopes in a Marine Conservation Zone by Autonomous Underwater Vehicle. *Conserv. Biol.* **2019**, *33*, 1174–1186. [CrossRef] [PubMed]
48. European Environment. *Agency Marine Protected Areas-Designed to Conserve Europe's Marine Life, Marine Protected Areas Are a Globally Recognised Tool for Managing and Enhancing Our Marine Ecosystems*; European Environment Agency (EEA): Copenhagen, Denmark, 2018.
49. Furlong, M.E.; Paxton, D.; Stevenson, P.; Pebody, M.; McPhail, S.D.; Perrett, J. Autosub Long Range: A Long Range Deep Diving AUV for Ocean Monitoring. In Proceedings of the 2012 IEEE/OES Autonomous Underwater Vehicles, AUV 2012, Southampton, UK, 24–27 September 2012. [CrossRef]
50. Smale, D.A.; Kendrick, G.A.; Harvey, E.S.; Langlois, T.J.; Hovey, R.K.; Van Niel, K.P.; Waddington, K.I.; Bellchambers, L.M.; Pember, M.B.; Babcock, R.C.; et al. Regional-Scale Benthic Monitoring for Ecosystem-Based Fisheries Management (EBFM) Using an Autonomous Underwater Vehicle (AUV). *ICES J. Mar. Sci.* **2012**, *69*, 1108–1118. [CrossRef]
51. Robbins, I.C.; Kirkpatrick, G.J.; Blackwell, S.M.; Hillier, J.; Knight, C.A.; Moline, M.A. Improved Monitoring of HABs Using Autonomous Underwater Vehicles (AUV). *Harmful Algae* **2006**, *5*, 749–761. [CrossRef]
52. Ramos, P.; Cruz, N.; Matos, A.; Neves, M.V.; Pereira, F.L. Monitoring an Ocean Outfall Using an AUV. In Proceedings of the Oceans Conference Record (IEEE), Honolulu, HI, USA, 5–8 November 2001; Volume 3, pp. 2009–2014. [CrossRef]
53. Marini, S.; Gjeci, N.; Govindaraj, S.; But, A.; Sportich, B.; Ottaviani, E.; Márquez, F.; Sánchez, P.J.B.; Pedersen, J.; Clausen, C.V.; et al. ENDURUNS: An Integrated and Flexible Approach for Seabed Survey Through Autonomous Mobile Vehicles. *J. Mar. Sci. Eng.* **2020**, *8*, 633. [CrossRef]
54. Jones, D.O.B.; Gates, A.R.; Huvenne, V.A.I.; Phillips, A.B.; Bett, B.J. Autonomous Marine Environmental Monitoring: Application in Decommissioned Oil Fields. *Sci. Total Environ.* **2019**, *668*, 835–853. [CrossRef]
55. Huvenne, V.A.I.; Robert, K.; Marsh, L.; Lo Iacono, C.; Le Bas, T.; Wynn, R.B. ROVs and AUVs. In *Submarine Geomorphology*; Springer Geology: Cham, Swizerland, 2018; pp. 93–108. [CrossRef]
56. Jawhar, I.; Mohamed, N.; Al-Jaroodi, J.; Zhang, S. An Architecture for Using Autonomous Underwater Vehicles in Wireless Sensor Networks for Underwater Pipeline Monitoring. *IEEE Trans. Ind. Inform.* **2019**, *15*, 1329–1340. [CrossRef]
57. He, Y.; Wang, D.B.; Ali, Z.A. A Review of Different Designs and Control Models of Remotely Operated Underwater Vehicle. *Meas. Control.* **2020**, *53*, 1561–1570. [CrossRef]
58. Griffiths, G. *Technology and Applications of Autonomous Underwater Vehicles-Google Libri*; CRC Press: Boca Raton, FL, USA, 2002; Volume 2.
59. Wynn, R.B.; Evans, A.J.; Griffiths, G.; Jones, V.A.I.; Palmer, A.R.; Dove, M.R.; Boyd, J.A. NERC-MAREMAP Report to Defra: AUVs and Gliders for MPA Mapping and Monitoring. Available online: https://eprints.soton.ac.uk/372785/1/DEFRA_MB0118%2528Wynn%2529_FINAL.pdf (accessed on 31 July 2021).
60. Verfuss, U.K.; Aniceto, A.S.; Harris, D.V.; Gillespie, D.; Fielding, S.; Jiménez, G.; Johnston, P.; Sinclair, R.R.; Sivertsen, A.; Solbø, S.A.; et al. A Review of Unmanned Vehicles for the Detection and Monitoring of Marine Fauna. *Mar. Pollut. Bull.* **2019**, *140*, 17–29. [CrossRef]
61. Majid, M.H.A.; Arshad, M.R. Design of an Autonomous Surface Vehicle (ASV) for Swarming Application. In Proceedings of the Autonomous Underwater Vehicles 2016, AUV 2016, Tokyo, Japan, 6–9 November 2016; pp. 230–235. [CrossRef]
62. Rodriguez-Ortiz, C.D. Automated Bathymetry Mapping Using an Autonomous Surface Craft. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA, USA, 1996.
63. Pahl, J.; Voß, S. *Maritime Load Dependent Lead Times-An Analysis*; Springer: Cham, Swizerland, 2017; Volume 10572, ISBN 9783319684956.
64. Odetti, A.; Bruzzone, G.; Altosole, M.; Viviani, M.; Caccia, M. SWAMP, an Autonomous Surface Vehicle Expressly Designed for Extremely Shallow Waters. *Ocean. Eng.* **2020**, *216*, 108205. [CrossRef]
65. Aguzzi, J.; Chatzievangelou, D.; Marini, S.; Fanelli, E.; Danovaro, R.; Flögel, S.; Lebris, N.; Juanes, F.; De Leo, F.C.; Del Rio, J.; et al. New High-Tech Flexible Networks for the Monitoring of Deep-Sea Ecosystems. *Environ. Sci. Technol.* **2019**, *53*, 6616–6631. [CrossRef] [PubMed]
66. Wang, F.; Zhu, J.; Chen, L.; Zuo, Y.; Hu, X.; Yang, Y. Autonomous and In Situ Ocean Environmental Monitoring on Optofluidic Platform. *Micromachines* **2020**, *11*, 69. [CrossRef]
67. Zhang, Y.; Ryan, J.P.; Kieft, B.; Hobson, B.W.; McEwen, R.S.; Godin, M.A.; Harvey, J.B.; Bellingham, J.G.; Birch, J.M.; Scholin, C.A.; et al. Targeted Sampling by Autonomous Underwater Vehicles. *Front. Mar. Sci.* **2019**, *6*, 415. [CrossRef]

68. Rountree, R.A.; Aguzzi, J.; Marini, S.; Fanelli, E.; De Leo, F.C.; Del Rio, J.; Juanes, F. Towards an optimal design for ecosystem-level ocean observatories. In *Oceanography and Marine Biology*; Taylor & Francis: Abingdon, UK, 2020.
69. Hwang, J.; Bose, N.; Fan, S. AUV Adaptive Sampling Methods: A Review. *Appl. Sci.* **2019**, *9*, 3145. [CrossRef]
70. Roper, D.T.; Phillips, A.B.; Harris, C.A.; Salavasidis, G.; Pebody, M.; Templeton, R.; Amma, S.V.S.; Smart, M.; McPhail, S. Autosub Long Range 1500: An Ultra-Endurance AUV with 6000 Km Range. In *OCEANS 2017-Aberdeen*; IEEE: Piscataway, NJ, USA, 2017; pp. 1–5. [CrossRef]
71. Roper, D.; Harris, C.A.; Salavasidis, G.; Pebody, M.; Templeton, R.; Prampart, T.; Kingsland, M.; Morrison, R.; Furlong, M.; Phillips, A.B.; et al. Autosub Long Range 6000: A Multiple-Month Endurance AUV for Deep-Ocean Monitoring and Survey. *IEEE J. Ocean. Eng.* **2021**, *46*, 1179–1191. [CrossRef]
72. Davis, R.E.; Eriksen, C.C.; Jones, C.P. Autonomous Buoyancy-Driven Underwater Gliders. In *The Technology and Applications of Autonomous Underwater Vehicles*; CRC Press: London, UK, 2002; pp. 37–58.
73. Webb, D.C.; Simonetti, P.J.; Jones, C.P. SLOCUM: An Underwater Glider Propelled by Environmental Energy. *IEEE J. Ocean. Eng.* **2001**, *26*, 447–452. [CrossRef]
74. Imlach, J.; Mahr, R. Modification of a Military Grade Glider for Coastal Scientific Applications. In Proceedings of the OCEANS 2012 MTS/IEEE: Harnessing the Power of the Ocean, Hampton Roads, VA, USA, 14–19 October 2012. [CrossRef]
75. ODYSSEA | Operating a Network of Integrated Observatory Systems in the Mediterranean Sea. Available online: https://odysseaplatform.eu/ (accessed on 24 September 2021).
76. Sherman, J.; Davis, R.E.; Owens, W.B.; Valdes, J. The Autonomous Underwater Glider "Spray". *IEEE J. Ocean. Eng.* **2001**, *26*, 437–446. [CrossRef]
77. Osse, T.J.; Eriksen, C.C. The Deepglider: A Full Ocean Depth Glider for Oceanographic Research. In Proceedings of the Oceans Conference Record (IEEE), Vancouver, BC, Canada, 29 September–4 October 2007. [CrossRef]
78. Guidetti, P. The Importance of Experimental Design in Detecting the Effects of Protection Measures on Fish in Mediterranean MPAs. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2002**, *12*, 619–634. [CrossRef]
79. Sciberras, M.; Jenkins, S.R.; Kaiser, M.J.; Hawkins, S.J.; Pullin, A.S. Evaluating the Biological Effectiveness of Fully and Partially Protected Marine Areas. *Environ. Evid.* **2013**, *2*, 4. [CrossRef]
80. Hayes, K.R.; Hosack, G.R.; Lawrence, E.; Hedge, P.; Barrett, N.S.; Przeslawski, R.; Caley, M.J.; Foster, S.D. Designing Monitoring Programs for Marine Protected Areas Within an Evidence Based Decision Making Paradigm. *Front. Mar. Sci.* **2019**, *6*, 746. [CrossRef]
81. Farrell, J.A.; Pang, S.; Li, W. Chemical Plume Tracing via an Autonomous Underwater Vehicle. *IEEE J. Ocean. Eng.* **2005**, *30*, 428–442. [CrossRef]
82. Sensichips: Learning Microsensors. Available online: https://sensichips.com/ (accessed on 24 September 2021).
83. Bria, A.; Cerro, G.; Ferdinandi, M.; Marrocco, C.; Molinara, M. An IoT-Ready Solution for Automated Recognition of Water Contaminants. *Pattern Recognit. Lett.* **2020**, *135*, 188–195. [CrossRef]
84. Bourelly, C.; Bria, A.; Ferrigno, L.; Gerevini, L.; Marrocco, C.; Molinara, M.; Cerro, G.; Cicalini, M.; Ria, A. A Preliminary Solution for Anomaly Detection in Water Quality Monitoring. In Proceedings of the 2020 IEEE International Conference on Smart Computing, Bologna, Italy, 14–17 September 2020; Institute of Electrical and Electronics Engineers Inc.: Piscataway, NJ, USA, 2020; pp. 410–415.
85. Aguzzi, J.; Chatzievangelou, D.; Company, J.B.; Thomsen, L.; Marini, S.; Bonofiglio, F.; Juanes, F.; Rountree, R.; Berry, A.; Chumbinho, R.; et al. The Potential of Video Imagery from Worldwide Cabled Observatory Networks to Provide Information Supporting Fish-Stock and Biodiversity Assessment. *ICES J. Mar. Sci.* **2020**, *77*, 2396–2410. [CrossRef]
86. Danovaro, R.; Aguzzi, J.; Fanelli, E.; Billett, D.; Gjerde, K.; Jamieson, A.; Ramirez-Llodra, E.; Smith, C.; Snelgrove, P.; Van Dover, C. An Ecosystem-Based Deep-Ocean Strategy. *Science* **2017**, *355*, 452–454. [CrossRef]
87. Danovaro, R.; Fanelli, E.; Aguzzi, J.; Billett, D.; Carugati, L.; Corinaldesi, C.; Dell'Anno, A.; Gjerde, K.; Jamieson, A.J.; Kark, S.; et al. Ecological Variables for Developing a Global Deep-Ocean Monitoring and Conservation Strategy. *Nat. Ecol. Evol.* **2020**, *4*, 181–192. [CrossRef]
88. Gaughan, P.J.; Kolar, H.R. Implementing a Smartbay on the West Coast of Ireland. *J. Ocean. Technol.* **2010**, *5*, 55–70.
89. Leocádio, A.; Weetman, A.; Wieland, K. (Eds.) *Using Underwater Television Surveys to Assess and Advise on Nephrops Stocks*; International Council for the Exploration of the Sea. ICES Cooperative: UK, 2018; ISBN 9788774822127.
90. JNCC. *Joint Nature Conservation Committee Scientific Advice on Possible Offshore Marine Conservation Zones Considered for Consultation in 2015*; JNCC: Peterborough, UK, 2014.
91. Bell, E.; Clements, A.; Dobby, H.; Doyle, J.; Feekings, J.; Leocádio, A.; Lordan, C.; Weetman, A.; Wieland, K. Using Underwater Television Surveys to Assess and Advise on Nephrops Stocks. In *ICES Cooperative Research Report*; International Council for the Exploration of the Sea: Copenhagen, Denmark, 2018.
92. Cristini, L.; Lampitt, R.S.; Cardin, V.; Delory, E.; Haugan, P.; O'Neill, N.; Petihakis, G.; Ruhl, H.A. Cost and Value of Multidisciplinary Fixed-Point Ocean Observatories. *Mar. Policy* **2016**, *71*, 138–146. [CrossRef]
93. Locascio, J.; Mann, D.; Wilcox, K.; Luther, M. Incorporation of Acoustic Sensors on a Coastal Ocean Monitoring Platform for Measurements of Biological Activity. *Mar. Technol. Soc. J.* **2018**, *52*, 64–70. [CrossRef]

94. Aguzzi, J.; Doya, C.; Tecchio, S.; De Leo, F.C.; Azzurro, E.; Costa, C.; Sbragaglia, V.; Del Río, J.; Navarro, J.; Ruhl, H.A.; et al. Coastal Observatories for Monitoring of Fish Behaviour and Their Responses to Environmental Changes. *Rev. Fish Biol. Fish.* **2015**, *25*, 463–483. [CrossRef]
95. Thomsen, L.; Aguzzi, J.; Costa, C.; De Leo, F.; Ogston, A.; Purser, A. The Oceanic Biological Pump: Rapid Carbon Transfer to Depth at Continental Margins during Winter. *Sci. Rep.* **2017**, *7*, 10763. [CrossRef] [PubMed]
96. Ocean Networks Canada. Available online: https://www.oceannetworks.ca/ (accessed on 24 September 2021).
97. Ten Years (2006–2016) of Oceanographic Temperature, Salinity, Pressure, Density and Dissolved Oxygen Data from the Saanich Inlet Cabled Observatory-Ocean Networks Canada. Available online: https://www.oceannetworks.ca/ (accessed on 24 September 2021).
98. Blondel, P.; Hatta, A.A.Z. Acoustic Soundscapes and Biodiversity–Comparing Metrics, Seasons and Depths with Data from the Neptune Ocean Observatory Offshore British Columbia. In Proceedings of the UACE2017-4th Underwater Acoustics Conference and Exhibition ACOUSTIC, Skiathos, Greece, 11 September 2017; pp. 763–768.
99. Hendricks, B.; Wray, J.L.; Keen, E.M.; Alidina, H.M.; Gulliver, T.A.; Picard, C.R. Automated Localization of Whales in Coastal Fjords. *J. Acoust. Soc. Am.* **2019**, *146*, 4672. [CrossRef] [PubMed]
100. Orlov, A. Possible Ways of Exchange between Asian and American Ichthyofaunas in the North Pacific Ocean. *ICES CM* **2003**, Q:09 (2003b). Available online: https://www.researchgate.net/publication/228961925_Possible_ways_of_exchange_between_Asian_and_American_ichthyofaunas_in_the_North_Pacific_Ocean (accessed on 31 July 2021).
101. Hanselman, D.; Heifetz, J.; Echave, K.; Dressel, S. Move It or Lose It: Movement and Mortality of Sablefish Tagged in Alaska. *Can. J. Fish. Aquat. Sci.* **2014**, *72*, 238–251. [CrossRef]
102. Ocean Observatories Initiative–A New Era of Oceanography. Available online: https://oceanobservatories.org/ (accessed on 24 September 2021).
103. EMSO–Observing the Ocean to Save the Earth. Available online: http://emso.eu/ (accessed on 24 September 2021).
104. Favali, P.; Beranzoli, L. Seafloor Observatory Science: A Review. *Geophys* **2006**, *49*, 515–567. [CrossRef]
105. Best, M.; Favali, P.; Beranzoli, L.; Cannat, M.; Cagatay, N.; Dañobeitia, J.J.; Delory, E.; De Stigter, H.; Ferré, B.; Gillooly, M.; et al. EMSO: A Distributed Infrastructure for Addressing Geohazards and Global Ocean Change. *Oceanography* **2014**, *27*, 167–169. [CrossRef]
106. Ruhl, H.A.; André, M.; Beranzoli, L.; Çağatay, M.N.; Colaço, A.; Cannat, M.; Dañobeitia, J.J.; Favali, P.; Géli, L.; Gillooly, M.; et al. Societal Need for Improved Understanding of Climate Change, Anthropogenic Impacts, and Geo-Hazard Warning Drive Development of Ocean Observatories in European Seas. *Prog. Oceanogr.* **2011**, *91*, 1–33. [CrossRef]
107. KM3NeT-Opens a New Window on Our Universe. Available online: https://www.km3net.org/ (accessed on 24 September 2021).
108. Breton, L.; Collaboration, N. KM3NeT: Next-generation neutrino telescope in the Mediterranean Sea. *Nucl. Instrum. Methods Phys. Res. Sect. A Accel. Spectrometers Detect. Assoc. Equip.* **2019**, *936*, 204–207. [CrossRef]
109. JERICO-S3 | Joint European Research Infrastructure for Coastal Observatories. Available online: https://www.jerico-ri.eu/ (accessed on 24 September 2021).
110. Puillat, I.; Farcy, P.; Durand, D.; Karlson, B.; Petihakis, G.; Seppälä, J.; Sparnocchia, S. Progress in Marine Science Supported by European Joint Coastal Observation Systems: The JERICO-RI Research Infrastructure. *J. Mar. Syst.* **2016**, *162*, 1–3. [CrossRef]
111. Cotroneo, Y.; Aulicino, G.; Ruiz, S.; Sánchez Román, A.; Torner Tomàs, M.; Pascual, A.; Fusco, G.; Heslop, E.; Tintoré, J.; Budillon, G. Glider Data Collected during the Algerian Basin Circulation Unmanned Survey. *Earth Syst. Sci. Data* **2019**, *11*, 147–161. [CrossRef]
112. First Call | JERICO Research Infrastructure. Available online: https://www.jerico-ri.eu/ta/call-program/first-call/ (accessed on 24 September 2021).
113. Van Kranenburg, R. *The Internet of Things: A Critique of Ambient Technology and the All-Seeing Network of RFID*; Institute of Network Cultures: Amsterdam, The Netherlands, 2008.
114. Yang, C.; Shen, W.; Wang, X. The Internet of Things in Manufacturing: Key Issues and Potential Applications. *IEEE Syst. Man Cybern. Mag.* **2018**, *4*, 6–15. [CrossRef]
115. IERC-European Research Cluster on the Internet of Things. Available online: http://internet-of-things-research.eu/ (accessed on 24 September 2021).
116. Li, C.Z.E.; Deng, Z.W. The Embedded Modules Solution of Household Internet of Things System and The Future Development. *Procedia Comput. Sci.* **2020**, *166*, 350–356. [CrossRef]
117. DG INFSO; EPoSS. Internet of Things: A Roadmap for the Future. *INFSO D* **2008**, *4*, 3–27.
118. Rose, K.; Eldridge, S.; Chapin, L. The Internet of Things: An Overview Understanding the Issues and Challenges of a More Connected World. *Proc. Internet Soc. (ISOC)* **2015**, *57*, 1–53.
119. Tarkoma, S.; Katasonov, A. Internet of Things Strategic Research Agenda. In Finnish Strategic Centre for Science, Technology and Innovation. Available online: http://www.internetofthings.fi/ (accessed on 12 December 2021).
120. Fang, S.; Xu, L.D.; Zhu, Y.; Ahati, J.; Pei, H.; Yan, J.; Liu, Z. An Integrated System for Regional Environmental Monitoring and Management Based on Internet of Things. *IEEE Trans. Ind. Inform.* **2014**, *10*, 1596–1605. [CrossRef]
121. Fleisch, E. What is the internet of things? An economic prospective. *Econ. Manag. Financ. Mark.* **2010**, *5*, 125–157.
122. Ray, P.P. A Survey on Internet of Things Architectures. *J. King Saud Univ. -Comput. Inf. Sci.* **2018**, *30*, 291–319. [CrossRef]

123. Mallon, S. IoT Is the Most Important Development of the 21st Century. Available online: https://www.smartdatacolletive.com/iot-most-important-development-of-21st-century/ (accessed on 12 December 2021).
124. Sarika, A.K.; Vinit, D.; Durafe, M.A. A Review Paper on Internet of Things and It's Applications. *Int. Res. J. Eng. Technol.* **2019**, *6*, 1623–1630.
125. Madakam, S.; Ramaswamy, R.; Tripathi, S. Internet of Things (IoT): A Literature Review. *Comput. Commun.* **2015**, *3*, 164–173. [CrossRef]
126. Eysenbach, G. What Is E-Health? *J. Med. Internet Res.* **2001**, *3*, e20. [CrossRef]
127. Ahmed, B.S.; Bures, M.; Frajtak, K.; Cerny, T. Aspects of Quality in Internet of Things (IoT) Solutions: A Systematic Mapping Study. *IEEE Access* **2019**, *7*, 13758–13780. [CrossRef]
128. Tziortzioti, C.; Amaxilatis, D.; Mavrommati, I.; Chatzigiannakis, I. IoT Sensors in Sea Water Environment: Ahoy! Experiences from a Short Summer Trial. *Electron. Notes Theor. Comput. Sci.* **2019**, *343*, 117–130. [CrossRef]
129. Zeinab, K.; Elmustafa, S. Internet of Things Applications, Challenges and Related Future Technologies. *World Sci. News* **2017**, *67*, 126–148.
130. Al Nuaimi, E.; Al Neyadi, H.; Mohamed, N.; Al-Jaroodi, J. Applications of Big Data to Smart Cities. *J. Internet Serv. Appl.* **2015**, *6*, 15. [CrossRef]
131. Khan, R.; Khan, S.U.; Zaheer, R.; Khan, S. Future Internet: The Internet of Things Architecture, Possible Applications and Key Challenges. In Proceedings of the 10th International Conference on Frontiers of Information Technology, FIT 2012, Islamabad, Pakistan, 17–19 December 2012; pp. 257–260.
132. Hong, W.J.; Shamsuddin, N.; Abas, E.; Apong, R.A.; Masri, Z.; Suhaimi, H.; Gödeke, S.H.; Noh, M.N.A. Water Quality Monitoring with Arduino Based Sensors. *Environments* **2021**, *8*, 6. [CrossRef]
133. Domingo, M.C. An Overview of the Internet of Things for People with Disabilities. *J. Netw. Comput. Appl.* **2012**, *35*, 584–596. [CrossRef]
134. Xu, G.; Shen, W.; Wang, X. Applications of Wireless Sensor Networks in Marine Environment Monitoring: A Survey. *Sensors* **2014**, *14*, 16932–16954. [CrossRef]
135. Sanchez-Iborra, R.; Liaño, I.G.; Simoes, C.; Couñago, E.; Skarmeta, A.F. Tracking and Monitoring System Based on LoRa Technology for Lightweight Boats. *Electronics* **2019**, *8*, 15. [CrossRef]
136. Al-Zaidi, R.; Woods, J.; Al-Khalidi, M.; Alheeti, K.M.A.; McDonald-Maier, K. Next Generation Marine Data Networks in an IoT Environment. In Proceedings of the 2017 2nd International Conference on Fog and Mobile Edge Computing, FMEC 2017, Valencia, Spain, 8–11 May 2017; pp. 50–55. [CrossRef]
137. Ebrahimi, S.H.; Ossewaarde, M.; Need, A. Smart Fishery: A Systematic Review and Research Agenda for Sustainable Fisheries in the Age of AI. *Sustainability* **2021**, *13*, 6037. [CrossRef]
138. Kritzer, J.P. Influences of At-Sea Fishery Monitoring on Science, Management, and Fleet Dynamics. *Aquac. Fish.* **2020**, *5*, 107–112. [CrossRef]
139. Sala, E.; Mayorga, J.; Costello, C.; Kroodsma, D.; Palomares, M.; Pauly, D.; Sumaila, U.; Zeller, D. The Economics of Fishing the High Seas. *Sci. Adv.* **2018**, *4*. [CrossRef]
140. Bartholomew, D.C.; Mangel, J.C.; Alfaro-Shigueto, J.; Pingo, S.; Jimenez, A.; Godley, B.J. Remote Electronic Monitoring as a Potential Alternative to On-Board Observers in Small-Scale Fisheries. *Biol. Conserv.* **2018**, *219*, 35–45. [CrossRef]
141. Luan, J.; Zhang, C.; Xu, B.; Xue, Y.; Ren, Y. The Predictive Performances of Random Forest Models with Limited Sample Size and Different Species Traits. *Fish. Res.* **2020**, *227*, 105534. [CrossRef]
142. Gloaguen, P.; Mahévas, S.; Rivot, E.; Woillez, M.; Guitton, J.; Vermard, Y.; Etienne, M.P. An Autoregressive Model to Describe Fishing Vessel Movement and Activity. *Environmetrics* **2015**, *26*, 17–28. [CrossRef]
143. Mutalipassi, M.; Esposito, R.; Ruocco, N.; Viel, T.; Costantini, M.; Zupo, V. Bioactive Compounds of Nutraceutical Value from Fishery and Aquaculture Discards. *Foods* **2021**, *10*, 1495. [CrossRef]
144. Franceschini, S.; Mattei, F.; D'Andrea, L.; Di Nardi, A.; Fiorentino, F.; Garofalo, G.; Scardi, M.; Cataudella, S.; Russo, T. Rummaging through the Bin: Modelling Marine Litter Distribution Using Artificial Neural Networks. *Mar. Pollut. Bull.* **2019**, *149*, 110580. [CrossRef]
145. Yang, X.; Zhang, S.; Liu, J.; Gao, Q.; Dong, S.; Zhou, C. Deep Learning for Smart Fish Farming: Applications, Opportunities and Challenges. *Rev. Aquac.* **2021**, *13*, 66–90. [CrossRef]
146. Kylili, K.; Hadjistassou, C.; Artusi, A. An Intelligent Way for Discerning Plastics at the Shorelines and the Seas. *Environ. Sci. Pollut. Res.* **2020**, *27*, 42631–42643. [CrossRef]
147. Cantorna, D.; Dafonte, C.; Iglesias, A.; Arcay, B. Oil Spill Segmentation in SAR Images Using Convolutional Neural Networks. A Comparative Analysis with Clustering and Logistic Regression Algorithms. *Appl. Soft Comput.* **2019**, *84*, 105716. [CrossRef]
148. Liu, S.; Liu, Y.; Alabia, I.; Tian, Y.; Ye, Z.; Yu, H.; Li, J.; Cheng, J. Impact of Climate Change on Wintering Ground of Japanese Anchovy (Engraulis Japonicus) Using Marine Geospatial Statistics. *Front. Mar. Sci.* **2020**, *7*, 604. [CrossRef]
149. Song, D.; Zhen, Z.; Wang, B.; Li, X.; Gao, L.; Wang, N.; Xie, T.; Zhang, T. A Novel Marine Oil Spillage Identification Scheme Based on Convolution Neural Network Feature Extraction from Fully Polarimetric SAR Imagery. *IEEE Access* **2020**, *8*, 59801–59820. [CrossRef]
150. Al-Ruzouq, R.; Gibril, M.B.A.; Shanableh, A.; Kais, A.; Hamed, O.; Al-Mansoori, S.; Khalil, M.A. Sensors, Features, and Machine Learning for Oil Spill Detection and Monitoring: A Review. *Remote Sens.* **2020**, *12*, 3338. [CrossRef]

151. Pittenger, R.; Anderson, B.; Benetti, D.D.; Dayton, P. *Sustainable Marine Aquaculture: Fulfilling the Promise; Managing the Risks*; Marine Aquaculture Task Force: Takoma Park, MD, USA, 2007.
152. Goldburg, R.J.; Elliott, M.S.; Nayor, R.L. Marine Aquaculture in the United States: Environmental Impacts and Policy Options. In *Pew Oceans Commission*; PEWOC: Arlington, VA, USA, 2001.
153. Braaten, B.R. Cage Aquaculture and Environmental Impacts. In *Aquacultural Engineering and Environment*; Research Signpost: Trivandrum, India, 2007; pp. 49–92.
154. Goldburg Future Seascapes, Fishing, and Fish Farming. *Front. Ecol. Environ.* **2005**, *3*, 21–28. [CrossRef]
155. Neill, W.H.; Brandes, T.S.; Burke, B.J.; Craig, S.R.; Dimichele, L.V.; Duchin, K.; Edwards, R.E.; Fontaine, L.P.; Gatlin, D.M.; Hutchins, C.; et al. Ecophys.Fish: A Simulation Model of Fish Growth in Time-Varying Environmental Regimes. *Rev. Fish. Sci.* **2004**, *12*, 233–288. [CrossRef]
156. Holmer, M. Environmental Issues of Fish Farming in Offshore Waters: Perspectives, Concerns and Research Needs. *Aquac. Environ. Interact.* **2010**, *1*, 57–70. [CrossRef]
157. Wu, R.S.S. The Environmental Impact of Marine Fish Culture: Towards a Sustainable Future. *Mar. Pollut. Bull.* **1995**, *31*, 159–166. [CrossRef]
158. Pearson, T.H.; Black, K.D. The Environmental Impacts of Marine Fish Cage Culture. In *Environmental Impacts of Aquaculture*; Sheffield Academic Press: UK, 2000; pp. 1–31. Available online: https://www.cabdirect.org/cabdirect/abstract/20013012517 (accessed on 31 July 2021).
159. Hargrave, B.T. Far-Field Environmental Effects of Marine Finfish Aquaculture. A Scientific Review of the Potential Environmental Effects of Aquaculture in Aquatic Ecosystems. *Can. Tech. Rep. Fish. Aquat. Sci.* **2003**, *1*, 1–35.
160. Grigorakis, K.; Rigos, G. Aquaculture Effects on Environmental and Public Welfare–The Case of Mediterranean Mariculture. *Chemosphere* **2011**, *85*, 899–919. [CrossRef]
161. Mustafa, F.H.; Bagul, A.H.B.P.; Senoo, S.; Shapawi, R. A Review of Smart Fish Farming Systems. *J. Aquac. Eng. Fish. Res.* **2016**, *2*, 193–200. [CrossRef]
162. Huntingford, F.A.; Adams, C.; Braithwaite, V.A.; Kadri, S.; Pottinger, T.G.; Sandøe, P.; Turnbull, J.F. Current Issues in Fish Welfare. *J. Fish Biol.* **2006**, *68*, 332–372. [CrossRef]
163. Conte, F.S. Stress and the Welfare of Cultured Fish. *Appl. Animal Behav. Sci.* **2004**, *86*, 205–223. [CrossRef]
164. Black, K.D. The environmental interactions associated with fish culture. In *Biology of Farmed Fish*; Sheffield Academic Press: Cambridge, MA, USA, 1998; pp. 284–326.
165. Pennell, W.; Barton, B.A. Principles of Salmonid Culture. In *Developments in Aquaculture and Fisheries Science*; Elsevier: Amsterdam, The Netherlands, 1996.
166. Beyan, C.; Browman, H.I. Setting the Stage for the Machine Intelligence Era in Marine Science. *ICES J. Mar. Sci.* **2020**, *77*, 1267–1273. [CrossRef]
167. Stien, L.H.; Gytre, T.; Torgersen, T.; Sagen, H.; Kristiansen, T.S. A System for Online Assessment of Fish Welfare in Aquaculture. *ICES CM* **2008**, R:18. Available online: https://imr.brage.unit.no/imr-xmlui/handle/11250/102545 (accessed on 31 July 2021).
168. Neeraja, Y.; Scholar, U. An IOT Based Remote Aquaculture Monitoring System. *Int. J. Eng. Trends Appl. (IJETA)* **2014**, *5*, 188.
169. Halpin, T. *Conceptual Schema & Relational Database Design*; WytLytPub: Hoboken, NJ, USA, 1999.
170. Zhang, Y.; Hua, J.; Wang, Y. Bin Application Effect of Aquaculture IOT System. In *Applied Mechanics and Materials*; Trans Tech Publications Ltd.: Stafa-Zurich, Switzerland, 2013; Volume 303–306, pp. 1395–1401. [CrossRef]
171. Li, D.; Fu, Z. *Aquaculture Digital Integrated Systeme*; Electronic Industry Press: Beijing, China, 2010.
172. Li, D.; Fu, Z.; Ma, L. Intelligent Aquaculture Information System Design and Preliminary Realizationtle. *Agric. Eng. J.* **2000**, *4*, 135–138.
173. Cario, G.; Casavola, A.; Gjanci, P.; Lupia, M.; Petrioli, C.; Spaccini, D. Long Lasting Underwater Wireless Sensors Network for Water Quality Monitoring in Fish Farms. In Proceedings of the OCEANS 2017-Aberdeen 2017, Aberdeen, UK, 19–22 June 2017; pp. 1–6. [CrossRef]
174. An, J. *The Freshwater Fish Feed Expert System Research Based on the WEB*; China Agricultural University: Guangzhou, China, 2002.
175. Xu, G. National Intelligent Information Technology in Agriculture Popularization and Application of Basic Operation Mechanism Research Summary. *Hunan Agric. Sci.* **2004**, *2*, 57–59.
176. Vikas, M.; Dwarakish, G.S. Coastal Pollution: A Review. *Aquat. Procedia* **2015**, *4*, 381–388. [CrossRef]
177. Pirotta, V.; Grech, A.; Jonsen, I.D.; Laurance, W.F.; Harcourt, R.G. Consequences of Global Shipping Traffic for Marine Giants. *Front. Ecol. Environ.* **2019**, *17*, 39–47. [CrossRef]
178. Er-Raioui, H.; Bouzid, S.; Marhraoui, M.; Saliot, A. Hydrocarbon Pollution of the Mediterranean Coastline of Morocco. *Ocean. Coast. Manag.* **2009**, *52*, 124–129. [CrossRef]
179. Orfanidis, S.; Panayotidis, P.; Stamatis, N. Ecological Evaluation of Transitional and Coastal Waters: A Marine Benthic Macrophytes-Based Model. *Mediterr. Mar. Sci.* **2001**, *2*, 45–66. [CrossRef]
180. Boudouresque, C.F.; Mayot, N.; Pergent, G. The Outstanding Traits of The Functioning of the *Posidonia Oceanica* Seagrass Ecosystem. *Biol. Mar. Medit.* **2006**, *13*, 109–113.
181. Micheli, F.; Levin, N.; Giakoumi, S.; Katsanevakis, S.; Abdulla, A.; Coll, M.; Fraschetti, S.; Kark, S.; Koutsoubas, D.; Mackelworth, P.; et al. Setting Priorities for Regional Conservation Planning in the Mediterranean Sea. *PLoS ONE* **2013**, *8*, e59038. [CrossRef] [PubMed]

182. Notarbartolo di Sciara, G.; Zanardelli, M.; Jahoda, M.; Panigada, S.; Airoldi, S. The Fin Whale Balaenoptera Physalus (L. 1758) in the Mediterranean Sea. *Mammal Rev.* **2003**, *33*, 105–150. [CrossRef]
183. Azzellino, A.; Panigada, S.; Lanfredi, C.; Zanardelli, M.; Airoldi, S.; Notarbartolo di Sciara, G. Predictive Habitat Models for Managing Marine Areas: Spatial and Temporal Distribution of Marine Mammals within the Pelagos Sanctuary (Northwestern Mediterranean Sea). *Ocean. Coast. Manag.* **2012**, *67*, 63–74. [CrossRef]
184. Trasviña-Moreno, C.A.; Blasco, R.; Marco, Á.; Casas, R.; Trasviña-Castro, A. Unmanned Aerial Vehicle Based Wireless Sensor Network for Marine-Coastal Environment Monitoring. *Sensors* **2017**, *17*, 460. [CrossRef] [PubMed]

marine drugs

MDPI

Review

Genome Mining as New Challenge in Natural Products Discovery

Luisa Albarano [1,2,†], Roberta Esposito [1,2,†], Nadia Ruocco [1] and Maria Costantini [1,*]

[1] Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; luisa.albarano@szn.it (L.A.); roberta.esposito@szn.it (R.E.); nadia.ruocco@gmail.com (N.R.)

[2] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Napoli, Italy

* Correspondence: maria.costantini@szn.it; Tel.: +39-081-583-3315; Fax: +39-081-764-1355

† These authors contributed equally to this work.

Received: 4 March 2020; Accepted: 3 April 2020; Published: 9 April 2020

Abstract: Drug discovery is based on bioactivity screening of natural sources, traditionally represented by bacteria fungi and plants. Bioactive natural products and their secondary metabolites have represented the main source for new therapeutic agents, used as drug leads for new antibiotics and anticancer agents. After the discovery of the first biosynthetic genes in the last decades, the researchers had in their hands the tool to understand the biosynthetic logic and genetic basis leading to the production of these compounds. Furthermore, in the genomic era, in which the number of available genomes is increasing, genome mining joined to synthetic biology are offering a significant help in drug discovery. In the present review we discuss the importance of genome mining and synthetic biology approaches to identify new natural products, also underlining considering the possible advantages and disadvantages of this technique. Moreover, we debate the associated techniques that can be applied following to genome mining for validation of data. Finally, we review on the literature describing all novel natural drugs isolated from bacteria, fungi, and other living organisms, not only from the marine environment, by a genome-mining approach, focusing on the literature available in the last ten years.

Keywords: bacteria; fungi; genome mining; natural products; synthetic biology

1. Introduction on Bioactive Natural Products Isolation

Nature is an important source of bioactive products and their derivatives (secondary metabolites), which form part of many important drugs formation widely used in the clinic field [1]. In fact, as reported in Newman and Cragg [2], over the last 30 years the great majority of anticancer, anti-infective, and anti-bacterial drugs are represented by natural products and their derivatives, produced by all organisms (from bacteria to plants, invertebrate, and other animals) with different chemical structure and leading to several biological activities [3,4]. Furthermore, these secondary metabolites have influenced the development of several drugs, including antibacterial, anticancer, and anti-cholesterol agents [5]. Several of these bioactive products are derived from microorganisms, such as fungi and bacteria [6], which have represented an important source of antibiotics and many other medicines [7,8]. In particular many bacteria deriving from the marine environment, particularly those found in association with marine invertebrates (such as sponges), are able to produce secondary metabolites with potential anticancer and antifungal roles because of their cytotoxic properties [9,10]. Considering the great problem of the antimicrobial resistance increase and its high impact on human health, there is an important need of searching for new natural products that could therefore remedy this issue [11,12]. For these reasons, in the past decade, genomic science has been used to identify the possible drug

targets and to find novel genes cluster for the biosynthesis of natural products [13]. The development of the genome sequencing technologies to find novel metabolites has surely drown the attention of pharmaceutical industries, which had by now lost interest in natural products due to the advent of combinatorial chemistry [14]. The advent of based-genome sequencing techniques, especially with establishment of genome mining, has allowed to obtain new natural drugs in a faster and cheaper way.

Genome Mining

The term "genome mining" are associated to every bioinformatics investigation used to detect not only the biosynthetic pathway of bioactive natural products, but also their possible functional and chemical interactions [15]. Specifically, the genome mining involves the identification of previously uncharacterized natural product biosynthetic gene clusters within the genomes of sequenced organisms, sequence analysis of the enzymes encoded by these gene clusters, together with the experimental identification of the products of the gene clusters (Figure 1; [16]).

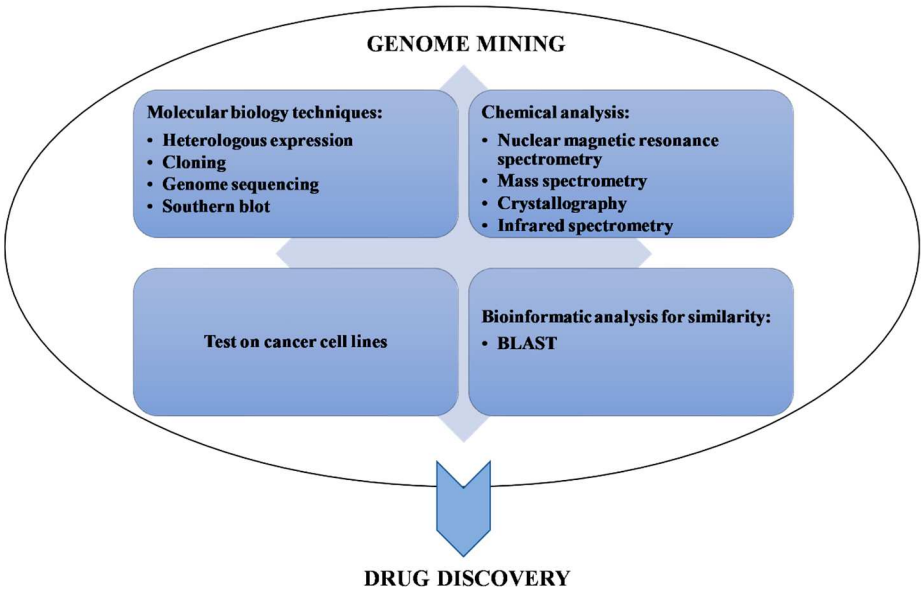

Figure 1. Associated techniques (categorized as molecular biology techniques, chemical analysis, cellular biology techniques, and bioinformatic analysis) to genome mining for validation of data, leading together to drug discovery.

Genome mining is entirely dependent on computing technology and bioinformatics tools. About this point, a huge amount of data, consisting of DNA sequences and their annotations, are now deposited in publicly accessible databases. The storage and handling of these resources relies on the continued development of computers and the networks. Once all the genes within a new genome are identified, they can be compared with those of known functions in the public databases. Both raw and annotated genomic data, as well as bioinformatics tools, for sequence comparisons are freely available through the different websites. It also important to keep in mind that it is now a mandatory publication prerequisite of most scientific journals that sequence data from research involving novel DNA sequences is deposited in a publicly accessible database.

In the case for which the sequences of many proteins, encoding for enzymes, involved in natural product biosynthesis are deposited in these databases, it is relatively easy to identify pathways in which they are involved by sequence comparisons. The availability of these synthesis enzymes and the pathways in which they operate, together with the sequence comparisons with genes from which they arise, can certainly be used to identify homologs, and potentially the pathways, in the new organism under analysis. However, it is important to consider that many enzymes are similar in sequences but

follow chemical processes that are slightly different, leading to a different pathway or very different final end product.

Furthermore, genome mining has a strong support by synthetic biology, consisting in the design and the construction of new biological, as for examples enzymes, genetic circuits and/or the redesign of existing biological systems. These combined approaches are mainly used to detect novel natural products in bacteria and fungi probably because of operon organization of their synthesis genes [13], allowing the control of transcriptional levels and also the association of their potential metabolic function [17]. Moreover, the central role of genome mining consists in finding new biosynthetic gene clusters (BGCs). In fact, the BGCs encode for two class of enzymes, polyketide synthases (PKS) and non-ribosomal peptide synthases (NRPS), which are the two most important biosynthetic routes responsible for the formation of natural products [18]. This approach also provides the possibility to compare target gene clusters to known gene clusters useful for the prediction of their function and structure using different associated web database [5]. In fact, although the genome mining allowed to find and identify the gene clusters responsible for the production of natural product synthesis, in the last decade web tools and databases have been integrated to improve the performance of this approach [15]. This scientific progress has enabled the development of three important web tools: (i) "antibiotics and Secondary Metabolite Analysis SHell" (antiSMASH), its first version was issued in 2011 and it is a web server able to associate the gene clusters identification with a series of specific algorithms for compounds analysis [19]. Therefore, this approach performs the prediction of sequences and offers a more detailed analysis of identified gene clusters and consequently gives the predicted image of amino acid stereochemistry structure [5]. (ii) "PRediction Informatics for Secondary Metabolomes" (PRISM), open-web tool, consisting of a genomic prediction of secondary metabolomes. Using different algorithms that compare the new genetic information with 57 virtual enzymatic reactions (such as adenylation, acyltransferase, and acyl-adenylating), this approach provides the possibility of obtaining a correspondence between known natural drugs and possible new ones [20]. (iii) "Integrated Microbial Genomes Atlas of Biosynthetic gene Clusters" (IMG/ABC) [21], launched in 2015, is a large open web database of known predicted microbial BGCs able to associate BGCs with secondary metabolites (SMs) and analyze both BGCs and SMs. In this way, it offers the ability of finding similar function between BGCs present in database and BGCs to be identified [22].

Starting from these general considerations, in the present review we want to emphasize the significance of genome mining approach to identify new natural products, also underlining the possible advantages and disadvantages of this technique. Moreover, we debate the associated techniques that can be applied following to genome mining for validation of data. Finally, we review the literature describing all novel natural drugs found from bacteria, fungi and other living organisms by genome mining approach, focusing on the examples available in the literature of the last ten years.

2. The Significance Genome Mining in Drug Discovery

Approximately half of clinically approved drugs (including antibiotics) are represented by natural products and their derivatives. Recently, the development of new bioinformatics, genetics and analytic tools, has provided new strategies for the discovery of natural products of biotechnological interest known as "combinatorial biosynthesis approaches" [23,24]. These techniques, together with bioinformatic approaches, have shown that the ability of organisms (particularly microorganisms) to produce bioactive natural products has been underestimated [25]. These organisms have been deeper explored through the sequencing of their genome and the application of genome mining approaches [26]. Genome analysis has shed light the presence of numerous biosynthetic gene clusters that could be involved in the synthesis of other secondary metabolites defined cryptic or orphan for their unknown origin [25].

Genome mining aims at predicting the genes that encode for new natural compounds of biotechnological interest by using several bioinformatic approaches [21]. The importance of genome mining is based on the urgent need to discover new drug entities due to the increased incidence of

severe diseases (such as cancer) and the reduced efficacy of existing drugs [27]. Furthermore, the biosynthetic gene clusters contain elements that can be used to increase the production of both natural and engineered products by promoting costs reduction and their commercial use [26].

2.1. Strengths and Weaknesses of Genome Mining

As in the case of all approaches, also genome mining has strengths and weaknesses, summarized in Table 1. One of the advantages of using genome mining is to foster the detection of a large amount of bioactive natural compounds [6]. In addition, genome mining approach is relatively cheap and easy to apply in laboratory, and it requires no particular skills and/or experience of the operators [28]. Combining genome mining with genetic engineering techniques will make it possible to achieve maximum diversity of natural products [29]. This bioinformatic approach allows to predict the chemical structure of bioactive natural products, but forecasts are often difficult to formulate [28,29].

Table 1. Strengths and weakness in the use of genome mining.

Strengths	Weaknesses
Easy to apply for experimental procedures in laboratory	Not to predict biotechnological potential of the natural compounds
Cheap and easy to apply in laboratory	Only known biosynthetic gene clusters
To predict chemical structures of bioactive natural products	Difficulty to formulate chemical structures
No particular skills and/or experience of the operators	Too new approach that needs to be deepened

As reported in Wohlleben et al. [6], a great disadvantage of genome mining is that only known biosynthetic gene clusters can be identified [29]. Moreover, with this approach, it is not possible to predict the biological activities of the natural products identified [26]. However, genome mining is still an evolving technique [29], in fact, scientists are trying to improve this bioinformatic tool in order to reduce the limits of this approach.

2.2. Synthetic Biology and Other Experimental Techniques Associated with Genome Mining

Synthetic biology progresses have been possible thanks to the very recent advent of DNA sequencing and synthesis in molecular biology field. The distinguishable element of synthetic biology respect to the other traditional molecular biology approaches is represented by its focus on the design and construction of components which are core for example of enzymes and metabolic pathways [30]. These genomic assessments joined to microbial diversity provide the fundamental natural libraries for further engineering.

In this review we have not focused our attention on synthetic biology because a great number of reviews on the most obvious and popular applications of synthetic biology methodologies have been published from 2008 to today [30–45].

Natural product production using engineered microorganisms represent the more important application of synthetic biology in the biotechnological field for natural products. The most important of commercialized examples are represented by two compounds produced by fermentation in genetically modified yeast: The semisynthetic malaria drug artemisinin and the first consumer-market synthetic biology product, “natural” vanillin [46,47]. These successful application of synthetic biology opened new perspective in the exploration of microbes as sources of high-value compounds on an industrial scale.

Genome mining is followed by the identification of cryptic pathways using several strategies, known as “combinatorial biosynthesis”, which that can be used in order to create novel genetic combinations of structural biosynthetic genes. These methods consist of gene activation/inactivation and mutasynthesis approaches. Gene inactivation involves the creation of a mutant organism, in which

the biosynthetic gene cluster becomes inactive, thus eliminating the production of metabolites. The comparison between mutant organisms can be made by high-performance liquid chromatography/mass spectrometry (HPLC-MS), revealing the natural product absent in the mutant organism [26]. Therefore, gene inactivation needs as evidence of cluster involvement in compound biosynthesis [24]. Secondary metabolites come from precursors of primary metabolism, and their over-production is related to an enhanced protein synthesis [48]. However, in some cases, there are genes that produce specific precursors not provided by the primary metabolism. These precursors are usually used as starting units for example to the production of polyketides synthases (PKS) or non-ribosomal peptide synthetases (NRPS), which in turn produce natural compounds. Inactivation of genes involved in the biosynthesis of these precursors leads to non-productive mutants that can be used for the biosynthesis of new compounds by mutasynthesis or mutational biosynthesis [23,24,26]. If some genes are silent, it would be impossible to produce and test the biological activity of the natural product. It is therefore necessary to apply the activation of silent pathways under the control of a constitutive promoter or inactivating repressors [28]. In the final stages of metabolites biosynthesis, several enzymes such as, transferase, oxygenase, oxidase, peroxidase, and reductase, play a key role for further modifications [26].

Examples of Other Experimental Techniques

A method to identify new natural products with biotechnological potential combines the research of coding genes for a specific compound with the detection of bacterial resistance. This approach, called target-directed genome mining, relies on the identification of gene clusters without knowing the molecules produced [49].

Another method to identify a natural product is the one strain/many compounds (OSMAC) approach. This method is based on the systematic alteration of culture media or cultivation parameters to force the expression of cryptic genes. In addition, any metabolism deregulation system can be used to improve the production of secondary metabolites, leading to the discovery of new bioactive compounds. Many of these approaches involve the treatment of known chemicals that modify the structure of chromatin or the use of small molecules that re-shape and regulate secondary metabolism by inhibiting the synthesis of fatty acids [50].

Another technique associated with genome mining is the in vitro reconstruction of biosynthetic pathways that produce natural products. This technique can be used to generate highly pure intermediates, limiting side reactions such as the formation of toxic compounds and reducing protein-protein interactions [51].

Taking into account this background, we review on the new natural drugs found from bacteria, fungi and other living organisms by genome mining approach. We analyzed organisms that derive not only from marine environment but also from the terrestrial ones, considering that the genome mining and other techniques associated with it are still at the beginning for the discovery of bioactive compounds from the sea.

2.3. Bacteria

The first point that must be underlined is that the most of medicinal products described above derive from bacteria [6] (see Table 2). In fact, the available literature on genome mining mainly concerns these microorganisms. Specifically, soil and marine bacteria, such as actinomycetes, produce the greatest part of natural drugs identified in the last thirty years [52]. The actinomycetes can be isolated from various habitats, such as soil, sea deposits, sponges, corals, mollusks, seagrasses, and mangroves [53].

Table 2. Genome mining approaches applied to microorganisms.

Microorganism	Experimental Purpose	Associated Techniques	References
Actinomycetes	Identification of strains capable to produce halogen enzymes.	PCR screening and NMR spectroscopy	[54]
Streptomyces aizunensis NRRL B-11277	Elucidation of new antibiotic ECO-02301 structure	HPLC, MIC	[55]
Streptomyces roseosporus	Anti-infective agent arylomycin and its BGCs	IMS, MS and SST	[56]
Streptomyces roseosporus	Identification of stenothricin and its BGCs	MS/MS spectra, antiSMASH, NMR, BioMAP, Cytological profiling	[57]
Streptomyces exfoliatus UC5319, *Streptomyces arenae* TU469 and *Streptomyces avermitilis*	Biosynthetic gene clusters involved in the synthesis of pentalenolactone	Cloning, MS/MS spectra, H-NMR spectroscopy	[58]
Streptomycetes sp. M10	To determine biosynthetic gene clusters involved in the synthesis of natural products	PRC screening, BLASTP, antiSMASH, Artemis Release 12.0, RT-PCR, MALDI-TOF	[59]
Streptomyces sp., *Streptomyces roche*, *Streptomyces lividans* SBT5	Streptothricin and borrelidin biosynthetic gene clusters	Heterologous expression, HPLC, LC-MS, LEXAS method, antiSMASH	[60]
Streptomyces sp. Tü 6176:	BGCs of nataxazole	antiSMASH 2.0 heterologous expression, gene inactivation, antibiotic disc diffusion assay, test on cancer cell lines	[61]
Strepmomyces argillaceus ATCC12956	Argimycin biosynthetic gene cluster	AntiSMASH, test on cancer cell lines	[62]
Streptomyces sp. CBMAI 2042	Valinomycin biosynthetic gene cluster	Test on pathogens, *in silico* analyses	[63]
Bacillus, *Streptomyces*, *Micronospora*, *Paenibacillus*, *Kocuria*, *Verricosispora*, *Staphylococcus*, *Micrococcus*	Influence of isolation location on secondary metabolite production	Test on cancer cell lines, MS, GNPS	[64]
Streptomyces sp. MA37, *Norcardia brasiliensis*, *Actinoplanes* sp. N902-109	Identification of Fluorinases	overexpression of gene, vitro activity assay and 19F NMR	[65]
Streptomyces sp. PKU-MA00045	Aromatic polyketides	1H-NMR and 13C-NMR spectra, genome sequencing, BLAST	[53]
Streptomyces sp. SM17	Identification of Surugamide A	NCBI BLASTN, antiSMASH, NMR	[10]
Pseudovibrio sp. POLY-S9	BGCs of symbiotic bacteria and gene involved in symbiontic relationship	genome sequencing, antiSMASH	[66]
Vibrio harveyi	BGCs of spongosine and potential secondary metabolites	MS/MS-based molecular networking, nitric oxide assay, MLSA and BLAST, genome sequencing and antiSMASH	[67]
Planctomyces	Metabolic properties of these bacteria	antiSMASH, MicroArray	[68]
Diverse prokaryotic species	New aldolase enzymes	LC–MS, cloning, FPLC, HTS	[69]
Pseudoalteromonas luteviolacea	Violacein biosynthetic pathway	LC-MS/MS, antiSMASH	[8]
Anabaena variabilis PCC 7937, *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 6301	MAA biosynthetic gene cluster	MAA induction with radiation UVR, MAA extraction, HPLC, BLAST	[70]
Hapalosiphon welwitschii UH strain IC-52-3, *Westiella intricate UH* strain HT-29-1 and *Fischerella* sp. CC 9431	Hapalosine biosynthetic pathway	PCR screening, antiSMASH, Geneious version 6.1.7	[71]

Hornung et al. [54] applied genome mining to identify strains capable of producing halogenase enzymes, where halogenations represent an important feature for the biological activity of a great number of different natural products. *Escherichia coli* DH5a and *E. coli* XL1 Blue were used to identify the complete halogenase gene sequence and to build primer-specific probes for these genes. Moreover, genomic DNA was isolated from 550 strains of actinomycetes available in strain collections. Using specific primer probes, it has been demonstrated that some actinomycetes are able to produce halogen enzymes.

Furthermore, nuclear magnetic resonance spectroscopy (NMR spectroscopy) was applied to understand the structure of these molecules, revealing that they were not exactly like those already known in literature. *Streptomyces*, a type of actinomycetes gram-positive bacteria, have also extensively been studied.

In fact, McAlpine et al. [55] used the genome mining approach to identify new antibiotic ECO-02301, with a potent antifungal activity, from *Streptomyces aizunensis* NRRL B-11277 bacteria. This compound was active against several strains of human pathogenic fungi (*Candida albicans* ATCC10231, *Candida glabrata* ATCC 90030, *Candida lusitaniae* ATCC 200953, *Candida tropicalis* ATCC 200955, *Candida krusei* LSPQ 0309, *Saccaromyces cerevisiae* ATCC 9763, *Aspergillus fumigatus* ATCC 204305, *Aspergillus flavus* ATCC 204304, *Cryptococcus neoformans* ATCC 32045). To obtain the expression of this gene after the grown of bacteria in flask, the proteins were extracted and analyzed by high performance liquid chromatography (HPLC) monitored by a diode array detector (DAD) that detects the absorption in UV region and positive/negative mass spectrometry (MS) ions.

The analysis of the genome of *S. aizunensis* NRRL B-11277 helped the prediction of the structure of this compound with sufficient accuracy so to represent a guide for its isolation.

Furthermore, an anti-infective agent, called arylomycin, and its BGCs by *Streptomyces roseosporus* strains, were described using imaging mass spectrometry (IMS) and MS guided by genome mining approaches [56]. Specifically, *S. roseosporus* was co-cultured with two pathogen strains, *Staphylococcus aureus* and *Staphylococcus epidermidis*, and its genome has been sequenced. It was so demonstrated that *S. roseosporus* produced daptomycin, an antibiotic molecule. Moreover, they spotted *S. roseosporus* in the center of *S. aureus* and *S. epidermidis* cultures and after 36 hours of incubation, using IMS and MS, aptomycin ions have been not observed, but a cluster corresponding to the potassium adduct was found. These results suggested that *S. roseosporus* was also able to produce three additional antibiotics. Furthermore, to identify the biosynthetic gene cluster of these molecules, a peptide-genomic mining approach was applied, which relied on the short sequence tag (SST) from tandem very spectrometric data. With this approach, in fact, they established that these three molecules were arylomycins.

In a similar study, Liu et al. [57] demonstrated that *S. roseosporus*, in addition to the non-ribosomal peptide synthetase-derived molecular families and their gene subnetworks, todaptomycin, arylomycin, and napsamycin, was also able to produce stenothricin. Firstly, after DNA extraction, to identify the molecular network they reduced the complexity of analysis to 837 genes using MS/MS spectra with parent ion masses within 0.3 Da and compared to related MS/MS spectral patterns. It was possible to observe the already known genes ofarylomycin, napsamycin, daptomycin and their variants. However, they identified four genes for stenothricin but combining the MS/MS spectra to the amino-acid blocks found by antiSMASH, 21 genes clusters were found. Furthermore, to understand their biological activities, a screening platform (named BioMAP) was used and then the cytological profiling, evaluating this activity against 15 bacterial strains. These approaches revealed that the stenothricinis was active on both Gram-negative and Gram-positive bacteria.

Seo et al. [58] used the DNA extraction to isolate the antibiotic pentalenolactone biosynthetic gene clusters from the known pentalenolactone producers *Streptomyces exfoliatus* UC5319 and *Streptomyces arenae* TU469. By building probes based on the previously cloned *S. exfoliates* pentalenene synthase gene, the sequence of the *S. exfoliatus* Pen biosynthetic gene cluster were analyzed, revealing that the furthest upstream gene, designated as PenR, encoded a 153 aa MarR-family transcriptional regulator. Moreover, PenI, PenH, and PenF were also found, which were expected to catalyze the

oxidative conversion of pentalenene to 1-deoxy-11-oxopentalenic acid, as previously established for the othologous *Streptomyces avermitilis* proteins. Furthermore, the attention was pointed out on penE product, because it seems to be the key branch point that distinguished the pentalenolactone and neopentalenolactone biosynthetic pathways. PenE gene encoded a protein that is a homologue of the known Baeyer-Villiger monooxygenase from *S. avermitilis*, PtlE. The compounds PenD, PntD, and PtlD were characterized by mass spectrometry and H-NMR, also generating the deletion mutants with no production of pentalenolactone.

In another study, Tang et al. [49] analyzed, through bioinformatic approaches (BLASTP, Artemis Release 12.0), the genome of *Streptomycetes* sp. M10 discovering 20 biosynthetic gene clusters involved in the synthesis of natural products, such aspolyketides, NRPs, siderophores, lantibiotics, terpenoids. In addition, one of all gene clusters shared a partial similarity with candicidin/FR-008gene cluster, which in turn encoded for antifungal polyene assuming the potential role of this strain to produce this compound. Finally, to confirm this potential activity, the polyene was tested against the phytopathogen *Fulvia fulva* for its antifungal activity.

A high throughput genomic library expression analysis system (LEXAS) was applied for efficient, function-driven discovery of cryptic and new antibiotics from *Streptomyces*, known producers of several antibiotics [60]. Each BAC clone was transferred individually into an engineered antibiotic overproduction host, avoiding preference for smaller BACs. The LEXAS captured two known antibiotics, identified two novel lipopeptides and their BGC that was not produced/expressed in the native *Streptomyces rochei* strain, and revealed a cryptic BGC for unknown antibiotic. Specifically, in this research two new antibiotics streptothricins and borrelidin were found and for their validation these genes were expressed in the surrogate host *Streptomyces lividans* SBT5 by heterologous expression. Moreover, to analyze the antimicrobial activity, SBT5 products were tested against *Staphylococcus aureus* and *Bacillus mycoides*, showing an inhibition. In addition, they discovered two novel linear lipopeptides and their BGCs also adding the analysis of their structures by HPLC and liquid chromatography-mass spectrometry (LC-MS).

Thirty-eight secondary biosynthetic gene clusters of nataxazole (NAT) and its derivatives were identified from *Streptomyces* sp. Tü 6176, using in silico by genome mining and antiSMASH 2.0 [61]. In particular, the NAT entire BGC was described, consisting of 21 genes: 12 encoding for structural proteins, 4 for regulatory proteins, 4 probably involved in NAT secretion, and 1 with unknown function. Moreover, using the gene inactivation and heterologous expression of NAT cluster, it was established that secondary metabolite pathways were outside of NAT gene cluster (not a common in actinomycetes) despite they were involved in NAT biosynthesis. Furthermore, using antibiotic disc diffusion assay, an antibiotic activity was found only against *Staphylococcus albus* J1074, whereas the negative effect was absent in *Streptomyces lividans* JT46, *Micrococcus luteus* and *Escherichia coli*. Anticancer activity was tested against human tumor cell lines (HT29, A549, MDA-MB-231, AGS and A2780) including mouse cell line NIH/3T3 used as control. In this way, they demonstrated that these compounds have moderate activity against maleficent cells. In a similar study, Ye et al. (2017) used genome mining and antiSMASH 2.0 to identify the presence of 31 biosynthetic clusters in *Strepmomyces argillaceus* ATCC12956.

The most studied BGC between all found was the gene that encoded for argimycin P (renamed *arp* cluster). In addition, the pathway for the biosynthesis of *arp* was reconstructed by means of genetic engineering. Moreover, using in vitro tests on cells, no cytotoxic activity of this compound was found against 59 tumour cell lines. In another study, Paulo et al. [63] used in silico genome mining on strains of *Streptomyces* sp. CBMAI 2042, isolated from the branches of the plants *Citrus sinensis*. Moreover, this strain also prevented the proliferation of pathogens in citrus such as *Citrus xylella*, *Geotrichum candid* var. citri-Aurantii, and *Colletotrichum gloesporioides*. In particular, 35 biosynthetic gene clusters were found including the putative NRP biosynthetic gene cluster that encoded for valinomycin. In addition, by combining genome mining and molecular network, it was possible to reconstruct the origin of the biosynthetic pathway of cyclodepsipeptides, which have antibacterial, antiviral, and anticancer activity.

Furthermore, Purves et al. [64] applied the genome mining approach on bacteria extracted from two marine sediments (Antarctic and Scotland). They identified eight genera (*Bacillus*, *Streptomyces*, *Micromonospora*, *Paenibacillus*, *Kocuria*, *Verrucosispora*, *Staphylococcus*, and *Micrococcus*) and used 38 strains on which MS analysis was conducted. Thanks to this approach a great number of metabolites were identified, of which 1422 were Antarctic-specific, while 1501 were Scottish-specific secondary metabolites. Moreover, a molecular network was built up by Global Natural Products Social (GNPS) Molecular Networking, showing that only 8% of strains belonging to these locations displayed a similarity, implying a high degree of biogeographic influence upon secondary metabolite production. Organic extracts from these 38 selected strains were tested for cytotoxicity against epithelial colon adenocarcinoma cells (Caco-2) and human fibroblasts originating from foreskin (HS27). No effect on normal cell viability was observed, while seven extracts were bioactive against Caco-2 at 50 g/mL concentration. Direct observation revealed morphological changes, such as cell shrinkage and formation of apoptotic bodies. Moreover, Deng et al. [65] identified three new fluorinase enzymes from three bacterial strains, *Streptomyces* sp. MA37, *Nocardia brasiliensis*, and *Actinoplanes* sp. using the genome mining approach. These proteins were isolated and purified using overexpression of fluorinasegenes in *Escherichia coli*. Analyzing this product with in vitro activity assay, it revealed a high homology (about 85%) of its BGCs to the original one (called flA1) founded in *Streptomyces cattleya*. Finally, it was also assessed that *Streptomyces* sp. MA37produced some unidentified fluorometabolites.

As mentioned before, the actinomycetes are distributed in different marine habitats, being mainly associated to sponges. In fact, Jin et al. [53] have conducted genome mining experiments with *Streptomyces* sp. PKU-MA00045 isolated from sponges. Specifically, five new aromatic polyketides, fluostatins M–Q (1–5) were isolated using PCR-based genome mining method, and their chemical structures were clarified by ^{1}H-NMR and ^{13}C-NMR. The entire genome of *Streptomyces* sp. PKU-MA00045 was sequenced and compared to homologues in the published fluostatin gene clusters with BLAST, so identifying the BGCs of these new five compounds. In a similar experiment, Almeida et al. [10] used OSMAC approach to identify an octapeptidicsurugamide (Surugamide A) from *Streptomyces* sp. SM17, isolated from the marine sponge *Haliclona simulans*. The phylogenetic analysis with NCBI BLASTN demonstrated that this marine bacteria was phylogenetically linked to five strains of terrestrial *Streptomyces* bacteria: *Streptomyces albidoflavus* strain J1074, *Streptomyces albidoflavus* strain SM254, *Streptomyces sampsonii* strain KJ40, *Streptomyces* sp. FR-008 and *Streptomyces koyangensis* strain VK-A60T. Since *S. albidoflavus* strain J1074 was widely used as a model for various biotechnological studies, the secondary metabolites of the biosynthetic gene clusters were predicted by antiSMASH program, comparing the new BGCs with those already collected by *S. albidoflavus* strains. In this way, it was demonstrated that *Streptomyces* sp. SM17 produced different secondary metabolites. Moreover, using NMR technique it was possible to show that *Streptomyces* sp. SM17 was able to produce higher levels of Surugamide A than the *S. albidoflavus* strain J1074.

However, Anoop et al. [66] studied another bacterial strain *Pseudovibrio* sp. POLY-S9, isolated from intertidal marine sponge *Polymastia penicillus* sampled from the Atlantic coast of Portugal. In fact, after genome sequencing of this marine bacteria, new genes-related bioactive compounds were isolated, such as polyketide synthase, nonribosomal peptide synthetase and siderophore, using genome mining by antiSMASH. Moreover, several genes involved in symbiotic relationships, such as the ankyrin repeats, tetratrico peptide repeats and Sel1, were also identified. Another important finding of this study was represented by some genome plasticity elements of POLYS9, which allowed the survival of these bacteria and their adaptation to various habitats through the exchange of genetic material. Using MS/MS-based molecular networking analysis a bacterial strain was isolated from the Caribbean sponge *Tectitethya crypta*, able to produce spongosine, deoxyspongosine, spongothymidine, and spongouridine, generally referred as "spongonucleosides" [67].

Spongosine, a methoxyadenosine derivative, had several pharmacological applications, having anti-inflammatory activity (for their capability to inhibit the nitric oxide production in cells) and analgesic and vasodilation properties. After MLSA and BLAST analyses, this strain was identified

as *Vibrio harveyi*, and thanks the genomic DNA sequencing and antiSMASH platform, six potential secondary metabolite pathways were described.

Planctomycetes are ubiquitous bacteria that were usually found in marine, freshwater and soil habitats, even if it is possible to find them as free living organisms, or attached to abiotic and biotic surfaces, as for example to algal cells. Some strains also live as symbionts of prawns, marine sponges or termites [72]. For instance, Jeske et al. [68] applied the genome mining methods to define the metabolic properties of *Planctomyces*. First, they found 102 genes or gene clusters involved in the production of secondary metabolites by analyzing 13 genomes on antiSMASH database. Moreover, the genome analysis showed a close correlation between the length of BGCs and the amino acid sequence of the predicted secondary metabolites. Moreover, since most BGCs were transcriptional silent, the Phenotype MicroArray technology was applied on compounds secreted by *Planctomyces limnophilus* (limnic strain) and *Rhodopirellula baltica* (marine strain), confirming that there was a strong relationship between *Planctomycetes* and algae or plants, which in turn secrete compounds that might serve as trigger to stimulate the secondary metabolite production in *Planctomycetes*. Thus, this study provides strong evidences for the use of these bacteria for drug development.

In a different study, Guérard-Hélaine et al. [69] identified new aldolase enzymes, belonging the aldolase/transaldolase family, from 313 different prokaryote species. Comparing the sequence of 1148 proteins extracted from these strains to already known aldolases and transaldolases, 700 genes were selected. The overexpression of these genes and the following LC-MS analysis allowed the selection of 19 proteins of interest. After cloning of the corresponding genes and using fast protein liquid chromatography (FPLC), 18 enzymes were purified, including two aldolases and sixteen transaldolase. Moreover, the activity of these 18 enzymes was evaluated by high-throughput screening (HTS), revealing that six of those annotated as transaldolase showed aldolase activity. Maansson et al. [8] extracted DNA from 13 closely related strains identified as *Pseudoalteromonas luteviolacea*, isolated from all over the Earth, and analysed their potential to produce secondary metabolites. Specifically, antiSMASH analysis demonstrated that only 10 biosynthetic pathways were preserved in all strains, including glycosylated lantipeptide (RiPP1) and two bacteriocins (RiPP2 and RiPP3). All strains have maintained essential pathways, such as that responsible for the production of siderophores, homoserine lactones and violacein. Furthermore, bacteria were grown in culture media to stimulate the synthesis of secondary metabolites and the chemical structures of these compounds were analyzed by LC-MS/MS. Particular attention was paid on violacein pathway, showing the presence of an insert in the *bmp1* gene in the thioesterase domain probably responsible of *Pseuoalteromonas* color. Moreover, the varieties *Pseudoalteromonas* S4047-1, S4054 and CPMOR-1 produced indolmicin antibiotic. However, the biosynthetic pathway coding for the antibiotic indolmicin has never been characterized.

Cyanobacteria

Cyanobacteria were also studied for their interesting bioactive secondary metabolites. For example, they produce mycosporine and mycosporine-like amino acids (MAAs), which are antioxidant molecules that eliminate toxic oxygen radicals protecting cells from saline, drying or thermal stress in some organisms and may act as an intracellular nitrogen reservoir. These compounds were also found in many other organisms such as yeasts, fungi, algae, corals and lichens [73]. Applying genome mining approach and BLAST analysis, Singh et al. [70] demonstrated that among four strains of cyanobacteria (*Anabaena variabilis* PCC 7937, *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 6301) exposed to 72 hours of UV radiation, only *Anabaena variabilis* PCC 7937 was able to produce MAAs. HPLC analysis of these four cyanobacteria revealed the presence of a unique combination of two genes, predicted *DHQ synthase* (YP\324358) and *O-methyltransferase*; (YP\324357) in *A. variabilis* PCC7937, which were missing in other non-MAAs-synthesizing cyanobacteria. Micallef et al. [71] identified the gene cluster responsible for hapalosine synthesis and hapalosine biosynthetic pathway from the genomes of three cyanobacteria (*Hapalosiphon welwitschii* UH strain IC-52-3, *Westiella intricata* UH strain HT-29-1 and *Fischerella* sp. CC 9431), by using genome mining combined with

Geneious version 6.1.7 and antiSMASH. Single cyanobactin cluster of biosynthetic genes was identified only in the genome of *W. intricate* UH strain HT-29-1, demonstrating that there is structural diversity of cyanobacteria inside cyanobacteria strains. Moreover, only *Fischerella* sp. PCC 9339 encoded a microviridine gene cluster and they identified the MAA (*mys*) gene cluster in the strains *W. intricate* varieties UH HT-29-1, *H. welwitschii* UH strain IC-52-3, *Mastigocoleus testarum* BC008, *Fischerella muscicola* SAG1427–1 and *Chloroglopsis* sp. PCC 9212. Finally, the presence of the cluster of scytonemin genes within the genome of *Mastigocladopsis repens* PCC 10,914 was discovered, suggesting that this organism was able to bio-sintetizes cytonemin in order to protect the cells against UVA-radiation. The geosmin gene cluster was identified in *W. intricata* variety UH HT-29-1, *H. welwitschii* UH strain IC-52-3, *Fischerella* sp. PCC 9431, and *F. muscicola* SAG 1427–1.

2.4. Fungi

As described above, the most important sources of natural drugs are not only bacteria but also fungi [6]. In fact, many different natural products, such as penicillin, cephalosporin, ergotrate and the statins represent well-known fungal secondary metabolites for pharmacological applications [74]. For these organisms the genome mining also proved to be a useful method to find BGCs (Table 3). In a study of Bergmann et al. [75] a silent metabolic pathway was detected, which might code for the biosynthesis of polyketides or polypeptides in *Aspergillus nidulans.* In particular, considering that the cryptic gene cluster provided a putative activator gene called *apdR*, it was amplified and cloned into expression vector pAL4, which coded for inducible alcohol dehydrogenase promoter *alcAp* of *A. nidulans* and the pyr-4 gene of *Neurospora crassa* as a selectable marker.

Table 3. Genome mining approaches applied to fungi.

Fungi	Experimental Purpose	Associated Techniques	References
Aspergillus nidulans	Detection of silent metabolic pathway	Southern blot, HPLC, NMR, IR, and MS	[75]
Calcarisporium arbuscula	Silent metabolic pathway involved in natural product biosynthesis	genome sequencing, LC-MS, chromatographic and NMR analysis, HPLC	[76]
Aspergillus MF297-2	Identification of BGCs of ephacidin and notoamide	genome sequencing, BLAST, gene cloning, overexpression of protein, HPLC, LC-MS, 1H, and 13C NMR	[77]
Aspergillus oryzae and *Neosartorya fischeri*	Isolation of terpene synthases	heterologous expression, GC-MS, 1H- and 13C-NMR, LC-MS, and HR-MS	[78]

Using Southern blot analysis, it was demonstrated that under inducing conditions the *apdA* gene encoded the PKS-NRPS hybrid synthetase. Moreover, HPLC analysis displayed that this induced strains were able to produce two main products, Aspyridones A and B, and two minor compounds, whose structures was elucidated by NMR and MS. In a similar study, Mao et al. [76] revealed a silent metabolic pathway involved in natural product biosynthesis. In fact, after genome sequencing, 68 BGCs were identified, being in contrast to the two predominant metabolites normally produced, the F1-ATPase inhibitors 1 and 2. Since these BGCs are localized within the heterochromatic regions, a mutant strain was built deleting *hdaA* (gene of the histone H3 lysine 14 (K14) deacetylase). In this way, using metabolite extraction and LC-MS analysis, it was demonstrated that the mutant produced more compounds compared to wild strain. Moreover, after overexpression of these genes, ten compounds were isolated, of which four contained new structures, including the cyclic peptides arbumycin and arbumelin, the diterpenoid arbuscullic acid A, and the meroterpenoid arbuscullic acid B. However, Ye et al. [78] applied the genome mining approach to conduct a phylogenetic analysis of

fifteen bifunctional terpene synthases found in five fungal genomes. Specifically, the terpene BGCs sequence were different and synthetized sesterterpenes with new carbon skeletons, suggesting that these microorganisms were separated in five different clades. Moreover, two clades, *Aspergillus oryzae* and *Neosartorya fischeri*, did not produce terpene, hypothesizing that BGCs were silent in standard conditions. For these reasons, heterologous expression was performed in *A. oryzae* using *E. coli* plasmids and the extract was analyzed with GC-MS, ^{1}H- and ^{13}C-NMR elucidating the structure of four compounds, one of which known as sesterfisherolsynthase (*NfSS*) and previously found in *N. fischeri*. Furthermore, bioinformatic analysis showed that *NfSS* gene was encoded downstream of a cytochrome P450 monooxygenase (NfP450) and it was transformed by NfP450 to sesterfisheric acid. Finally, to identify NfP450 gene, double transformant with NfSS and NfP450 genes was prepared and the extract was examined by LC-MS and HR-MS indicating that NfP450 conducted a NfSS modification.

Furthermore, Ding et al. [77] have identified the first BGCs of the stephacidin and notoamide, belong to family of prenylated alkaloids, from *Aspergillus* sp. MF297-2. Specifically, after sequencing of genome the entirenotoamide and stephacidin gene cluster was identified by BLAST comparing sequence to gene *ftmA*, which was previously mined from an *Aspergillus fumigatus*. By bioinformatics analysis, 19 genes involved in notoamide biosynthesis were found to constitute this cluster. To understand the function, this cluster was cloned using *E. coli* DH5R and overexpressed into *E. coli* BL21. The proteins were purified with a single Ni-NTA column and analyzed with HPLC, LC-MS, ^{1}H- and ^{13}C-NMR. Two central pathway enzymes, *NotF* and *NotC*, were identified suggesting a scheme for the biosynthesis of stephacidin and notoamide metabolites.

2.5. Other Organisms

Several other organisms, completely unrelated to the marine environment, have been used as subject of genome mining approach, such as terrestrial microorganisms, plants, and animals (Liu et al. 2018; Table 4).

Table 4. Genome mining approaches applied to ants, copepods and plants.

Organism	Experimental Purpose	Associated Techniques	References
Atta cephalotes, *Camponotus floridanus* and *Harpegnathos saltator*	Defense- and neuropeptides in Social Ants	tBLASTn, GeneWise algorithm, ClustalW	[4]
Calanus sp., *Pontella* sp., *Oikopleura* sp., *Acartia* sp., *Acartia* sp. and *Corycaeus* sp.	Metabolic pathway from conversion from β-carotene to astaxanthin.	LC-UV method, HPLC, Hhpred database	[79]
Arabidopsis thaliana, *Capsella rubella*, *Brassica oleracea*, *Nicotiana benthamiana*, *Agrobacterium tumefaciens*	Sesterterpene biosynthetic gene cluster	plantiSMASH, heteroloug expression, GC-MS, cristallography	[80]

Gruber and Muttenthaler [4] applied genome mining to identify defense- and neuropeptides in the genomes of social ants of the subfamilies of Myrmicinae (*Atta cephalotes*), Formicinae (*Camponotus floridanus*) and Ponerina (*Harpegnathos saltator*); ants are difficult to manipulate for scientific purposes because of the size of their bodies and organs. Most interestingly, genes encoding for oxytocin/vasopressin-related peptides (inotocins) and their putative receptors were identified, using a publicly available matrix of tools, including the search for similarity with tBLASTn, prediction of gene structure using GeneWise algorithm and alignments of sequences by ClustalW.

Carotenoids cannot be synthesized de novo, but they must therefore be taken with food (such as algae) and get protective human health benefits as well. Free astaxanthin and its esterified forms are the main carotenoids present in crustaceans and in particular in copepods. Mojib et al. [79] aimed on understanding the metabolic and genetic basis of the blue phenotype between the blue pigmented organisms from the phylum Arthropoda, subclass Copepoda (*Acartia fossae*) and the phylum Chordata, class Appendicularia (*Oikopleura dioica*) in the Red Sea. Firstly, liquid chromatography-UV method was used to detect the carotenoids and mass spectrometry and HPLC were used to detect intermediate

metabolites, present at low concentrations. The chromatograms identified astaxanthin in all samples, while the fucoxanthin was not detected in any samples. In addition, other carotenoids, intermediate compounds for conversion from β-carotene to astaxanthin, were also identified. The metabolic pathway for each sample was reconstructed for the conversion from β-carotene to astaxanthin. The results showed that all the species followed the same metabolic pathways via almost the same intermediate metabolite formation. Echinenone, one of the intermediate metabolite was not detected in any of the samples but its hydroxylated form, the 3-idrossi chinenone, was detected in all samples, as well as lutein. Putative β-carotene hydroxylase of P450 family coding transcripts was identified in blue *A. fossae* by in silico transcriptome mining. Putative carotenoid-binding proteins after transcriptome/genome mining showing 100% homology to Apolipoprotein D (ApoD) and crustacyanin as predicted by HHpred database.

A customized version of the plantiSMASH genome mining algorithm was created to identify a sesterterpene synthase gene repertoire in some *Brassicaceae* plants, which synthesizes fungal-type sesterterpenes with diverse scaffolds, thus fueling the drug-discovery pipeline [80]. Sesterterpenoids are a rare terpene class with not well explored chemical structure and diversity, representing a potential new drug source. This study offered new insights on the origin of structural diversity for protein engineering, supporting the idea of convergent evolution for natural product biosynthesis.

3. General Conclusions

Many drugs used, for example, as anticancer, antibacterial, and anti-inflammatory agents in the clinical field are derived from natural products and their derivatives. In fact, these secondary metabolites are produced by all organisms (from bacteria to plants, invertebrates, and other animals) and show several biological activities useful in several biotechnological applications. However, the most important sources of natural drugs are microorganisms (mainly bacteria, also associated with marine organisms, such to sponges) and fungi. In the last decades, the great advances made in the field of molecular biology techniques, representing a good example the genome mining together with the synthetic biology, strongly push the identification of BGCs, encoding for enzymes involved in the biosynthesis of natural products. Taking together, these next-generation and highly sophisticated tools contribute to the emergence of a new generation of natural product research. These techniques are in their infancy for their application to marine environment, but there are in literature a lot of applications for the discovery of bioactive natural products for other environments. For this reason, we think that a review reporting all these examples could give strong support in pushing the applications of these new techniques in discovery bioactive compounds from the marine environment, also due to high level of biodiversity offered by the sea in comparison with the Earth. Genome mining, as well as synthetic biology and all the techniques to them associated, represent a new challenge in natural products discovery from the marine environment, without impact on the environment and with no use of destructive collection practices of marine organisms.

Author Contributions: M.C. conceived and designed the scheme of the review; L.A. and R.E. performed the bibliographic research and prepared the original draft; N.R. contributed to the preparation of the original draft; All authors have read and agree to the final version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Luisa Albarano was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship co-funded by the Stazione Zoologica Anton Dohrn and University of Naples Federico II. Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. Nadia Ruocco was supported by a research grant "Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ren, H.; Wang, B.; Zhao, H. Breaking the silence: New strategies for discovering novel natural products. *Curr. Opin. Biotechnol.* **2017**, *48*, 21–27. [CrossRef]
2. Newman, D.J.; Cragg, G.M. Marine-sourced anti-cancer and cancer pain control agents in clinical and late preclinical development. *Mar. Drugs* **2014**, 255–278. [CrossRef]
3. Gruber, C.W. Global cyclotide adventure: A journey dedicated to the discovery of circular peptides from flowering plants. *Pept. Sci.* **2010**, *94*, 565–572. [CrossRef]
4. Gruber, C.W.; Muttenthaler, M. Discovery of defense- and neuropeptides in social ants by genome-mining. *PLoS ONE* **2012**, *7*, 1–12. [CrossRef]
5. Boddy, C.N. Bioinformatics tools for genome mining of polyketide and non-ribosomal peptides. *J. Ind. Microbiol. Biotechnol.* **2014**, 443–450. [CrossRef]
6. Wohlleben, W.; Mast, Y.; Stegmann, E.; Ziemert, N. Antibiotic drug discovery. *Microb. Biotechnol.* **2016**, *9*, 541–548. [CrossRef]
7. Peláez, F. The historical delivery of antibiotics from microbial natural products—Can history repeat? *Biochem. Pharmacol.* **2006**, *71*, 981–990. [CrossRef]
8. Maansson, M.; Vynne, N.G.; Klitgaard, A.; Nybo, J.L.; Melchiorsen, J.; Nguyen, D.D.; Sanchez, L.M.; Ziemert, N.; Dorrestein, P.C.; Andersen, M.R.; et al. An integrated metabolomic and genomic mining workflow to uncover the biosynthetic potential of bacteria. *Am. Soc. Microbiol.* **2016**, *1*, 1–14. [CrossRef]
9. Gulder, T.A.M.; Moore, B.S. Chasing the treasures of the sea-bacterial marine natural products. *Curr. Opin. Microbiol.* **2009**, *12*, 252–260. [CrossRef]
10. Almeida, E.L.; Kaur, N.; Jennings, L.K.; Felipe, A.; Rinc, C.; Jackson, S.A.; Thomas, O.P.; Dobson, A.D.W. Genome mining coupled with OSMAC-based cultivation reveal differential production of Surugamide A by the marine sponge isolate *Streptomyces* sp. SM17 when compared to its terrestrial relative *S. albidoflavus* J1074. *Microorganisms* **2019**, *7*, 394. [CrossRef]
11. Tracanna, V.; De Jong, A.; Medema, M.H.; Kuipers, O.P. Mining prokaryotes for antimicrobial compounds: From diversity to function. *FEMS Microbiol. Rev.* **2017**, *41*, 417–429. [CrossRef]
12. Durand, G.A.; Raoult, D.; Dubourg, G. Antibiotic discovery: History, methods and perspectives. *Int. J. Antimicrob. Agents* **2019**, *53*, 371–382. [CrossRef]
13. Nett, M. Genome mining: Concept and strategies for natural product discovery. *Prog. Chem. Org. Nat. Prod.* **2014**, *99*, 199–245.
14. Challis, G.L. Genome mining for novel natural product discovery. *J. Med. Chem.* **2008**, *51*, 2618–2628. [CrossRef]
15. Ziemert, N.; Alanjary, M.; Weber, T. The evolution of genome mining in microbes—A review. *Nat. Prod. Rep.* **2016**, *33*, 988–1005. [CrossRef]
16. Trivella, D.B.B.; De Felicio, R. The tripod forbacterial natural product discovery: Genome mining, silent pathway induction, and mass spectrometry-based molecular networking. *mSystems* **2018**, *3*, e00160–e17. [CrossRef]
17. Koonin, E.V. Evolution of genome architecture. *Int. J. Biochem. Cell Biol.* **2009**, *41*, 298–306. [CrossRef]
18. Timmermans, M.L.; Paudel, Y.P.; Ross, A.C. Investigating the biosynthesis of natural products from marine proteobacteria: A survey of molecules and strategies. *Mar. Drugs* **2017**, *15*, 235. [CrossRef]
19. Blin, K.; Medema, M.H.; Kazempour, D.; Fischbach, M.A.; Breitling, R.; Takano, E.; Weber, T. antiSMASH 2.0—A versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Res.* **2013**, *41*, 204–212. [CrossRef]
20. Skinnider, M.A.; Dejong, C.A.; Rees, P.N.; Johnston, C.W.; Li, H.; Webster, A.L.H.; Wyatt, M.A.; Magarvey, N.A. Genomes to natural products PRediction Informatics for Secondary Metabolomes (PRISM). *Nucleic Acids Res.* **2015**, *43*, 9645–9662. [CrossRef]
21. Machado, H.; Tuttle, R.N.; Jensen, P.R. Omics-based natural product discovery and the lexicon of genome mining. *Curr. Opin. Microbiol.* **2017**, *39*, 136–142. [CrossRef]
22. Hadjithomas, M.; Chen, I.A.; Chu, K.; Huang, J.; Ratner, A.; Palaniappan, K.; Andersen, E.; Markowitz, V.; Kyrpides, N.C.; Ivanova, N. IMG-ABC: New features for bacterial secondary metabolism analysis and targeted biosynthetic gene cluster discovery in thousands of microbial genomes. *Nucleic Acids Res.* **2017**, *45*, 560–565. [CrossRef]

23. Helfrich, E.J.N.; Reite, S.; Piel, J. Recent advances in genome-based polyketide discovery. *Curr. Opin. Biotechnol.* **2014**, *29*, 107–115. [CrossRef]
24. Olano, C.; Méndez, C.; Salas, J.A. Molecular insights on the biosynthesis of antitumour compounds by actinomycetes. *Microb. Biotechnol.* **2011**, *4*, 144–164. [CrossRef]
25. Nett, M.; Ikeda, H.; Moore, B.S. Genomic basis for natural product biosynthetic diversity in the actinomycetes. *Nat. Prod. Rep.* **2009**, *26*, 1362–1384. [CrossRef]
26. Olano, C.; Méndez, C.; Salas, J.A. Strategies for the design and discovery of novel antibiotics using genetic engineering and genome mining. In *Antimicrobial Compounds: Current Strategies and New Alternatives*; Villa, T.G., Veiga-Crespo, P., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 1–25.
27. Mody, K.H.; Haldar, S. Genome mining for bioactive compounds. In *Springer Handbook of Marine Biotechnology*; Kim, S.K., Ed.; Springer Handbooks; Springer: Berlin/Heidelberg, Germany, 2015; pp. 531–539.
28. Scheffler, R.J.; Colmer, S.; Tynan, H.; Demain, A.L.; Gullo, V.P. Antimicrobials, drug discovery, and genome mining. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 969–978. [CrossRef]
29. Zerikly, M.; Challis, G.L. Strategies for the discovery of new natural products by genome mining. *ChemBioChem* **2009**, *10*, 625–633. [CrossRef]
30. Breitling, R.; Takano, E. Synthetic biology advances for pharmaceutical production. *Curr. Opin. Biotechnol.* **2015**, *35*, 46–51. [CrossRef]
31. Keasling, J.D. Synthetic biology for dynthetic chemistry. *ACS Chem. Biol.* **2008**, *3*, 64–76. [CrossRef]
32. Quin, M.B.; Schmidt-Dannert, C. Designer microbes for biosynthesis. *Curr. Opin. Biotechnol.* **2014**, *29*, 55–61. [CrossRef]
33. Singh, V. Recent advancements in synthetic biology: Current status and challenges. *Gene* **2014**, *535*, 1–11. [CrossRef]
34. Sleator, R.D. The synthetic biology future. *Bioengineered* **2014**, *5*, 69–72. [CrossRef]
35. Unkles, S.E.; Valiante, V.; Mattern, D.J.; Brakhage, A.A. Synthetic biology tools for bioprospecting of natural products in eukaryotes. *Chem. Biol.* **2014**, *21*, 1–7. [CrossRef]
36. Wilson, M.C.; Piel, J. Metagenomic approaches for exploiting uncultivated bacteria as a resource for novel biosynthetic enzymology. *Chem. Biol.* **2013**, *20*, 636–647. [CrossRef]
37. Wright, G. Synthetic biology revives antibiotics. *Nature* **2014**, *509*, S13.
38. Medema, M.H.; Breitling, R.; Bovenberg, R.; Takano, E. production in microorganisms. *Nat. Publ. Gr.* **2010**, *9*, 131–137.
39. Cobb, R.E.; Luo, Y.; Freestone, T.; Zhao, H. Drug discovery and development via synthetic biology. In *Synthetic Biology—Tools and Applications*; Zhao, H., Ed.; Elsevier: Oxford, UK, 2013; pp. 183–206.
40. Hranueli, D.; Starcevic, A.; Zucko, J.; Rojas, J.D.; Diminic, J.; Baranasic, D.; Gacesa, R.; Padilla, G.; Long, P.F.; Cullum, J. Synthetic biology: A novel approach for the construction of industrial microorganisms. *Food Technol. Biotechnol.* **2013**, *51*, 3–11.
41. Zakeri, B.; Lu, T.K. Synthetic biology of antimicrobial discovery. *ACS Synth. Biol.* **2012**. [CrossRef]
42. Cummings, M.; Breitling, R.; Takano, E. Steps towards the synthetic biology of polyketide biosynthesis. *FEMS Microbiol. Lett.* **2014**, *351*, 116–125. [CrossRef]
43. Genilloud, O. The re-emerging role of microbial natural products in antibiotic discovery. *Antonie Van Leeuwenhoek* **2014**, *106*, 173–188. [CrossRef]
44. Luo, Y.; Cobb, R.E.; Zhao, H. Recent advances in natural product discovery. *Curr. Opin. Biotechnol.* **2014**, *30*, 230–237. [CrossRef]
45. Porro, D.; Branduardi, P.; Sauer, M.; Mattanovich, D. Old obstacles and new horizons for microbial chemical production. *Curr. Opin. Biotechnol.* **2014**, *30*, 101–106. [CrossRef]
46. Paddon, C.J.; Keasling, J.D. Semi-synthetic artemisinin: A model for the use of synthetic biology in pharmaceutical development. *Nat. Rev. Microbiol.* **2014**, *12*, 355–367. [CrossRef]
47. Kurita, K.L.; Glassey, E.; Linington, R.G. Integration of high-content screening and untargeted metabolomics for comprehensive functional annotation of natural product libraries. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 11999–12004. [CrossRef]
48. Wang, G.; Hosaka, T.; Ochi, K.; Wang, G.; Hosaka, T.; Ochi, K. Dramatic activation of antibiotic production in *Streptomyces coelicolor* by cumulative drug resistance mutations. *Appl. Environ. Microbiol.* **2008**, *74*, 2834. [CrossRef]

49. Tang, X.; Li, J.; Milla, N.; Zhang, J.J.; Neill, E.C.O.; Ugalde, J.A.; Jensen, P.R.; Mantovani, S.M.; Moore, B.S. Identification of thiotetronic acid antibiotic biosynthetic pathways by target-directed genome mining. *ACS Chem. Biol.* **2015**, *10*, 2841–2849. [CrossRef]
50. Craney, A.; Ozimok, C.; Pimentel-elardo, S.M.; Capretta, A.; Nodwell, J.R. Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism. *Chem. Biol.* **2012**, *19*, 1020–1027. [CrossRef]
51. Kwon, T.; Lee, G.; Rhee, Y.; Park, H.; Chang, M.; Lee, S.; Lee, J.; Lee, T. Identification of nickel response genes in abnormal early developments of sea urchin by differential display polymerase chain reaction. *Ecotoxicol. Environ. Saf.* **2012**, 1–7.
52. Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.* **2016**, *79*, 629–661. [CrossRef]
53. Jin, J.; Yang, X.; Liu, T.; Xiao, H.; Wang, G.; Zhou, M.; Liu, F.; Zhang, Y.; Liu, D.; Chen, M.; et al. Fluostatins M–Q featuring a 6-5-6-6 ring skeleton and high oxidized A-rings from marine *Streptomyces* sp. PKU-MA00045. *Mar. Drugs* **2018**, *16*, 87. [CrossRef]
54. Hornung, A.; Bertazzo, M.; Dziarnowski, A.; Schneider, K.; Welzel, K.; Wohlert, S.; Holzenkämpfer, M.; Nicholson, G.J.; Bechthold, A.; Süssmuth, R.D.; et al. A genomic screening approach to the structure-guided identification of drug candidates from natural sources. *ChemBioChem* **2007**, *8*, 757–766. [CrossRef]
55. Mcalpine, J.B.; Bachmann, B.O.; Piraee, M.; Tremblay, S.; Alarco, A.; Zazopoulos, E.; Farnet, C.M. Microbial genomics as a guide to drug discovery and structural elucidation: ECO-02301, a novel antifungal agent, as an example. *J. Nat. Prod.* **2005**, *68*, 493–496. [CrossRef]
56. Liu, W.; Kersten, R.D.; Yang, Y.; Moore, B.S.; Dorrestein, P.C. Imaging mass spectrometry and genome mining via short sequence tagging identified the anti-infective agent arylomycin in Streptomyces roseosporus. *J. Am. Chem. Soc.* **2011**, *133*, 18010–18013. [CrossRef]
57. Liu, W.; Lamsa, A.; Wong, W.R.; Boudreau, P.D.; Kersten, R.; Peng, Y.; Moree, W.J.; Duggan, B.M.; Moore, B.S.; Gerwick, W.H.; et al. MS/MS-based networking and peptidogenomics guided genome mining revealed the stenothricin gene cluster in *Streptomyces roseosporus*. *J. Antibiot. Tokyo* **2014**, *67*, 99–104. [CrossRef]
58. Seo, M.; Zhu, D.; Endo, S.; Ikeda, H.; Cane, D.E. Genome mining in *Streptomyces*. Elucidation of the role of baeyer-villiger monooxygenases and non-heme iron-dependent dehydrogenase/oxygenases in the final steps of the biosynthesis of pentalenolactone and neopentalenolactone. *Biochemistry* **2011**, *50*, 1739–1754. [CrossRef]
59. Tang, J.; Liu, X.; Peng, J.; Tang, Y.; Zhang, Y. Genome sequence and genome mining of a marine-derived antifungal bacterium *Streptomyces* sp. M10. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 2763–2772. [CrossRef]
60. Xu, M.; Wang, Y.; Zhao, Z.; Gao, G.; Huang, S.-X.; Kang, Q.; He, X.; Lin, S.; Pang, X.; Deng, Z.; et al. Functional genome mining for metabolites encoded by large gene clusters using heterologous expression of a whole genomic BAC library in *Streptomyces*. *Appl. Environ. Microbiol.* **2016**, *82*, 5795–5805. [CrossRef]
61. Cano-prieto, C.; García-Salcedo, R.; Sánchez-Hidalgo, M.; Braña, A.F.; Fiedler, H.-P.; Méndez, C.; Salas, J.A.; Olano, C. Genome mining of *Streptomyces* sp. Tü 6176: Characterization of nataxazole biosynthesis pathway. *ChemBioChem* **2015**, *16*, 1461–1473. [CrossRef]
62. Ye, S.; Molloy, B.; Braña, A.F.; Zabala, D.; Olano, C.; Cortés, J.; Morís, F.; Salas, J.A.; Méndez, C. Identification by genome mining of a Type I Polyketide gene cluster from *Streptomyces argillaceus* involved in the biosynthesis of pyridine and piperidine alkaloids Argimycins P. *Front. Microbiol.* **2017**, *8*, 1–18. [CrossRef]
63. Paulo, B.S.; Sigrist, R.; Angolini, C.F.F.; Oliveira, L.G. De New cyclodepsipeptide derivatives revealed by genome mining and molecular networking. *Chem. Sel.* **2019**, *4*, 7785–7790.
64. Purves, K.; Macintyre, L.; Brennan, D.; Hreggviðsson, G.Ó.; Kuttner, E.; Ásgeirsdóttir, M.E.; Young, L.C.; Green, D.H.; Edrada-ebel, R.; Duncan, K.R. Using molecular networking for microbial secondary metabolite bioprospecting. *Metabolites* **2016**, *6*, 2. [CrossRef]
65. Deng, H.; Ma, L.; Bandaranayaka, N.; Qin, Z.; Mann, G.; Kyeremeh, K.; Yu, Y.; Shepherd, T.; Naismith, J.H.; O'Hagan, D. Identification of fluorinases from *Streptomyces* sp. MA37, *Norcardia brasiliensis*, and *Actinoplanes* sp. N902-109 by genome mining. *ChemBioChem* **2014**, *15*, 364–368. [CrossRef] [PubMed]
66. Anoop, A.; Antunes, A. Whole genome sequencing of the symbiont *Pseudovibrio* sp. from the intertidal marine sponge *Polymastia penicillus* revealed a gene repertoire for host-switching permissive lifestyle. *Genome Biol. Evol.* **2015**, *7*, 3022–3032.

67. Bertin, M.J.; Schwartz, S.L.; Lee, J.; Korobeynikov, A.; Dorrestein, P.C.; Gerwick, L.; Gerwick, W.H. Spongosine production by a *Vibrio harveyi* strain associated with the sponge *Tectitethya crypta*. *J. Nat. Prod.* **2014**, *78*, 493–499. [CrossRef]
68. Jeske, O.; Jogler, M.; Petersen, J.; Sikorski, J.; Jogler, C. From genome mining to phenotypic microarrays: *Planctomycetes* as source for novel bioactive molecules. *Antonie Van Leeuwenhoek* **2013**, *104*, 551–567. [CrossRef]
69. Guérard-Hélaine, C.; de Berardinis, V.; Besnard-Gonnet, M.; Darii, E.; Debacker, M.; Debard, A.; Fernandes, C.; Hélaine, V.; Mariage, A.; Pellouin, V.; et al. Genome mining for innovative biocatalysts: New dihydroxyacetone aldolases for the Chemist' s Toolbox. *ChemCatChem* **2015**, *7*, 1871–1879. [CrossRef]
70. Singh, S.P.; Klisch, M.; Sinha, R.P.; Häder, D. Genomics genome mining of mycosporine-like amino acid (MAA) synthesizing and non-synthesizing cyanobacteria: A bioinformatics study. *Genomics* **2010**, *95*, 120–128. [CrossRef]
71. Micallef, M.L.; Agostino, P.M.D.; Sharma, D.; Viswanathan, R.; Moffitt, M.C. Genome mining for natural product biosynthetic gene clusters in the Subsection V cyanobacteria. *BMC Genom.* **2015**, 1–20. [CrossRef]
72. Fuerst, J.A.; Sagulenko, E. Beyond the bacterium: Planctomycetes structure and function. *Nat. Rev. Microbiol.* **2011**, *9*, 13–18. [CrossRef]
73. Oren, A.; Gunde-cimerman, N. Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiol. Lett.* **2007**, *269*, 1–10. [CrossRef]
74. Keller, N.P.; Turner, G.; Bennett, J.W. Fungal secondary metabolism—from biochemistry to genomics. *Nat. Rev. Microbiol.* **2015**, *3*, 937–947. [CrossRef]
75. Bergmann, S.; Schümann, J.; Scherlach, K.; Lange, C.; Brakhage, A.A.; Hertweck, C. Genomics-driven discovery of PKS-NRPS hybrid metabolites from *Aspergillus nidulans*. *Nat. Chem. Biol.* **2007**, *3*, 213–217. [CrossRef]
76. Mao, X.; Xu, W.; Li, D.; Yin, W.; Chooi, Y.; Li, Y.; Tang, Y.; Hu, Y. Epigenetic genome mining of an endophytic fungus leads to the pleiotropic biosynthesis of natural products. *Angew. Commun.* **2015**, *54*, 7592–7596. [CrossRef]
77. Ding, Y.; De Wet, J.R.; Cavalcoli, J.; Li, S.; Greshock, T.J.; Miller, K.A.; Finefield, J.M.; Sunderhaus, J.D.; Mcafoos, T.J.; Tsukamoto, S.; et al. Genome-based characterization of two prenylation steps in the assembly of the Stephacidin and Notoamide anticancer agents in a marine-derived *Aspergillus* sp. *J. Am. Chem. Soc.* **2010**, *132*, 12733–12740. [CrossRef]
78. Ye, Y.; Minami, A.; Mándi, A.; Liu, C.; Taniguchi, T.; Kuzuyama, T.; Monde, K.; Gomi, K.; Oikawa, H. Genome mining for sesterterpenes using bifunctional terpene synthases reveals a unified intermediate of di/sesterterpenes. *J. Am. Chem. Soc.* **2015**, *137*, 11846–11853. [CrossRef]
79. Mojib, N.; Amad, M.; Thimma, M.; Aldanondo, N.; Kumaran, M.; Irigoien, X. Carotenoid metabolic profiling and transcriptome-genome mining reveal functional equivalence among blue-pigmented copepods and appendicularia. *Mol. Ecol.* **2014**, *23*, 2740–2756. [CrossRef]
80. Huang, A.C.; Kautsar, S.A.; Hong, Y.J.; Medema, M.H.; Bond, A.D.; Tantillo, D.J.; Osbourn, A. Unearthing a sesterterpene biosynthetic repertoire in the Brassicaceae through genome mining reveals convergent evolution. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 1–10. [CrossRef]

marine drugs

MDPI

Review

Sponges and Their Symbionts as a Source of Valuable Compounds in Cosmeceutical Field

Roberta Esposito [1,2,†], Nadia Ruocco [1,†], Thomas Viel [3], Serena Federico [1,2,3], Valerio Zupo [3,*] and Maria Costantini [1,*]

[1] Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy; roberta.esposito@szn.it (R.E.); nadia.ruocco@szn.it (N.R.); serena.federico@studenti.unina.it (S.F.)
[2] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Naples, Italy
[3] Stazione Zoologica Anton Dohrn, Department of Marine Biotechnology, Villa Dohrn, Punta San Pietro, 80077 Naples, Italy; thomas.viel7@gmail.com
* Correspondence: valerio.zupo@szn.it (V.Z.); maria.costantini@szn.it (M.C.)
† These authors contributed equally to this work.

Abstract: In the last decades, the marine environment was discovered as a huge reservoir of novel bioactive compounds, useful for medicinal treatments improving human health and well-being. Among several marine organisms exhibiting biotechnological potential, sponges were highlighted as one of the most interesting phyla according to a wide literature describing new molecules every year. Not surprisingly, the first marine drugs approved for medical purposes were isolated from a marine sponge and are now used as anti-cancer and anti-viral agents. In most cases, experimental evidence reported that very often associated and/or symbiotic communities produced these bioactive compounds for a mutual benefit. Nowadays, beauty treatments are formulated taking advantage of the beneficial properties exerted by marine novel compounds. In fact, several biological activities suitable for cosmetic treatments were recorded, such as anti-oxidant, anti-aging, skin whitening, and emulsifying activities, among others. Here, we collected and discussed several scientific contributions reporting the cosmeceutical potential of marine sponge symbionts, which were exclusively represented by fungi and bacteria. Bioactive compounds specifically indicated as products of the sponge metabolism were also included. However, the origin of sponge metabolites is dubious, and the role of the associated biota cannot be excluded, considering that the isolation of symbionts represents a hard challenge due to their uncultivable features.

Keywords: sponges; bacteria; fungi; anti-oxidant; anti-aging; skin whitening; anti-microbial; photoprotection

Citation: Esposito, R.; Ruocco, N.; Viel, T.; Federico, S.; Zupo, V.; Costantini, M. Sponges and Their Symbionts as a Source of Valuable Compounds in Cosmeceutical Field. *Mar. Drugs* **2021**, *19*, 444. https://doi.org/10.3390/md19080444

Academic Editor: Marialuisa Menna

Received: 6 July 2021
Accepted: 30 July 2021
Published: 2 August 2021

1. Introduction

Marine sponges represent a fascinating phylum of marine invertebrates, hosting a wide symbiotic community together with a huge production of secondary metabolites [1–7]. The sponge-associated biota may bring together a broad group of phylogenetic lineages, including archaea, bacteria, and fungi [8,9]. The relationships between sponges and their mutualistic symbionts are complex, and the production of bioactive secondary metabolites might have a possible defense role or be involved in the competition for space within benthic habitats [10,11]. On the whole, sponge symbionts were recognized to be responsible for the host metabolism and growth, chemical defense, and adaptation to biotic and abiotic stressors [2,12–14].

The discovery of marine bioactive metabolites as potential drugs for the pharmaceutical, nutraceutical, and cosmeceutical industries prompted several research projects relying on the identification of novel chemical moieties with innovative biological functions [15]. Recently, the cosmeceutical field has been fast-growing, since consumers have given greater

attention to creams and lotions containing natural compounds with pharmacological properties [16]. Cosmeceuticals are topical products containing some bioactive ingredients that mimic drug-like benefits by enhancing skin health-related function [16,17]. On a global scale, the cosmeceutical industry is gradually shifting to natural compounds for their biocompatible, safe, and eco-friendly properties [18]. The success of cosmeceutical productions primarily depends on safety; low costs; and the ability to maintain the active ingredient, deliver it in a biologically active form, and exert a biological effect through known mechanisms [19]. To overcome these latter issues, particularly related to unsuitable chemical properties, some encapsulation and nano-formulation methods were developed to greatly improve drug delivery and effectiveness [20–25].

Despite cosmeceuticals being historically retrieved from terrestrial plants [26–28], in the last decades, several of them were discovered in marine environments. In fact, the ocean represents a rich source of bioactive ingredients, such as vitamins, minerals, amino acids, proteins, lipids, polysaccharides, terpenoids, polyphenols, pigments, and enzymes, which find several applications in the cosmeceutical field [29]. Marine cosmeceuticals showed a broad range of beneficial activities, such as anti-oxidants, anti-UV, anti-aging, anti-tyrosinase (skin whitening), anti-microbial, wound healing, and emulsifying properties (Figure 1) [29–43].

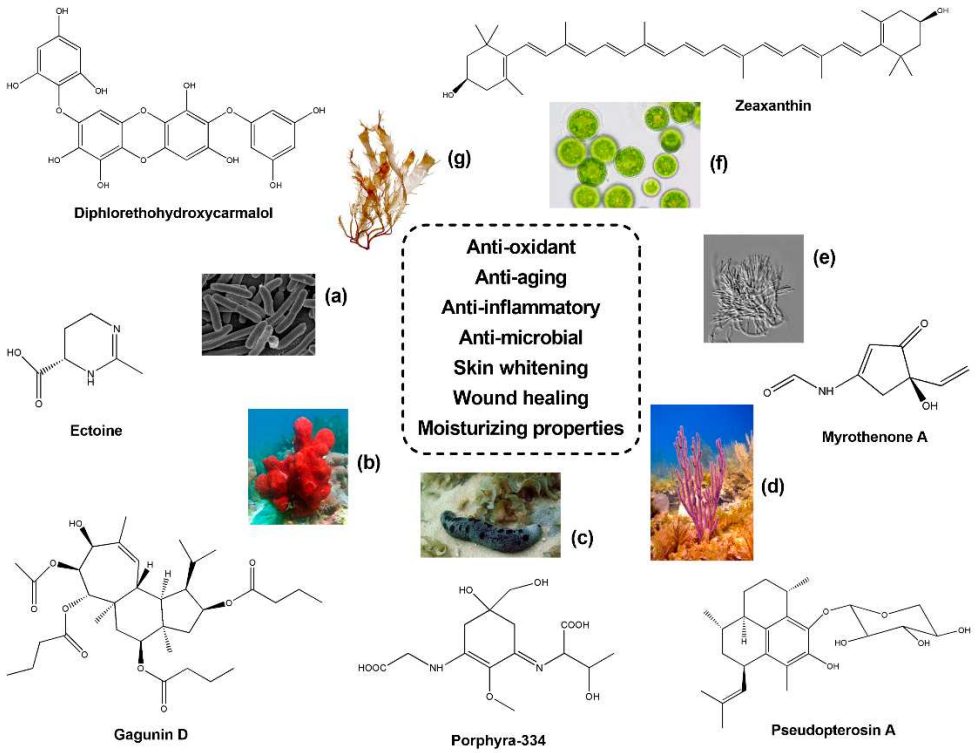

Figure 1. Examples of bioactive compounds isolated from bacteria (**a**), sponges (**b**), echinoderms (**c**), corals (**d**), fungi (**e**), micro- (**f**) and macro-algae (**g**) reported as suitable candidates for the formulation of cosmetics.

Recently, much attention has been paid to marine anti-oxidants, including mycosporines and mycosporine-like amino acids (MAAs), carotenoids and other compounds exhibiting multiple roles within cosmeceutical field [44,45]. Some examples are pigments (e.g., carotenoids), extremely abundant in the marine environment since they are produced by all autotrophic organisms (e.g., bacteria, archaea, algae and fungi). Carotenoids include carotenes (e.g., lycopene and α- and β-carotene) and xanthophylls (e.g., astaxanthin, fucoxanthin and lutein), which showed anti-oxidant activities [46] protecting skin from Reactive Oxygen Species (ROS) that are normally released within the cells after the natural oxidation induced by UV radiation and skin aging [42]. Since synthetic compounds

may exert toxic effects for human health and wellness [47], natural anti-oxidants were investigated for their potential use in cosmetics [48,49]. Anti-microbial and anti-fouling agents that protect against skin disease-related pathogens, such as *Staphylococcus epidermis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, were also described from various sources and considered useful tools for the formulation of cosmetic products and dermatological treatments [39,50–54]. Moreover, bioactive compounds with anti-tyrosinase activity found several applications in the cosmetic industry, since tyrosinase represents a key enzyme involved in melanin biosynthesis, and the block of its enzymatic activity might be used for skin whitening treatments, whose deployment is extremely popular in some countries [55]. Surfactants and emulsifiers, with both hydrophilic and hydrophobic groups, could also be used in the cosmetic field [56,57]. Several protein-polysaccharide complexes, glycolipids and lipopeptides isolated from marine microorganisms were studied for the production of biosurfactants and bioemulsifiers [58]. For instance, chitosan, due to its high water-binding capacity, was proposed as a skin moisturizer and delivery agent in cosmeceutical preparations of anti-aging products [59].

Recognized producers of marine cosmeceuticals are cyanobacteria, along with micro- and macro-algae [24,60–63], with several compounds under clinical trials or already approved for the market [64,65]. As mentioned before, sponge-associated microbiota produce a plethora of bioactive compounds with beneficial properties for human health [6]. Despite the great biotechnological relevance, so far, only a few studies have reviewed the potential applications of sponge symbiont metabolites in the cosmetic field focusing on specific sponge species [66] or grouping several taxa of marine organisms [29].

In the present review, we analyzed a collection of scientific literature on sponge symbiont-related compounds displaying interesting biological activities in the cosmeceutical field. In particular, we focused on bacteria and fungi, which are extremely abundant within sponge associated communities. Moreover, we also considered sponge metabolites, whose biological activities were found extremely suitable for cosmeceutical formulations.

2. Sponge Symbionts in Cosmeceutical Field

2.1. Bacteria

A variety of bioactive compounds described from marine bacteria such as polyketides, alkaloids, peptides, proteins, lipids, mycosporines and MAAs, glycosides, isoprenoids and hybrids, displayed surprising activities, such as photo-protective, anti-aging, anti-microbial, anti-oxidant, and moisturizing activities [58,67]. The interesting capability to produce some UV-absorbing compounds, including scytonemins (exclusively cyanobacteria), mycosporines, carotenoids and melanin, was explained through possible evolutionary mechanisms evolved to protect sponges from the harmful effects of UV radiation [68,69].

As reported in the introduction section, carotenoids, such as β-carotene and lycopene, exhibited a photoprotective activity, thus revealing several applications in cosmeceutical fields [70]. Dharmaraj and co-authors [71] investigated the carotenoid extract of a *Streptomyces strain* (AQBWWS1) associated to the sponge *Callyspongia diffusa* collected from the west coast of Kerala (India). Its chemical profile revealed the presence of lycopene, suggested as a potential ingredient for the preparation of cosmetic products [71].

A novel diapolycopenedioic acid xylosyl ester A, extracted from the marine sponge-derived bacterium *Rubritalea squalenifaciens* sp. nov., revealed a potent anti-oxidant activity in a $^1O_2$ suppression model with half maximal inhibitory concentration (IC_{50}) of 4.1 μg/mL [72]. The alkaloid Diazepinomicin was also isolated from the strain *Micromonospora* sp. RV115 associated to the sponge *Aplysina aerophoba* collected from the Mediterranean Sea. This molecule was able to protect the human kidney (HK-2) and human promyelocytic (HL-60) cell lines from toxicity and genomic damage induced by H_2O_2 [73]. The metabolites isolated from *Virgibacillus* sp. associated to the sponge *C. diffusa* (Gulf of Mannar) showed 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity with IC_{50} of 857.49 μg/mL. In addition, a clear hydroxyl and superoxide free radical scavenging activity was detected (IC_{50} = 471.07 μg/mL and 1353.28 μg/mL, respectively),

probably correlated to the presence of bioactive compounds such as alkaloids, terpenoids, reducing sugars and anthroquinones, detected by chemical analyses [74]. In similar works, two strains of *Vibrio* (P1Ma8 and P1Ma5) and several *Bacillus* sp. isolated from the sponges *Phorbas tenacior* and *Tedania anhelans*, respectively, displayed enhanced free radical scavenging activity evaluated by DPPH assay [75,76]. The anti-oxidant properties of a bioactive compound (Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-$C_7H_{10}N_2O_2$) extracted from a sponge-derived *Bacillus* sp. (Lakshadweep archipelago in India) was also studied using DPPH assay, nitric oxide (NO) and hydrogen peroxide (H_2O_2) scavenging activity, and total reducing power. The active compound was capable of scavenging H_2O_2 in a dose-dependent manner. Moreover, IC_{50} for NO and DPPH inhibition was 41.70 µg/mL and 15.025 µg/mL, respectively [77].

Bioactivity screening of one hundred bacterial bionts isolated from several Indian sponges led to the isolation of the GUVFCFM-3 strain, identified as *Chromohalobacter israelensis*. In particular, the methanol extract showed a significant percentage of DPPH (67.83%) and superoxide (65.87%) scavenging activities [78]. DPPH tests and quantification of total phenolic content (TPC) were also used to evaluate the anti-oxidant activity of *Pseudomonas* sp. extract associated to the marine sponge *Hyrtios aff. erectus* from the Red Sea. In particular, DPPH assay showed a 100% of inhibition at all quantities tested (50, 25, 12.5 and 6.25 mg) [79]. Moreover, Vijayan et al. [80] demonstrated that bacteria associated to darkly pigmented sponges (*Haliclona pigmentifera*, *Sigmadocia pumila*, *Fasciospongia cavernosa*, *Spongia officinalis* and *C. diffusa*) collected from the Gulf of Mannar in Indian ocean produced non-cytotoxic melanin, with anti-oxidant and photoprotective activities. Among bacterial strains demonstrating high production of melanin, *Vibrio alginolyticus*, isolated from *Haliclona pigmentifera*, *Sigmadocia pumila* and *S. officinalis*, protected mouse fibroblast cells (L929) from UV-induced intracellular reactive oxygen stress (IC_{50} = 9.0 µg/mL) and exerted no cytotoxicity on L929 cells and brine shrimps [80]. Sponge derived strains retrieved from Indonesian waters, HAL-08, HAL-13 and HAL-74 (*Haliclona* sp.) as well as PTR-21 (*Petrosia* sp.), were evaluated using the DPPH and ABTS (2,2′-azinobis3-ethylbenzothiazoline-6-sulfonate) methods. Among the isolates tested, the highest anti-oxidant activity was revealed by the crude extract of HAL-08 with IC_{50} values of 17.10 and 59.39 µg/mL for DPPH and ABTS radicals, respectively. In addition, PTR-21 appeared to be the most potent anti-aging agent tested on the viability of *Schizosaccharomyces pombe* [81]. The anti-oxidant activity of bacteria PTR-08, PTR-40, PTR-41, and PTR-47, identified as *Pseudomonas* sp., was also evaluated. PTR-08 extract exhibited the highest anti-oxidant properties with IC_{50} values of 9.25 and 235.53 µg/mL for DPPH and ABTS radicals, respectively. Interestingly, PTR-08 modulated yeast longevity of *Schizosaccharomyces pombe* promoting the anti-oxidant defence mechanisms correlated with intracellular oxidative stress [82]. The same authors examined the extract of another Indonesian bacteria (*Pseudoalteromonas flavipulchra*, named STILL-33) associated to the sponge *Stylotella* sp. STILL-33, which exhibited a high DPPH and ABTS degrading activity with IC_{50} values of 7.80 µg/mL (DPPH) and 31.50 µg/mL (ABTS) [83].

Some works, together with the anti-oxidant capabilities, evaluated the growth inhibition activity of specific pathogens commonly involved in skin infections. For instance, a chlorinated quinolone, Ageloline A, isolated from *Streptomyces* sp. SBT345, a bacterial symbiont of the Mediterranean sponge *Agelas oroides*, was investigated for its radical scavenging and anti-microbial properties. This compound exhibited anti-oxidant potential on a human leukemic cell line (HL-60) and was further able to reduce oxidative stress and genomic damage induced by 4-nitroquinoline-1-oxide (NQO). Moreover, Ageloline A inhibited the growth of *Chlamydia trachomatis* in a dose-dependent manner with an IC_{50} value of 2.14 µg/mL [84]. Anti-microbial activities against *E. coli* MTCC-1687, *P. aeruginosa* MTCC-1688, *B. subtilis* MTCC-441 and *S. aureus* MTCC-737 were also observed from a GSA10 strain associated to the sponge *Halichondria glabrata* (West coast of Mumbai, India). In addition, anti-oxidant properties were detected using DPPH scavenging and Total Radical-trapping Anti-oxidant Parameter (TRAP) assay. In particular, through TRAP assay,

the GSA10 acted as peroxyl scavengers, and the percentage of inhibition was proportional to the GSA10 concentrations [85]. In a recent work, the crude methanolic extract and the fractions of *Bacillus* 2011SOCCUF3 strain isolated from the sponge *S. officinalis* (Cortiou and Riou, France) exhibited anti-oxidant and anti-microbial activities. In particular, DPPH assay showed a dose-dependent scavenging activity, with a percentage inhibition of 38.9-49.1% (10–50 mg/mL), and agar-well diffusion method revealed a high inhibitory effect against *C. albicans* at a concentration range of 2.5–20 mg/mL [86].

Anti-aging and skin whitening properties from the crude extracts of bacterial symbionts from *Scopalina hapalia* (South-east coasts of Mayotte) were evaluated on several targets, including elastase, tyrosinase, catalase, sirtuin 1 (Sirt1), cyclin-dependent kinase 7 (CDK7), fyn kinase, and proteasome [66]. In particular, the isolate SH-82 (*Micromonospora fluostatini*) exhibited sufficient inhibition of elastase activity, whereas SH-89 exerted significant anti-melanogenic properties by tyrosinase inhibition (58.33%). The most potent activators of Sirt1 activity were shown by SH-82 and SH-100 (*Bacillus licheniformis*) extracts. Moreover, four *Bacillus* strains and three extracts of *Salinispora arenicola* exhibited appreciable anti-oxidant and CDK7 inhibitory activities, respectively. Surprising results were reported from *S. arenicola* (SH-78-EA-SM) and *B. licheniformis* (SH-04-EA-SM), inhibiting Fyn activity at the three concentrations tested (0.033, 0.0033 and 0.00033 µg/mL). In contrast, the crude extracts of SH-45, SH-54, SH-78, and SH-99 exhibited a slight activity, detected only at the highest concentration (0.033 µg/mL). On the whole, the authors have proposed these sponge-derived bacteria as suitable sources of new skin whitening and anti-aging agents [66].

As mentioned above (see the introduction section), microbial biosurfactants displayed suitable properties for skincare formulations [57]. For instance, Dhasayan et al. [87] evaluated the moisturizing features of several strains isolated from the Indian sponge *C. diffusa*. In particular, the MB-30 (*Halomonas* sp.) and MB-I9 (*Alcaligenes* sp.) exhibited the highest emulsification activity after 48 h of incubation, whereas MB-7 (*Bacillus subtilis*) and MB-101 (*Bacillus amyloliquefaciens*) isolates showed the same properties after 72 h of incubation, suggesting that the bioactive compounds are probably secreted during the stationary phase of growth [87]. Moreover, in a recent work, a bacterial strain (*Bacillus niabensis*, My-30) associated to the sponge *Mycale ramulosa* (Gulf of California) showed a clear activity in the collapsing drop test and emulsification properties with high stability for 24 h, compared to the control (Sodium Dodecyl Sulfate, SDS). Moreover, supernatants of My-30 demonstrated a promising antifouling activity, with Minimum Inhibitory Concentration (MIC) values of 1–2% (*v/v*), against *Bacillus subtilis*, *Micrococcus* sp., and *Sagittula stellata* [88].

The cosmeceutical compounds discussed above are listed in Table 1.

Table 1. Bacterial source, sponge host, compound/extract, biological activity, and reference are reported.

Source	Sponge Host	Extract/Compound	Biological Activity	Reference
R. squalenifaciens	*Halichondria okadai*	Diapolycopenedioic acid xylosyl ester A	Anti-oxidant	[72]
Streptomyces	*C. diffusa*	Carotenoid extracts	Anti-aging	[71]
Micromonospora sp. RV115	*A. aerophoba*	Diazepinomicin	Anti-oxidant	[73]
Virgibacillus sp.	*C. diffusa*	Ethyl acetate extracts	Anti-oxidant	[74]
Vibrio (P1Ma8 and P1Ma5)	*P. tenacior*	CH_2Cl_2/MeOH (1:1) extracts	Anti-oxidant	[75]
Bacillus sp.	*T. anhelans*	Ethyl acetate extracts	Anti-oxidant	[76]
Bacillus sp.	Sponges from Lakshadweep archipelago	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-$C_7H_{10}N_2O_2$	Anti-oxidant	[77]
Halomonas sp. MB-30 and *Alcaligenes* sp. MB-I9	*C. diffusa*	Isolates of bacteria	Biosurfactants	[87]
C. israelensis	*Callyspongia fibrosa*	Ethyl acetate extracts	Anti-oxidant	[78]
Streptomyces sp. SBT345	*A. oroides*	Ageloline A	Anti-oxidant	[84]
Pseudomonas sp.	*H. aff. Erectus*	Ethyl acetate extracts	Anti-oxidant	[79]

Table 1. *Cont.*

Source	Sponge Host	Extract/Compound	Biological Activity	Reference
V. alginolyticus	*H. pigmentifera*, *S. pumila* and *S. officinalis*	Melanin extracts	Anti-oxidant	[80]
GSA10	*H. glabrata*	Ethyl acetate extracts	Anti-oxidant	[85]
HAL-08, HAL-13, HAL-74 and PTR-21	*Haliclona* sp. and *Petrosia* sp.	Ethyl acetate extracts	Anti-oxidant	[81]
PTR-08, PTR-40, PTR-41, and PTR-47	*Petrosia* sp.	Ethyl acetate extracts	Anti-oxidant	[82]
P. flavipulchra STILL-33	*Stylotella* sp.	Ethyl acetate extracts	Anti-oxidant and anti-aging	[83]
B. niabensis (My-30)	*M. ramulosa*	Isolates of bacteria	Biosurfactants	[88]
Bacillus 2011SOCCUF3	*S. officinalis*	Methanol crude extracts	Anti-oxidant and anti-microbial	[86]
SH-82 (*M. fluostatini)*	*S. hapalia*	Ethyl acetate and methanol extracts	Anti-oxidant	[66]

2.2. Fungi

So far, several fungal isolates showed pharmacological activities, including anti-bacterial, anti-oxidant, anti-proliferative, and so on [89–93]. Since 1998, a research group published some results about an amylase isolated from the fungus *Mucor* sp. associated to the sponge *Spirastrella* sp. Amylases are enzymes involved in cellular and metabolic pathways, useful for the pharmaceutical, cosmeceutical (as a detergent), and nutraceutical industries [94]. A well-known example is Circumdatin, a benzodiazepine alkaloid that has been isolated from a fungus of the genus *Exophiala* (Bogil Island, Korea). Due to its UVA photo protection, with higher effectiveness than common sunscreen agents, this molecule is now used in commercial products [95]. Similarly, in a recent work, some fungal meroterpenoids described from the fungus *Penicillium brasilianum* WZXY-m122-9, were isolated from an unidentified marine sponge collected in the South of China. Among the chemically characterized compounds, Brasilianoid A was capable of up-regulating *filaggrin* and *Caspase-14* expression and increasing the viability (up to 77%) of UVB irradiated human keratinocytes (HaCaT). The authors considered this compound to be a promising candidate for the treatment of dermatological diseases and skin protection from UVB damages [96].

Concerning the anti-oxidant activity, two extracellular polysaccharides, ENP1 and ENP2, were isolated from the fermentation fluid of the sponge-derived marine fungus *Epicoccum nigrum* (Hainan, China). Both molecules exhibited, in vitro, a slight anti-oxidant capacity by hydroxyl, superoxide, and DPPH assay, with low half maximal effective concentration (EC_{50}) values (280–1570 μg/mL). However, ENP2 was found to be the most active, since a higher radical scavenging activity was measured at all concentrations tested [97]. The same properties were detected from an aromatic polyketide isolated from *Aspergillus versicolor*, a fungus cultured in laboratory conditions after rinsing some tissues excised from the Korean sponge *Petrosia* sp. (Jeju Island in South Korea). By comparing it to standard anti-oxidants, this compound displayed anti-oxidant properties at increasing concentrations (5–100 μg/mL) by DPPH assay and inhibition of lipid peroxidation, higher than butylated hydroxytoluene (BHT) [98]. Bioassay-guided fractionations led to the isolation of other anti-oxidant compounds from the sponge-derived fungus *Penicillium citrinum* SpI080624G1f01 (Ishigaki Island, Japan). The DPPH radical scavenging activity of a sorbicillinoid derivative, named JBIR-124, was found particularly interesting, with an IC_{50} value of 13 μg/mL [99]. In a similar work, DPPH combined to Thiobarbituric acid (TBARS) and NO assay, showed significant anti-oxidative and anti-inflammatory activities of the crude extracts obtained from three fungi, *Chaetomium globosum*, *Gymnascella dankaliensis* and *Nigrospora oryzae*. These fungal strains were isolated from the sponge *Hippospongia communis*, collected between the West coast of Alexandria and the borders

of Libya. In particular, *C. globosum* and *G. dankaliensis* displayed a significant inhibition of lipid peroxidation (93%) and DPPH scavenging activity (59%), respectively, whereas *N. oryzae* was the most effective in inhibiting NO species [100]. Extensive chemical analyses also allowed the identification of more than twenty Spiro-Phthalides and Isocoumarins from the fungus *Setosphaeria* sp. SCSIO41009 associated to the sponge *Callyspongia* sp. retrieved from Chinese waters. Among them, 7-O-demethylmonocerin evidenced a strong scavenging activity on DPPH radicals, with IC_{50} value (11.2 μg/mL) comparable to ascorbic acid [101]. From the ethyl acetate extract of the Chinese strain *Aspergillus europaeus* WZXY-SX-4-1, isolated from the marine sponge *Xestospongia testudinaria*, six polyketide derivatives were separated through chromatographic method. Bioactivity screening showed that three Benzophenones exhibited the most potent scavenging activity against DPPH radicals (IC_{50} = 1.7–5.4 μg/mL) as compared to the positive control (trolox) [102]. A fungus species identified as *Aspergillus unguis* RSPG_204 was isolated from the sponge *Agelas* sp. (Hurghada coast, Red Sea, Egypt). The mycelia extract and culture supernatants exhibited significant superoxide anion scavenging activity, while extremely low anti-tyrosinase capabilities were detected. Interestingly, the biological activity was corroborated by chemical analyses revealing several bioactive compounds in the supernatants of static cultures and mycelial extract [103]. Recently, a marine fungus of the same genus (*Aspergillus terreus*), living as symbiont of the marine sponge *Phakellia fusca*, was found to produce four butenolide derivatives. DPPH assay revealed moderate anti-oxidant properties (IC_{50} = ~14–36 μg/mL) with promising application in the cosmeceutical field [104].

Henriquez et al. [105] sampled eleven marine sponges from the Fildes Bay (King George Island, Antarctica), belonging to the genera *Tedania* sp., *Hymeniacidon* sp., *Dendrilla* sp., and three unidentified ones grouped in the order Poecilosclerida. Through sequence analysis of the ITS1-5.8S-ITS2 region, 24 genotypes linked to the four taxonomic classes Leotiomycetes, Dothiodeomycetes, Eurotiomycetes, and Sordiaromycetes were identified. Among the fungal extracts tested for their anti-bacterial activity on *P. aeruginosa* and *S. aureus* ATCC25922, more than half of them showed inhibitory activity against one of the bacterial strains analyzed. Interestingly, several fungal isolates with the same ITS genotype showed completely different activities. The anti-oxidant capacity, evaluated for all the extracts, revealed a wide range of activities that ranged from very low to extremely high for three isolates of the genus *Epicoccum* (F09T15-3, F09-T15-6) and an unknown one (F09-T18-16) [105].

Moderate anti-bacterial and anti-oxidant activities were instead observed from furan, cyclopentenone and tyrosol derivatives isolated from the fungus species *Hypocrea koningii* PF04 (South China) and Acremostrictin, a tricyclic lactone identified from the culture broth of *Acremonium strictum* (Gagu-do, Korea) [106–108]. In particular, Hypofurans A/B, Hypocrenones A/B/C, and Hypocrol A displayed low inhibitory activity on *S. aureus* ATCC25923 and *E. coli* [107,108]. Moreover, Hypocrol A and Trichodenol B revealed a slight anti-oxidant capacity, with IC_{50} values of 48.5 and 97.4 μg/mL, respectively [108]. Similarly, Acremostrictin reduced DPPH radicals on H_2O_2-induced HaCaT cells in a dose-dependent manner with IC_{50} of 529.2 μg/mL [106]. Since low activities have been detected, these compounds clearly reported unsuitable cosmeceutical features.

The bioactivity of twenty-two fungi associated to several sponge species (*Agelas citrina*, *Stelligera rigida*, *Oscarella lobularis*, *Celtodoryx girardae*, *Madracis miriabilis*, *Cliona celata* and *Spongosorites difficilis*) collected from the Red Sea was also tested for their anti-microbial and anti-oxidant capacities. An anti-microbial assay was carried out upon agar plates containing the bacterial pathogens *S. aureus*, *P. aeroginosa* and *C. albicans*. Among fungi under analysis, the most promising anti-microbial activities against all pathogens tested were ascribed to *Aspergillus oryzae* and *Cladosporium cladosporioides*. Regarding the anti-oxidant properties, the fresh mycelium was found more effective than the culture filtrate extract, with *Aspergillus fumigatus* reporting the highest percentage of DPPH scavenging activity (59.7%) [109]. A different study conducted on the sponge *Amphimedon* sp. (Yongxin Island, China) brought to the isolation of the fungus *Peyronellaea glomerata*. Chromatographic

separation of the ethyl acetate extract revealed five Isocoumarins, Peyroisocumarins A-D and Isocitreoisocoumarinol, plus thirteen analogs. The anti-bacterial assays were applied to different organisms, including *S. aureus* and *E. coli*. In particular, Alternariol slightly inhibited the growth of *S. aureus* (MIC = 16 μM). On the other hand, through Antioxidant Response Element (ARE)-driven luciferase reporters, a significant regulation of the nuclear factor E2-related factor 2 (Nrf2), a transcription factor that responds to oxidative stress, was observed in Peyroisocumarins A and B with chlorination at side chain. Hence, these two compounds were suggested by the authors as potential leads for anti-oxidant agents [110].

Several biological activities were also found in the extracts obtained from the sponge-derived *Aspergillus sydowii* strain W4-2 and an unidentified fungus named FS1 (Red Sea, Egypt). The supernatant of crude extracts obtained from fungal static cultures showed a high DPPH free radical scavenging activity, plus a moderate tyrosinase inhibitory capacity of *A. sydowii*. Moreover, FS1 demonstrated anti-bacterial properties against *S. aureus*, *C. albicans* and *P. aeruginosa* [111]. Interestingly, another *A. sidowii* strain isolated from the Indonesian sponge KN-15-3 also demonstrated significant anti-bacterial activity on Multi-Drug Resistant *S. aureus* and *E. coli* bacteria [112]. Contrary to the results reported by El-Hady and collaborators [103,111], the ethyl acetate extract of fungal strains (*Penicillium* sp., *Aspergillus niger* and *Trichophyton megninii*) isolated from another Indonesian sponge (*Haliclona fascigera*) displayed considerable anti-tyrosinase activity. In particular, all fungi inhibited tyrosinase functionality, and only one strain of the genus *Penicillium* was found extremely active (IC_{50} = 26 μg/mL). Overall, these species were pointed out as potential sources of tyrosinase inhibitors and skin-whitening agents [113].

The sole anti-biofilm activity against *S. epidermidis* was instead evaluated in the dipeptide cis-cyclo(Leucyl-Tyrosyl) isolated from the symbiotic ascomycete *Penicillium* sp. F37. Interestingly, the dipeptide was able to reduce biofilm formation (~60–85%) at 0.25, 0.5 and 1 mg/mL without blocking bacterial growth, which was then inhibited at higher doses (2 mg/mL). The anti-biofilm activity was corroborated by Scanning Electron Microscopy (SEM), showing a clear attachment of bacteria in untreated biofilms with a visible production of exopolysaccharides (EPS) [114].

The anti-oxidant and anti-aging properties of the culture filtrate (ACCB) obtained from the sponge-associated *Aspergillus chavalieri* TM2-S6 (Tel Aviv-Jaffa, Israeli Mediterranean coast) were also tested on primary normal human dermal fibroblasts (NHDF) through bioassays and molecular approaches [115]. Chemical analyses on the ACCB ethyl acetate extract revealed two abundant compounds, named Tetrahydroauroglaucin and Flavoglaucin. To correlate the chemical composition to the biological activity of ACCB, ATP assay was performed on H_2O_2 treated NHDF cells. Experimental results showed that the incubation with ACCB at 0.05 μg/mL increased cell viability, in comparison to the samples without ACCB. Gene expression analysis corroborated the anti-oxidant capacity of ACCB through the up-regulation of *glutathione peroxidase-1* (*GPX-1*), *superoxide dismutase-1* (*SOD-1*) and *erythroid 2 like 2* (*NRF2*) genes. Moreover, ACCB promoted cell proliferation and extracellular matrix organization, since the expression levels of six key genes involved in these processes, *collagen type I alpha 1 chain* (*COL1A1*), *collagen type III alpha 1 chain* (*COL3A1*), *matrix metallopeptidase 14* (*MMP14*), *CD44 molecule* (*CD44*), *vascular endothelial growth factor A* (*VEGFa*) and *transforming growth factor beta 3* (*TGFB3*), significantly increased. Interestingly, the mRNA levels of *sirtuin 1* (*SIRT1*) and *sirtuin 2* (*SIRT2*), implicated in skin aging, were also found up-regulated in H_2O_2-induced NHDF cells. Combining these results, the authors concluded that ACCB stimulated cell proliferation, anti-oxidant response and extracellular matrix organization as well as reduced aging, thus proposing ACCB as a perfect candidate for the formulation of cosmetic products [115].

The aforementioned biological activities of fungi isolated from marine sponges are schematically reported in Table 2.

Table 2. Fungal source, sponge host, compound/extract, biological activity and reference are reported.

Source	Sponge Host	Extract/Compound	Biological Activity	Reference
Exophiala	*H. panicea*	Circumdatin	Anti-UV	[95]
A. strictum	Unidentified marine sponge of the class Choristida	Acremostrictin	Anti-microbial and anti-oxidant	[106]
E. nigrum JJY-40	Unidentified marine sponge	ENP1, ENP2	Anti-oxidant	[97]
A. versicolor	*Petrosia* sp.	Aromatic polyketide	Anti-oxidant	[98]
P. citrinum SpI080624G1f01	Unidentified marine sponge	JBIR-124	Anti-oxidant	[99]
C. globosum, *G. dankaliensis* and *N. oryzae*	*H. communis*	Ethyl acetate extract	Anti-oxidant and anti-inflammatory	[100]
Penicillium sp. F37	*A. corrugata*	Cis-cyclo(Leucyl-Tyrosyl)	Anti-biofilm	[114]
A. sydowii strain W4-2 and unidentified fungus FS1	*Agelas* sp. and *Amphimedon viridis*	Crude extract of static cultures	Anti-oxidant, anti-tyrosinase and anti-microbial	[111]
F09T15-3, F09-T15-6, F09-T18-16	*Tedania* sp., *Hymeniacidon* sp., *Dendrilla* sp. and three Poecilosclerida	Ethyl acetate extract of culture medium	Anti-oxidant	[105]
H. koningii PF04	*P. fusca*	Hypofurans A/B and Hypocrenones A/B/C	Anti-microbial	[107]
A. unguis RSPG_204	*Agelas* sp.	Several metabolites from mycelia and culture supernatant extracts	Anti-oxidant and anti-tyrosinase	[103]
H. koningii PF04	*P. fusca*	Hypocrol A and Trichodenol B	Anti-microbial and anti-oxidant	[108]
A. oryzae, *C. cladosporioides*, *A. fumigatus*	*A. citrina*, *S. rigida*, *O. lobularis*, *C. girardae*, *M. miriabilis*, *C. celata* and *S. difficilis*	Mycelia and culture filtrate extracts	Anti-microbial and antioxidant	[109]
P. glomerata	*Amphimedon* sp.	Alternariol and Peyroisocumarins A and B	Anti-microbial and anti-oxidant	[110]
A. sydowii	KN-15-3	Culture extract	Anti-microbial	[112]
A. europaeus WZXY-SX-4-1	*X. testudinaria*	Eurobenzophenone C, 3-de-O-methylsulochrin and 14-de-Omethyl-5-methoxysulochrin	Anti-oxidant	[102]
Setosphaeria sp. SCSIO41009	*Callyspongia* sp.	7-O-demethylmonocerin	Anti-oxidant	[101]
A. terreus	*P. fusca*	Butyrolactone I, Butyrolactone II, 5-[(3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-yl)-methyl]-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone and Aspernolide A	Anti-oxidant	[104]
P. brasilianum WZXY-m122-9	Unidentified marine sponge	Brasilianoid A	Anti-UV	[96]
Penicilium sp., *A. niger* and *T. megninii*	*H. fascigera*	Ethyl acetate extract	Anti-tyrosinase	[113]
A. chavalieri TM2-S6	*Axinella* sp.	Tetrahydroauroglaucin and Flavoglaucin	Anti-aging and anti-oxidant	[115]

3. Sponges

The isolation of sponge-associated microbiota was reported as a complicated step within drug discovery pipelines, since the majority of them are uncultivable in laboratory conditions [116]. For this reason, a considerable amount of literature focused on activity screenings without investigating whether the bioactive metabolites were produced by symbiotic bacteria or the sponge hosts.

Among the natural compounds with possible applications in cosmeceutical fields, free radical scavenging activity was particularly retrieved. Avarol and its derivatives, isolated from the Mediterranean sponge *D. avara* (Bay of Naples, Italy), were investigated for their anti-oxidant, anti-inflammatory and anti-proliferative properties, and the results were compared to the well-known activity of Avarol. In particular, DPPH assay and ROS generation in stimulated human neutrophils revealed that avarol-3′-thiosalicylate (TA) was the most active (DPPH, IC_{50} = 15.9 μg/mL), with ROS scavenging capability even higher (IC_{50} = 1.2 μg/mL) when compared to Avarol (IC_{50} = 1.7 μg/mL). Interestingly, the same compound also inhibited prostaglandin E2 (PGE_2) production in HaCaT cell line. Therefore, the authors suggested that the sponge derived compound could potentially block the inflammatory events associated to psoriasis [117]. This latter hypothesis was later explored through in vitro experiments revealing a considerable reduction of (i) PGE_2 and (ii) TNF-α levels in human monocytes and (iii) NFκB binding to DNA in HaCaT cells. Since a crosstalk between TNF-α and NFκB was detected in patients with psoriasis, a role of TA compound in the treatment of psoriatic patients has been corroborated [118].

The ethylacetate extracts from the sponges *Rhabdastrella globostellata* and *Spirastrella inconstans* (Gulf of Mannar) were investigated for their anti-oxidant activity in vivo at different concentrations (2, 4, 6, 8 and 10 mg/kg). The oral administration in rats of the sponge extracts increased the hepatic activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes [119]. Moreover, the dichloromethane and methanol extracts of the sponges *Fascaplysinopsis reticulata*, *Callyspongia siphonella*, *Niphates furcata*, *Callyspongia* sp., *Callyspongia clavata* and *Pseudosaberites clavatus* harvested from the North coast of the Persian Gulf were also investigated for their free radical scavenging capabilities by DPPH and Hydroxyl Radical Scavenging assays. In particular, the methanol extract of the sponge *P. clavatus* displayed the best anti-oxidant activity on DPPH (IC_{50} = 234 μg/mL), while both extracts of *N. furcata* and *F. reticulate* were clearly inhibited OH radicals (~70–80%) [120]. Similarly, the anti-oxidant activity was evaluated in the total extracts of six sponges collected from Indonesia using DPPH assay. The authors found that *Aaptos suberitoides* induced the highest activity ($IC_{50} < 3 \times 10^4$ μg/mL), while *F. reticulata*, *Acanthella* sp., *Petrosia contignata* and *Xestospongia exigua* exerted only a slight anti-oxidant effect with IC_{50} values less than 1×10^5 μg/mL [121]. The methanol extracts of eleven sponge species collected from six geographical sites in Turkey were evaluated for their anti-oxidant properties through DPPH, NO and superoxide radical scavenging activities. The DPPH and superoxide assays revealed a significant dose-dependent radical scavenging activity, with the sponge *Dysidea avara* being the most promising among all specimens under analysis (DPPH, IC_{50} = 92.8 μg/mL; O_2^-, 34.1 μg/mL). Concerning NO radicals, a moderate activity was recorded, with the sole methanolic extract of *Ciocalypta carbolloi* displaying anti-oxidant capacities (700.7 μg/mL) that were higher when compared to the control (quercetin). Interestingly, the authors noticed that the biological activity of sponge extracts was clearly correlated to the location, since the samples collected from Kemer revealed the highest anti-oxidative properties [122]. On the contrary, Botic et al. [123] observed an anti-oxidative capability of different Antarctic sponges of the genus *Latrunculia* through photochemiluminescence assay. The significant variation among samples was explained by a probable changing in the symbiotic community, which was not influenced by the geographical site but rather was species specific [123]. Puupehenol, a meroterpenoid isolated from the organic extract of the Hawaiian Deep-Water sponge *Dactylospongia* sp. was found to be both an anti-oxidant and anti-microbial compound. In fact, a significant radical scavenging activity, detected by Ferric Reducing Antioxidant Power (FRAP) Assay, and a moderate growth inhibition of the Gram-positive bacteria *Staphylococcus aureus* was detected [124]. Anti-oxidant activity was also demonstrated in the crude extract of the sponge *Ircinia spinulosa* collected from the Atlantic Moroccan coast. DPPH assay revealed considerable free radical scavenging capabilities (25.25%) of the crude extract, together with a content of polyphenols, flavonoids and tannins [125]. Tannins and flavonoids were also found in the sponges *A. suberitoides*, *Dactylospongia elegans*, *Stylissa massa* and *Haliclona* sp.

(Red Sea, Egypt). The anti-oxidant activity, evaluated by the phosphomolybdenum method, revealed that, among the samples analyzed, the hexane extract of *D. elegans* and the ethyl acetate extract of *A. suberitoides* induced the highest anti-oxidant properties in comparison to the ascorbic acid [126]. Recently, DPPH and 2.2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid assays also displayed a significant anti-oxidant activity (93% and 99%, respectively) of *H.* aff. *erectus* (Red Sea, Egypt) extract at 1 mg, plus a considerable content of carotenoids (1.976 mg/g) [127]. Moreover, 141 extracts of other sponge samples collected from Mauritius were investigated by DPPH and FRAP assays. The two sponges *Axinella donnani* and *Pseudosuberites* sp. were found the most promising, with significant radical scavenging activities, measured as 92.15% (DPPH) and 10.57 Fe^{2+}/g of extract (FRAP), respectively [128]. The anti-oxidant capacity was also analyzed in the protein extract and the ammonium sulfate fractions of the sponge *Niphates* sp. collected from the sponge reefs of Spermonde waters (South Sulawesi). Among the samples investigated, the DPPH radical scavenging was mostly observed in the crude extract (IC_{50} = 5.05 µg/mL), probably due to a higher glutathione content enhancing the anti-oxidant potentialities [129].

The anti-inflammatory and photoprotective role of Topsentin, a bis(indolyl)imidazole alkaloid identified from the Korean sponge *Spongosorites genitrix*, was evaluated by a multi-approach study [130]. Human keratinocytes HaCaT cells were irradiated with UVB rays and then treated with increasing concentrations of Topsentin (1.25, 2.5, 5 and 10 µM) to measure the protective effect of the sponge derived compound. Western blot and ELISA assay revealed a dose-dependent reduction of cicloxygenase 2 (COX-2) proteins and PGE_2 in cell supernatants (IC_{50} = 0.4 µg/mL) after Topsentin treatment. These data were corroborated by gene expression analyses, showing a significant down-regulation of *COX-2 miR-4485* (a miRNA involved in UVB-induced skin inflammation) and the correlated *tumour necrosis factor alpha induced protein* (*TNF-α IP2*). As expected, Topsentin exposure also reduced PGE_2 levels in human skin models and visibly restored the tissue layer after the damage of UVB radiation [130].

Topic formulations with skin whitening properties have also found huge applications in the cosmetic industry. Concerning sponge-derived compounds, experiments of immunofluorescence on murine melanoma B16 cells treated with the anti-tumour compound Geoditin A, isolated from the sponge *Geodia japonica* (South China Sea), revealed anti-melanogenic and skin whitening properties. Increasing concentrations (0.6, 1.25 and 5 µg/mL) of Geoditin A induced a dose-dependent reduction of melanin within the cytosol and Golgi apparatus, and, similarly, a depletion of tyrosinase was observed into the endoplasmic reticulum (ER). The decrease of tyrosinase activity after Geoditin A treatment was corroborated through the detection on immunoblotting of melanogenic proteins [131]. Similarly, Gagunin D (GD) (see Figure 1), a diterpenoid isolated from the sponge *Phorbas* sp. (Gagu-Do, Korea), was revealed as a potent anti-melanogenic compound [132]. In particular, treatments of GD at increasing concentrations significantly reduced the production of melanin (IC_{50} = 5.7 µg/mL) in Melan-a cells, with higher effects in comparison to the commercial skin whitening agent arbutin. This result was also confirmed by Real time qPCR on melanogenesis-related genes revealing a significant down-regulation of *PAX3*, *SOX10*, *MITF*, *tyrosinase*, *TRP-1* and *TRP-2*. Moreover, GD exposure at 10 µM on UVB irradiated human skin models demonstrated a considerable reduction of melanin biosynthesis [132].

The methanol, ethanol and hexane extracts obtained from *Acanthella cavernosa*, a sponge collected from Bali (Indonesia), were rather explored for anti-microbial and anti-biofilm properties against *Propionibacterium acnes* [133], a common pathogen inducing the inflammatory events connected to acne issues. In particular, the ethanol extract displayed MIC and Minimum Bactericidal Concentration (MBC) values of 125 and 250 µg/mL, respectively, and a considerable inhibition of *P. acnes* biofilm at 250 µg/mL (45%). These results, combined to a slight anti-oxidant activity, suggested a possible application of these sponge extracts as cosmetic ingredients for preventing acne infections [133]. The anti-microbial activity of seven sponge extracts was also evaluated through agar well

diffusion on *S. epidermis*, *S. aureus* and *P. aeruginosa* [134], three microbes that normally constitute skin microflora. Five sponge samples retrieved from Indian waters revealed anti-microbial activity, with *Neopetrosia exigua* extract being the most promising against target organisms plus *C. albicans* showing significant anti-fungal properties. Moreover, all methanol extracts exerted anti-oxidant effects by DPPH assay, particularly *Hyrtios erecta* (IC_{50} = 32.5 µg/mL), followed by *N. exigua* (IC_{50} = 36.6 µg/mL) and *X. testudinaria* (IC_{50} = 46.7 µg/mL) species [134]. In a recent work, bioassay-guided fractionations from the CH_2Cl_2-MeOH extract of the sponge *Haliclona* sp. collected in the Indian Ocean led to the identification of several long-chain highly oxygenated polyacetylenes, named Osirisynes A, B, E, G, H and I. These latter compounds were all tested for catalase and sirtuin 1 activation and CDK7, proteasome, Fyn kinase, tyrosinase, and elastase inhibition, which are considered suitable targets for studying aging-related diseases. In particular, Osirisyne B was found the most effective, with a significant blockage of Fyn kinase (IC_{50} = 14.7 µg/mL), CDK7 kinase (IC_{50} = 7.3 µg/mL), and proteasome (IC_{50} = 0.2 µg/mL) [135].

Marine natural compounds with wound healing properties were also identified as suitable sources for cosmetic manufacturing. Fibroblasts normally produce several compounds in the extracellular matrix, such as glycosaminoglycans (GAGs) or collagen, with the specific capability to adsorb the excessive exudate within tissue wounds and promote skin repair [136]. Advances in chemical extraction methods allowed the isolation of collagen and other similar substances from marine invertebrates, including sponges [137]. One of the first studies observed that collagen, extracted from the sponge *Condrosia reniformis* (Aegean Sea), slightly influenced skin pH and hydration, revealing promising results [138]. Then, in a different research, four sponge samples, *Spongia lamella*, *Spongia officinalis*, *Hippospongia communis* and *Sarcotragus spinosulus* collected from Sardinian beaches (Western Mediterranean Sea), were also found to produce considerable quantities of natural GAGs with good water adsorbing capabilities [139]. Moreover, a recent work evaluated the anti-oxidant, photoprotective and wound healing properties of collagen hydrolysate (MHC) fractions from the Mediterranean sponge *C. reniformis* [140]. DPPH and Nitro Blue Tetrazolium (NBT)/riboflavin assays displayed a high ROS and superoxide anion scavenging activity at 50 µg/mL and 100 µg/mL compared to controls. In addition, the exposure to MHC fractions increased the mRNA levels of *collagen 1A* (*Col1A*) in L929 murine fibroblasts and enhanced cell growth in UV flashed L929 (~8–40%) and HaCaT (~14–32%) cells, depending on the UV dose. "Scratch" tests showed good skin repair properties, particularly on keratinocytes, where cell proliferation near the wound edges was clearly visible at 6 h and 24 h of treatment, with ~22% of wound extension [140].

The chemical compounds and biological activities described in this section are summarized in Table 3.

Table 3. Sponge species and genera, compound/extract, biological activity and reference are reported.

Sponge Species and Genera	Extract/Compound	Biological Activity	Reference
C. reniformis	Collagen	Wound healing	[138]
D. avara	Avarol-3′-thiosalicylate	Anti-oxidant and anti-inflammatory	[117]
D. avara	Avarol-3′-thiosalicylate	Anti-oxidant and anti-inflammatory	[118]
R. globostellata and *S. inconstans*	Ethyl acetate extracts	Anti-oxidant	[119]
G. japonica	Geoditin A	Skin whitening	[131]
A. suberitoides, *D. elegans*, *S. massa* and *Haliclona* sp.	Hexane and ethyl acetate extracts	Anti-oxidant	[126]
F. reticulata, *C. siphonella*, *N. furcata*, *Callyspongia* sp., *C. clavata* and *P. clavatus*	Dichloromethane and methanol extracts	Anti-oxidant	[120]
F. reticulata, *Acanthella* sp., *P. contignata*, *X. exigua* and *A. suberitoides*	Total extracts	Anti-oxidant	[121]

Table 3. *Cont.*

Sponge Species and Genera	Extract/Compound	Biological Activity	Reference
D. avara and *C. carbolloi*	Methanol extracts	Anti-oxidant	[122]
Latrunculia bocagei and *Latrunculia biformis*	Fatty acids extracts	Anti-oxidant	[123]
Dactylospongia sp.	Puupehenol	Anti-oxidant and anti-microbial	[124]
A. cavernosa	Methanol, ethanol and hexane extracts	Anti-acne	[133]
Phorbas sp.	Gagunin D	Skin whitening	[132]
I. spinulosa	Crude extract	Anti-oxidant	[125]
S. lamella, *S. officinalis*, *H. communis* and *S. spinosulus*	Glycosaminoglycans	Wound healing	[139]
N. exigu, *H. erecta* and *X. testudinaria*	Methanol extracts	Anti-oxidant and anti-fungal	[134]
C. reniformis	Collagen hydrolysate fractions	Wound healing and anti-oxidant	[140]
Haliclona sp.	Osirisynes A, B, E, G, H and I	Anti-aging	[135]
S. genitrix	Topsentin	Anti-inflammatory	[130]
H. aff. *erectus*	Crude extract	Anti-oxidant	[127]
A. donnani and *Pseudosuberites* sp.	Crude extract	Anti-oxidant	[128]
Niphates sp.	Protein extract and the ammonium sulfate fractions	Anti-oxidant	[129]

4. Conclusions

Despite the fact that Porifera has historically been considered as a basal phylum among marine organisms, researchers have discovered that they can represent a real possibility for improving the life quality of entire human communities in the future. In the present review, we analyzed several bioactive compounds isolated from sponges and their associated microorganisms and symbionts with suitable features for cosmeceutical applications. We emphasized that those compounds isolated from sponges might derive from their symbiotic community. Nevertheless, it must be considered that bacteria and fungi isolated and cultivated in a laboratory are merely marine opportunistic microbes and, with high probability, not specifically associated to sponges.

The above-mentioned compounds exerted several activities such as anti-oxidant, anti-inflammatory, anti-microbial, anti-aging, skin-whitening, wound healing and moisturizing properties. In particular, most of them exhibited anti-oxidant properties, whose biological function might be extremely useful for preventing skin aging. However, this could be due to the experimental procedures adopted, since free radical scavenging properties are normally detected through colorimetric assays (e.g., DPPH), which are relatively fast, cheap, and easy to apply.

Among thirty-seven papers published in the last ten years on bacteria and sponges, a considerable number of compounds were purified and chemically characterized, such as Ganunin D, Osirisynes, Topsentin and Ageloline A, although the majority of the works relied on the investigation of the crude extracts. On the contrary, almost all papers on fungi reported isolated compounds, as in the case of 7-O-demethylmonocerin, Brasilianoid A, Tetrahydroauroglaucin and Flavoglaucin, which were then correlated to the biological activity. Among bacterial and fungal symbionts, *Bacillus*, *Penicillium* and *Aspergillus* were found to be the most abundant genera with interesting features, since the crude extracts and/or molecules displayed suitable biological activities. Moreover, an important finding may be represented by the isolation of non-toxic and bio-compatible melanin from sponge associated bacteria for its promising application in UV-protective products. Regarding sponges, the relevance of *C. reniformis*, which is due to the discovery of a natural collagen and which has potential wound healing properties, must also be remarked upon.

Despite there being numerous promising candidates of bioactive and beneficial compounds investigated in sponges and their symbionts, only a few examples of commercial products were found, as in the case of Circumdatins, a fungus-derived molecule which is now used in sunscreen formulations. Several steps are still extremely long and not well standardized, so more efforts are needed to improve the (i) isolation and characterization of the products (sometimes very difficult due to the uniqueness of the chemical structures of some compounds of marine origin), (ii) chemical modification techniques, (iii) evaluation of their pharmacological properties and safety aspect, and (iv) estimation of the product quality. For this reason, the pipeline that goes from the isolation of a potential cosmetic product to the evaluation of its safety for human usage should certainly be improved, at least as concerns those molecules isolated from sponges and their symbionts.

Author Contributions: Conceptualization, R.E., N.R. and M.C.; performed bibliographic research, R.E., N.R., T.V. and S.F.; writing—original draft preparation, R.E. and N.R.; T.V. and S.F. contributed to the writing of some paragraphs; review and editing, V.Z. and M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. Nadia Ruocco was supported by the research grants on "Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Thomas, T.R.A.; Kavlekar, D.P.; LokaBharathi, P.A. Marine drugs from sponge-microbe association—A review. *Mar. Drugs* **2010**, *8*, 1417–1468. [CrossRef]
2. Thacker, R.W.; Freeman, C.J. Sponge-microbe symbioses: Recent advances and new directions. *Adv. Mar. Biol.* **2012**, *62*, 57–111.
3. Webster, N.S.; Taylor, M.W. Marine sponges and their microbial symbionts: Love and other relationships. *Environ. Microbiol.* **2012**, *14*, 335–346. [CrossRef] [PubMed]
4. Mehbub, M.F.; Lei, J.; Franco, C.; Zhang, W. Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Mar. Drugs* **2014**, *12*, 4539–4577. [CrossRef] [PubMed]
5. Kumar, M.S.; Pal, A.K. A review of bioactive compounds from marine organisms with special mention on the potential of marine sponges in pharmacological applications. *J. Mar. Biol. Assoc. India* **2016**, *58*, 87–96. [CrossRef]
6. Bibi, F.; Faheem, M.; Azhar, E.; Yasir, M.; Alvi, S.; Kamal, M.; Ullah, I.; Naseer, M. Bacteria from marine sponges: A source of new drugs. *Curr. Drug Metab.* **2016**, *17*. [CrossRef]
7. Perdicaris, S.; Vlachogianni, T.; Valavanidis, A. Bioactive natural substances from marine sponges: New developments and prospects for future pharmaceuticals. *Nat. Prod. Chem. Res.* **2013**, *1*, 3. [CrossRef]
8. Simister, R.L.; Deines, P.; Botté, E.S.; Webster, N.S.; Taylor, M.W. Sponge-specific clusters revisited: A comprehensive phylogeny of sponge-associated microorganisms. *Environ. Microbiol.* **2012**, *14*, 517–524. [CrossRef] [PubMed]
9. Moitinho-Silva, L.; Nielsen, S.; Amir, A.; Gonzalez, A.; Ackermann, G.L.; Cerrano, C.; Astudillo-Garcia, C.; Easson, C.; Sipkema, D.; Liu, F.; et al. The sponge microbiome project. *Gigascience* **2017**, *6*, 1–13. [CrossRef] [PubMed]
10. Tianero, M.D.; Balaich, J.N.; Donia, M.S. Localized production of defence chemicals by intracellular symbionts of *Haliclona* sponges. *Nat. Microbiol.* **2019**, *4*, 1149–1159. [CrossRef] [PubMed]
11. Yang, Q.; Zhang, W.; Franco, C.M.M. Response of sponge microbiomes to environmental variations. In *Symbiotic Microbiomes of Coral Reefs Sponges and Corals*; Li, Z., Ed.; Springer Nature: Basingstoke, UK, 2019; pp. 181–247. ISBN 9789402416121.
12. Freeman, C.J.; Thacker, R.W. Complex interactions between marine sponges and their symbiotic microbial communities. *Limnol. Oceanogr.* **2011**, *56*, 1577–1586. [CrossRef]
13. Guzman, C.; Conaco, C. Gene expression dynamics accompanying the sponge thermal stress response. *PLoS ONE* **2016**, *11*, 1–15. [CrossRef]
14. Marty, M.J.; Vicente, J.; Oyler, B.L.; Place, A.; Hill, R.T. Sponge symbioses between *Xestospongia deweerdtae* and *Plakortis* spp. are not motivated by shared chemical defense against predators. *PLoS ONE* **2017**, *12*, 1–20. [CrossRef] [PubMed]
15. Shinde, P.; Banerjee, P.; Mandhare, A. Marine natural products as source of new drugs: A patent review (2015–2018). *Expert Opin. Ther. Pat.* **2019**, *29*, 283–309. [CrossRef] [PubMed]

16. Draelos, Z.D. Cosmeceuticals: What's real, what's not. *Dermatol. Clin.* **2019**, *37*, 107–115. [CrossRef] [PubMed]
17. Ahsan, H. The biomolecules of beauty: Biochemical pharmacology and immunotoxicology of cosmeceuticals. *J. Immunoass. Immunochem.* **2019**, *40*, 91–108. [CrossRef]
18. Alves, T.F.R.; Morsink, M.; Batain, F.; Chaud, M.V.; Almeida, T.; Fernandes, D.A.; Silva, C.F.; Souto, E.B. Synthetic polymers in cosmetic formulations. *Cosmetics* **2020**, *7*, 75. [CrossRef]
19. Sharad, J. Cosmeceuticals. In *Advances in Integrative Dermatology*; França, K., Lotti, T., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2019; pp. 393–411.
20. Khezri, K.; Saeedi, M.; Maleki Dizaj, S. Application of nanoparticles in percutaneous delivery of active ingredients in cosmetic preparations. *Biomed. Pharmacother.* **2018**, *106*, 1499–1505. [CrossRef]
21. Bilal, M.; Iqbal, H.M.N. New insights on unique features and role of nanostructured materials in cosmetics. *Cosmetics* **2020**, *7*, 24. [CrossRef]
22. Ben Haddada, M.; Gerometta, E.; Chawech, R.; Sorres, J.; Bialecki, A.; Pesnel, S.; Spadavecchia, J.; Morel, A.L. Assessment of antioxidant and dermoprotective activities of gold nanoparticles as safe cosmetic ingredient. *Colloids Surf. B Biointerfaces* **2020**, *189*, 110855. [CrossRef]
23. Kesavan Pillai, S.; Kleyi, P.; de Beer, M.; Mudaly, P. Layered double hydroxides: An advanced encapsulation and delivery system for cosmetic ingredients-an overview. *Appl. Clay Sci.* **2020**, *199*, 105868. [CrossRef]
24. Poulose, N.; Sajayan, A.; Ravindran, A.; Sreechithra, T.V.; Vardhan, V.; Selvin, J.; Kiran, G.S. Photoprotective effect of nanomelanin-seaweed concentrate in formulated cosmetic cream: With improved antioxidant and wound healing properties. *J. Photochem. Photobiol. B Biol.* **2020**, *205*, 111816. [CrossRef]
25. Genç, Y.; Bardakci, H.; Yücel, Ç.; Karatoprak, G.Ş.; Akkol, E.K.; Barak, T.H.; Sobarzo-Sánchez, E. Oxidative stress and marine carotenoids: Application by using nanoformulations. *Mar. Drugs* **2020**, *18*, 423. [CrossRef]
26. Gupta, R.K.; Soni, P.; Shrivastava, J.; Rajput, P.; Parashar, S.; Kalpana, B.; Nidana, R.; Vikriti, E. Cosmeceutical role of medicinal plants/herbs: A review on commercially available cosmetic ingredients. *Int. J. Innov. Sci. Technol.* **2018**, *3*, 70–73. [CrossRef]
27. Joshi, L.S.; Pawar, H.A. Herbal cosmetics and cosmeceuticals: An overview. *Nat. Prod. Chem. Res.* **2015**, *3*, 170. [CrossRef]
28. Charles Dorni, A.I.; Amalraj, A.; Gopi, S.; Varma, K.; Anjana, S.N. Novel cosmeceuticals from plants—An industry guided review. *J. Appl. Res. Med. Aromat. Plants* **2017**, *7*, 1–26. [CrossRef]
29. Alves, A.; Sousa, E.; Kijjoa, A.; Pinto, M. Marine-derived compounds with potential use as cosmeceuticals and nutricosmetics. *Molecules* **2020**, *25*, 2536. [CrossRef] [PubMed]
30. Pallela, R.; Na-Young, Y.; Kim, S.K. Anti-photoaging and photoprotective compounds derived from marine organisms. *Mar. Drugs* **2010**, *8*, 1189–1202. [CrossRef] [PubMed]
31. Felician, F.F.; Xia, C.; Qi, W.; Xu, H. Collagen from marine biological sources and medical applications. *Chem. Biodivers.* **2018**, *15*, e1700557. [CrossRef] [PubMed]
32. Manandhar, B.; Wagle, A.; Seong, S.H.; Paudel, P.; Kim, H.R.; Jung, H.A.; Choi, J.S. Phlorotannins with potential anti-tyrosinase and antioxidant activity isolated from the marine seaweed *Ecklonia Stolonifera*. *Antioxid.* **2019**, *8*, 240. [CrossRef] [PubMed]
33. Couteau, C.; Coiffard, L. Phycocosmetics and other marine cosmetics, specific cosmetics formulated using marine resources. *Mar. Drugs* **2020**, *18*, 322. [CrossRef] [PubMed]
34. Pilevneli, A.D.; Konuklugil, B. Marine derived tyrosinase inhibitors. *Ege J. Fish. Aquat. Sci.* **2020**, *37*, 427–436. [CrossRef]
35. Solano, F. Photoprotection and skin pigmentation: Melanin-related molecules and some other new agents obtained from natural sources. *Molecules* **2020**, *25*, 1537. [CrossRef]
36. Chang, T.S. Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity. *Materials* **2012**, *5*, 1661–1685. [CrossRef]
37. Balakrishnan, D.; Kandasamy, D.; Nithyanand, P. A review on antioxidant activity of marine organisms. *Int. J. Chem. Tech. Res.* **2014**, *6*, 3431–3436.
38. Balboa, E.M.; Conde, E.; Soto, M.L.; Pérez-Armada, L.; Domínguez, H. Cosmetics from marine sources. In *Springer Handbook of Marine Biotechnology*; Kim, S.K., Ed.; Elsevier: Amsterdam, The Netherlands, 2015; Volume 44, pp. 1015–1042.
39. Taofiq, O.; Heleno, S.A.; Calhelha, R.C.; Alves, M.J.; Barros, L.; Barreiro, M.F.; González-Paramás, A.M.; Ferreira, I.C.F.R. Development of mushroom-based cosmeceutical formulations with anti-inflammatory, anti-tyrosinase, antioxidant, and antibacterial properties. *Molecules* **2016**, *21*, 1372. [CrossRef]
40. Brunt, E.G.; Burgess, J.G. The promise of marine molecules as cosmetic active ingredients. *Int. J. Cosmet. Sci.* **2016**, *40*, 1–15. [CrossRef]
41. Chrapusta, E.; Kaminski, A.; Duchnik, K.; Bober, B.; Adamski, M.; Bialczyk, J. Mycosporine-like amino acids: Potential health and beauty ingredients. *Mar. Drugs* **2017**, *15*, 326. [CrossRef] [PubMed]
42. Galasso, C.; Corinaldesi, C.; Sansone, C. Carotenoids from marine organisms: Biological functions and industrial applications. *Antioxidants* **2017**, *6*, 96. [CrossRef]
43. Núñez-Pons, L.; Avila, C.; Romano, G.; Verde, C.; Giordano, D. UV-protective compounds in marine organisms from the southern ocean. *Mar. Drugs* **2018**, *16*, 336. [CrossRef]
44. Vílchez, C.; Forján, E.; Cuaresma, M.; Bédmar, F.; Garbayo, I.; Vega, J.M. Marine carotenoids: Biological functions and commercial applications. *Mar. Drugs* **2011**, *9*, 319–333. [CrossRef]

45. Wada, N.; Sakamoto, T.; Matsugo, S. Mycosporine-like amino acids and their derivatives as natural antioxidants. *Antioxidants* **2015**, *4*, 603–646. [CrossRef]
46. Birben, E.; Sahiner, U.; Sackesen, C.; Erzurum, S.; Kalayci, O. Oxidative stress and antioxidant defense. *Science* **1997**, *22*, 161–168. [CrossRef] [PubMed]
47. Younes, I.; Rinaudo, M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs* **2015**, *13*, 1133–1174. [CrossRef] [PubMed]
48. Sansone, C.; Brunet, C. Marine algal antioxidants. *Antioxidants* **2020**, *9*, 206. [CrossRef] [PubMed]
49. Tripathi, V.C.; Horam, S.; Singh, A.; Lata, M.; Reddy, T.J.; Arockiaraj, J.; Pasupuleti, M. The discovery of antioxidants in marine microorganisms and their protective effects on the hepatic cells from chemical-induced oxidative stress. *Free Radic. Res.* **2020**, *54*, 150–161. [CrossRef]
50. Siavash, H.C.; Fadzilah, A.A.M. Cosmeceutical values, antimicrobial activities and antioxidant properties of cashew leaves extract. *Afr. J. Biotechnol.* **2011**, *10*, 4573–14582. [CrossRef]
51. Baldisserotto, A.; Malisardi, G.; Scalambra, E.; Andreotti, E.; Romagnoli, C.; Vicentini, C.B.; Manfredini, S.; Vertuani, S. Synthesis, antioxidant and antimicrobial activity of a new phloridzin derivative for dermo-cosmetic applications. *Molecules* **2012**, *17*, 13275–13289. [CrossRef]
52. Herman, A.; Herman, A.P.; Domagalska, B.W.; Młynarczyk, A. Essential oils and herbal extracts as antimicrobial agents in cosmetic emulsion. *Indian J. Microbiol.* **2013**, *53*, 232–237. [CrossRef] [PubMed]
53. Adegoke, T.; Arotupin, D.; Ekundayo, T. Antimicrobial activities of some commercial cosmetics on selected cutaneous microflora. *J. Adv. Microbiol.* **2017**, *4*, 1–9. [CrossRef]
54. Kim, J.W.; Yu, H.; Park, K.M.; Chang, P.S. Antimicrobial characterization of erythorbyl laurate for practical applications in food and cosmetics. *J. Chem.* **2020**, *2020*, 1–8. [CrossRef]
55. Pillaiyar, T.; Manickam, M.; Namasivayam, V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 403–425. [CrossRef] [PubMed]
56. McClements, D.J.; Gumus, C.E. Natural emulsifiers—Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv. Colloid Interface Sci.* **2016**, *234*, 3–26. [CrossRef] [PubMed]
57. Adu, S.A.; Naughton, P.J.; Marchant, R.; Banat, I.M. Microbial biosurfactants in cosmetic and personal skincare pharmaceutical formulations. *Pharmaceutics* **2020**, *12*, 1099. [CrossRef]
58. Corinaldesi, C.; Barone, G.; Marcellini, F.; Dell'Anno, A.; Danovaro, R. Marine microbial-derived molecules and their potential use in cosmeceutical and cosmetic products. *Mar. Drugs* **2017**, *15*, 118. [CrossRef]
59. Kong, S.Z.; Li, J.C.; Li, S.D.; Liao, M.N.; Li, C.P.; Zheng, P.J.; Guo, M.H.; Tan, W.X.; Zheng, Z.H.; Hu, Z. Anti-aging effect of chitosan oligosaccharide on d-galactose-induced subacute aging in mice. *Mar. Drugs* **2018**, *16*, 181. [CrossRef]
60. Jahan, A.; Ahmad, I.Z.; Fatima, N.; Ansari, V.A.; Akhtar, J. Algal bioactive compounds in the cosmeceutical industry: A review. *Phycologia* **2017**, *56*, 410–422. [CrossRef]
61. Pereira, L. Seaweeds as source of bioactive substances and skin care therapy—Cosmeceuticals, algotheraphy, and thalassotherapy. *Cosmetics* **2018**, *5*, 68. [CrossRef]
62. Hamidi, M.; Safarzadeh Kozani, P.; Safarzadeh Kozani, P.; Pierre, G.; Michaud, P.; Delattre, C. Marine bacteria versus microalgae: Who is the best for biotechnological production of bioactive compounds with antioxidant properties and other biological applications? *Mar. Drugs* **2020**, *18*, 28. [CrossRef]
63. Thiyagarasaiyar, K.; Goh, B.H.; Jeon, Y.J.; Yow, Y.Y. Algae metabolites in cosmeceutical: An overview of current applications and challenges. *Mar. Drugs* **2020**, *18*, 323. [CrossRef]
64. Martins, A.; Vieira, H.; Gaspar, H.; Santos, S. Marketed marine natural products in the pharmaceutical and cosmeceutical industries: Tips for success. *Mar. Drugs* **2014**, *12*, 1066–1101. [CrossRef]
65. Calado, R.; Leal, M.C.; Gaspar, H.; Santos, S.; Marques, A.; Nunes, M.L.; Vieira, H. How to succeed in marketing marine natural products for nutraceutical, pharmaceutical and cosmeceutical markets. In *Grand Challenges in Biology and Biotechnology*; Rampelotto, P.H., Trincone, A., Eds.; Springer International Publishing: Manhattan, NY, USA, 2018; pp. 317–403. ISBN 9783319690759.
66. Hassane, C.S.; Fouillaud, M.; Le Goff, G.; Sklirou, A.D.; Boyer, J.B.; Trougakos, I.P.; Jerabek, M.; Bignon, J.; de Voogd, N.J.; Ouazzani, J.; et al. Microorganisms associated with the marine sponge *Scopalina hapalia*: A reservoir of bioactive molecules to slow down the aging process. *Microorganisms* **2020**, *8*, 1262. [CrossRef]
67. Imhoff, J.F.; Labes, A.; Wiese, J. Bio-mining the microbial treasures of the ocean: New natural products. *Biotechnol. Adv.* **2011**, *29*, 468–482. [CrossRef] [PubMed]
68. Gao, Q.; Garcia-Pichel, F. Microbial ultraviolet sunscreens. *Nat. Rev. Microbiol.* **2011**, *9*, 791–802. [CrossRef] [PubMed]
69. Morabito, K.; Shapley, N.C.; Steeley, K.G.; Tripathi, A. Review of sunscreen and the emergence of non-conventional absorbers and their applications in ultraviolet protection. *Int. J. Cosmet. Sci.* **2011**, *33*, 385–390. [CrossRef]
70. Young, A.J.; Lowe, G.L. Carotenoids—antioxidant properties. *Antioxidants* **2018**, *7*, 28. [CrossRef] [PubMed]
71. Dharmaraj, S.; Ashokkumar, B.; Dhevendaran, K. Food-grade pigments from *Streptomyces* sp. isolated from the marine sponge *Callyspongia diffusa*. *Food Res. Int.* **2009**, *42*, 487–492. [CrossRef]

72. Shindo, K.; Asagi, E.; Sano, A.; Hotta, E.; Minemura, N.; Mikami, K.; Tamesada, E.; Misawa, N.; Maoka, T. Diapolycopenedioic acid xylosyl esters A, B, and C, novel antioxidative glyco-C_{30}-carotenoic acids produced by a new marine bacterium Rubritalea Squalenifaciens. *J. Antibiot. (Tokyo)* **2008**, *61*, 185–191. [CrossRef]
73. Abdelmohsen, U.R.; Szesny, M.; Othman, E.M.; Schirmeister, T.; Grond, S.; Stopper, H.; Hentschel, U. Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated *Micromonospora* strain RV115. *Mar. Drugs* **2012**, *10*, 2208–2221. [CrossRef]
74. Arunachalam, K.; Amirtham Jacob Appadorai, R. Antioxidant potential and biochemical evaluation of metabolites from the marine bacteria *Virgibacillus* sp. associated with the sponge *Callyspongia diffusa*. *Free Radic. Antioxid.* **2013**, *3*, 47–51. [CrossRef]
75. Dupont, S.; Carré-Mlouka, A.; Descarrega, F.; Ereskovsky, A.; Longeon, A.; Mouray, E.; Florent, I.; Bourguet-Kondracki, M.L. Diversity and biological activities of the bacterial community associated with the marine sponge *Phorbas tenacior* (Porifera, Demospongiae). *Lett. Appl. Microbiol.* **2014**, *58*, 42–52. [CrossRef]
76. Balakrishnan, D.; Bibiana, A.S.; Vijayakumar, A.; Santhosh, R.S.; Dhevendaran, K.; Nithyanand, P. Antioxidant activity of bacteria associated with the marine sponge *Tedania anhelans*. *Indian J. Microbiol.* **2015**, *55*, 13–18. [CrossRef]
77. Mohan, G.; Centre, N.; Sustainable, F.; Management, C.; Kumar, T.T.A. Marine natural product, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- ($C_7H_{10}N_2O_2$) of antioxidant properties from *Bacillus* species at Lakshadweep archipelago. *J. Coast. Life Med.* **2014**, *2*, 636–641. [CrossRef]
78. Velho-Pereira, S.; Parvatkar, P.; Furtado, I.J. Evaluation of antioxidant producing potential of halophilic bacterial bionts from marine invertebrates. *Indian J. Pharm. Sci.* **2015**, *77*, 183–189.
79. El-Moneam, N.M.A.; El-Assar, S.A.; Shreadah, M.A.; Nabil-Adam, A. Isolation, identification and molecular screening of *Pseudomonas* sp. metabolic pathways NRPs and PKS associated with the Red Sea Sponge, *Hyrtios* aff. erectus, Egypt. *J. Pure Appl. Microbiol.* **2017**, *11*, 1299–1311. [CrossRef]
80. Vijayan, V.; Jasmin, C.; Anas, A.; Parakkaparambil Kuttan, S.; Vinothkumar, S.; Perunninakulath Subrayan, P.; Nair, S. Sponge-associated bacteria produce non-cytotoxic melanin which protects animal cells from photo-toxicity. *Appl. Biochem. Biotechnol.* **2017**, *183*, 396–411. [CrossRef] [PubMed]
81. Prastya, M.E.; Astuti, R.I.; Batubara, I.; Wahyudi, A.T. Antioxidant, antiglycation and in vivo antiaging effects of metabolite extracts from marine sponge-associated bacteria. *Indian J. Pharm. Sci.* **2019**, *81*, 344–354. [CrossRef]
82. Prastya, M.E.; Astuti, R.I.; Batubara, I.; Takagi, H.; Wahyudi, A.T. Chemical screening identifies an extract from marine *Pseudomonas* sp.-PTR-08 as an anti-aging agent that promotes fission yeast longevity by modulating the Pap1–ctt1$^+$ pathway and the cell cycle. *Mol. Biol. Rep.* **2020**, *47*, 33–43. [CrossRef]
83. Prastya, M.E.; Astuti, R.I.; Batubara, I.; Takagi, H.; Wahyudi, A.T. Natural extract and its fractions isolated from the marine bacterium *Pseudoalteromonas flavipulchra* STILL-33 have antioxidant and antiaging activities in *Schizosaccharomyces pombe*. *FEMS Yeast Res.* **2020**, *20*, 1–14. [CrossRef]
84. Cheng, C.; Othman, E.M.; Reimer, A.; Grüne, M.; Kozjak-Pavlovic, V.; Stopper, H.; Hentschel, U.; Abdelmohsen, U.R. Ageloline A, new antioxidant and antichlamydial quinolone from the marine sponge-derived bacterium *Streptomyces* sp. SBT345. *Tetrahedron Lett.* **2016**, *57*, 2786–2789. [CrossRef]
85. Phadale, R.; Kumar, M.S. Characterization of an antimicrobial and antioxidant compound from a marine bacterium GSA10 associated with the sponge *Halichondria glabrata*. *J. Microbiol. Biotechnol. Food Sci.* **2018**, *7*, 651–658. [CrossRef]
86. Odekina, P.A.; Agbo, M.O.; Omeje, E.O. Antimicrobial and antioxidant activities of novel marine bacteria (*Bacillus* 2011SOCCUF3) isolated from marine sponge (*Spongia officinalis*). *Pharm. Sci.* **2020**, *26*, 82–87. [CrossRef]
87. Dhasayan, A.; Selvin, J.; Kiran, S. Biosurfactant production from marine bacteria associated with sponge *Callyspongia diffusa*. *3 Biotech* **2015**, *5*, 443–454. [CrossRef]
88. Alemán-Vega, M.; Sánchez-Lozano, I.; Hernández-Guerrero, C.J.; Hellio, C.; Quintana, E.T. Exploring antifouling activity of biosurfactants producing marine bacteria isolated from gulf of California. *Int. J. Mol. Sci.* **2020**, *21*, 6068. [CrossRef]
89. Agrawal, S.; Adholeya, A.; Barrow, C.J.; Deshmukh, S.K. Marine fungi: An untapped bioresource for future cosmeceuticals. *Phytochem. Lett.* **2018**, *23*, 15–20. [CrossRef]
90. Vitale, G.A.; Coppola, D.; Esposito, F.P.; Buonocore, C.; Ausuri, J.; Tortorella, E.; de Pascale, D. Antioxidant molecules from marine fungi: Methodologies and perspectives. *Antioxidants* **2020**, *9*, 1183. [CrossRef]
91. Jeewon, R.; Luckhun, A.B.; Bhoyroo, V.; Sadeer, N.B.; Mahomoodally, M.F.; Rampadarath, S.; Puchooa, D.; Venkateswara Sarma, V.; Sundara, S.; Durairajan, K.; et al. Pharmaceutical potential of marine fungal endophytes. In *Endophytes and Secondary Metabolites*; Jha, S., Ed.; Springer Nature: Basingstoke, UK, 2019; pp. 1–23.
92. Devi, R.; Kaur, T.; Guleria, G.; Rana, K.L.; Kour, D.; Yadav, N.; Yadav, A.N.; Saxena, A.K. Fungal secondary metabolites and their biotechnological applications for human health. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 147–161.
93. Tanod, W.A.; Muliadin, M.; Adel, Y.S.; Dewanto, D.K. Potential marine-derived fungi isolated from sponges produce new and beneficial compounds. *J. Fish. Mar. Aquat. Sci.* **2020**, *2*, 52–66.
94. Mohapatra, B.R.; Bapuji, M. Characterization of acetylcholinesterase from *Arthrobacter ilicis* associated with the marine sponge (*Spirastrella* sp.). *J. Appl. Microbiol.* **1998**, *84*, 393–398. [CrossRef]
95. Zhang, D.; Yang, X.; Kang, J.S.; Choi, H.D.; Son, B.W. Circumdatin I, a new ultraviolet-A protecting benzodiazepine alkaloid from a marine isolate of the fungus *Exophiala*. *J. Antibiot. (Tokyo)* **2008**, *61*, 40–42. [CrossRef]

96. Zhang, J.; Yuan, B.; Liu, D.; Gao, S.; Proksch, P.; Lin, W. Brasilianoids A-F, new meroterpenoids from the sponge-associated fungus *Penicillium brasilianum*. *Front. Chem.* **2018**, *6*, 1–13. [CrossRef] [PubMed]
97. Sun, H.H.; Mao, W.J.; Jiao, J.Y.; Xu, J.C.; Li, H.Y.; Chen, Y.; Qi, X.H.; Chen, Y.L.; Xu, J.; Zhao, C.Q.; et al. Structural characterization of extracellular polysaccharides produced by the marine fungus *Epicoccum nigrum* JJY-40 and their antioxidant activities. *Mar. Biotechnol.* **2011**, *13*, 1048–1055. [CrossRef] [PubMed]
98. Li, J.L.; Lee, Y.M.; Hong, J.; Bae, K.S.; Choi, J.S.; Jung, J.H. A new antioxidant from the marine sponge-derived fungus *Aspergillus versicolor*. *Nat. Prod. Sci.* **2011**, *17*, 14–18.
99. Kawahara, T.; Takagi, M.; Shin-Ya, K. JBIR-124: A novel antioxidative agent from a marine sponge-derived fungus *Penicillium citrinum* SpI080624G1f01. *J. Antibiot. (Tokyo)* **2012**, *65*, 45–47. [CrossRef] [PubMed]
100. Abdel-Monem, N.; Abdel-Azeem, A.M.; El Ashry, E.S.H.; Ghareeb, D.A.; Nabil-Adam, A. Assessment of secondary metabolites from marine-derived fungi as antioxidant. *Open J. Med. Chem.* **2013**, *3*, 60–73. [CrossRef]
101. Pang, X.; Lin, X.; Yang, J.; Zhou, X.; Yang, B.; Wang, J.; Liu, Y. Spiro-phthalides and Isocoumarins isolated from the marine-sponge-derived fungus *Setosphaeria* sp. SCSIO41009. *J. Nat. Prod.* **2018**, *81*, 1860–1868. [CrossRef]
102. Du, X.; Liu, D.; Huang, J.; Zhang, C.; Proksch, P.; Lin, W. Polyketide derivatives from the sponge associated fungus *Aspergillus europaeus* with antioxidant and NO inhibitory activities. *Fitoterapia* **2018**, *130*, 190–197. [CrossRef]
103. El-Hady, F.K.A.; Abdel-aziz, M.S.; Shaker, K.H.; El-shahid, Z.A.; Ibrahim, L.S. Antioxidant, acetylcholinesterase and α-glucosidase potentials of metabolites from the marine fungus. *Int. J. Pharm. Sci. Rev. Res.* **2015**, *30*, 272–278.
104. Sun, Y.; Liu, J.; Li, L.; Gong, C.; Wang, S.; Yang, F.; Hua, H.; Lin, H. New butenolide derivatives from the marine sponge-derived fungus *Aspergillus terreus*. *Bioorganic Med. Chem. Lett.* **2018**, *28*, 315–318. [CrossRef]
105. Henríquez, M.; Vergara, K.; Norambuena, J.; Beiza, A.; Maza, F.; Ubilla, P.; Araya, I.; Chávez, R.; San-Martín, A.; Darias, J.; et al. Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. *World J. Microbiol. Biotechnol.* **2014**, *30*, 65–76. [CrossRef] [PubMed]
106. Julianti, E.; Oh, H.; Jang, K.H.; Lee, J.K.; Lee, S.K.; Oh, D.C.; Oh, K.B.; Shin, J. Acremostrictin, a highly oxygenated metabolite from the marine fungus *Acremonium strictum*. *J. Nat. Prod.* **2011**, *74*, 2592–2594. [CrossRef]
107. Ding, L.J.; Gu, B.B.; Jiao, W.H.; Yuan, W.; Li, Y.X.; Tang, W.Z.; Yu, H.B.; Liao, X.J.; Han, B.N.; Li, Z.Y.; et al. New furan and cyclopentenone derivatives from the sponge-associated fungus *Hypocrea koningii* PF04. *Mar. Drugs* **2015**, *13*, 5579–5592. [CrossRef] [PubMed]
108. Ding, L.J.; Yuan, W.; Li, Y.X.; Liao, X.J.; Sun, H.; Peng, Q.; Han, B.N.; Lin, H.W.; Li, Z.Y.; Yang, F.; et al. Hypocrol A, a new tyrosol derivative from a sponge-derived strain of the fungus *Hypocrea koningii*. *Nat. Prod. Res.* **2016**, *30*, 1633–1638. [CrossRef] [PubMed]
109. Sayed, M.A.; El-rahman, T.M.A.A.; El-diwany, A.I.; Product, M.; Sayed, S.M. Biodiversity and bioactivity of red sea sponge associated endophytic fungi. *Int. J. Adv. Res. Eng. Appl. Sci.* **2016**, *5*, 1–15.
110. Zhao, Y.; Liu, D.; Proksch, P.; Yu, S.; Lin, W. Isocoumarin derivatives from the sponge-associated fungus *Peyronellaea glomerata* with antioxidant activities. *Chem. Biodivers.* **2016**, *13*, 1186–1193. [CrossRef] [PubMed]
111. El-Hady, F.K.A.; Abdel-Aziz, M.S.; Shaker, K.H.; El-Shahid, Z.A. Tyrosinase, acetylcholinesterase inhibitory potential, antioxidant and antimicrobial activities of sponge derived fungi with correlation to their GC/MS analysis. *Int. J. Pharm. Sci. Rev. Res.* **2014**, *26*, 338–345.
112. Trianto, A.; Widyaningsih, S.; Radjasa, O.; Pribadi, R. Symbiotic fungus of marine sponge *Axinella* sp. producing antibacterial agent. *Conf. Ser. Earth Environ. Sci.* **2017**, *55*, 012005. [CrossRef]
113. Handayani, D.; Andalas, U.; Sandrawati, N.; Andalas, U.; Syafni, N.; Putra, D.P. Tyrosinase inhibitory activity of ethyl acetate extracts from marine sponge-derived fungi *Haliclona fascigera*. *Biosci. Res.* **2019**, *16*, 2369–2373.
114. Scopel, M.; Abraham, W.R.; Henriques, A.T.; MacEdo, A.J. Dipeptide cis-cyclo(Leucyl-Tyrosyl) produced by sponge associated *Penicillium* sp. F37 inhibits biofilm formation of the pathogenic *Staphylococcus epidermidis*. *Bioorganic Med. Chem. Lett.* **2013**, *23*, 624–626. [CrossRef] [PubMed]
115. Letsiou, S.; Bakea, A.; Le Goff, G.; Lopes, P.; Gardikis, K.; Weis, M.; Benayahu, Y.; Ouazzani, J. Marine fungus *Aspergillus chevalieri* TM2-S6 extract protects skin fibroblasts from oxidative stress. *Mar. Drugs* **2020**, *18*, 460. [CrossRef]
116. Laport, M.S. Isolating bacteria from sponges: Why and how? *Curr. Pharm. Biotechnol.* **2018**, *18*, 1224–1236. [CrossRef]
117. Amigó, M.; Terencio, M.C.; Mitova, M.; Iodice, C.; Payá, M.; De Rosa, S. Potential antipsoriatic avarol derivatives as antioxidants and inhibitors of PGE2 generation and proliferation in the HaCaT cell line. *J. Nat. Prod.* **2004**, *67*, 1459–1463. [CrossRef]
118. Amigó, M.; Payá, M.; De Rosa, S.; Terencio, M.C. Antipsoriatic effects of avarol-3′-thiosalicylate are mediated by inhibition of TNF-α generation and NF-kB activation in mouse skin. *Br. J. Pharmacol.* **2007**, *152*, 353–365. [CrossRef]
119. Chairman, K.; Singh, A.J.A.R.; Alagumuthu, G. Cytotoxic and antioxidant activity of selected marine sponges. *Asian Pac. J. Trop. Dis.* **2012**, *2*, 234–238. [CrossRef]
120. Seradj, H.; Moein, M.; Eskandari, M.; Maaref, F. Antioxidant activity of six marine sponges collected from the Persian gulf. *Iran. J. Pharm. Sci.* **2012**, *8*, 249–255.
121. Abdillah, S.; Nurhayati, A.P.D.; Nurhatika, S.; Setiawan, E.; Heffen, W.L. Cytotoxic and antioxidant activities of marine sponge diversity at Pecaron Bay Pasir Putih Situbondo East Java, Indonesia. *J. Pharm. Res.* **2013**, *6*, 685–689. [CrossRef]
122. Aktas, N.; Genc, Y.; Gozcelioglu, B.; Konuklugil, B.; Sebnem Harput, U. Radical scavenging effect of different marine sponges from mediterranean coasts. *Rec. Nat. Prod.* **2013**, *7*, 96–104.

123. Botić, T.; Cör, D.; Anesi, A.; Guella, G.; Sepčić, K.; Janussen, D.; Kersken, D.; Knez, Ž. Fatty acid composition and antioxidant activity of Antarctic marine sponges of the genus *Latrunculia*. *Polar Biol.* **2015**, *38*, 1605–1612. [CrossRef]
124. Hagiwara, K.; Garcia Hernandez, J.E.; Harper, M.K.; Carroll, A.; Motti, C.A.; Awaya, J.; Nguyen, H.Y.; Wright, A.D. Puupehenol, a potent antioxidant antimicrobial meroterpenoid from a Hawaiian deep-water *Dactylospongia* sp. sponge. *J. Nat. Prod.* **2015**, *78*, 325–329. [CrossRef]
125. Rhandour, Z.; Tarbaoui, M.; Oumam, M.; Elamraoui, B.; Bennamara, A.; Abourriche, A. Determination of polyphenols, tannins, flavonoids and antioxidant activity in extracts of two genus *Ircinia* marine sponges of Atlantic Morrocan Coast. *Front. Mar. Sci.* **2016**, *3*. [CrossRef]
126. Rivera, A.P.; Uy, M.M. In vitro antioxidant and cytotoxic activities of some marine sponges collected off misamis oriental coast, Philippines. *E-J. Chem.* **2012**, *9*, 354–358. [CrossRef]
127. Nabil-Adam, A.; Shreadah, M.A.; El Moneam, N.M.A.; El-Assar, S.A. Various in vitro bioactivities of secondary metabolites isolated from the sponge *Hyrtios* aff. erectus from the red sea coast of Egypt. *Turk. J. Pharm. Sci.* **2020**, *17*, 127–135. [CrossRef] [PubMed]
128. Oogarah, P.N.; Ramanjooloo, A.; Rovisham, J.; Doorga, S.; Meyepa, C.; Wilhelmus, R.; Van Soest, M.; Edgard, D.; Marie, P. Assessing antioxidant activity and phenolic content of marine sponges from mauritius waters. *Int. J. Pharmacogn. Phytochem. Res.* **2020**, *12*, 123–131. [CrossRef]
129. Warsidah, M.; Sofiana, M.S.J.; Safitri, I.; Sapar, A.; Aritonang, A.B.; Muttalib, Y.S.; Fadly, D. Protein isolation from sponge *Niphates* sp. as an antibacterial and antioxidant. *Syst. Rev. Pharm.* **2020**, *11*, 518–521. [CrossRef]
130. Hwang, J.; Kim, D.; Park, J.S.; Park, H.J.; Shin, J.; Lee, S.K. Photoprotective activity of Topsentin, a bis(indole) alkaloid from the marine sponge *Spongosorites genitrix*, by regulation of COX-2 and Mir-4485 expression in UVB-irradiated human keratinocyte cells. *Mar. Drugs* **2020**, *18*, 87. [CrossRef]
131. Cheung, F.W.K.; Guo, J.; Ling, Y.H.; Che, C.T.; Liu, W.K. Anti-melanogenic property of geoditin A in murine B16 melanoma cells. *Mar. Drugs* **2012**, *10*, 465–476. [CrossRef]
132. Lee, H.Y.; Jang, E.J.; Bae, S.Y.; Jeon, J.E.; Park, H.J.; Shin, J.; Lee, S.K. Anti-melanogenic activity of Gagunin D, a highly oxygenated diterpenoid from the marine sponge *Phorbas* sp., via modulating tyrosinase expression and degradation. *Mar. Drugs* **2016**, *14*, 212. [CrossRef]
133. Yanti, C.; Vendy, V.; Hwang, J.K. In vitro anti-acne activity of marine sponge *Acanthella cavernosa* extracts. *Int. J. Biol. Pharm. Res.* **2015**, *6*, 388–392.
134. Chander, M.P.; Vijayachari, P. Antimicrobial and anti-oxidant potentials of marine sponges of South Andaman, India. *Bangladesh J. Pharmacol.* **2018**, *13*, 13–15. [CrossRef]
135. Campos, P.E.; Herbette, G.; Chendo, C.; Clerc, P.; Tintillier, F.; Voogd, N.J.D.; Papanagnou, E.D.; Trougakos, I.P.; Jerabek, M.; Bignon, J.; et al. Osirisynes G-I, new long-chain highly oxygenated polyacetylenes from the Mayotte marine sponge *Haliclona* sp. *Mar. Drugs* **2020**, *18*, 350. [CrossRef]
136. Ghatak, S.; Maytin, E.V.; MacK, J.A.; Hascall, V.C.; Atanelishvili, I.; Moreno Rodriguez, R.; Markwald, R.R.; Misra, S. Roles of proteoglycans and glycosaminoglycans in wound healing and fibrosis. *Int. J. Cell Biol.* **2015**, *2015*, 1–20. [CrossRef]
137. Pallela, R.; Ehrlich, H.; Bhatnagar, I. Marine sponges: Chemico-biological and biomedical applications. In *Marine Sponges: Chemicobiological and Biomedical Applications*; Pallela, R., Ehrlich, H., Eds.; Springer: New Delhi, India, 2016; pp. 1–381. ISBN 9788132227946.
138. Swatschek, D.; Schatton, W.; Kellermann, J.; Müller, W.E.G.; Kreuter, J. Marine sponge collagen: Isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. *Eur. J. Pharm. Biopharm.* **2002**, *53*, 107–113. [CrossRef]
139. Langasco, R.; Cadeddu, B.; Formato, M.; Lepedda, A.J.; Cossu, M.; Giunchedi, P.; Pronzato, R.; Rassu, G.; Manconi, R.; Gavini, E. Natural collagenic skeleton of marine sponges in pharmaceutics: Innovative biomaterial for topical drug delivery. *Mater. Sci. Eng. C* **2017**, *70*, 710–720. [CrossRef] [PubMed]
140. Pozzolini, M.; Millo, E.; Oliveri, C.; Mirata, S.; Salis, A.; Damonte, G.; Arkel, M.; Scarfì, S. Elicited ROS scavenging activity, photoprotective, and wound-healing properties of collagen-derived peptides from the marine sponge *Chondrosia reniformis*. *Mar. Drugs* **2018**, *16*, 465. [CrossRef] [PubMed]

marine drugs

MDPI

Review

Marine Demospongiae: A Challenging Treasure of Bioactive Compounds

Roberta Esposito [1,2,†], Serena Federico [1,†], Marco Bertolino [3], Valerio Zupo [1,*] and Maria Costantini [1,*]

[1] Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; roberta.esposito@szn.it (R.E.); serena.federico@szn.it (S.F.)
[2] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cin-thia 21, 80126 Naples, Italy
[3] Department of Earth, Environment and Life Sciences (DISTAV), Università degli Studi di Genova, Corso Europa 26, 16132 Genova, Italy; marco.bertolino@unige.it
* Correspondence: valerio.zupo@szn.it (V.Z.); maria.costantini@szn.it (M.C.)
† These authors contributed equally to this work.

Abstract: In the last decades, it has been demonstrated that marine organisms are a substantial source of bioactive compounds with possible biotechnological applications. Marine sponges, in particular those belonging to the class of Demospongiae, have been considered among the most interesting invertebrates for their biotechnological potential. In this review, particular attention is devoted to natural compounds/extracts isolated from Demospongiae and their associated microorganisms with important biological activities for pharmacological applications such as antiviral, anticancer, antifouling, antimicrobial, antiplasmodial, antifungal and antioxidant. The data here presented show that this class of sponges is an exciting source of compounds, which are worth developing into new drugs, such as avarol, a hydroquinone isolated from the marine sponge *Disidea avara*, which is used as an antitumor, antimicrobial and antiviral drug.

Keywords: Demospongiae; bacteria; fungi; diverse bioactivities

Citation: Esposito, R.; Federico, S.; Bertolino, M.; Zupo, V.; Costantini, M. Marine Demospongiae: A Challenging Treasure of Bioactive Compounds. *Mar. Drugs* **2022**, *20*, 244. https://doi.org/10.3390/md20040244

Academic Editors: Angelo Fontana and Margherita Gavagnin

Received: 28 February 2022
Accepted: 29 March 2022
Published: 31 March 2022

1. Introduction

1.1. Natural Products from Marine Organisms

The discovery of marine-derived natural products is a promising, comparatively new field, which started with the isolation of unusual nucleoside derivatives in the sponge *Tectitethya crypta* (de Laubenfels, 1949) (ex. *Tethya crypta*) in the 1950s by Bergmann and Feeney [1,2]. In the early 1960s, research on marine natural products was driven by chemical studies and a few compounds were tested for relevant bioactivity [3]. An example is represented by the production of a pyrrole antibiotic by a marine bacterium *Pseudomonas bromoutilis* [4]. However, the utilization of marine organisms as sources of bioactive metabolites started seriously at the end of 1960s [5] with the isolation of prostaglandin derivatives from the Caribbean gorgonian *Plexaura homomalla* (Esper, 1794) [6]. In the 80s, effective collaborations were established between marine chemists and pharmacologists, and the investigations were focused on the toxins active in the membranes of the central nervous system, ion channel effectors, anticancer and antiviral agents, iser promoters and anti-inflammatory agents [7]. In the 90s, the pharmaceutical and biotechnological industries focused on the chemical libraries of both natural products and the synthetic compounds produced by combinatorial methods [8]. Invertebrates, mainly sponges, tunicates, bryozoans and shellfish, provided several marine natural products, which could be used for clinical or preclinical studies [9]. In fact, the research led to the discovery of many anticancer compounds from marine sponges, which have not yet been tested on humans, except for the Eribulin mesylate (an analogue of halichondrin B), which has been tested on women with breast cancer [10]. Many clinical trials were made through experimental models,

such as the mice and zebrafish, representing a step forward in the evaluation of possible adverse effects not detectable by in in vitro tests [11]. The discovery of marine natural products has accelerated in the last two decades with the number of new compounds discovered each year increasing from 20 to more than 200 [12]. It has been assessed that more than 15,000 marine natural products (MNPs) have been discovered [13–15] since 2010, with 8368 new compounds recorded in the decade of 2001–2010. This constitutes over half of all the compounds discovered since 1951 [16]. Among the marine organisms investigated, marine sponges (Porifera) are recognized as the richest sources of MNPs, with about 9398 compounds to date, contributing to nearly 30.7% of all marine natural products discovered so far, according to a database of MNPs [17–22]. This makes sponges the most prolific marine producers of compounds, with more than 200 new compounds reported each year in the last decade [23]. With this myriad of MNPs already available, several studies have revealed a broad spectrum of biological activities for these compounds, including anticancer, antiviral, antibacterial, antifungal, antiprotozoal, antihelmintic, anti-inflammatory, immunosuppressive, neurosuppressive, neuroprotective, antifouling and a range of other bioactivities [14]. In addition, as infectious microorganisms evolve and develop resistance to existing pharmaceuticals, marine sponges are providing novel leads against bacterial, fungal and viral diseases [23,24]. The annual discovery of marine natural products continued at a constant level of about 500 products in the late 1990s [12], but this number increased from 600 to over 1000 compounds per year from 2008 to 2010, a significant increase which was partly driven by new developments in modern analytical technology and instruments, especially the development of the high resolution nuclear magnetic resonance (NMR) and mass spectrometry (MS) coupled with high-performance liquid chromatography (LC) and gas chromatography (GC) [12].

Several efforts have been made in the last years to identify antitumor compounds for therapeutic applications, for example, screening methods at the National Cancer Institute (NCI-NIH/USA), which aimed at identifying antitumor agents with selective cytotoxic activity against tumor cells [25]. Furthermore, several companies, as in the case of Pharma-Mar, performed the pharmacological evaluation and, ultimately, the commercialization of bioactive compounds [26].

In this review, we analyzed a set of scientific publications on sponges and sponge symbiont-related compounds that demonstrate interesting biotechnological applications in pharmacological field. In particular, we focused on Demospongiae, which are the largest class, encompassing 81% of all living sponges with almost 7,000 species worldwide [27]. Considering the abundance of molecules isolated from Demospongiae, we think that this class can be considered a challenging treasure of bioactive compounds, from which several others will be identified.

1.2. Description of the Class Demospongiae

The class Demospongiae, together with Calcarea, Hexactinellida and Homoscleromorpha, belong to the phylum Porifera. Demospongiae are the class that includes the largest number of extant species [28,29]. They exhibit multiple shapes, are able to colonize any aquatic environment (marine, brackish and fresh waters) and have a wide distribution, both geographic (from polar to tropical waters) and bathymetric (from intertidal zones to depths of thousands of meters) [21]. They prefer hard substrates, but several species are also capable of living on soft bottoms, due to the presence of stems or bundles of spicules, which allow them to affix themselves to the substrate, while still remaining distant from the sediment [30,31]. Other species are able to live under the sediment, from which they release only rising, finger-like growths, ending in an osculum (habitus psammobiotic). Others, indicated as "free" specimens, are devoid of any anchoring structure and can live floating above the sediment, without attaching themselves.

Most demosponges are characterized by an aquiferous system, made of canals and choanocyte chambers (the leuconoid condition). The aquiferous system permeates the body of the sponge and pumps enough water to carry out an essential replacement. They

filter heterotrophic bacteria, heterotrophic eukaryotes, phytoplankton and debris, within a size range of 0.1–50 µm. This class also includes the so called "carnivorous sponges", which lack an aquiferous system (e.g., the family Cladorhizidae). Microorganisms that resist the sponge's digestive process and survive its immune response can successfully inhabit the sponges [22]. Demosponges host a rich symbiotic community (Eubacteria, but also Archaea) and in some cases they reach 60% of the total biomass of the sponge [32].

Demosponge skeletons can be made up of siliceous spicules, either isolated or in conjunction with an organic collagen skeleton. Collagen can be dispersed or can give rise to sponge fibers and filaments. A few taxa, unrelated to each other, have no skeleton other than a diffused fibrillar collagen. Other minor groups have developed a hypercalcified basal skeleton with or without free spicules.

The spicules can be divided into megasclere and microsclere. The former are monaxial or tetraxial (never triaxial), while the latter is characterized by various shapes. The spicules are produced inside specialized cells (sclerocytes) and contain an organic axial filament, with a triangular or hexagonal section, around which hydrated silica are periodically deposited, giving rise to a concentric arrangement. It is generally assumed that spicule growth is a bidimensional process: the increase in length is affected by the elongation of the filament, whereas the increase in width is determined by the apposition of the silica [27]. In the class Demospongiae, the organic axial filament, which functions as a template for silica deposition, is constituted by peculiar proteins called silicateins. The potential number of spicule types in a species of sponges appears to be genetically fixed, but the environmental conditions, specifically, the availability of silicon, may determine whether a genetically determined spicule type is finally expressed [29]. In Demospongiae the cellular elements, remarkably different, are never syncytial (unlike those of the class Hexactinellida, which possess a choanosyncytium made by choanocytes fused to form a continuous cytoplasmic compartment). The reproduction of demosponges can be sexual (both oviparous and viviparous with the production of larvae, mostly of the parenchymella type) or asexual, occurring by fragmentation, budding and gemmulation.

1.3. Demospongiae as Sources of Beneficial Compounds

The Demospongiae (demosponges) are the group of sponges encompassing most of the existing species, and they are an opulent source of biologically active specialized metabolites with potential biotechnological applications because of their antiviral, antitumor, antimicrobial, antiplasmodial, antifungal and antifouling [33–35] (see Figure 1).

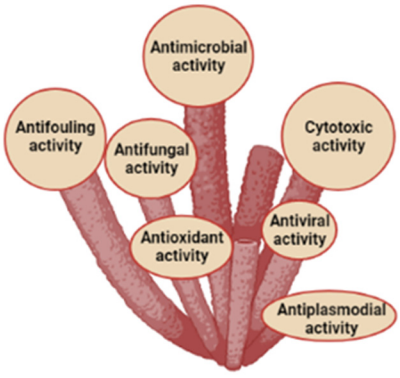

Figure 1. Graphical representation of sponges and their associated biota activities reported for the pharmacological application. The scale of the bubble is relative to the number of papers found. This image was created in Biorender.com (accessed on 1 January 2022).

Specialized metabolites are not usually involved in processes like the growth, development or reproduction of an organism. They are generated as result of the organism adapting to its neighbouring environment and/or are produced to act as a possible defence mechanism against predators and to improve the fitness of the organism [36,37]. Marine

natural products originate from sponges or sponges-associated biota (archaea, bacteria and fungi) [38]. Our knowledge about the heterogeneity of the sponge-associated biota is still incipient, and a large number of the features of sponge–associated biota are still unexplored. The exploration of biotechnological potentials of biota associated with sponges has been limited due to the difficulties in cultivating sponges and the microbes associated with sponges [38]. However, it is possible to perform a genome mining approach applied to all uncultured organisms to detect biosynthetic pathways of bioactive natural products, as well as their possible functional and chemical interactions [39]. In Figure 2, some examples of natural compounds isolated from the Demospongiae are depicted, which will be discussed in the following paragraphs.

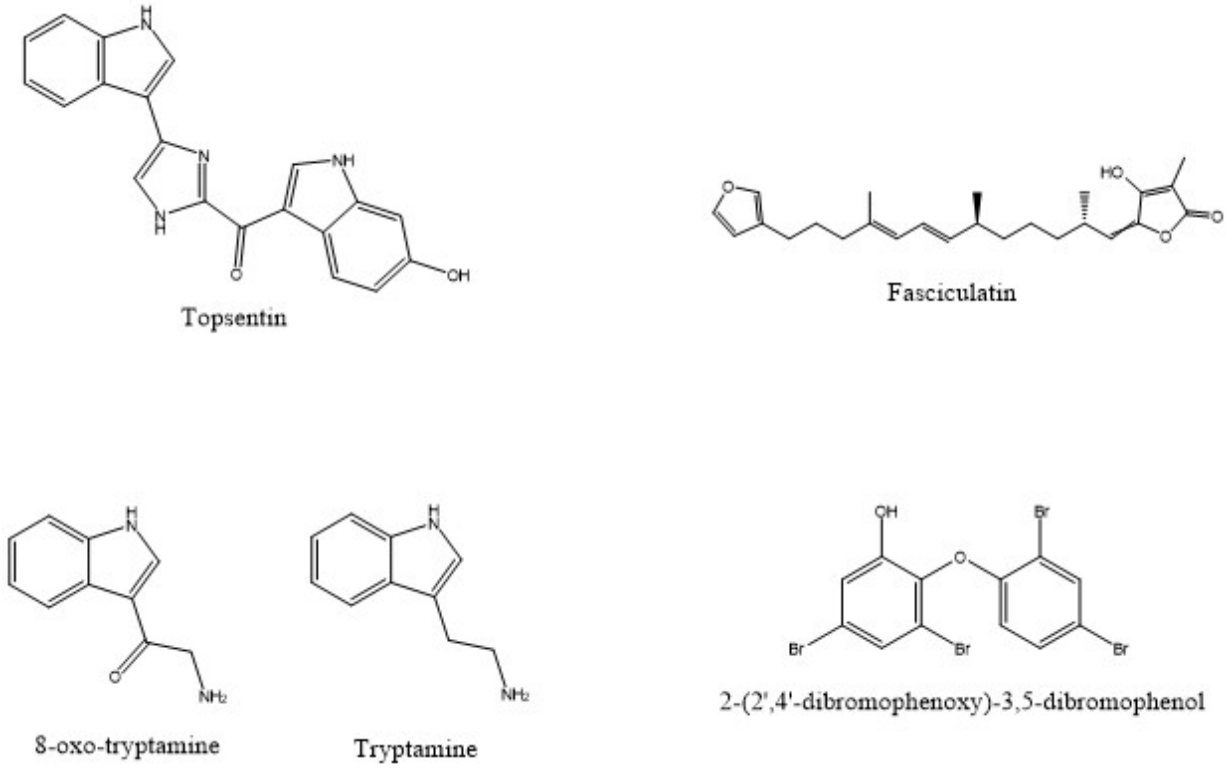

Figure 2. Examples of natural products isolated from some sponges belonging to the class of Demospongiae.

2. Biotechnological Activities of Compounds Isolated from Demospongiae or Their Associated Microorganisms

2.1. Cytotoxic Activity

Cancer is the second deadliest illness and has obtained enormous attention from researchers, who are trying to understand mechanisms of this disease and to find new drugs for therapy [40]. Marine sponges and their sponge-associated organisms represent precious sources of natural products with cytotoxic activity [41–43]. Two indole alkaloids, topsentin (see Figure 1) and bromotopsentin, were isolated from different species of sponges belonging to the genus *Spongosorites* (*Spongosorites* sp. and *Spongosorites ruetzleri*, Van Soest and Stentoft, 1988) and were tested on different cancer cell lines. In particular, these products showed cytotoxic activity against HCT8 (adenocarcinoma colorectal), A549 (lung carcinoma), T47D (breast carcinoma) and P388 (mouse lymphoma) with an IC_{50} of 3.0 μg/mL for the last cell line and 20 μg/mL for all other cancer cell lines [44]. Interestingly, cacospongionolide, a sesterterpene isolated from the marine sponge *Cacospongia mollior* (Schidt, 1862), collected in the northern Adriatic, showed potent antitumor activity in the brain shrimp assay with LD_{50} (lethal dose) of 0.1 μg/mL [45]. In a similar study, a polycyclic alkaloid (saraine A) isolated from the Mediterranean sponge *Reniera sarai*,

previously characterized by Cimino et al. [46], tested for its cytoxic activity on the brine shrimp *Artemia salina*, showed a LD_{50} value of 46.7 µg/mL [47]. Petroleum ether and total methanolic extracts isolated from *Negombata magnifica* (Keller, 1889), collected in the Red Sea, showed anticancer activity against a human liver carcinoma cell line (HepG2) with an IC_{50} value of 5 and 10 µg/mL, respectively. Moreover, all concentrations triggered lower toxicity than positive control (palmitic acid) [48]. Similarly, aqueous ethanol extract from the marine sponge *N. magnifica*, collected along the Gulf of Aqaba in the Red Sea, had anti-tumor effects against MCF-7 (breast cancer) and CACO-2 (colon cancer) with an IC_{50} of 0.37 and 1.09 µg/mL, respectively [49]. Geodiamolide H3, obtained from *Geodia* sp., collected in Macqueripe Bay (Trinidad), showed in vitro cytotoxicity, calculated as total growth inhibition (TGI), against a number of human cancer cell lines: HOP-92 (non-small cell lung cancer, 0.118 µM), SF-268 (central nervous system, 0.153 µM), OV Car-4 (ovarian cancer, 0.0186 µM), A498 and UO-31 (renal cancer cells, 0.0948 µM and 0.185 µM, respectively) and MDA-MB-23 and HS 578T (breast cancer cells, 0.433 µM and 0.245 µM, respectively) [50]. Other studies on the *Geodia* genus [51] demonstrated that methanolic extracts obtained from the marine sponge *Geodia cydonium* (Jameson, 1811), collected in Gulf of Naples, manifested an anti-inflammatory effect on a MCF-7 cancer cell line, inducing a reduction in the levels of VEGF and five proinflammatory cytokines (CXCL10, CCL2, CXCL8, IFN-γ and TNF-α) in a dose-dependent manner. Furthermore, this extract showed a growth inhibition in three breast cancer cell lines, MDA-MB231, MCF-7 and MDA-MB468, with an IC_{50} of 44, 67 and 70 µg/mL, respectively, after 48 h of incubation [52]. Also, oxysterol and 4′-methylheptyl-benzoate isolated from the marine sponge *Hyrtios erectus* (Keller, 1889) displayed significant cytotoxic activity against breast adenocarcinoma (MCF-7) with IC_{50} values of 2.4 and 3.8 µM, respectively. The first compound, also showed an antiproliferative effect on HepG2 (hepatocellular carcinoma cells) with an IC_{50} value of 1.3 µM [53]. In an analogous study, a furanosesterterpene (fasciculation, see Figure 1) was isolated from the marine sponge *Ircinia variabilis* (Schimdt, 1862), collected from the Atlantic Coast of Morocco, and its biological activity was determined [54]. Achievements completed showed that this compound produced a dose-dependent growth inhibitory effect on MCF-7, SF-268 (CNS cancer) and NCI-H460 (non-small cell lung cancer) cell lines measured as GI_{50} (concentrations of compound, which cause 50% inhibition of tumor cell growth), corresponding to 47.11 ± 0.93, 72.45 ± 2.19 and 64.49 ± 0.84 µM, respectively, compared to the positive control doxorubicin and cyclosporin. Methanolic crude extracts from *Agelas oroides* (Schimdt, 1864) and *Petrosia ficiformis* (Poiret, 1789), collected in the Mediterranean Sea, influenced LAN5 and SK-N-BE(2)-C (human neuroblastoma cells) survival in a different way, using the concentrations of 5, 10 and 20 µg/mL of extract for 15 and 30 min. In fact, the extract of *A. oroides* was vastly more cytotoxic for two cell lines after 30 min, while the extract of *P. ficiformis* had already induced necrosis after 15 min [55]. Moreover, the cytotoxic effect of the extract from *A. oroides* differed considerably depending on seasons and depths, the greatest effect resulting from sponges collected from the site "Paraggi" in winter at −20 m [56]. In a similar work, Di Bari et al. [57] assessed the biological activity of aqueous extracts from *Tethya aurantium* (Pallas, 1766), *Tethya citrina* (Sarà & Melone, 1965), *Hymeniacidon perlevis* (Montagu, 1814), *I. variabilis*, *Chondrilla nucula* (Schimdt, 1862), *Aplysina aerophoba* (Nardo, 1843) and *Sarcotragus spinosulus* (Linnaeus, 1759), collected in the southern Adriatic Sea, on macrophages THP-1, CaCo-2 (epithelial cells), BHK-21 (fibroblasts and primary rat astrocytes) and ASTRO (astrocytes), demonstrating that the extracts from *T. citrina* and *H. perlevis* were the most cytotoxic in comparison to the other extracts analysed. In fact, ASTRO cells viability, after treatment with 30 µg/mL of extract from *T. citrina*, was of 20%, while BHK-21 cells viability treated with 30 µg/mL of extract from *H. perlevis* was 40%. Gukulenin A is a bis-tropolone tetraterpenoid obtained from the marine sponge *Phorbas gukhulensis* (Sim & Kim, 2004), which induced apoptotic cell death in A2780, SKOV3, OVCAR-3 and TOV-21G (human ovarian cancer cells) in a dose-dependent manner. The strongest cytotoxic effect was found on the ovarian carcinoma cell line A2780 at the concentration of 5 µM [58].

Matsumoto et al. [59] purified lectin from associated microorganisms with a black demosponge *Halichondria okadai* (Kadota, 1922), sampled in Japan. Lectins are carbohydrate-binding proteins and have many roles such as cell growth regulation, anti-infectious estates and the support of natural immunity with the help of their binding to specific oligosaccharides to create glycoconjugates. In this case, the lectin killed the Jurkat leukemia T cells and the K562 (erythroleukemia cells) in a dose-dependent manner, showing 40% and 50% cell death, respectively.

However, as mentioned in the introduction, sponge-associated biota also has a definite biotechnological role, exhibiting several bioactivities [60,61]. For instance, Pagliara and Carocco [62] isolated eight cyanobacterial strains (*Synechoccus* sp. red and blue-green types, *Cyanobium* sp., *Leptolyngbya* cfr. *minuta*, *Leptolyngbya* cfr. *ectocarpii*, *Leptolyngbya* sp. 1, 2 and 3) from *P. ficiformis*. They demonstrated that the aqueous extracts of strains, ITAC101, ITAC104 and ITAC102, belonging to *Leptolyngbya* genus, were the most toxic on *A. salina* nauplii with an LC_{50} of 6440, 10270 and 12270 µg/mL, respectively, after 24 h of exposure. Moreover, ITAC103 and ITAC104 extracts induced a delay in the development and an increment in deformed embryo production of *Paracentrotus lividus*. In the following study, Pagliara et al. [63] split eight cyanobacterial strains (*Cyanobium* sp., *Synechoccus* sp., *Pseudoanabaena* sp. 1, 2, *Leptolyngbya ectocarpi*, *Halomicronema* cf. *metazoicum*, *H. metazoicum*) isolated from the same sponge and evaluated their biological activity, testing their aqueous cell supernatants on HeLa (cervical adenocarcinoma), SH-SY5Y (neuroblastoma) and B-104-1-1 (glioblastoma). The strain ITAC106 (*Pseudanabaena* sp. 1) showed the strongest cytotoxic activity on all cell lines analysed at a concentration of 150 µg/mL. In a similar study, petrocidin A, a new cyclic dipeptide isolated from the solid culture of *Streptomyces* sp. SBT348, which had previously been recovered from the Mediterranean sponge *P. ficiformis*, displayed significant cytotoxic effects towards acute promyelocytic leukemia (HL-60) and human colon adenocarcinoma (HT-29) cell lines with the IC_{50} values of 3.9 and 5.3 µg/mL, respectively, measured with MTT assay, using as positive control 5-Flurouracil [64]. Strepoxazine A, a new phenoxazine analogue isolated from the solid culture of sponge-associated *Streptomyces* sp. SBT345, which had earlier been isolated from the Mediterranean sponge *A. oroides*, exhibited a potent cytotoxic effect against HL-60 cells (human promyelocytic leukemia) with an IC_{50} at 16 µg/mL [65]. Another study has been carried out on isolates from specimens of sponges belonging to the genus *Haliclona*. Handayani et al. [66] prepared twenty extracts of fungi derived from the marine sponge *Haliclona fascigera* (Hentschel, 1912), collected from West Sumatera, testing their biological activity using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay and using doxorubicin as a positive control. The fungal extract of WR6 (*Trichophyton* sp.) showed the highest cytotoxicity with the IC_{50} values of 163.37, 118.3, 67.1 and 47.4, µg/mL against Vero cells, HeLa (HeLa as cervix cell line), T47D (human ductal breast epithelial tumor cell line) and WiDr (colon adenocarcinoma cell line), respectively, compared with the IC_{50} values (43.74, 1.25, 10.05 and 0.28 µg/mL, respectively) of doxorubicin. Several studies revealed the importance of kinase inhibitors from marine sponges, demonstrating the key role of these proteins in cell regulation, controlling cell differentiation, proliferation, metabolism, DNA damage repair and cell motility. The deregulation of kinase has been identified as a priority due to an ever-expanding list of diseases, including cancer, central nervous system disorders and metabolic diseases [67]. For example, penazetidine A, isolated from sponge *Penares sollasi*, and hymenialdisines 4 and 5, isolated from marine sponge *Stylotella aurantium*, exhibited activity against PKC (protein kinase C) with the IC_{50} values of 0.03, 0.8 and 1.3 µM, respectively [68,69]. The cytotoxic compounds examined above are listed in the Table 1.

Table 1. Source, sponge host, extract/compound, cell line/organism tested and corresponding reference are reported.

Source	Associated Organisms	Extract/Compound	Cell Line/Organism Tested	Reference
Spongosorites sp. and *S. ruetzleri*		Topsentin and bromotopsentin	P388, HCT8, A549, T47	[44]
C. mollior		Cacospongiolide	Shrimp	[45]
R. sarai		Saraine A	*A. salina*	[47]
N. magnifica		Petroleum ether and total methanolic extracts	HepG2	[48]
N. magnifica		Aqueous ethanol extract	CACO-2 and MCF-7	[49]
Geodia sp.		Geodiamolide H 3	HOP 92, SF-268, OV Car-4, A498, UO-31, MDA-MB-231, HS 578T	[50]
G. cydonium		Methanolic extract	MCF-7, MDA-MB231, MDA-MB468	[52]
H. erectus		Oxysterol and 4′-methylheptyl benzoate	MCF-7 and HepG2	[53]
I. variabilis		Fasciculatin	MCF-7, NCI-H460 and SF-268	[54]
A. oroides and *P. ficiformis*		Methanolic extract	LAN5 and SK-N-BE(2)-C	[55]
T. aurantium, *T. citrina*, *H. perlevis*, *I. variabilis*, *C. nucula*, *A. aerophoba and S. spinosulus*		Aqueous extract	THP-1, CaCo-2 and BHK-21	[57]
P. gukhulensis		Gukulenin A	A2780, SKOV3, OVCAR-3 and TOV-21G	[58]
H. okadai		Lectin	Jurkat leukemia T and K562	[59]
P. ficiformis	*Synechoccus* sp. red and blue-green types, *Cyanobium* sp., *Leptolyngbya* cfr. Minuta, *Leptolyngbya* crf. *ectocarpii*, *Leptolyngbya* sp. 1, 2 and 3	Aqueous extract	*A. salina* and *P. lividus*	[62]
P. ficiformis	*Cyanobium* sp., *Synechoccus* sp., *Pseudoanabaena* sp. 1, 2, *L. ectocarpi*, *Halomicronema* cf. *metazoicum*, *H. metazoicum*	Aqueous cell supernatans	HeLa, SH-SY5Y and B-104-1-1	[63]
P. ficiformis	*Streptomyces* sp. SBT348	Petrocidin A	HL-60 and HT-29	[64]
A. oroides	*Streptomyces* sp. SBT345	Strepoxazine A	HL-60	[65]
H. fascigera	*Trichophyton* sp.	Ethil acetate extract	WiDr, T47D and HeLa	[66]
P. sollasi		Penazetidine A	PKC	[68]
S. aurantium		Hymenialdisines 4 and 5	PKC	[69]

2.2. Antibacterial and Antiviral Activities

Specialized metabolites produced by sponges or sponge-associated biota are bioactive and indispensable for their survival in the marine environment, hence, they have potential for pharmacological applications, including antimicrobial and antiviral activities [70,71]. Sponges do not have a specific immune system but possess eosinophilic granular cells that can perform a non-specific response to a variety of dangers. This information was the foundation of the study conducted by Krylova et al. [72], which obtained pure eosinophilic amoebocyte (EA) fractions from the marine sponge *Halisarca dujardini* (Johnston, 1842), sampled from the Kandalaksha Bay, White Sea. Interestingly, only part of the subfraction showed antimicrobial activity against *Escherichia coli* and *Listeria monocytogenes* [72]. Several ethyl acetate extracts from marine sponges showed interesting activity against *E. coli*; for example, a fraction of the marine species *Aplysina fistularis* (Pallas, 1766) (sampled in Bahía de Mochima, Venezuela) with a MIC (minimum inhibitory concentration) value higher than 16 µg/mL. Instead, other fractions from the same sponge showed activity specifically on *Staphylococcus aureus* with MIC of 0.125, 128 and 256 µg/mL, respectively [73]. Similarly, manzamenones M extracted from a marine sponge belonging to the genus *Plakortis* (Okinawan Sea) showed antimicrobial activity against the same two bacterial strains (*E. coli* MIC = 32.0 µg/mL, *S. aureus* MIC = 16.0 µg/mL) and *Cryptococcus neoformans* (MIC = 4.0 µg/mL) as well. From the same sponge species another manzamenone (N) was also isolated and tested, showing biological activity against *E. coli* (MIC = 32.0 µg/mL) and *C. neoformans* (MIC = 32.0 µg/mL) [74]. Plakortide N and plakortide F free acid isolated from sponges of the genus *Plakortis* (sampled in Jamaica) showed, similarly to its Venezuelan counterpart, activity against *C. neoformans* with an IC_{50} ranging from 2.5 to 5.5 µg/mL, using amphotericin B and ciprofloxacin as positive controls [75].

A methanol extract from the demosponge *Xestospongia testudinaria* (Lamarck, 1815), collected in Pasir Putih (Indonesia), showed antimicrobial activity against several microbes like *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella tiphy*, *Pseudomonas aeruginosa* MDR (Multidrug Resistant) and *S. aureus* MRSA (Methicillin resistant), using the agar diffusion method [76]. Further studies on specimens of the same sponge sampled in India demonstrated that a methanol extract of this sponge was also effective against *Staphylococcus epidermidis* [77]. Interesting compounds such as 1-monoamphilectine and 8,15- diisocyano-11(20)-amphilectene obtained from the extract of the marine sponge *Hymeniacidon* sp., sampled from the Mona Island, demonstrated their effectiveness on the *Mycobacterium tuberculosis* (H37Rv) with MIC values of 15.3 and 3.2 µg/mL [78].

Even though it might appear improbable, some extracts from marine sponges demonstrated higher efficiency against several Gram-positive and Gram-negative bacteria as compared to positive antibacterial positive controls. For instance, ethyl extracts from the marine sponges *Axinella damicornis* (Esper, 1794) and *A. oroides* (sampled from the Tunisian Mediterranean coast, Monastir) were more effective than the antibiotic gentamycin (10 µg) against several distinct human pathogens. *A. damicornis* demonstrated wider potentiality, as it was efficient on several bacterial strains, with a growth inhibition diameter (mm) of 21 for *S. epidermidis*, 17 for *S. aureus*, 26 *Micrococcus luteus*, and 20 for *Enterococcus feacalis*; Gram-negative bacteria: 12 for *P. aeruginosa*, 20 for *E. coli*, 16 for *Salmonella thyphymerium* and 20 for *L. monocytogenes*. In contrast, *A. oroides* was efficient only on some of those strains, with growth inhibition diameter (mm) of 13 for *S. epidermidis*, 17 for *S. aureus*, 18 for *M. luteus*, and Gram-negative bacteria 13 for *E. coli* and 12 for *S. thyphymerium*, compared with positive control gentamycin [79].

Powerful antibiotics were extracted and characterized from the sponge *Dysidea granulosa* (Bergquist, 1965), then tested against several different human pathogens, such as *Klebsiella pneumoniae*, with encouraging results. The recorded MIC of the compound named as 2-(2′,4′-dibromophenoxy)-3,5-dibromophenol (see Figure 1) was 0.1 µg/mL. The importance of this result is due to the fact that the positive controls available and used in the treatment of this pathogen (ciprofloxacin, cefoxitin and imipenem) are efficient at high concentrations (MIC = 0.125 µg/mL, MIC = 0.25 µg/mL and MIC= 0.25 µg/mL,

respectively) [80]. Interestingly, recent studies were directed towards the extraction of compounds useful for the human health and against human pathogens from environments which are considered harsh for the humans. A pioneer study directed by Kosgahakumbura et al. [81] was focused on the extraction of bioactive compounds from the marine sponge *Stryphnus fortis* (Vosmaer, 1885). Many different peptides were purified but only one, named "peptide C", demonstrated modest antimicrobial activity against *S. aureus* with MIC = 36.14 μM [81]. Another study was performed on two fractions, namely, A (aqueous extract) and B (methanol extract), isolated from the sponge *Suberites iona*, sampled from the Persian Arabic Gulf (PAG), which lives in hyperthermic and hypersalinic conditions. These fractions demonstrated activity not only against *S. aureus* but also against *Enterococcus faecalis* [82]. Interesting results were obtained by Tsujii and Rinehar [44], testing two indole alkaloids (topsentin and bromotopsentin) extracted from several samples of sponges belonging to the genus *Spongosorites*, collected in the Bahamas. These compounds were found to be active as antiviral agents against the *Herpes simplex* virus 1(HSV-1), vesicular stomatitis virus (VSV), and the *Coronavirus* A-59. A halistanol-enriched fraction (TSH fraction) and its compounds 1 (halistanol sulfate) and 2 (halistanol sulfate C) isolated from the sponge *Petromica citrina* (Muricy, Hajdu, Minervino, Madeira & Peixinho, 2001), collected in Brazil, showed anti-herpes activity through the reduction of viral infectivity, inhibition of virus entry into the cells and by the impairment of levels of ICP27 and gD proteins of HSV-1 [83]. In a similar study, El-Damhougy et al. [49] demonstrated that a crude extract from the marine sponge *Grayella cyathophora* (Carter, 1869), collected along the Gulf of Aqaba in the Red Sea, showed a high cytotoxic effect compared to Vero cells with the hepatitis A virus. A polycyclic alkaloids (saraine 2), isolated from sponge *R. sarai*, showed interesting antibacterial activity against *S. aureus* with MIC of 50.0 μg/mL [47].

In recent years, a number of new compounds with variegated activities have been detected through the cultivation of sponge-associated microorganisms [84–86]. For instance, several bacterial strains were isolated from the marine Demospongiae *Hymeniacidon perlevis*, collected on the Nanji Island (Eastern China Sea, China), and their ethyl acetate extracts were tested on human and plant pathogens, and the ones that showed significant antimicrobial activity were named as NJ6-3-1 and NJ6-3-2 and successively identified as *Pseudoalteromonas piscicida* and *Bacillus megaterium*, respectively. The extract of the former strain was efficient against *Bacillus subtilis*, *E. coli*, *S. aureus*, *Agrobacterium tumefaciens* and the yeast *Saccharomyces cerevisiae*; while the extract of the latter strain demonstrated activity against *B. subtilis*, *A. tumefaciens*, *S. aureus* and the yeast *S. cerevisiae* [87]. Frequently, the isolated bacterial species belong to the genus *Bacillus*, as is the case for the strain "2011SOCCUF3", isolated from the marine sponge *Spongia officinales* (Linnaeus, 1759), subfractions of which were active against as *S. aureus* (MIC = 247 μg/mL), *S. tiphy* (MIC = 83 μg/mL), *P. aeruginosa* (MIC = 162 μg/mL) and *E. coli* (628 μg/mL), compared with the positive controls (ciprofloxacin and fluconazole) [88]. Similarly, from the sponge *Halichondria glabrata* (Keller, 1891) (collected in Mumbai) the strain GSA10 was isolated and successively classified for its similarities with pG1 *Bacillus amyloliquefaciens*. However, its ethyl acetate extracts were tested and were active against human pathogens such as *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* [89]. In a similar way, *Bacillus* sp. Was isolated from samples of the marine sponge *Dysidea fragilis*, from the Agatti Island in the Lakshadweep archipelago, and its purified molecule (Pyrrolo(1,2-a)pyrazine-1,4-dione, hexahydro) was tested against several model bacterial pathogens, such as *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Flavobacterium* sp., *Proteus mirabilis* and *Citrobacter brackii*, and showed a LC_{50} of 31.25 μg/mL, using antibiotic amoxicillin as a positive control [90]. Another pathogen which affects aquaculture stocks is *Vibrio anguillarum*, isolated from the marine sponge *Erylus deficiens* (Topsent, 1927) [71]. Bacteria isolated from *P. ficiformis*, sampled from the Portofino Promontory (Ligurian Sea), were tested for their potential production of antibiotic compounds against *S. aureus*. Two strains were identified as *Rhodococcus erythropolis* and the other one belonged to the genus *Pseudomonas* [91]. Recently, Koch et al. [92] studied two sponges sampled from the North East Atlantic, *Pheronema carpenteri* (Thomson, 1869)

and *Hertwigia* sp., from which several bacterial strains were isolated and tested for their antimicrobial activity. From the sponge *P. carpenter*, strains of *Bacillus altitudinis*, *Streptomyces* sp., *Brevundimonas* sp., *Microbacterium maritypicum* were isolated, while from the *Hertwigia* sp. was isolated from the species *Delftia acidovorans*. All these bacteria were active against *S. aureus*, *E. coli* and *M. luteus*. Many other bacterial strains belonging to phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes were isolated from specimens of *Suberites carnosus* (Johnston, 1842) and *Leucosolenia* sp., sampled from Ireland (Lough Hyne, Co. Cork), and tested for their antimicrobial activity against many pathogens (*B. subtilis* IA40, *E. coli* NCIMB 12212, *S. aureus* NCIMB 9518, *K. marxianus* CB86556). Antibacterial activity was higher among the isolates obtained from the sponge *S. carnosus* than from the isolates separated from *Leucosolenia* sp. [93]. In a study conducted by Halloran et al. [94], three additional species of sponges (*Polymastia boletiformis* (Lamarck, 1815), *Axinella dissimilis* (Bowerbank, 1866) and *Haliclona simulans* (Johnston, 1842)) were collected from the same area. From these specimens seventy-three different bacterial strains, all belonging to *Pseudovibrio* spp., were isolated and tested for their antibacterial effect against an ample group of pathogens. The strongest antibacterial activity was discovered against *E. coli*, *S. Typhimurium*, *B. subtilis*, *S. aureus*, methicillin-resistant *Staphylococcus aureus*, *S. aureus* VISA (vancomycin intermediate), heterogenous vancomycin intermediate *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridioides difficile*. A minor activity was observed when the test was carried out on *Yersinia enterocolitica*, *B. cereus*, *Enterococcus faecium*, vancomycin-resistant *Enterococcus* (VRE) and *L. monocytogenes* [94]. From samples belonging to *Ircinia* sp., collected in the North Bay (Andaman), many bacteria (*Vibrio* sp., *Bacillus* sp., *Aeromonas* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Enterococcus* sp., *Streptococcus* sp., *Neisseria* sp., *Citrobacter* sp., *Veillonella* sp. and *Klebsiella* sp.) associated with this sponge were isolated and tested for their antimicrobial activity and measured by disc diffusion assay, using erythromycin and ciprofloxacin as positive controls, against Gram-negative and Gram-positive pathogens (*Aeromonas hydrophila*, *Enterococcus durans*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Streptococcus lentus* and *Rolstonia solanacearum*). Most activity was recorded against Gram-positives (41.54%), whereas minor activity was found against Gram-negative bacteria (12.82%). The bacterial metabolites were differed significantly among them. Some of the isolates were specific for a single pathogen. In particular, the highest percentage was active just against 2–3 organisms, while only some isolates showed a wider spectrum of activity against 4–5 different pathogens [95].

Demospongiae members are not only found in the marine environment but can also be found in freshwater lakes, as is the case for the sponge *Ochridaspongia rotunda* (Arndt, 1937), an endemic species of the Lake Ohrid, in Europe. Interestingly, its methanol extract resulted in biological activity against several bacterial strains (*Enterobacter cloacae*, *E. coli*, *L. monocytogenes*, *Mariniluteicoccus flavus*, *Bacillus cereus*, *P. aeruginosa*, *Salmonella typhimurium* and *S. aureus)*, with a MIC between 7.5 and 15.0 μg/mL and MBC (minimum bactericidal concentration) between 15 and 30 μg/mL. Moreover, this extract was more effective than streptomycin and ampicillin (positive controls) [96]. The aforementioned antibacterial and antiviral activities of demosponges or demosponge-associated biota are schematically summarized in Table 2.

Table 2. Source, sponge host, extract/compound, cell line/organism tested and reference are reported.

Source	Associated Organisms	Isolated Compound	Cell Line/Organism Tested	Reference
H. dujardini		Eosinophilic amoebocytes (EA) fraction	*E. coli* and *L. monocytogenes*	[72]
A. fistularis		Ethyl acetate extract	*S. aureus*	[73]
Plakortis sp.		Manzamenones M and N	*E. coli*, *S. aureus* and *C. neoformans*	[74]
P. angulospiculatus		Plakortide N and F	*C. neoformans*	[75]

Table 2. *Cont.*

Source	Associated Organisms	Isolated Compound	Cell Line/Organism Tested	Reference
X. testudinaria		Methanol extract	*S. aureus*, *E.coli*, *K. Pneumoniae*, *S. tiphy*, *P. aeruginosa MDR* and *S. aureus MRSA*	[76]
X. testudinaria		Methanol extract	*S. epidermidis*	[77]
Hymeniacidon sp.		1 Monoamphilectine and 8,15-diisocyano-11(20)-amphilectene	*M. tuberculosis (H37Rv)*	[78]
A. dormicons and *A. orides*		Ethyl acetate extract	*S. epidermidis*, *S. aureus*, *M. luteus*, *E. feacalis*, *E. coli*, *P. Aeruginosa*, *S. thyphymerium* and *L. monocytogenes.*	[79]
D. granulosa		2-(2′,4′-dibromophenoxy)-3,5-dibromophenol	*K. pneumoniae*	[80]
S. fortis		Peptide C	*S. aureus*	[81]
S. luna		Aqueous extractA and methanol extract B	*S. aureus* and *E. faecalis*	[82]
H. perleve	*P. piscicida (NJ6-3-1) and B. megaterium (NJ6-3-2)*	Ethyl acetate extract	*B. subtilis*, *S. aureus*, *E. coli*, *A. tumefaciens* and *S. cerevisiae*	[87]
S. officinales	Bacillus 2011SOCCUF3	Methanol extract	*S. aureus*, *S. tiphy*, *P. aeruginosa* and *E. coli*	[88]
H. glabrata	*Bacillus amyloliquefaciens*	Ethyl acetate extract	*E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*	[89]
D. fragilis	*Bacillus* sp.	Pyrrolo(1,2-a)pyrazine-1,4-dione,hexahydro	*V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *Flavobacterium* sp., *P. mirabilis*, *C. brackii*, *A. salmonicida* and *Edwardsiella* sp.	[90]
E. deficiens	Proteobacteria, Actinobacteria and Firmicutes phyla	Aqueous extract	*V. anguillarum*	[71]
P. ficiformis	*Rhodococcus* sp. and *Pseudomonas* sp.	Bacterial isolates	*S. aureus*	[91]
P. carpenteri and *Hertwigia* sp.	*B. altitudinis*, *Streptomyces* sp., *Brevundimonas* sp., *M. maritypicum* and *D. acidovorans*	Bacterial isolates	*S. aureus*, *E. coli* and *M. luteus*	[92]
S. carnosus and *Leucosolenia* sp.	Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes phyla	Bacterial isolates	*E. coli* NCIMB 12212, *B. subtilis* IA40, *S. aureus* NCIMB 9518, *K. marxianus* CB86556	[93]

Table 2. *Cont.*

Source	Associated Organisms	Isolated Compound	Cell Line/Organism Tested	Reference
P. boletiformis, *A. dissimilis* and *H. simulans*	*Pseudovibrio* spp.	Bacterial isolates	*E. coli*, *S. Typhimurium*, *B. subtilis*, *S. aureus*, *S. aureus* MRSA, *S. aureus* VISA, hVISA, *C. perfringens*, *C. difficile*, *Y. enterocolitica*, *B. cereus*, *E. faecium*, *Enterococcus* (VRE) and *L. monocytogenes*	[94]
Ircinia sp.	*Vibrio* sp., *Aeromonas* sp., *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Streptococcus* sp., *Enterococcus* sp., *Neisseria* sp., *Veillonella* sp., *Citrobacter* sp. and *Klebsiella* sp.	Bacterial isolates	*A. hydrophila*, *B. subtilis*, *E. durans*, *S. lentus*, *K. pneumoniae* and *R. solanacearum*	[95]
O. rotunda		Methanol extract	*B. cereus*, *E. Cloacae*, *E. Coli*, *L. Monocytogenes*, *M. Flavus*, *P. Aeruginosa*, *S. Typhimurium* and *S. aureus*	[96]

2.3. Antifouling Activity

The Woods Hole Oceanographic Institute [97] refers to fouling as the process by which "plants and animals grow on the surface of submerged artificial structures and not natural objects". Fouling has always been the cause of worldwide economic losses by reducing boats speed and increasing fuel consumption. In addition, the losses could also be extended to aquaculture systems where fouling can erode and degrade equipment, and can also cause mass mortalities in farming plants [98,99]. Nowadays, there is the urge to implement the knowledge and study of new compounds that can replace the obsolete biocides, which were in use for a long period of time and are now banned from the market (e.g., Tributyltin and derivatives) but are still used for navy vessels [98,100]. Marine organisms such as sponges (or, indirectly, their symbionts) naturally produce antifouling compounds, which are useful to avoid larvae from marine organisms and various bacterial strains from attaching to the surface of their bodies, eventually blocking their pores and preventing filtering activity and so leading the animals to starvation [98,100].

Diatoms are among the organisms involved in microfouling. A study conducted by Tsoukatou et al. [101] demonstrated the antifouling activity of extracts of sponges belonging to the genus *Ircinia* on three diatom species: *Amphora coffeaformis* (AC2078), *Phaeodactylum tricornutum* (DIA12) and *Cylindrotheca closterium* (DIA6). The ability to inhibit the development of diatoms was evaluated by the addition of sponge extracts in concentrations of 30 µg/mL to a flask in which the diatoms were being cultured. Interesting results were obtained, showing that an aqueous extract of *Ircinia variabilis* was active on all three diatom species (inhibition rates varied between 1–30%), while its ethanol extract was more effective against the first two species. The ethanolic extract of another sponge belonging to the genus *Ircinia* (*I. spinosula* Schmidt, 1862) was tested on the same diatom species and demonstrated biological activity against *A. coffeaformis* (inhibition between 31–59%) and *P. tricornutum* and *C. closterium* (between 1–30%) [101]. Other monocellular organisms capable of inducing fouling are bacteria such as *Vibrio carchariae*, which may be responsible for the death of a large number of marine fish and invertebrates in aquaculture systems, leading to huge eco-

nomic losses [102,103]. The MIC values of 1 μg/mL were recorded when testing tryptamine (see Figure 1) extracted from the sponge *Fascaplysinopsis reticulata* (Hentschel, 1912) on these bacteria [99]. In the same study, two novel compounds, (6-bromo-8,1′-dihydro-isoplysin A and 5,6-dibromo-8,1′-dihydro-isoplysin A), were extracted, characterized and tested against *Vibrio natrigens*, which is one of the major biofilm producers, able to corrode artificial surfaces when immersed. These two products showed encouraging activity with the MIC values of 0.01 and 1.00 μg/mL, respectively [99]. Besides *Vibrio*, other bacterial strains are involved in the fouling processes, such as *Bacillus cereus*, *Bacillus pumilus*, *Bacillus megaterium*, *Pseudoalteromonas haloplanktis*, *Pseudomonas chlororaphis*, *Pseudomonas putida* and *Pseudomonas aeruginosa*. For this reason, Mol et al. [104] investigated the effectiveness of aqueous and ethyl acetate extracts of the marine sponge *Haliclona exigua* (Kirkpatrick, 1900) on these bacterial strains (high activity at concentrations of 100 μg/disc), using penicillin-G and streptomycin as positive controls. Macroalgae are also included in the macrofouling organisms, but to date not much is known about the potentiality of using sponge extracts against macroalgae fouling. Tsoukatou et al. [101] pointed out that dichloromethane extracts from *I. oros* (Schimdt, 1864) and *I. spinosula* are the best inhibitors of the adherence of macroalgae (*Enteromorpha intestinalis*, *Ulva lactuca* and *Sargassum muticum*) to natural substrates compared with positive control TBTO (bis tributyltin oxide).

Among the organisms that very frequently constitute macrofouling are barnacles, covering the ship hulls and their cooling system and leading to supplementary economic expenses in addition to those that are normally required for boat upkeep [98]. Marine sponges such as *Lissodendoryx isodictyalis* (Carter, 1882) (also called garlic sponge for its characteristic garlicky odour) do not present any fouling organisms on their surface. This remarkable absence has been also observed in other sponges [104,105], and this led to the idea that they are able to produce useful compounds that prevent fouling. Specimens of *L. isodictyalis* were collected from the Core Sound near Straits, North Carolina, at depths less than 1 meter below the low tide, and their ethyl acetate extracts were tested for settlement inhibition against the barnacle *Balanus amphitrite*. The effective concentration (EC_{50}) was 100 μg/mL [105]. *L. isodictyalis* extracts were not the only ones effective against this *Balanus* species. In fact, kalihinenes X, Y and Z (diterpenes) extracted from the marine sponge Acanthella cavernosa (Dendy, 1922), collected on Yakushima Island, were also active with an EC_{50} of 0.49, 0.45 and 1.1 μg/mL, respectively [106]. In addition, from the marine sponge *Neopetrosia chaliniformis* (Thiele, 1899) (ex *Haliclona exigua*), collected in the Gulf of Mannar (India), the ethyl acetate and aqueous extracts inhibited the settlement of the B. amphitrite larvae with the EC_{50} of 6.55 μg/mL and 6.57 μg/mL, respectively [104]. Similarly, extracts of sponges belonging to *Callyspongia* spp. and Callyspongia (*Cladochalina*) plicifera (Lamarck, 1814), collected in Hong Kong and the Bahamas, respectively, showed not only activity against *B. amphitrite*, but also against the polychaete *Hydroides elegans* [107], which is another frequent fouler of boats' hulls. In an analogous way, several sponge compounds extracted have displayed interesting activity against *Balanus improvises*, another barnacle species affecting the man-made submersed surfaces, for example barretin and 8,9-dihydrobarretin, isolated from the marine sponge *Geodia barretti* (Bowerbank, 1858), collected in the Atlantic Ocean. These natural compounds were able to inhibit the larval settlement of barnacles at concentrations of 1.9 and 19 μM, respectively [100]. Similar inhibition of barnacle larval settlement was exhibited by already known compounds such as bastadins 3, 4, and 9, extracted from the marine sponge *Ianthella basta* (Pallas, 1766), and aplysamine-2 from *Pseudoceratina purpurea* (Carter, 1880) (concentrations between 1 and 10 μM) and new compounds as Bastadin-16, hemibastadin-1 from *I. basta* and psammaplin A from *Aplysinella rhax* (de Laubenfels, 1954) inhibited larval settlement at doses of 10 μM [98].

Mussels are also among the most common macrofoulers which can be found attached to boats' chains and buoys. Some examples represented by mussels, such as *Perna perna* and the acetone/dichloromethane extracts of several marine sponges collected in Brazil, including *Tethya rubra* (Samaai & Gibbons, 2005), *Tethya maza* (Selenka, 1878), *Hymeniacidon heliophila* (Wilson, 1911) and *Petromica citrina*, proved to be useful in inhibiting the

power of byssus attachment with statistically significant results [108]. Furthermore, a marine sponge belonging to the genus *Haliclona*, collected in Palau, was an effective repellent against the blue mussel *Mytilus edulis and Mytilus galloprovincialis*. Specifically, this activity was attributed to two hexapeptides extracted from these sponges [109]. The compounds isolated from Demospongiae with antifouling activity examined in this section are summarized in Table 3.

Table 3. Source, extract/compound, pathogens tested and corresponding references are reported.

Source	Extract/Compound	Pathogens Tested	Reference
I. variabilis, *I. spinosula* and *I. oros*	Aqueous, ethanol and dichloromethane extract	*A. coffeaformis*, *P. tricornutum*, *C. Closterium*, *E. intestinalis*, *U. lactuca* and *S. muticum*	[101]
F. reticulata	Tryptamine and 6-bromo-8,1′-dihydro-isoplysin A and 5,6-dibromo-8,1′-dihydro-isoplysin A	*V.carchariae* and *V. natrigens*	[99]
N. chaliniformis	Ethyl acetate and aqueous extracts	*B. cereus*, *B. pumilus*, *B. megaterium*, *P. haloplanktis*, *P. chlororaphis*, *P. putida*, *P. aeruginosa* and *B. amphitrite*	[104]
L. isodictyalis	Ethyl acetate extract	*B. amphitrite*	[105]
A. cavernosa	Kalihinenes X, Y and Z	*B. amphitrite*	[106]
Callyspongia spp. and *C. plicifera*	Dichloromethane extract	*B. amphitrite* and *H. elegans*	[107]
G. barretti	Barretin and 8,9-dihydrobarretin	*B. improvisus*	[100]
I. basta, *P. purpurea* and *A. rhax*	Bastadins 3, 4, 9, bastadin-16, hemibastadin-1, aplysamine-2, psammaplin A	*B. improvisus*	[98]
T. rubra, *T. maza*, *H. heliophile and P. citrina*	Acetone/dichloromethane extract	*P. perna*	[108]
Haliclona sp.	Two hexapeptides	*M. edulis galloprovincialis*	[109]

2.4. Other Miscellaneous Activities

Due to the widespread emergence and the resistance of human pathogens to available drugs, there is a need to detect and develop new compounds. Malaria is one of the most infectious diseases in the world that frequently causes death in children and pregnant women. Rough estimates indicate 209 million cases in 2019 alone [110]. The rich and diversified marine environment has provided us with many compounds useful for biotechnological applications and for this reason it is important to once again search this enormous reservoir for antimalarial compounds and more. In fact, a few studies have been carried out on marine sponges to assess their antimalarial, antileishmanial and antitrypanosomal activities. For instance, several compounds were isolated from the marine sponge *Plakortis simplex* (Schulze, 1880) and were then tested for their antimalarial activity against two cloroquine-resistant (CQ-R) and chloroquine-sensitive (CQ-S) strains of *Plasmodium falciparum*. Besides an unknown compound, these compounds were named plakortin and plakortide Q; these three compounds exhibited consistent antimalarial activity against both strains, even though they were less efficient against the CQ-R strain [111]. In a similar study, monoamphilectine A, a diterpenoid β-lactam alkaloid, was purified from the marine sponge *Hymeniacidon* sp., sampled in Puerto Rico. It showed potent antimalarial activity with an IC_{50} value of 0.60 μM [78]. From the marine Demospongiae *Monachora unguiculata* (Dendy, 1922), collected in Madagascar, two new compounds named as ptilomycalin F and fromiamycalin were isolated, exhibiting interesting activity against *P. falciparum* with the IC_{50} values of 0.23 and 0.24 μM, respectively [112]. In a further study,

a new compound named as 8-oxo-tryptamine (see Figure 1) and a mixture of two already known compounds (*E*)-6-bromo-20-demethyl-30-*N*-methylaplysinopsin and (*Z*)-6-bromo-20-demethyl-30-*N*-methylaplysinopsin, extracted from the marine sponge *F. reticulata*, exhibited antiplasmodial activity against *P. falciparum* (IC_{50} values 8.8 and 8.0 µg/mL), using artemisinin as a positive control with an IC_{50} of 0.006 ± 0.002 µg/mL. [99]. Moreover, unidentified marine bacteria were isolated from the marine sponge *Hyattella intestinalis* (Lamarck, 1814), collected in Thondi, and tested for their antiplasmodial activity. In particular, two ethyl acetate extracts of bacterial colonies named THB20 and THB34 by Inbaneson and Rayikumar [113] displayed significant antimalarial activity with the IC_{50} values of 41.88 and 42.36 µg/mL, respectively, compared with positive control chloroquine (IC_{50} of 19.59 µg/mL). A glycoprotein, named pachymatismin, isolated from the sponge *Pachymatisma johnstonii* (Bowerbank, 1842), collected along the French coast, showed cytotoxic activity with the IC_{50} of 1 µg/mL, inducing alterations in the cell shape, phospholipase A_2 activity and the invasion capacity of the parasite (*Leishmania brazieliensis* and *L. mexicana*) [114]. This is the first compound isolated from marine sponge with antileishmanial activity. From the marine sponge *Haliclona exigua* (Kirkpatrick, 1900), from Tamil Nadu coast of India, an alkaloide named araguspongin C was isolated, demonstrating strong activity against *L. donovani* with an IC_{50} of 8.2 µg/mL in vitro and 31.2 µg/mL in vivo [115]. In another study, the compound hyrtiodoline A, isolated from the marine sponge *Hyrtios* sp., sampled from the Red Sea, exhibited potent antitrypanosomal activity against *Trypanosoma brucei* with the IC_{50} value of 7.48 µM [116].

Fungal diseases represent another increasing worldwide danger to human health. However, only a few antifungal drugs are currently available for the treatment of life-threatening fungal infections [117]. Candidosis is among the most common fungal infection in humans (accounting an estimated 40 million infections per year) affecting human mucosae. Due to its wide diffusion, this infection represents a problem in immunocompromised patients [118]. For this reason, finding novel compounds able to eliminate this pathogen and cure its infections is of fundamental importance. Sponge extracts can manifest specific activity against several strains of *Candida*. This is the case for the ethanol extracts from the sponge *A. oroides*, which was effective against *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Candida glabrata Candida tropicalis* and *Candida dubliniensis* [79]. Similarly, Untenospongin B, extracted from the marine sponge *Hippospongia communis* (Lamarck, 1814), collected off the Atlantic coast of Morocco, exhibited interesting antifungal activity against *Candida tropicalis* (R2 CIP 1275.81), *Candida albicans* (ATCC 10231), *Fusarium oxysporum* (CIP 108.74) and *Aspergillus niger* (CIP 1082.74), using amphotericin B as a positive control [119]. Wide antifungal activity was also found in several bacterial strains isolated from two marine sponges, *S. carnosus* and *Leucosolenia* sp., sampled at a depth of 15 meters, off Lough Hyne, Co. Cork, in Ireland. All bacteria isolated were tested for their antifungal activity against *C. albicans* (Sc5314), *C. glabrata* (CBS138), *Aspergillus fumigatus* (Af293) and *Kluyveromyces marxianus* (CB86556). Most activity stemmed from the bacteria belonging to the genera *Pseudoalteromonas*, *Bacillus*, *Vibrio* and *Staphylococcus*, isolated from *Leucosolenia* sp. (15% of the total isolates), while only 4% of the bacterial strains from *S. carnosus* were active [93]. Another compound extracted from the symbiont *Bacillus sp.* (2011SOCCUF3), isolated from *Spongia officinalis* (Linnaeus, 1759), showed a strong antifungal activity. The vacuum liquid chromatography (VLC) fractions obtained from this bacterium showed specific activity against *C. albicans* (MIC = 108 µg/mL) [88]. Similarly, against *C. albicans*, a methanol extract from the marine Demospongia *Neopetrosia exigua* (Kirkpatrick, 1900) showed encouraging activity [77], as well as for the "peptide C" extracted from the deep sea marine sponge *S. fortis* (MIC = 18.07 µM [81]. A study conducted by Mohammed et al. [75] planned to test compounds of Plakortide N and Plakortide F, extracted from the sponge *Plakortis angulospigulatus* (Carter, 1879), sampled from Jamaica, on various *Candida* species (*C. glabrata*, *C. albicans*, *C. krusei*), showing IC_{50} values ranging from 0.25 to 3.0 µg/mL. Similarly, two different compounds extracted from a sponge belonging to the genus *Plakortis*, sampled from the Okinawan Sea, were named Menzamenone M and Menzamenone N.

Both of them showed interesting activity against *C. albicans* [74]. Similar activity against *C. albicans* was found in 86.6% of the aqueous extracts of the bacteria belonging to Proteobacteria, Actinobacteria and Firmicutes phyla, isolated from the marine sponge *Erylus deficiens* (Topsent, 1927), using amphotericin B (0.19, 0.39, 0.78 and 1.56 µg/mL) and rifampicin (62500, 125000, 250000 and 500000 µg /mL) as positive controls [71].

In cases where interesting activity is not detected this does not mean that the sponge species will never develop such activity, as the production of compounds could be linked to the conditions in which the sponges are actually growing. This can be seen in a case reported by Kibungu et al. [120], where the crude extract obtained from the marine sponge *Psammaplysilla* sp., sampled from Phillips Reef, South Africa, showed seasonal variation in antifungal activity. In fact, only ethyl acetate extracts performed from sponge samples collected in spring exhibited bioactivity against *C. albicans* compared to the positive controls fluconazole, itraconazole and voriconazole.

Sponges can also help findings in new biotechnologies for regenerative medicine. Re-generative medicine currently needs innovative biomaterials with low immunogenicity and toxicity and good mechanical properties. However, skin and bones from bovine or porcine wastes continues to be the primary source of proteins for regenerative medicine. Recently, the scientific community has shown a strong interest in the marine collagen isolated from fish and various marine invertebrates, including sponges, used in tissue engineering [121,122]. Collagen extracts from *Chondrosia reniformis* (Nardo, 1847) do not cause toxicity in mammalian cells, but positive effects on the proliferation of L929 fibroblasts, HaCat keratinocytes and RAW 264.7 macrophages. Moreover, the fractions M4 and M5 from this sponge revealed promising wound-healing properties, facilitating either cell migration or proliferation at the site of the damage to epidermal and dermal cells. So, these data suggested that extracts could be exploited for cosmetic or regenerative medicine purposes, facilitating cell migration or proliferation at the site of the wounded epidermal and dermal cells [121]. Pozzolini et al. [122] isolated collagen filaments from the marine sponges *Ircinia oros* and *Sarcotragus foetidus* (Schimdt, 1862), collected in the Ligurian Sea, and tested them on HaCat keratinocytes and L929 fibroblasts. Additionally, in this study, the extracts were effective for wound-healing when compared with the positive controls hydrogen peroxide and quartz. The products and biological activities described in this section are summarized in Table 4.

Table 4. Source, extract/compound, activity and corresponding reference are reported.

Source	Sponge Host	Extract/Compound	Activity	Reference
P. simplex		Plakortin and Plakortide Q	Antiplasmodial	[111]
Hymeniacidon sp.		Monoamphilectine A	Antiplasmodial	[78]
M. unguiculata		Ptilomycalin F and Fromiamycalin	Antiplasmodial	[112]
F.reticulata		8-oxo-tryptamine and (*E*)-6-bromo-20-demethyl-30-*N*-methylaplysinopsin and (*Z*)-6-bromo-20-demethyl-30-*N*-methylaplysinopsin	Antiplasmodial	[99]
H. intestinalis	Bacterial colonies THB20 and THB34	Ethyl acetate extract	Antiplasmodial	[113]
P. johnstonii		Pachymatismin	Antileishmanial	[114]
H. exigua		Araguspongin C	Antileishmanial	[115]
Hyrtios sp.		Hyrtiodoline A	Antitrypanosomial	[116]
A. oroides		Ethanol extract	Antifungal	[79]

Table 4. *Cont.*

Source	Sponge Host	Extract/Compound	Activity	Reference
H. communis		Untenospongin B	Antifungal	[119]
S. carnosus and *Leucosolenia* sp.	Psedoalteromonas, Bacillus, Vibrio and Staphylococcus phyla	Isolated of bacteria	Antifungal	[93]
S. officinalis	*Bacillus* 2011SOCCUF3	Methanol extract	Antifungal	[88]
N. exigua		Methanolic extract	Antifungal	[77]
S. fortis		Peptide C	Antifungal	[81]
P. angulospigulatus		Plakortide N and Plakortide F	Antifungal	[75]
Plakortis sp.		Menzamenone M and Menzamenone N	Antifungal	[74]
E. deficiens	Proteobacteria, Actinobacteria and Firmicutes phyla	Aqueous extract	Antifungal	[71]
Psammaplysilla sp. 1		Ethyl acetate extract	Antifungal	[120]
C. reniformis		Collagen extract	Wound-healing	[121]
I. oros and *S. foetidus*		Collagen filaments	Wound-healing	[122]

3. Conclusions

As amply demonstrated by reviewing the available data, marine Demospongiae represent a class of sponges with great biotechnological potential. This important role in drug discovery is mainly due to the diverse range of specialized metabolites, which are isolated from different environmental and geographic conditions. In particular, the majority of natural products from Demospongiae have been isolated since 2000, so the data reported is quite recent, with a large increase in the last 10 years. Terpenes and alkaloids are the major reported chemical classes among the natural products isolated from Demospongiae, even if most reported results concern activities of total extracts or fractions not yet chemically identified. Interestingly, 35% and 30% of the compounds have properties showing antimicrobial and cytotoxic activities, respectively, as reported in Figure 3.

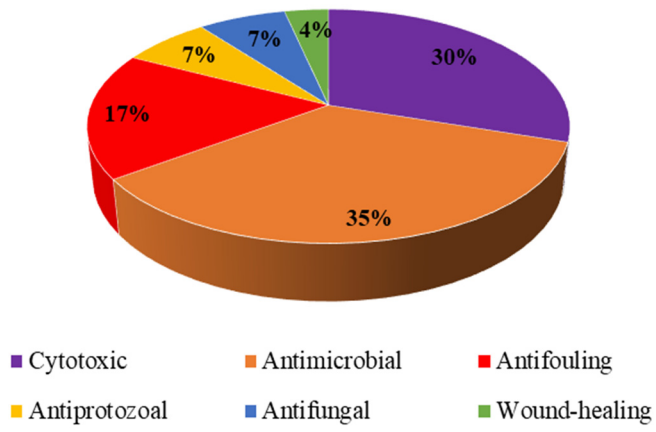

Figure 3. Different bioactive extracts/compounds isolated from Demospongiae.

Another major activity seen in this class (17%), concerns the antifouling properties of some specialized metabolites, while a very low percentages of extracts/fractions demonstrate antiplasmodial, wound-healing and antifungal activities. In conclusion, the increas-

ing number of bioactive extracts/fractions should push researchers towards additional investigation on sponges belonging to the class Demospongiae.

Author Contributions: Conceptualization, R.E., S.F., V.Z. and M.C.; performed bibliographic research, R.E. and S.F.; writing—original draft preparation, R.E. and S.F.; M.B. contributed to the writing of the paragraph on the description of the Demospongiae class; review and editing, V.Z. and M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374).

Acknowledgments: Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. Serena Federico was supported by the research grant "Antitumor Drugs and Vaccines from the Sea (ADViSE)", project (PG/2018/0494374).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bergmann, W.; Feeney, R.J. The isolation of a new thymine pentoside from Sponges. *J. Am. Chem. Soc.* **1950**, *72*, 2809–2810. [CrossRef]
2. Bergmann, W.; Feeney, R.J. Contributions to the study of marine products. XXXII. the nucleosides of sponges. I. *J. Org. Chem.* **1951**, *16*, 981–987. [CrossRef]
3. Carté, B.K. Potential of marine biomedical natural products research and medical applications. *Oxf. J.* **1996**, *46*, 271–286.
4. Burkholder, P.R.; Pfister, R.M.; Leitz, F.H. Production of a pyrrole antibiotic by a marine bacterium. *Appl. Microbiol.* **1966**, *14*, 649–653. [CrossRef] [PubMed]
5. Proksch, P.; Edrada, R.A.; Ebel, R. Drugs from the seas—Current status and microbiological implications. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 125–134. [CrossRef]
6. Weinheimer, A.J.; Spraggins, R.L. The occurrence of two new prostaglandin derivatives (15-epi-PGA2 and its acetate, methyl ester) in the Gorgonian *Plexaura Homomalla*. *Tetrahedron Lett.* **1969**, *10*, 5185–5188. [CrossRef]
7. Ireland, C.; Copp, B.; Foster, M.; McDonald, L.; Radisky, D.; Swersey, J. Biomedical potential of Marine natural products. In *Pharmaceutical and Bioactive Natural Products*; Springer: Boston, MA, USA, 1993; pp. 1–43.
8. Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. *J. Med. Chem.* **1994**, *37*, 1385–1401. [CrossRef]
9. Alonso, D.; Khalil, Z.; Satkunanthan, N.; Livett, B. Drugs from the Sea: Conotoxins as drug leads for neuropathic pain and other neurological conditions. *Mini-Rev. Med. Chem.* **2003**, *3*, 785–787. [CrossRef]
10. Twelves, C.; Cortes, J.; Vahdat, L.; Wanders, J.; Akerele, C.; Kaufman, P. Phase III trials of eribulin mesylate (E7389) in extensively pretreated patients with locally recurrent or metastatic breast cancer. *Clin. Breast Cancer* **2010**, *10*, 160–163. [CrossRef]
11. Munekata, P.E.S.; Pateiro, M.; Conte-Junior, C.A.; Domínguez, R.; Nawaz, A.; Walayat, N.; Fierro, E.M.; Lorenzo, J.M. Marine alkaloids: Compounds with in vivo activity and chemical synthesis. *Mar. Drugs* **2021**, *19*, 374. [CrossRef]
12. Hu, G.P.; Yuan, J.; Sun, L.; She, Z.G.; Wu, J.H.; Lan, X.J.; Zhu, X.; Lin, Y.C.; Chen, S.P. Statistical research on marine natural products based on data obtained between 1985 and 2008. *Mar. Drugs* **2011**, *9*, 514–525. [CrossRef] [PubMed]
13. Faulkner, D.J. Marine natural products. *Nat. Prod. Rep.* **2002**, *19*, 1–49. [CrossRef] [PubMed]
14. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2006**, *23*, 26–78. [CrossRef] [PubMed]
15. Faulkner, D. Marine natural products. *Nat. Prod. Rep.* **2000**, *17*, 7–55. [CrossRef]
16. Mehbub, M.F.; Lei, J.; Franco, C.; Zhang, W. Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Mar. Drugs* **2014**, *12*, 4539–4577. [CrossRef]
17. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2010**, *27*, 165–237. [CrossRef]
18. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2011**, *28*, 196–268. [CrossRef]
19. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2012**, *29*, 144–222. [CrossRef]
20. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2013**, *30*, 237–323. [CrossRef]
21. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2014**, *31*, 160–258. [CrossRef]

22. Lyu, C.; Chen, T.; Qiang, B.; Liu, N.; Wang, H.; Zhang, L.; Liu, Z. CMNPD: A comprehensive marine natural products database towards facilitating drug discovery from the ocean. *Nucleic Acids Res.* **2021**, *49*, D509–D515. [CrossRef] [PubMed]
23. Laport, M.; Santos, O.; Muricy, G. Marine sponges: Potential sources of new antimicrobial drugs. *Curr. Pharm. Biotechnol.* **2009**, *10*, 86–105. [CrossRef] [PubMed]
24. Sagar, S.; Kaur, M.; Minneman, K.P. Antiviral lead compounds from marine sponges. *Mar. Drugs* **2010**, *8*, 2619–2638. [CrossRef] [PubMed]
25. Nagle, D.; Zhou, Y.; Mora, F.; Mohammed, K.; Kim, Y. Mechanism targeted discovery of antitumor Marine natural products. *Curr. Med. Chem.* **2004**, *11*, 1725–1756. [CrossRef] [PubMed]
26. Gomes, N.G.M.; Dasari, R.; Chandra, S.; Kiss, R.; Kornienko, A. Marine invertebrate metabolites with anticancer activities: Solutions to the "supply problem". *Mar. Drugs* **2016**, *14*, 98. [CrossRef]
27. Morrow, C.; Cárdenas, P. Proposal for a revised classification of the Demospongiae (Porifera). *Front. Zool.* **2015**, *12*, 1–27. [CrossRef]
28. Borchiellini, C.; Manuel, M.; Alivon, E.; Boury-Esnault, N.; Vacelet, J.; Le Parco, Y. Sponge paraphyly and the origin of Metazoa. *J. Evol. Biol.* **2001**, *14*, 171–179. [CrossRef]
29. Hooper, J.N.A.; Van Soest, R.W.M. Systema Porifera. A guide to the classification of sponges. In *Systema Porifera*; Springer: Boston, MA, USA, 2002; pp. 1–7.
30. Beaulieu, S.E. Life on glass houses: Sponge stalk communities in the deep sea. *Mar. Biol.* **2001**, *138*, 803–817. [CrossRef]
31. Beaulieu, S.E. Colonization of habitat islands in the deep sea: Recruitment to glass sponge stalks. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **2001**, *48*, 1121–1137. [CrossRef]
32. Hill, M.; Hill, A.; Lopez, N.; Harriott, O. Sponge-specific bacterial symbionts in the Caribbean sponge, *Chondrilla nucula* (Demospongiae, Chondrosida). *Mar. Biol.* **2006**, *148*, 1221–1230. [CrossRef]
33. Taylor, M.W.; Radax, R.; Steger, D.; Wagner, M. Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 295–347. [CrossRef] [PubMed]
34. Kautsar, S.A.; Blin, K.; Shaw, S.; Navarro-Muñoz, J.C.; Terlouw, B.R.; Van Der Hooft, J.J.J.; Van Santen, J.A.; Tracanna, V.; Suarez Duran, H.G.; Pascal Andreu, V.; et al. MIBiG 2.0: A repository for biosynthetic gene clusters of known function. *Nucleic Acids Res.* **2020**, *48*, D454–D458. [CrossRef] [PubMed]
35. Medema, M.H.; de Rond, T.; Moore, B.S. Mining genomes to illuminate the specialized chemistry of life. *Nat. Rev. Genet.* **2021**, *22*, 553–571. [CrossRef] [PubMed]
36. Dewick, P. Secondary metabolism: The building blocks and construction mechanisms. In *Medicinal Natural Products: A Biosynthetic Approach*; John Wiley & Sons, Ltd.: Hobokon, NJ, USA, 2002; pp. 7–38.
37. Ghisalberti, E. Detection and isolation of bioactive natural products. In *Bioactive Natural Products: Detection, Isolation and Structural Detection*; CRS Press: London, UK, 2008; pp. 11–76.
38. Wang, G. Diversity and biotechnological potential of the sponge-associated microbial consortia. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 545–551. [CrossRef]
39. Albarano, L.; Esposito, R.; Ruocco, N.; Costantini, M. Genome mining as new challenge in natural products discovery. *Mar. Drugs* **2020**, *18*, 199. [CrossRef]
40. Nagai, H.; Kim, Y.H. Cancer prevention from the perspective of global cancer burden patterns. *J. Thorac. Dis.* **2017**, *9*, 448–451. [CrossRef]
41. Khan, S.; Al-Fadhli, A.A.; Tilvi, S. Discovery of cytotoxic natural products from Red Sea sponges: Structure and synthesis. *Eur. J. Med. Chem.* **2021**, *220*, 113491. [CrossRef]
42. Di, L.; Mannelli, C.; Esposito, F.P.; Sangiovanni, E.; Pagano, E.; Mannucci, C.; Polini, B.; Ghelardini, C.; Agli, M.D.; Izzo, A.A.; et al. Pharmacological activities of extracts and compounds isolated from mediterranean sponge sources. *Pharmaceuticals* **2021**, *14*, 1329.
43. Mioso, R.; Marante, F.J.T.; Bezerra, R.D.S.; Borges, F.V.P.; Santos, B.V.D.O.; De Laguna, I.H.B. Cytotoxic compounds derived from marine sponges. A review (2010–2012). *Molecules* **2017**, *22*, 8. [CrossRef]
44. Tsujii, S.; Rinehart, K.L.; Gunasekera, S.P.; Cross, S.S.; Lui, M.S.; Pomponi, S.A.; Cristina Diaz, M.; Kashman, Y. Topsentin, Bromotopsentin, and Dihydrodeoxybromotopsentin: Antiviral and antitumor bis(Indolyl)imidazoles from Caribbean deep-sea sponges of the Family Halichondriidae. Structural and synthetic studies. *J. Org. Chem.* **1988**, *53*, 5446–5453. [CrossRef]
45. De Rosa, S.; De Stefano, S.; Zavodnik, N. Cacospongionolide: A new antitumoral sesterterpene, from the marine sponge *Cacospongia Mollior*. *J. Org. Chem.* **1988**, *53*, 5020–5023. [CrossRef]
46. Cimino, G.; De Stefano, S.; Scognamiglio, G.; Sodano, G.; Trivellone, E. Sarains: A new class of alkaloids from the marine sponge *Reniera sarai*. *Bull. Soc. Chim. Belg.* **1986**, *95*, 783–800. [CrossRef]
47. Caprioli, V.; Cimino, G.; De Giulio, A.; Madaio, A.; Scognamiglio, G.; Trivellone, E. Selected biological activities of saraines. *Comp. Biochem. Physiol.—Part B Biochem.* **1992**, *103*, 293–296. [CrossRef]
48. Rady, H.M.; Hassan, A.Z.; Salem, S.M.; Mohamed, T.K.; Esmaiel, N.N.; Ez-El-Arab, M.A.; Ibrahim, M.A.; Fouda, F.K. Induction of apoptosis and cell cycle arrest by *Negombata magnifica* sponge in hepatocellular carcinoma. *Med. Chem. Res.* **2016**, *25*, 456–465. [CrossRef]
49. Ka, E.; El-naggar, H.A.; Ibrahim, H.A.H.; Mansour, A.B.; Fekry, M.A.S. Biological activities of some marine sponge extracts from Aqaba Gulf, Red Sea, Egypt. *Int. J. Fish. Aquat. Stud.* **2017**, *5*, 652–659.

50. Tinto, W.F.; Lough, A.J.; McLean, S.; Reynolds, W.F.; Yu, M.; Chan, W.R. Geodiamolides H and I, further cyclodepsipeptides from the marine sponge *Geodia* sp. *Tetrahedron* **1998**, *54*, 4451–4458. [CrossRef]
51. Costantini, S.; Romano, G.; Rusolo, F.; Capone, F.; Guerriero, E.; Colonna, G.; Ianora, A.; Ciliberto, G.; Costantini, M. Anti-inflammatory effects of a methanol extract from the marine sponge *Geodia cydonium* on the human breast cancer MCF-7 cell line. *Mediat. Inflamm.* **2015**, *2015*, 204975. [CrossRef]
52. Costantini, S.; Guerriero, E.; Teta, R.; Capone, F.; Caso, A.; Sorice, A.; Romano, G.; Ianora, A.; Ruocco, N.; Budillon, A.; et al. Evaluating the effects of an organic extract from the mediterranean sponge *Geodia cydonium* on human breast cancer cell lines. *Int. J. Mol. Sci.* **2017**, *18*, 2112. [CrossRef]
53. Hawas, U.W.; Abou El-Kassem, L.T.; Abdelfattah, M.S.; Elmallah, M.I.Y.; Eid, M.A.G.; Monier, M.; Marimuthu, N. Cytotoxic activity of alkyl benzoate and fatty acids from the red sea sponge *Hyrtios erectus*. *Nat. Prod. Res.* **2018**, *32*, 1369–1374. [CrossRef]
54. Rifai, S.; Fassouane, A.; Pinho, P.M.; Kijjoa, A.; Nazareth, N.; Nascimento, M.S.J.; Herz, W. Cytotoxicity and inhibition of lymphocyte proliferation of fasciculatin, a linear furanosesterterpene isolated from *Ircinia variabilis* collected from the Atlantic Coast of Morocco. *Mar. Drugs* **2005**, *3*, 15–21. [CrossRef]
55. Ferretti, C.; Marengo, B.; De Ciucis, C.; Nitti, M.; Pronzato, M.A.; Marinari, U.M.; Pronzato, R.; Manconi, R.; Domenicotti, C. Effects of *Agelas oroides* and *Petrosia ficiformis* crude extracts on human neuroblastoma cell survival. *Int. J. Oncol.* **2007**, *30*, 161–169. [CrossRef] [PubMed]
56. Ferretti, C.; Vacca, S.; De Ciucis, C.; Marengo, B.; Duckworth, A.R.; Manconi, R.; Pronzato, R.; Domenicotti, C. Growth dynamics and bioactivity variation of the Mediterranean demosponges *Agelas oroides* (Agelasida, Agelasidae) and *Petrosia ficiformis* (Haplosclerida, Petrosiidae). *Mar. Ecol.* **2009**, *30*, 327–336. [CrossRef]
57. Di Bari, G.; Gentile, E.; Latronico, T.; Corriero, G.; Fasano, A.; Marzano, C.N.; Liuzzi, G.M. Comparative analysis of protein profiles of aqueous extracts from marine sponges and assessment of cytotoxicity on different mammalian cell types. *Environ. Toxicol. Pharmacol.* **2014**, *38*, 1007–1015. [CrossRef] [PubMed]
58. Ahn, J.H.; Woo, J.H.; Rho, J.R.; Choi, J.H. Anticancer activity of gukulenin a isolated from the marine sponge *Phorbas gukhulensis* in vitro and in vivo. *Mar. Drugs* **2019**, *17*, 126. [CrossRef]
59. Matsumoto, R.; Fujii, Y.; Kawsar, S.M.A.; Kanaly, R.A.; Yasumitsu, H.; Koide, Y.; Hasan, I.; Iwahara, C.; Ogawa, Y.; Im, C.H.; et al. Cytotoxicity and glycan-binding properties of an 18 kDa lectin isolated from the marine sponge *Halichondria okadai*. *Toxins* **2012**, *4*, 323–338. [CrossRef]
60. Agrawal, S.; Adholeya, A.; Deshmukh, S.K. The pharmacological potential of non-ribosomal peptides from marine sponge and tunicates. *Front. Pharmacol.* **2016**, *7*, 333. [CrossRef]
61. Zhang, H.; Zhao, Z.; Wang, H. Cytotoxic natural products from marine sponge-derived microorganisms. *Mar. Drugs* **2017**, *15*, 68. [CrossRef]
62. Pagliara, P.; Caroppo, C. Cytotoxic and antimitotic activities in aqueous extracts of eight cyanobacterial strains isolated from the marine sponge *Petrosia ficiformis*. *Toxicon* **2011**, *57*, 889–896. [CrossRef]
63. Pagliara, P.; Barca, A.; Verri, T.; Caroppo, C. The marine sponge *Petrosia ficiformis* harbors different cyanobacteria strains with potential biotechnological application. *J. Mar. Sci. Eng.* **2020**, *8*, 638. [CrossRef]
64. Cheng, C.; Othman, E.M.; Stopper, H.; Edrada-Ebel, R.A.; Hentschel, U.; Abdelmohsen, U.R. Isolation of petrocidin a, a new cytotoxic cyclic dipeptide from the marine sponge-derived bacterium *Streptomyces* sp. SBT348. *Mar. Drugs* **2017**, *15*, 383. [CrossRef]
65. Cheng, C.; Othman, E.M.; Fekete, A.; Krischke, M.; Stopper, H.; Edrada-, R.; Mueller, M.J.; Hentschel, U.; Abdelmohsen, U.R.; Cheng, C.; et al. Strepoxazine A, a new cyctotoxic phenoxazin from the marine sponge- derived bacterium *Streptomyces* sp. SBT345. *Tetrahedron Lett.* **2016**, *57*, 4196–4199. [CrossRef]
66. Handayani, D.; Rasyid, W.; Rustini, Z.E.; Hertiani, T. Cytotoxic activity screening of fungal extracts derived from the west sumatran marine sponge *Haliclona fascigera* to several human cell lines: Hela, WiDr, T47D and Vero. *J. Appl. Pharm. Sci.* **2018**, *8*, 055–058. [CrossRef]
67. Skropeta, D.; Pastro, N.; Zivanovic, A. Kinase inhibitors from marine sponges. *Mar. Drugs* **2011**, *9*, 2131–2154. [CrossRef] [PubMed]
68. Alvi, K.A.; Jaspars, M.; Crews, P.; Strulovici, B.; Oto, E. Penazetidine A, an alkaloid inhibitor of protein kinase C. *Bioorgan. Med. Chem. Lett.* **1994**, *4*, 2447–2450. [CrossRef]
69. Patil, A.D.; Freyer, A.J.; Killmer, L.; Hofmann, G.; Johnson, R.K. Z-axinohydantoin and debromo-z-axinohydantoin from the sponge *Stylotella aurantium*: Inhibitors of protein kinase C. *Nat. Prod. Lett.* **1997**, *9*, 201–207. [CrossRef]
70. Piel, J. Metabolites from symbiotic bacteria. *Nat. Prod. Rep.* **2009**, *26*, 338–362. [CrossRef]
71. Graça, A.P.; Viana, F.; Bondoso, J.; Correia, M.I.; Gomes, L.; Humanes, M.; Reis, A.; Xavier, J.R.; Gaspar, H.; Lage, O.M. The antimicrobial activity of heterotrophic bacteria isolated from the marine sponge *Erylus deficiens* (Astrophorida, Geodiidae). *Front. Microbiol.* **2015**, *6*, 389. [CrossRef]
72. Krylova, D.D.; Aleshina, G.M.; Kokryakov, V.N.; Ereskovsky, A.V. Antimicrobial properties of mesohylar granular cells of *Halisarca Dujardini* Johnston, 1842 (Demospongiae, Halisarcida). *Boll. Mus. Ist. Biol. Univ. Genova* **2003**, *68*, 399–404.
73. Morales, T.; Cubero, J.; Lanz, Z.; Gomez-Guinan, Y.; Segnini-Bravo, M. Actividad antimicrobiana de extractos organicos aislados de Aplysina fistularis (Demospongiae: Aplysinidae). *Rev. Biol. Trop.* **2000**, *48*, 199–206.

74. Kubota, T.; Ishiguro, Y.; Takahashi-Nakaguchi, A.; Fromont, J.; Gonoi, T.; Kobayashi, J. Manzamenones L-N, new dimeric fatty-acid derivatives from an Okinawan marine sponge *Plakortis* sp. *Bioorganic Med. Chem. Lett.* **2013**, *23*, 244–247. [CrossRef]
75. Mohammed, R.; Peng, J.; Kelly, M.; Yousaf, M.; Winn, E.; Odde, S.; Bie, Z.; Xie, A.; Doerksen, R.J.; Hamann, M.T. Polyketide-peroxides from a species of Jamaican plakortis (Porifera: Demospongiae). *Aust. J. Chem.* **2010**, *63*, 877–885. [CrossRef]
76. Parama Cita, Y.; Kamal Muzaki, F.; Radjasa, O.K.; Sudarmono, P. Screening of antimicrobial activity of sponges extract from Pasir Putih, East Java (Indonesia). *J. Mar. Sci. Res. Dev.* **2017**, *7*, 2. [CrossRef]
77. Chander, M.; Vijayachari, P. Antimicrobial and antioxidant potentials of marine sponges of South Andaman, India. *Bangladesh J. Pharmacol.* **2018**, *13*, 13–15. [CrossRef]
78. Avilés, E.; Rodríguez, A. Monamphilectine a, a potent antimalarial β-lactam from a marine sponge *Hymeniacidon* sp: Isolation, structure, semisythesis, and bioactivity. *Org. Lett.* **2010**, *12*, 5290–5293. [CrossRef]
79. Touati, I.; Chaieb, K.; Bakhrouf, A.; Gaddour, K. Screening of antimicrobial activity of marine sponge extracts collected from Tunisian coast. *J. Mycol. Med.* **2007**, *17*, 183–187. [CrossRef]
80. Sun, S.; Canning, C.B.; Bhargava, K.; Sun, X.; Zhu, W.; Zhou, N.; Zhang, Y.; Zhou, K. Polybrominated diphenyl ethers with potent and broad spectrum antimicrobial activity from the marine sponge *Dysidea*. *Bioorgan. Med. Chem. Lett.* **2015**, *25*, 2181–2183. [CrossRef]
81. Kosgahakumbura, K.N.M.L.; Hettiarachchi, C.; Jayasinghe, R.; Cárdenas, P.; Gunasekera, S. Isolation of cysteine-rich peptides from the deep-sea marine sponge *Stryphnus fortis* and determination of its antimicrobial effect. In Proceedings of the International conference on Frontiers in Chemical Technology, Colombo, Sri Lanka, 20–22 July 2020; p. 55.
82. Giraldes, B.W.; Goodwin, C.; Al-Fardi, N.A.A.; Engmann, A.; Leitão, A.; Ahmed, A.A.; Ahmed, K.O.; Abdulkader, H.A.; Al-Korbi, H.A.; Al Easa, H.S.S.; et al. Two new sponge species (Demospongiae: Chalinidae and Suberitidae) isolated from hyperarid mangroves of Qatar with notes on their potential antibacterial bioactivity. *PLoS ONE* **2020**, *15*, e0232205. [CrossRef]
83. Guimarães, T.D.R.; Quiroz, C.G.; Rigotto, C.; De Oliveira, S.Q.; De Almeida, M.T.R.; Bianco, É.M.; Moritz, M.I.G.; Carraro, J.L.; Palermo, J.A.; Cabrera, G.; et al. Anti HSV-1 activity of halistanol sulfate and halistanol sulfate C isolated from Brazilian marine sponge *Petromica citrina* (Demospongiae). *Mar. Drugs* **2013**, *11*, 4176–4192. [CrossRef]
84. Jayatilake, G.S.; Thornton, M.P.; Leonard, A.C.; Grimwade, J.E.; Baker, B.J. Metabolites from an antarctic sponge-associated bacterium, *Pseudomonas aeruginosa*. *J. Nat. Prod.* **1996**, *59*, 293–296. [CrossRef]
85. Mitova, M.; Tommonaro, G.; De Rosa, S. A novel cyclopeptide from a bacterium associated with the marine sponge *Ircinia muscarum*. *J. Biosci.* **2003**, *58*, 740–745. [CrossRef]
86. Suzumura, K.; Yoko, T.; Funatsu, M.; Nagai, K.; Tanaka, K.; Zhang, H.; Suzuki, K. YM-266183 and YM-266184, novel thiopeptide antibiotics produced by *Bacillus cereus* isolated from a marine sponge. *J. Antibiot.* **2003**, *56*, 129–134. [CrossRef] [PubMed]
87. Zheng, L.; Chen, H.; Han, X.; Lin, W.; Yan, X. Antimicrobial screening and active compound isolation from marine bacterium NJ6-3-1 associated with the sponge *Hymeniacidon perleve*. *World J. Microbiol. Biotechnol.* **2005**, *21*, 201–206. [CrossRef]
88. Odekina, P.A.; Agbo, M.O.; Omeje, E.O. Antimicrobial and antioxidant activities of novel marine bacteria (*Bacillus* 2011SOCCUF3) Isolated from marine Ssponge (*Spongia officinalis*). *Pharm. Sci.* **2020**, *26*, 82–87. [CrossRef]
89. Phadale, R.; Kumar, M.S. Characterization of an antimicrobial and antioxidant compound from a marine bacterium Gsa10 associated with the sponge *Halichondria glabrata*. *J. Microbiol. Biotechnol. Food Sci.* **2018**, *7*, 651–658. [CrossRef]
90. Mohan, G.; Thangappanpillai, A.K.; Ramasamy, B. Antimicrobial activities of secondary metabolites and phylogenetic study of sponge endosymbiotic bacteria, *Bacillus* sp. at Agatti Island, Lakshadweep Archipelago. *Biotechnol. Rep.* **2016**, *11*, 44–52. [CrossRef]
91. Chelossi, E.; Milanese, M.; Milano, A.; Pronzato, R.; Riccardi, G. Characterisation and antimicrobial activity of epibiotic bacteria from *Petrosia ficiformis* (Porifera, Demospongiae). *J. Exp. Mar. Biol. Ecol.* **2004**, *309*, 21–33. [CrossRef]
92. Koch, M.J.; Hesketh-Best, P.J.; Smerdon, G.; Warburton, P.J.; Howell, K.; Upton, M. Impact of growth media and pressure on the diversity and antimicrobial activity of isolates from two species of hexactinellid sponge. *Microbiology* **2021**, *167*, 001123. [CrossRef]
93. Flemer, B.; Kennedy, J.; Margassery, L.M.; Morrissey, J.P.; O'Gara, F.; Dobson, A.D.W. Diversity and antimicrobial activities of microbes from two Irish marine sponges, *Suberites carnosus* and *Leucosolenia* sp. *J. Appl. Microbiol.* **2012**, *112*, 289–301. [CrossRef]
94. O' Halloran, J.A.; Barbosa, T.M.; Morrissey, J.P.; Kennedy, J.; O' Gara, F.; Dobson, A.D.W. Diversity and antimicrobial activity of *Pseudovibrio* spp. from Irish marine sponges. *J. Appl. Microbiol.* **2011**, *110*, 1495–1508. [CrossRef]
95. Krishnan, P. Antimicrobial activity of *Ircinia* sp.; a marine sponge and its associated bacteria from Andaman Coast. *Adv. Anim. Vet. Sci.* **2014**, *2*, 37–41. [CrossRef]
96. Pejin, B.; Talevski, A.; Ciric, A.; Glamoclija, J.; Nikolic, M.; Talevski, T.; Sokovic, M. In vitro evaluation of antimicrobial activity of the freshwater sponge *Ochridaspongia rotunda* (Arndt, 1937). *Nat. Prod. Res.* **2014**, *28*, 1489–1494. [CrossRef] [PubMed]
97. Hole, W. *Marine Fouling and its Prevention*; US Naval Institute: Annapolis, MD, USA, 1952.
98. Ortlepp, S.; Sjögren, M.; Dahlström, M.; Weber, H.; Ebel, R.; Edrada, R.A.; Thoms, C.; Schupp, P.; Bohlin, L.; Proksch, P. Antifouling activity of bromotyrosine-derived sponge metabolites and synthetic analogues. *Mar. Biotechnol.* **2007**, *9*, 776–785. [CrossRef] [PubMed]
99. Campos, P.E.; Pichon, E.; Moriou, C.; Clerc, P.; Trépos, R.; Frederich, M.; De Voogd, N.; Hellio, C.; Gauvin-Bialecki, A.; Al-Mourabit, A. New antimalarial and antimicrobial tryptamine derivatives from the marine sponge *Fascaplysinopsis reticulata*. *Mar. Drugs* **2019**, *17*, 167. [CrossRef]

100. Sjögren, M.; Goransson, U.; Johnson, A.; Dahlstrom, M.; Anderson, R.; Bergman, J.; Jonsson, P.; Bohlin, L. Antifouling activity of a dibrominated cyclopeptide from the marine sponge *Geodia barretti*. *J. Nat. Prod.* **2004**, *67*, 368–372. [CrossRef] [PubMed]
101. Tsoukatou, M.; Hellio, C.; Vagias, C.; Harvala, C.; Roussis, V. Chemical defense and antifouling activity of three Mediterranean sponges of the genus Ircinia. *Z. Fur Naturforsch.-Sect. C J. Biosci.* **2002**, *57*, 161–171. [CrossRef]
102. Lee, K.K.; Liu, P.C.; Chuang, W.H. Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. *Mar. Biotechnol.* **2002**, *4*, 267–277. [CrossRef]
103. Nicolas, J.; Basuyaux, O.; Mazurie, J.; Thébault, A. *Vibrio carchariae*, a pathogen of the abalone *Haliotis tuberculata*. *Dis. Aquat. Organ.* **2002**, *50*, 35–43. [CrossRef]
104. Limna Mol, V.P.; Raveendran, T.V.; Parameswaran, P.S. Antifouling activity exhibited by secondary metabolites of the marine sponge, *Haliclona exigua* (Kirkpatrick). *Int. Biodeterior. Biodegrad.* **2009**, *63*, 67–72. [CrossRef]
105. Sears, M.A.; Gerhart, D.J.; Rittschof, D. Antifouling agents from marine sponge *Lissodendoryx isodictyalis* carter. *J. Chem. Ecol.* **1990**, *16*, 791–799. [CrossRef]
106. Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. Antifouling kalihinenes from the marine sponge *Acanthella cavernosa*. *Tetrahedron Lett.* **1995**, *36*, 8637–8640. [CrossRef]
107. Qian, P.Y.; Dobretsov, S.; Dahms, H.U.; Pawlik, J. Antifouling activity and microbial diversity of two congeneric sponges *Callyspongia* spp. from Hong Kong and the Bahamas. *Mar. Ecol. Prog. Ser.* **2006**, *324*, 151–165. [CrossRef]
108. Ribeiro, S.; Rogers, R.; Rubem, A.; Da Gama, B.; Muricy, G.; Pereira, R. Antifouling activity of twelve demosponges from Brazil. *Braz. J. Biol.* **2013**, *73*, 501–506. [CrossRef] [PubMed]
109. Sera, Y.; Adachi, K.; Fujii, K.; Shizuri, Y. A new antifouling hexapeptide from a Palauan sponge, *Haliclona* sp. *J. Nat. Prod.* **2003**, *66*, 719–721. [CrossRef] [PubMed]
110. World Malaria Report. 2020. Available online: https://www.who.int/publications/i/item/9789240015791 (accessed on 1 January 2022).
111. Campagnuolo, C.; Fattorusso, E.; Romano, A.; Taglialatela-Scafati, O.; Basilico, N.; Parapini, S.; Taramelli, D. Antimalarial polyketide cycloperoxides from the marine sponge *Plakortis simplex*. *Eur. J. Org. Chem.* **2005**, *6*, 5077–5083. [CrossRef]
112. Campos, P.E.; Wolfender, J.L.; Queiroz, E.F.; Marcourt, L.; Al-Mourabit, A.; Frederich, M.; Bordignon, A.; De Voogd, N.; Illien, B.; Gauvin-Bialecki, A. Unguiculin A and ptilomycalins E-H, antimalarial guanidine alkaloids from the marine sponge *Monanchora unguiculata*. *J. Nat. Prod.* **2017**, *80*, 1404–1410. [CrossRef]
113. Inbaneson, S.J.; Ravikumar, S. In vitro antiplasmodial activity of marine sponge Hyattella intestinalis associated bacteria against *Plasmodium falciparum*. *Asian Pac. J. Trop. Biomed.* **2011**, *1*, S100–S104. [CrossRef]
114. Le Pape, P.; Zidane, M.; Abdala, H.; Moré, M.T. A glycoprotein isolated from the sponge, *Pachymatisma johnstonii*, has anti-leishmanial activity. *Cell Biol. Int.* **2000**, *24*, 51–56. [CrossRef]
115. Dube, A.; Singh, N.; Saxena, A.; Lakshmi, V. Antileishmanial potential of a marine sponge, *Haliclona exigua* (Kirkpatrick) against experimental visceral leishmaniasis. *Parasitol. Res.* **2007**, *101*, 317–324. [CrossRef]
116. Shady, N.H.; Fouad, M.A.; Ahmed, S.; Pimentel-Elardo, S.M.; Nodwell, J.R.; Kamel, M.S.; Abdelmohsen, U.R. A new antitrypanosomal alkaloid from the Red Sea marine sponge *Hyrtios* sp. *J. Antibiot.* **2018**, *71*, 1036–1039. [CrossRef]
117. El-Hossary, E.M.; Cheng, C.; Hamed, M.M.; El-Sayed Hamed, A.N.; Ohlsen, K.; Hentschel, U.; Abdelmohsen, U.R. Antifungal potential of Marine natural products. *Eur. J. Med. Chem.* **2017**, *126*, 631. [CrossRef]
118. Naglik, J.R.; Richardson, J.P.; Moyes, D.L. *Candida albicans* pathogenicity and epithelial immunity. *PLoS Pathog.* **2014**, *10*, 8–11. [CrossRef] [PubMed]
119. Rifai, S.; Fassouane, A.; Kijjoa, A.; Van Soest, R. Antimicrobial activity of untenospongin b, a metabolite from the marine sponge *Hippospongia communis* collected from the atlantic coast of Morocco. *Mar. Drugs* **2004**, *2*, 147–153. [CrossRef]
120. Kibungu, W.C.; Fri, J.; Clarke, A.M.; Otigbu, A.; Akum Njom, H. Seasonal variation in antimicrobial activity of crude extracts of *Psammaplysilla* sp. 1 from Phillips Reef, South Africa. *Int. J. Microbiol.* **2021**, *2021*, 7568493. [CrossRef] [PubMed]
121. Pozzolini, M.; Millo, E.; Oliveri, C.; Mirata, S.; Salis, A.; Damonte, G.; Arkel, M.; Scarfì, S. Elicited ROS scavenging activity, photoprotective, and wound-healing properties of collagen-derived peptides from the marine sponge *Chondrosia reniformis*. *Mar. Drugs* **2018**, *16*, 465. [CrossRef]
122. Pozzolini, M.; Tassara, E.; Dodero, A.; Castellano, M.; Vicini, S.; Ferrando, S.; Aicardi, S.; Cavallo, D.; Bertolino, M.; Petrenko, I.; et al. Potential biomedical applications of collagen filaments derived from the marine demosponges *Ircinia oros* (Schmidt, 1864) and sarcotragus foetidus (Schmidt, 1862). *Mar. Drugs* **2021**, *19*, 563. [CrossRef]

International Journal of
Molecular Sciences

MDPI

Review

Bioactive Compounds from Marine Sponges and Algae: Effects on Cancer Cell Metabolome and Chemical Structures

Roberta Esposito [1,2], Serena Federico [1,†], Francesca Glaviano [2,3,†], Emanuele Somma [3,4,†], Valerio Zupo [3,*] and Maria Costantini [1,*]

1 Stazione Zoologica Anton Dohrn, Department of Ecosustainable Marine Biotechnology, Via Ammiraglio Ferdinando Acton, 80133 Napoli, Italy
2 Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Napoli, Italy
3 Stazione Zoologica Anton Dohrn, Department of Ecosustainable Marine Biotechnology, Ischia Marine Centre, 80077 Ischia, Italy
4 Department of Life Sciences, University of Trieste, 34127 Trieste, Italy
* Correspondence: vzupo@szn.it (V.Z.); maria.costantini@szn.it (M.C.)
† These authors contributed equally to this work.

Abstract: Metabolomics represent the set of small organic molecules generally called metabolites, which are located within cells, tissues or organisms. This new "omic" technology, together with other similar technologies (genomics, transcriptomics and proteomics) is becoming a widely used tool in cancer research, aiming at the understanding of global biology systems in their physiologic or altered conditions. Cancer is among the most alarming human diseases and it causes a considerable number of deaths each year. Cancer research is one of the most important fields in life sciences. In fact, several scientific advances have been made in recent years, aiming to illuminate the metabolism of cancer cells, which is different from that of healthy cells, as suggested by Otto Warburg in the 1950s. Studies on sponges and algae revealed that these organisms are the main sources of the marine bioactive compounds involved in drug discovery for cancer treatment and prevention. In this review, we analyzed these two promising groups of marine organisms to focus on new metabolomics approaches for the study of metabolic changes in cancer cell lines treated with chemical extracts from sponges and algae, and for the classification of the chemical structures of bioactive compounds that may potentially prove useful for specific biotechnological applications.

Keywords: algae; cancer; sponges; marine eukaryotes; metabolism

Citation: Esposito, R.; Federico, S.; Glaviano, F.; Somma, E.; Zupo, V.; Costantini, M. Bioactive Compounds from Marine Sponges and Algae: Effects on Cancer Cell Metabolome and Chemical Structures. *Int. J. Mol. Sci.* **2022**, *23*, 10680. https://doi.org/10.3390/ijms231810680

Academic Editor: Antonio González-Sarrías

Received: 9 July 2022
Accepted: 8 September 2022
Published: 14 September 2022

1. Introduction: A New "Omics" Technology: Metabolomics

Metabolomics (or metabonomics [1]) is used to define the large-scale study of small organic molecules, commonly known as metabolites (small molecules of 1 kilodalton, KDa), present within cells, biofluids, tissues or organisms. All of these small molecules taken together, and their interactions within a biological system, are known as the metabolome. This new discipline, together with the other omics techniques, helps reaching a complete view of cellular processes. The aim of this approach is to sketch, in cooperation with other omics technologies (genomics, transcriptomics and proteomics), a network of interactions that can describe, in detail, the state of the cell. This is called "interactome analysis" [2]. In fact, while genomics is the study of DNA and genetic information within a cell, transcriptomics is the study of RNA and differences in mRNA expression; proteomics is the large-scale study of proteins produced in an organism, system or biological context. In turn, metabolomics completes these studies, analyzing the substrates and products of metabolism as they are influenced by genetic and environmental factors (Figure 1).

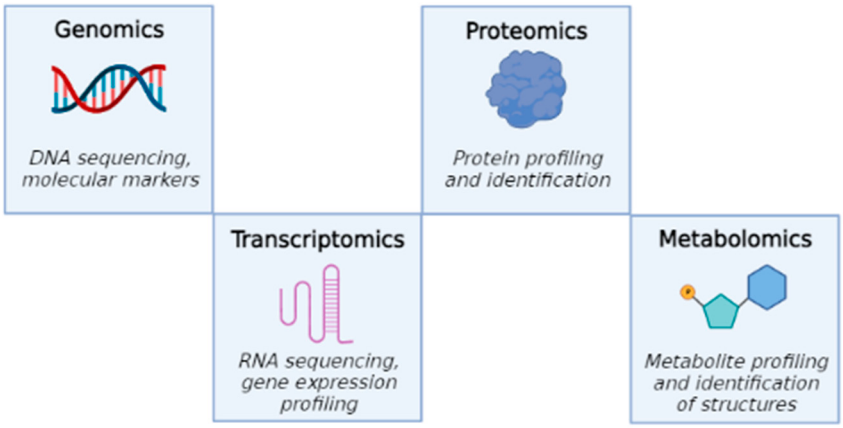

Figure 1. Four major "omics" fields, starting from genomics to metabolomics.

Metabolomics represents a powerful approach, because metabolites and their concentrations directly reflect the underlying biochemical activity and state of cells and/or tissues, producing the molecular phenotype. The set of these small molecules, such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols and alkaloids, represents the metabolome, through which the final or intermediate products of a biochemical process can be studied in order to build a metabolic pathway [2]. This area of research produces a "photo" of a cell, which can help identify its current phase in the cell cycle, and help determine whether or not it is facing environmental stress or if it is correctly performing its physiological role [3]. In fact, metabolomics approaches are extensively used i. to evaluate responses to environmental stress; ii. to investigate toxicology, drug discovery [4] and nutrition [5]; iii. to study the global effects of genetic manipulation and cancer; iv. to discover natural products; and v. to compare different stages of growth [6–11] (Figure 2).

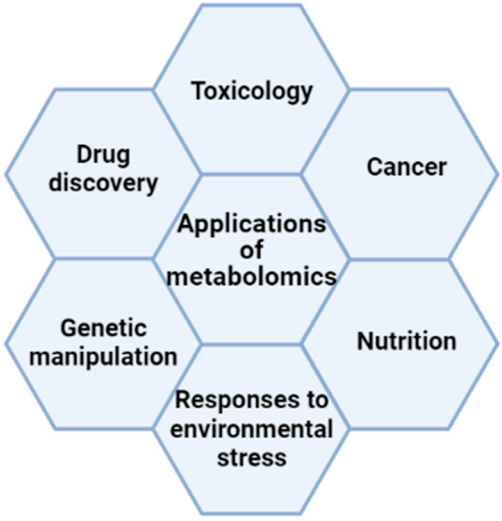

Figure 2. The main applications of metabolomics and their relationships.

The concept of the metabolic profile first appeared in literature in the 1950s, but only in the following three decades was this area of research completely developed. Despite this observation, metabolomics has only recently aroused the interest of researchers, thanks to the development of advanced technologies for the quantification of metabolites, such as gas chromatography (GC) and mass spectrometry (MS) [3]. Initially, studies mainly concerned metabolites of specific compounds such as pharmaceutical products [12,13]. Later, in this research area, studies on classes of compounds were included, such as catecholamines and oxylipins [14–17]. In particular, quantitative determination of some metabolites of

catecholamines was performed with metabolomics approaches. These metabolites were used as biological markers for diagnosis, for evaluation of therapeutic responses, and for early recognition of tumor relapses derived from the neural crest (neuroblastoma, pheochromocytoma), carcinoid tumors and melanoma [14]. In addition, metabolomics approaches were used to identify oxygenated fatty acid derivatives, called oxylipins, mainly produced from diatoms. Oxylipins have a negative effect on reproduction and on the development of different marine invertebrates, such as copepods [18] and sea urchins [19], but these molecules also have a cytotoxic effect on several cancer cell lines [20]. Metabolomic research is primarily carried out in complex matrices such as blood, cells, plants or extracts of other marine organisms [21]. Therefore, appropriate sample preparation and analysis techniques are necessary for a rapid and simultaneous determination of various compounds (Figure 3) [22].

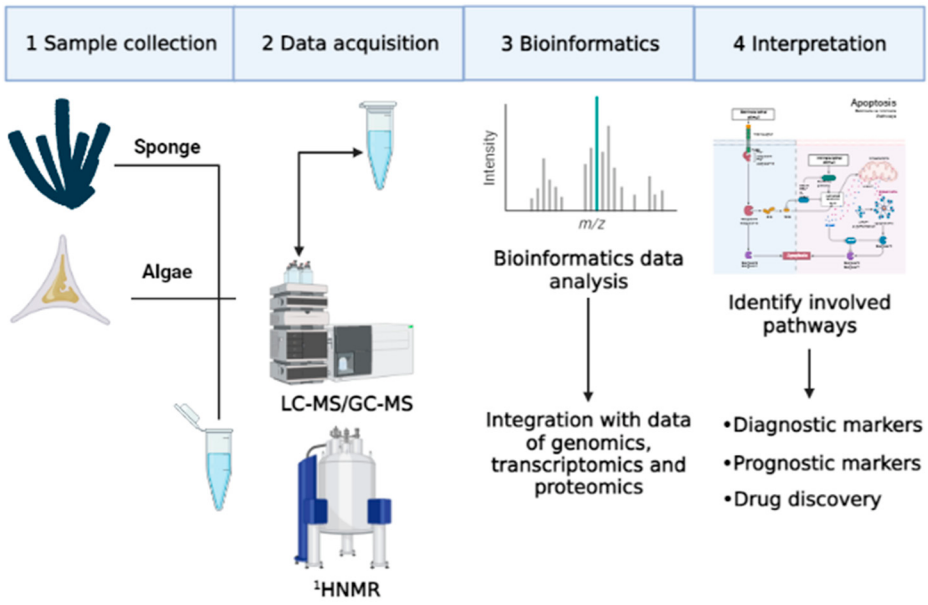

Figure 3. Main steps of metabolomics technologies.

2. Main Metabolomics Methodologies

Different methods can be applied to prepare, to extract and to analyse samples. In this paragraph, we explain the most used techniques to treat marine specimens in order to avoid rapid alteration of their metabolic profile and to stop metabolic reactions.

2.1. Sample and Extraction Techniques

Obtaining a broad coverage of the metabolome is difficult, due to a wide range of physico-chemical properties exhibited by small molecules. For this reason, various techniques are used to evaluate the set of metabolites [23]. Once samples are collected, it is recommended to treat them with liquid nitrogen or specific solvents [24–26]. Since extracts, for example, from marine organisms, contain a high percentage of salt and lipids that interfere with the most common analytical methods used in metabolomics, such as LC-MS and HPLC, they must be eliminated. The most used methods for this purpose are solid-phase extraction with Diaion HP-20 Resins, pre-equilibrated with methanol [27], or C18 and PS-DVB SPE cartridges [28,29], Sephadex LH-20 with a mobile phase of methanol and dichloromethane (1:1) or C18 SPE cartridges that are highly lipophilic [27]. After sample collection and preparation, separation techniques, such as liquid chromatography (LC) in its high performance (HPLC) or ultra-performance (UPLC) forms, gas chromatography (GC) and detection techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) are used [23–25]. Further, acquired data are processed in order to create a numerical matrix, which can be used for statistical or multivariate analysis [25]. Generally,

the treatment of Nuclear Magnetic Resonance (NMR) data is simpler than that required for LC-MS data, consisting of phase correction; baseline adjustment; shift adjustment and binning that divides an NMR spectrum into many regions or bins to reduce the effects of pH, composition and ionic strength of sample [30]; fuzzy warping (an algorithm that can be used to establish correspondence between the most intense peaks of the spectra to be aligned) [31]; peak alignment using a genetic algorithm [32]; and normalization [33]. For LC–MS data, different software are available for data handling, some of which are open access, such as OpenMS [34], MZMine 2 [35], XCMS [36] and MS-DIAL [37]. *t*-test, analysis of variance, principal component analysis (PCA), and partial least squares (PLS) and orthogonal-PLS (OPLS) analyses are the most used statistical methods in metabolomics studies [38,39]. In the following paragraphs, the most important separation and detection techniques are summarized.

2.2. Separation Techniques

Several separation techniques are routinely applied according to the characteristics of the putative compounds to be identified. Among them, Gas Chromatography (GC) is an excellent separation technique that was considerably improved with the introduction in 1979 of fused-silica capillary columns that resulted in higher resolution, higher efficiency, better reproducibility and smaller sample size [40]. In addition, the combination of gas chromatography with a mass spectrometer may be a highly sensitive approach. However, GC is limited to small compounds, which are thermally stable, volatile or can be rendered chemically volatile, for instance, by trimethylation [41]. Detection in GC analysis may be limited to certain compounds unless Mass Spectrometry (MS) is the method of choice, however, even when MS is used, some of the compounds may not be ionized sufficiently to be detected at low levels unless they are derivatized with an ionisable moiety [42]. HPLC and UHPLC are powerful tools for metabolomic studies that enable the separation and characterization of metabolite similarities. These techniques represent efficient separation technologies, which can be used to determine different groups of compounds, hydrophilic as well as hydrophobic, salts, acids, bases, etc. [6]. HPLC, unlike GC, is not limited to the separation of thermally stable volatile compounds or large molecules. The separation of each group within the HPLC is a function of the solute properties that determine the column type (stationary phase) and mobile phase to be used for successful separation [43]. These modes include RP (reverse phase), normal phase, ion exchange, chiral, size exclusion, hydrophilic interaction chromatography (HILIC) and mixed modes [42,44].

2.3. Detection Techniques

MS and NMR are the most widely used analytical techniques in metabolomics [45]. MS provides a mix of rapid, sensitive and selective qualitative and quantitative analyses with good skill to identify metabolites [46]. Mass spectrometers act by ion formation, which entails the separation of ions according to their mass-to-charge (m/z) ratio, and the detection of separated ions [9,47]. MS is rapidly gaining interest in metabolomics, though it is more often associated with other techniques, such as chromatography [48]. In fact, MS, which was widely developed over the last decades, holds a distinguished position in the field of determination, quantification and separation of mixtures of compounds. Recent advances in MS-based metabolomics produced the potential to quantify the levels of hundreds of metabolites that are intermediate or final products of cellular processes [49]. Thanks to its high sensitivity, selectivity and wide range of covered metabolites, MS has become the most widely used technique in metabolomics studies [2]. Another advantage of MS is derived from its reproducible quantitative analysis and its power to analyse samples with extremely high molecular complexity [50,51]. The objectives of developing MS for metabolomics range from the structural characterization of important metabolites to the detection of metabolite variations [52]. Moreover, MS can be applied to analyse biological samples, either by direct-injection or following chromatographic separations [3,53,54]. Recent progress and improvements in mass accuracy and precision

have drastically increased the range of metabolites that can be analysed by MS, and have enhanced the accuracy of compound identification [48]. Usually, both of the above mentioned methods are used in metabolomics studies in order to identify and quantify all metabolites of the biological system under analysis [55,56]. Additionally, NMR provides users a vision of intact molecules at the atomic level and enables the viewing not only of 1-H atoms, but also many other kinds of atoms (^{13}C, ^{15}N) or biologically reactive groups, including phosphate atoms (^{31}P) [57–60]. In addition, this technique needs minimal or no sample preparation and it is non-destructive, unlike MS [9,61]. Jeremy Nicholson was a pioneer in the use of NMR spectroscopy in the toxicological field [62]. Therefore, it is suitable for studies of cell extracts, and for cell cultures and tissues in vitro or in vivo. Among other advantages, it is highly reproducable and has the ability to identify unknown metabolites [63,64]. Our metabolomics work exclusively used ^{1}H-NMR for the analyses of marine samples, but other nuclides (i.e., ^{13}C, ^{31}P, ^{15}N, ^{19}F, and ^{2}H) may provide more information about this set of metabolites. The main limits of this technique concern the spectral resolution and sensitivity, which can be improved by high intensity magnetic field [57,65]. Furthermore, as compared to LC-MS and GC-MS, ^{1}H-NMR spectroscopy is almost 100 times less sensitive. This means that a typical ^{1}H-NMR-based metabolomics study only gives back information on 50–200 identified metabolites with concentrations >1 μM, while a typical LC-MS study can render information on more than 1000 identified metabolites with concentrations of >10 to 100 nM [57].

3. Metabolomics Applied to Marine Organisms in Cancer Studies

Cancer is one of the deadliest human diseases, able to alter the metabolism of a cell [66]. It is well known that cancer metabolism is different from that of normal tissue, and an important hypothesis published in the 1950s by Otto Warburg suggested that cancer cells use anaerobic metabolism as a source of energy [67]. In fact, the best-studied aspect of cancer metabolism is the central carbon metabolism and the relationships between glycolysis, the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation [68]. One assumption is that cancer mainly converts pyruvate to lactate, rather than fuelling the TCA cycle, even in aerobic conditions [69,70]. The exploration of the cancer metabolome is the best way to understand the phenotypic changes associated to biological functions. Thanks to the metabolomics approach, it is possible to identify a range of metabolites involved in the process of carcinogenesis [71]. Moreover, a metabolomics approach is used for the discovery of biomarkers, and consequently, to improve the diagnosis and prognosis of many cancers, such as colorectal, breast, gastric, pancreatic and liver cancer [72]. In metabolomics, there are several approaches: fingerprinting, footprinting, profiling, flux analysis and target analysis. The first approach includes the screening of all metabolites within a biological system. Footprinting (mainly related to in vitro cell system) investigates metabolites from the environment around the system under analysis and shows information about metabolic exchange. Profiling is used to identify chemical compounds, for example lipids, also using standards for analyses. The fourth approach, called "flux analysis", is the detection of one compound, usually isotope-labelled carbon, through a specific pathway or set of pathways, to determine the destiny of the compound. Target analysis provides a comparison of one or a few target metabolites, whose concentrations can change depending on the environmental conditions [72,73].

According to an analysis of literature, in the last years, the marine environment has shown to be the most promising source of bioactive compounds against cancer. In particular, sponges and algae are likely to be the marine organisms from which the largest number of natural compounds with antiproliferative activity could be isolated. During the past 50 years, sponges are considered a gold mine for the discovery of bioactive natural products [74,75]. In fact, the first marine-derived anticancer compound, the cytarabine or Ara-C, which was approved in 1969 and is still used to treat acute myelocytic leukemia and non-Hodgkin's lymphoma, was isolated from the Caribbean sponge *Tethya crypta* [76]. In 2010 another anticancer agent, Eribulin, was isolated from the sponge *Halichondria*

okadai [77], developed from the polyether metabolite halichondrin B and commercialized as Halaven [78]. Algae, especially microalgae, have the same importance as a source of natural products, being easily cultivated in large-scale closed ensuring a theoretically limitless supply of biomass. Bioactive compounds of algal origin can be sourced directly from primary metabolism (e.g., proteins, fatty acids, vitamins and pigments) or can be synthesized from secondary metabolism [79]. Potent sunscreens against ultraviolet (UV)-induced cell damage were isolated from Spirulina, Chlorella and Dunaliella [80–83], as well as antioxidant carotenoids (astaxanthin, lutein, zeaxanthin, canthaxanthin and b-carotene) from *Dunaliella salina* [84–88].

In the frame of evolutive processes, several marine organisms, such as macro- and microalgae, sponges and fishes, developed appropriate defence mechanisms. They are based on the use of a variety of natural weapons, i.e., molecules that allow them to survive in a hostile environment characterized by stressful conditions. This is due to variable salinity, pressure, temperature and light, as well as to the need to avoid microbial and/or viral attacks [19]. These compounds, playing key ecological roles, are characterized by specific biological and potential biotechnological activities (anti-cancer, anti-inflammatory, anti-oxidant, anti-microbial, anti-hypertensive) worth explotation for pharmacological purposes. Sponges and algae represent promising resources for cancer treatment [20,21]. Consequently, our attention was focused on these two groups of marine organisms, in order to show how metabolomics have, in the past few years, aided in the exploitation of these organisms for several applications against cancer. In particular, we highlighted how metabolomics has been used with two different approaches, starting from the chemical extracts from sponges and algae (Figure 4).

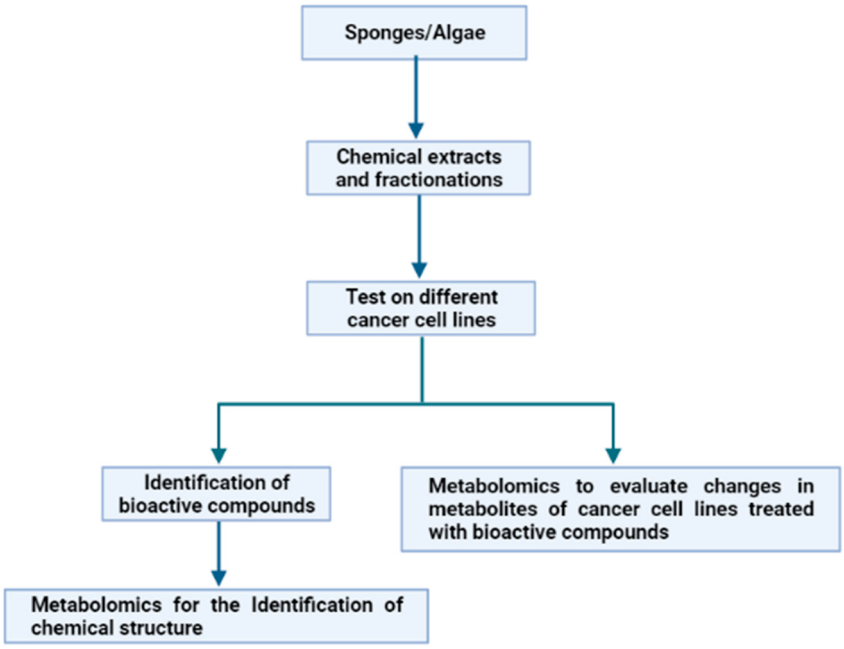

Figure 4. Experimental workflow of the two different applications of metabolomics to the study of marine extracts for anticancer applications.

The first approach involves the use of bioassay-guided fractionation of chemical extracts from the marine organisms under analysis on different cancer cell lines, and, once an active fraction has been identified, metabolomics approaches are applied to elucidate the chemical structure of the potentially bioactive compounds. Otherwise, in the second approach, the bioassay-guided fractionation of chemical extracts is also performed. However, once a cancer cell line on which the active fractions have antiproliferative effects has been identified, metabolomics approaches are applied to define the changes in metabolites of treated cells. To date, the data reported in literature (see below) demonstrated that

the first approach is the most used and fewer applications have been reported on the second approach, because, at the moment, the second approach represents a novelty in the pharmacological field.

3.1. Structure Elucidation of Bioactive Molecules from Sponges

Marine sponges (phylum Porifera) have been largely demonstrated to be one of the richest sources of exclusive secondary metabolites with relevant bioactivity, by means of bioassays [89,90]. Many scientists are trying to districate the complex network of causes and factors influencing the appearance of neoplastic cells [91,92]. Metabolomics represents a powerful tool for seeking potentially new and sustainable bioactive compounds in different species of sponges. In fact, two compounds (Stylissamide A and Stylissoside A) from the marine sponge *Stylissa carteri* sampled in the Red Sea, were extracted and analysed using LC-MS and ^{1}H-NMR. Further, these compounds were tested on two cancer cell lines. The IC_{50} values for Stylissamide A on MCF7 (breast cancer) and on HepG2 (liver cancer) were of 21.1 μM and 36.8 μM, respectively, while the IC_{50} values for Stylissoside A were of 27.5 μM on MCF7 and 30.5 μM on HepG2 [93]. Similar metabolomic approaches (by LC-MS) led to the isolation and characterization of two compounds from another sponge species, *Callyspongia siphonella*. The molecules were characterized by using ^{1}H-NMR and named 5-bromotrisindoline and 6-bromotrisindoline (see Figure 5 for chemical structure). In particular, 5-bromotrisindoline was effective against HT29 (colon carcinoma), OVCAR3 (ovarian carcinoma) and MM.1S (multiple myeloma) with IC_{50} of 8, 7 and 9 μM, respectively while 6-bromotrisindoline was effective against the same cancer cell lines with IC_{50} 12.5, 9 and 11 μM, respectively [94]. Some sponges living in extreme environmental conditions can also produce interesting compounds, which can be active against human cancer cell lines. This is the case of a specimen belonging to the sponge *Haliclona rosea*, collected in shallow water at a hydrothermal vent site. The extract of this sponge was analysed using the MS technique. This made possible the identification of several 3-alkyl-pyridine alkaloids (3-APA) responsible for the reduction of the 70–90% of cell viability of SKBR3 breast cancer cell line [95] at the concentration of 33 μg/mL in dichloromethane/methanol extracts. In particular, Cyclostellettamine P, one out of thirteen 3-APA compounds, was characterized, for the first time, by ion mobility mass spectrometry (IMS) in time-aligned parallel (TAP) fragmentation mode. This new technique permits the separation of ionic species as they drift through a gas phase under the influence of an electric field. Then, ions isolated in this way were subjected to subsequent fragmentation in the "trap" region of the IMS device [96]. LC-MS led to the identification of 20 compounds that could be responsible for the cytotoxic activity of the crude extract of *Coscinoderma* sp. on cancer cells. Crude extracts alone showed moderate activity, but were enhanced when the extract was encapsulated in liposomes. In this case, the IC_{50} values were notably lower compared to the positive control (Doxorubicin). The tested cell lines were HepG2 (IC_{50} = 2.2 μg/mL), MCF7 (IC_{50} = 4.1 μg/mL) and CaCo2 (colon cancer, IC_{50} = 1.7 μg/mL) [97]. A new compound named Geodiataurine (see Figure 5 for chemical structure) has been isolated from the marine sponge *Geodia macandrewii*, thanks to a complex combination of two techniques for the generation of metabolomics data: UHPLC and MS. The cytotoxic activity of this compound was tested against a melanoma cancer cell line (A2058) and showed weak cytotoxic activity (IC_{50} = 8.5 μM) [98]. Similarly, a sponge coming from the deep-sea Antarctic zone belonging to the species *Latrunculia biformis* caught the attention of Li et al. [99]. The authors revealed, thanks to ^{1}H-NMR and MS analyses, the presence of known and unknown compounds. However, only three of them showed cytotoxic activity on the HCT-116 colon cancer cell line. The first tested compound was already known: (−)-discorhabdin L, and it showed IC_{50} equal to 0.33 μg/mL. The other two interesting compounds were new. They are two new discorhabdin analogs, i.e., (−)-1-acetyl-discorhabdin L and (+)-1-octacosatrienoyl-discorhabdin L, exhibiting IC_{50} of 1.1 μg/mL and 25.6 μg/mL, respectively [99]. Metabolite analyses of the marine sponge *Theonella swinhoei* performed by MS and ^{1}H-NMR showed the presence of theonellamides. In particular, Theopalauamide was an already known

compound whose cytotoxic activity was evaluated on the HTC-116 colon carcinoma cell line (IC_{50} = 2.8 μM), while two new compounds (5-*cis*-Apoa-theopalauamide reported in Figure 5 and theonellamide K) exhibited a cytotoxic activity on the same cell line, with IC_{50} of 21.8 and 3.5 μM, respectively [100].

We should consider, however, that sponges are holobionts that live in symbiosis with many bacterial species. These latter can represent up to 35% of the sponge's weight [101]. Most of the symbiotic bacteria able to produce bioactive compounds are potential candidates for biotechnological applications [102–104]. From the marine sponge *Petrosia ficiformis* sampled in the Mediterranean Sea (Milos, Greece), a bacterial strain of Streptomyces sp. (SBT348) has been isolated. Thanks to a metabolomic approach performed through LC-MS analyses, it was possible to isolate a known compound, namely Petrocidin A (see Figure 5 for chemical strcture), which had cytotoxic effect towards HL-60 (human promyelocytic cell) and HT29 cell lines with IC_{50} 3.9 and 5.3 μg/mL, respectively. In addition, from the Streptomyces sp. (SBT348), a new compound whose structure has been elucidated (2,3-Dihydroxybenzamide, Figure 5) showed activity against the same cell lines with different IC_{50} values of 5.5 μg/mL and 3.8 μg/mL, respectively [105].

In another study, from samples of the Red Sea sponge *Callyspongia* sp., a strain of *Nocardiopsis* sp. (UR67) has been isolated, from which Nocardiotide A has been detected and characterized through MS analyses. Surprisingly, this compound showed activity against several different cancer cell lines, such as the CT26 (murine colon carcinoma, IC_{50} = 12 μM/mL), HeLa (human cervix carcinoma, IC_{50} = 11 μM/mL) and MM.1S (IC_{50} = 8 μM/mL) cancer cell lines [106]. Two bacterial strains belonging to Nocardia sp. (UR 86) and *Nocardiopsis* sp. (UR 92) were isolated from another sponge belonging to *Amphimedon* sp., coming from the Red Sea. These bacterial strains were cultured in different culture conditions and their crude extracts were analysed with an MS approach. The extract of *Nocardia* sp. (UR86) caused cellular inhibition of several cancer cell lines, such as HepG2, MCF7 and CaCo2, with IC_{50} values of 3.1 μg/mL, 3.9 μg/mL and 14.4 μg/mL, respectively. Similarly, extracts of *Nocardiopsis* sp. (UR92) were active against the same cancer cell lines with IC_{50} values of 3.7 μg/mL, 14.7 μg/mL and 14.3 μg/mL, respectively [107]. A newer approach is present in the research recently conducted by Hifnawy et al. [108]. They co-cultured two Actinomycetes to stimulate them to produce metabolites that would not have been produced if the two strains were cultured separately. These two actinomycetes were isolated from two sponges: *Micromonospora* sp. was isolated from *Callyspongia* sp., while *Actinokineospora* sp. was isolated from *Spheciospongia vagabunda*. Several compounds were detected using LC–MS metabolomics analyses, but only one was noteworthy in terms of its cytotoxic effect: the *N*-(2-hydroxyphenyl)-acetamide (see Figure 5). This was active against several cancer cell lines, such as HCT116 (colorectal carcinoma), HePG-2 (hepatocellular carcinoma) and MCF7 (mammary gland), with IC_{50} values ranging from 10 to 36 μM [108]. Extracts/compounds from sponges and their cytotoxic activities are summarized in Table 1.

Table 1. Sponge species, compound/extract, cell lines tested, metabolomics techniques and corresponding reference are reported.

Sponge	Compound/Extract	Cell Lines	Metabolomics	Reference
S. carteri	Stylissamide A and Stylissoside A	MCF7 and HepG2	LC-MS	[93]
C. siphonella	5-bromotrisindoline and 6-bromotrisindoline	HT29, OVCAR3 and MM.1S	LC-MS	[94]
H. rosea	3-alkyl pyridine alkaloids	SKBR3	MS	[95]
Coscinoderma sp.	Crude extract	HepG2, MCF7 and Caco2	LC-MS	[97]
G. macandrewii	Geodiataurine	A2058	UHPLC and MS	[98]
L. biformis	(−)-discorhabdin L, (−)-1-acetyl-discorhabdin L and (+)-1-octacosatrienoyl-discorhabdin L	HCT116	1H-NMR and MS	[99]

Table 1. *Cont.*

Sponge	Compound/Extract	Cell Lines	Metabolomics	Reference
T. swinhoei	Theopalauamide, 5-*cis*-Apoa-theopalauamide and Theonellamide K	HCT116	1D and 2D NMR; MS	[100]
P. ficiformis	Petrocidin A and 2,3-Dihydroxybenzamide	HL60 and HT29	LC-MS	[105]
Callyspongia sp.	Nocardiotide A	CT26, HeLa and MM.1S	MS	[106]
Amphimedon sp.	Crude extract	HepG2, MCF7 and Caco2	MS	[107]
Callyspongia sp. and *S. vagabunda*	*N*-(2-hydroxyphenyl)-acetamide	HCT116, HepG2 and MCF7	LC–MS	[108]

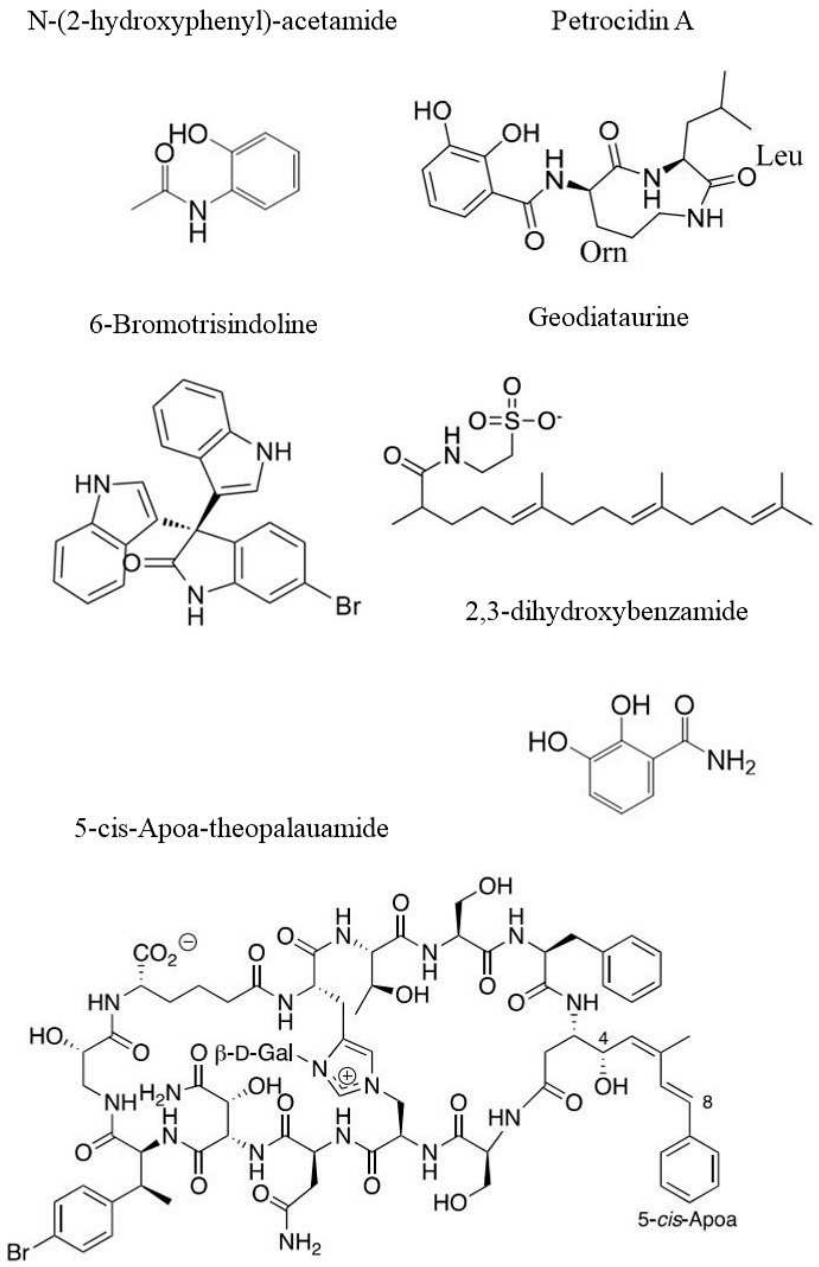

Figure 5. Examples of chemical structures of natural compounds from sponges: *N*-(2-hydroxyphenyl)-acetamide [108], Petrocidin A and 2,3-Dihydroxybenzamide [105], 6-Bromotrisindoline [94], Geodiataurine [98] and 5-*cis*-Apoa-theopalauamide [100].

3.2. Structure Elucidation of Bioactive Molecules from Algae

Algae are a complex and heterogeneous group of photosynthetic organisms, showing an extraordinary biological diversity represented by more than 166,000 species [109]. It is convenient to divide them into micro- (unicellular) and macro- (multicellular) algae according to their structure, evolution, ecological properties and sizes. These organisms produce and store a huge variety of metabolites, which include biologically active compounds (e.g., pigments, proteins and polysaccharides, antioxidants and polyunsaturated fatty acids), and several secondary metabolites produced in response to the pressures received in a wide range of environments, characterized by different conditions of temperature, light and salinity [110], among others. Bioactive molecules extracted both from microalgae and macroalgae show cytotoxic, antiviral and anti-inflammatory effects, with high potential for use in various medical fields. Some of those compounds are effective as therapeutic agents against cancer, showing high specificity for target molecules [111,112]. Moreover, seaweeds represent an excellent source of bioactive compounds because they are easy to cultivate, allowing for the production of larger biomasses that can be used for industrial purposes. Among the edible species, having an historical importance as source of food for human consumption, there are the seaweeds belonging to the genus Ulva, which have a range of health-promoting bioactive components. In particular, ulvan, a polysaccharide contained in its cell walls, is mainly composed of sulfated rhamnose, uronic acids (glucuronic acid and iduronic acid) and xylose. It showed cytotoxic activity against cancer cells [113,114]. In fact, according to Than et al. [114], ulvan extracted from *Ulva lactuca* had strong effect at various concentrations (0.8, 4, 20 and 100 µg/mL) against HepG2, MCF7 and Hela cancer cell lines. They also assessed the value of IC_{50} on the above three cell lines, being 29.7 µg/mL, 25.1 µg/mL and 36.3 µg/mL, respectively. In conclusion, ulvan showed a significant cytotoxic activity in a dose-dependent manner and it can be developed as a promising cancer-fighting compound. In the same work, the researchers determined the fine structure of the ulvan, using ^{1}H-NMR and MS methods. Similarly, Mofeed et al. [115] tested an organic extract of *U. lactuca* and *Ulva fasciata* on MCF7 and HTC-116 (colorectal carcinoma) cell lines at different concentrations (12.5, 25, 50 and 100 µg/mL), highlighting a significant dose-dependent response after 48 h of exposure. They also tested the extracts of three additional species of seaweeds, which, similarly, exhibited cytotoxic activity: two red algae, namely *Amphiroa anceps* and *Corallina mediterranea*, and the fucales *Sargassum filipendula*. The extracts were analysed by means of GC-MS. Moreover, various researches have been carried out on seaweed belonging to order Fucales, such as *Fucus vesiculosus*. Geisen et al. [116] reported the inhibition of the cellular cycle in several cancer cell lines of pancreatic ductal adenocarcinoma (Panc1, PancTU1 and Panc89) and pancreatic adenosquamous carcinoma (Colo 357), testing fractions from a hydrophilic extract, after separation through HPLC. This effect seems related to the up-regulation of cell cycle inhibitors, showing an alteration of the expression levels of proteins and mRNA. Additionally, in the case of *F. vesiculosus*, Zenthoefer et al. [117] analysed the effect of six crude extracts, each of which was analysed by ^{1}H-NMR spectroscopy techniques, revealing a characteristic fingerprint that was significantly correlated with the activity. In particular, the acetonic crude extract (FvT_A) showed the strongest activity against Panc89 and PancTu1, with an inhibitory rate of 80.3% and 82.6%, respectively. It is worth observing that the particular attention paid to some brown algae is due to the production and storage of a sulphated polysaccharide called Fucoidan, which is well known as a promising compound to be applied for cancer treatment. Among various species of brown algae, one of the best known is *Cladosiphon okamuranus*, an edible alga that is commonly cultured in Japan. This alga largely produces an accessory pigment named fucoxanthin, which is mainly metabolised and transformed into fucoxanthinol by the digestive enzymes of the gastrointestinal tracts of consumers. Both compounds are well known and studied because they exert an anti-proliferative effect [118]. Rokkaku et al. [119] extracted fucoxanthin and fucoxanthinol by the seaweed *C. okamuranus* using HPLC and MS. The results showed potentially anti-osteosarcoma properties, which appear to be at least partially attributable to the inhibition of Akt and AP-1 signal pathways

in human and mouse osteosarcoma cancer cell lines (Saos-2, MNNG/HOS, 143B and LM8). In addition, various sulphated compounds extracted from algal biomasses have aroused interest for their biotechnological applications. In particular, Shao et al. [120] demonstrated the activity of three sulphated polysaccharides extracted by *U. fasciata* (UFP), *Gloiopeltis furcata* (GFP) and *Sargassum henslouianum* (SHP). The polysaccharides were extracted after ultrasonic disruption applying Radial Flow Chromatography (RFC) separation and then tested on MKN45 (gastric cancer) and DLD (intestinal cancer) cell lines. After incubating with those three extracts for 24 h at different concentrations (from 0.125 to 1.00 mg/mL), the inhibitive effects on MKN45 cancer cells were observed. In particular, SHP exhibited the strongest cytotoxic effect, with a growth inhibition percentage of almost 50% at the concentration of 1.00 mg/mL. In contrast, all samples showed low percentages of growth inhibition on DLD cancer cells, at all concentrations.

After the aforementioned works, dealing with various macroalgae, hereafter we explore studies focused on the use of microalgae as a source of anti-cancer compounds. The study of Abreu et al. [121] investigated the effectiveness of ^{1}H-NMR and MS metabolomic approaches to record the variation of metabolites naturally present in the dinoflagellate *Amphidinium carterae* in a long-term culture. Specifically, among other compounds of interest, as fatty acids and carotenoids, they focused on the amphidinol family and their content variation in relationship to different levels of daily irradiance and nutrients in anf/2 medium. Three concentrations of methanolic extract (10, 30, and 100 µg/mL) were tested on four cancer cell lines: namely A549, HT29, MDA-MB-231 and PSN-1, showing a high antiproliferative activity against all four tumor cell lines (80%). Similarly, other studies investigated the chemical composition of the extracts of various microalgae along with their anticancer effects. Arslan et al. [122] analysed a crude extract of *Isochrysis galbana* through ^{1}H-NMR and GC-MS and tested its cytotoxic effect on four cell lines: chronic myelogenous leukemia K562, human acute T lymphoblastic leukaemia MOLT-4, human Caucasian histiocytic lymphoma U937, Caucasian promyelocytic leukemia HL60 and human Burkitt's lymphoma Raji cancer cells, showing the highest cytotoxicity (about 24.07%) at a concentration of 500 µg/mL against Raji cells. An interesting approach to the assay of natural products against cancer cell lines was reported by Karakas et al. [123]. They tested crude extracts of two microalgae (*Chlorella protothecoides* and *Nannochloropsis oculata*) against A172 (brain glioblastoma) and HCT116 cell lines. Assays on cells were carried out, testing not only the crude extract at increasing concentrations (25, 50, 100 µg/mL), but also three micro- and nano-particles loaded with the extracts, represented by PVA: Chitosan solution (PCH) and PVA: NaAlg solution (PNA) (produced through electrospraying techniques), and NPAL (obtained using the microemulsion method). The particles PCH and PNA were prepared by loading with concentrations of 70, 35 and 17.5 µg/mL, while NPAL particles obtained by the microemulsion method were loaded with 50, 25 and 12.5 µg/mL. Each particle was preliminarily tested on HUVEC cell lines (non-cancerous cells) in order to ensure absence of cytotoxicity against normal cells. Crude extracts were analysed with GC-MS. The results of the assay showed cytotoxic effects of both microalgal extracts and encapsulated microalgal extracts on two cancer cell lines while they did not have cytotoxic effects on healthy cells. This study showed that microalgal extracts have cytotoxic effects on cancer cells and did not lose their cytotoxic effects after encapsulation. In a similar study, Hussein et al. [124] analyzed the effects of crude extracts of *Tetraselmis suecica* in conjunction with an innovative compound, through the application of silver nanoparticles adopted as a carrier, against MCF7, mammary carcinoma 4 T1 and normal Vero cell-lines. The cytotoxicity assays were carried out by separately testing the crude extract of the microalgae, the silver nanoparticles AgNPs. In conclusion, the co-application of the two. *T. suecica* single application only showed the IC_{50} of 46.77 µg/mL on MCF7 and 83.17 µg/mL on 4 T1 cells. The AgNPs single application displayed the highest cytotoxicity according to a dose-dependent pathway after 72 h treatments with an IC_{50} of 5.3 µg/mL on MCF7, 17.78 µg/mL on 4 T1, and 25.11 µg/mL on Vero cells. Besides, the AgNPs-*T. suecica* co-application reached the IC_{50} of 6.60 µg/mL and

53.7 µg/mL, respectively, while the *T. suecica*-CHL single application only showed the IC_{50} of 46.77 µg/mL and 83.17 µg/mL against MCF7 and 4 T1, respectively. Moreover, they analysed the crude extract of *T. suecica* through GC-MS. In another study, Hussein et al. [125] demonstrated that the highest cytotoxic activity against MCF7 cells was exhibited by the synergic application of Tamoxifen (TMX, anti-estrogen drug) and *Nannochloropsis oculata*'s water extract with IC_{50} of 15.8 µg/mL, TMX-*T. suecica*'s ethanolic extract with IC_{50} of 16.9 µg/mL, TMX-*Chlorella* sp.'s chloroform extract with IC50 of 13.4 µg/mL, while TMX-*N. oculata*'s chloroform extract, TMX-*T. suecica*'s ethanolic extract and TMX-*Chrolella* sp.'s ethanolic extract showed cytotoxic effect against 4 T1 cells with IC_{50} of 15.4, 13.8 and 16.9 µg/mL, respectively. Moreover, the synergistic application of TMX-algae's extracts maintains an antiproliferative effect on cancer cell lines and reduced the toxicity on normal Vero cells. In addition, after ^{1}H-NMR analyses, isoleucine was found only in the ethanolic extract of *Chlorella* sp., glycerol only in the ethanolic extract of *T. suecica* and in the chloroform extract of *Chlorella* sp., while xanthine was found only in the chloroform extract of *Chlorella* sp. These metabolites can help reduce the toxicity of non-cancerous Vero cells. Fayyad et al. [126] tested various concentrations of hot methanolic extracts of *Spirulina platensis* to identify the most active chemical compounds and also to check the cytotoxic effect on cancer cell lines L20 B (mice intestine carcinoma) and MCF7 (breast cancer) after 24 h and 48 h exposure. GC mass analysis showed that the active chemical compounds in the extracts contained alkaloids, terpenes, phenols, resins, saponines, flavones, steroids, proteins and amino acids. Moreover, this extract exhibited the highest growth inhibition by testing the 12.5 mg/mL concentration against L20 B (32.5%) and against MCF7 (71.5%). These percentages increased after 48 h application (35.5% against L20 B and 78% against MCF7). Other studies, rather than testing the entire extract from microalgae, worked on different fractions obtained with distinct methods. El-Baz et al. [127] extracted carotenoid and polar fractions from both *Haematococcus pluvialis* and *Dunaliella salina* and tested them on HePG2, MCF7, HCT116, and A549 cancer cell lines. Moreover, carotenoids of *H. pluvialis* identified using LC-MS showed high cytotoxic activity against the HCT116 (100% inhibition at 0.1 mg/mL) and mild activity against both MCF7 and HePG2 lines, while the carotenoid-rich fraction of *D. salina* showed moderate cytotoxic activity on the MCF7 and HePG2 cancer cell lines.

Savio et al. [128] obtained hydrophilic and lipophilic fractions from the diatoms *Phaeodactylum tricornutum* and *Staurosirella pinnata* that were analysed with ^{1}H-NMR. They performed a bioactivity assay on human immortalised keratinocytes HaCaT and human melanoma CHL-1 cell lines in a 24 h dose-response test with different concentrations (0.2, 0.4, 0.8, 1.6, 3.2 and 10.0 mg/mL). The most important result showed that *S. pinnata* extract had an important dose-dependent effect on CHL-1. Extracts and compounds from the macro- and micro-algae analysed in this section are shown in Table 2.

Table 2. Algal species, compound or extract, cell lines tested, metabolomics techniques and corresponding reference are reported.

Algae	Compound/Extract	Cell Lines	Metabolomics	Reference
U. lactuca	Ulvan	HepG2, MCF7 and Hela	^{1}H-NMR-MS	[114]
U. fasciata, *U. lactuca*, *A. anceps*, *C. mediterranea* and *S. filipendula*	Organic extract	MCF7 and HTC116	GC-MS	[115]
F. vesiculosus	Hydrophilic extract	Panc1, PancTU1, Panc89 and Colo 357	HPLC	[116]
F. vesiculosus	Crude extracts	Panc89 and PancTU1	^{1}H-NMR	[117]
C. okamuranus	Fucoxanthin and fucoxanthinol	Saos-2, MNNG/HOS, 143 B and LM8	HPLC-MS	[119]

Table 2. *Cont.*

Algae	Compound/Extract	Cell Lines	Metabolomics	Reference
U. fasciata, *G. furcata* and *S. henslouianum*	Sulphated polysaccharides	MKN45 and DLD	RFC	[120]
A. carterae	Methanolic extract	A549, HT29, MDA-MB-231 and PSN-1	^{1}H-NMR and MS	[121]
I. galbana	Crude extract	K562, MOLT-4, U937, HL60 and Burkitt's lymphoma	^{1}H-NMR, GC-MS	[122]
C. protothecoides and *N. oculata*	Crude extract	A172 and HTC116	GC-MS	[123]
T. suecica	Crude extract	A172 and HTC116	GC-MS	[124]
N. oculata, *T. suecica* and *Chlorella* sp.	Water, ethanolic and methanolic extracts	MCF7 and 4 T1	^{1}H-NMR	[125]
S. platensis	Methanolic extract	L20 B and MCF7	GC-MS	[126]
H. pluvialis and *D. salina*	Carotenoid fractions and polar fractions	HepG2, MCF7, HCT116 and A549	LC-MS	[127]
P. tricornutum and *S. pinnata*	Hydrophilic and lipophilic fractions	CHL-1	^{1}H-NMR	[128]

3.3. Metabolite Changes in Treated Cancer Cell Lines

As mentioned above, a few studies report analyses of metabolite changes after treatment of cancer cells with extracts/molecules isolated from sponges or algae. An interesting method to study cytotoxic activity is to provide a metabolic profiling of the treated cells to understand at which level the compound is interacting. This was the aim of the research conducted by Costantini et al. [129]. The studied sponge was *Geodia cydonium* and it was tested on three breast cancer cell lines, MCF7, MDA-MB-231 and MDA-MB-468. An organic extract of this sponge had a cytotoxic effect with IC_{50} of 37, 44 and 70 µg/mL, respectively, on three cancer cell lines analysed, whereas it was inactive on normal breast cells (MCF-10A). After the treatment, the metabolomic profile was studied through ^{1}H-NMR spectra. Interestingly, the active fraction was able to interfere with the glycolysis, lipid and amino acid metabolism of the tumor cells, enabling them to support their bioenergetic and macromolecular synthesis. In particular, the proton resonances related to the metabolites identified in three breast cancer cells were studied. The spectral region from 0.5 to 3 ppm showed the presence of signals from alanine, arginine, aspartate, glutamate, glutamine, isoleucine, lactate, leucine, lipids, lysine, proline, threonine and valine. The spectral region from 3 to 5.5 ppm was mainly composed of signals corresponding to choline, -glucose, -glucose, glycine, glycero-phosphocholine and phosphocholine. The 5.5–8.5 ppm region contained the resonances of histidine, phenylalanine and tyrosine. They also showed an increasing level of lactate after treatment in all three cell lines, and a decrease of α-glucose, β-glucose, choline, glycerophosphocholine, glutamine, glutamate and lipids. Other metabolites were also reduced: proline in MCF-7 cells, threonine in MDA-MB231 and asparagine and lysine in MDA-MB468 cells, whereas glycine was reduced in both MDA-MB231 and MDA-MB468 cells.

A decrease of pro-inflammatory cytokines (VEGF, CXCL10 and IL-8) levels was detected, as well, along with an increase of anti-inflammatory cytokines levels (IL-4 and IL-10). Finally, the chemical entities present in this fraction were analyzed by liquid chromatography high-resolution mass spectrometry combined with molecular networking.

An additional interesting approach to the brown macroalga *F. vesiculosus* was performed by Heavisides et al. [130], who investigated seasonal variations in the metabolome of this inhabitant of the Baltic Sea and its potential relation to the bioactivity profile. The authors developed an optimised protocol in order to extract algal biomass monthly for a full year, employing UHPLC-MS for untargeted metabolome analysis. Simultaneously,

these crude extracts were screened to evaluate their cytotoxic activity on A-549 (lung adenocarcinoma), MB-231 (breast carcinoma) and Panc1 cell lines, demonstrating that an organic extract of the alga sampled in June exhibited caspase 3/7 activity of 2.2 for the Panc1 cancer cell line. The extracts were also tested for their general toxicity on human keratinocyte (HaCaT) cell lines, against which no activity was recorded, implying a lack of general toxicity for normal cells. In addition, results of this study showed the variation of many compounds according to the sampling month; for example, the highest content in phlorotannin was recorded during the summer period. Literature analyses reference the detection of the antiproliferative effect of *F. vesiculosus* due to phlorotannins [117], fucoidan [131] and fucoxanthin [132]. This study highlighted the importance of the impact of the sampling season on the bioactivity and metabolite profile.

4. Conclusions

Among the "omics" platforms, metabolomics has a great power to aid cancer research, thanks to the possibility for rapid analysis of tissue or biofluid samples. In fact, by coupling metabolite profiling and organism biology, it is possible to provide significant impacts for the discovery of new compounds and define their possible biotechnological applications for blocking the progression of cancer. Metabolic profiling is usually referred to a quantitative study of a group of metabolites that is associated with a given pathway. As demonstrated by the data reported in this review, metabolomics has been extensively used in the last years, with the aim to isolate bioactive compounds from marine organisms (mainly from sponges and algae) against cancer, defining their chemical structures. A few examples, in contrast, may be reported on the use of a metabolomics approach to study how treatment with chemical extracts from sponges and algae could induce metabolite changes in treated cancer cell lines. For this reason, we think that this is a strength of our review, pushing the scientific world to invest in research projects aimed to test marine natural products in metabolomic studies. In fact, we think that this represents a very important application for cancer research, helping in the understanding of changes in the metabolic pathways induced by natural compounds from the sea. This is possible because of a high diversity of marine natural products, which represent promising opportunities for drug discovery and the development of marine biotechnologies. In addition, high-throughput techniques, such as metabolomics, are extremely useful for rapidly exploring the chemical diversity of marine environments with respect to the classical approaches, being also able to detect metabolites when present at low concentrations. Metabolite identification remains the main metabolomics bottleneck, and together with bioinformatic tools, such as molecular networks, it can lead to the annotation of unknown metabolites, leading to the discovery of new compounds. Furthermore, understanding the ecological and biological factors that contribute to the production of a certain metabolite can be extremely useful for selecting and optimizing the extraction of bioactive compounds, enhancing their yields and elucidating gene clusters associated with the biosynthetic pathways to which they belong.

Author Contributions: Conceptualization, R.E., V.Z. and M.C.; performed bibliographic research, R.E., S.F., F.G. and E.S.; original draft preparation, R.E., S.F., F.G. and E.S.; contributed to coordinating the drafting of the manuscript and putting together the various parts of the text written by the other co-authors, R.E.; review and editing, V.Z. and M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Antitumor Drugs and Vaccines from the Sea (ADViSE) project (PG/2018/0494374).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. Serena Federico was supported by the research grant "Antitumor Drugs and Vaccines from the Sea (ADViSE)", project (PG/2018/0494374). Francesca Glaviano was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship at the StazioneZoologica Anton Dohrn. Emanuele Somma was supported by a PhD fellowship cofunded by the StazioneZoologica Anton Dohrn and the University of Trieste. All individuals included in this section have consented to the acknowledgement.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wilson, I.D.; Theodoridis, G.; Virgiliou, C. A perspective on the standards describing mass spectrometry-based metabolic phenotyping (metabolomics/metabonomics) studies in publications. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2021**, *1164*, 122515. [CrossRef] [PubMed]
2. Zhang, A.; Sun, H.; Wang, P.; Han, Y.; Wang, X. Modern analytical techniques in metabolomics analysis. *Analyst.* **2012**, *137*, 293–300. [CrossRef] [PubMed]
3. Rochfort, S. Metabolomics reviewed: A new "omics" platform technology for systems biology and implications for natural products research. *J. Nat. Prod.* **2005**, *68*, 1813–1820. [CrossRef] [PubMed]
4. Wishart, D.S. Applications of metabolomics in drug discovery and development. *Drugs R D* **2008**, *9*, 307–322. [CrossRef] [PubMed]
5. Wishart, D.S. Metabolomics: Applications to food science and nutrition research. *Trends Food Sci. Technol.* **2008**, *19*, 482–493. [CrossRef]
6. Wang, X.; Sun, H.; Zhang, A.; Sun, W.; Wang, P.; Wang, Z. Potential role of metabolomics apporoaches in the area of traditional Chinese medicine: As pillars of the bridge between Chinese and Western medicine. *J. Pharm. Biomed. Anal.* **2011**, *55*, 859–868. [CrossRef]
7. Zhang, A.; Sun, H.; Wang, Z.; Sun, W.; Wang, P.; Wang, X. Metabolomics: Towards understanding traditional Chinese medicine. *Planta Med.* **2010**, *76*, 2026–2035. [CrossRef]
8. Sreekumar, A.; Poisson, L.M.; Rajendiran, T.M.; Khan, A.P.; Cao, Q.; Yu, J.; Laxman, B.; Mehra, R.; Lonigro, R.J.; Li, Y.; et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* **2009**, *457*, 910–914. [CrossRef]
9. Dunn, W.B.; Ellis, D.I. Metabolomics: Current analytical platforms and methodologies. *Trends Anal. Chem.* **2005**, *24*, 285–294. [CrossRef]
10. Goulitquer, S.; Potin, P.; Tonon, T. Mass spectrometry-based metabolomics to elucidate functions in marine organisms and ecosystems. *Mar. Drugs* **2012**, *10*, 849–880. [CrossRef]
11. Kim, H.K.; Choi, Y.H.; Verpoorte, R. NMR-based plant metabolomics: Where do we stand, where do we go? *Trends Biotechnol.* **2011**, *29*, 267–275. [CrossRef] [PubMed]
12. Williams, M.C.; Helton, E.D.; Goldzieher, J.W. Chromatographiic profiling and identification of ethynyl and non-ethynyl compounds. *Steroids* **1975**, *25*, 229–244. [CrossRef]
13. Krstulovic, A.M.; Matzura, C.T.; Bertani-Dziedzic, L.; Cerqueira, S.; Gitlow, S.E. Endogenous levels of free and conjugated urinary 3-methoxy-4-hydroxyphenylethyleneglycol in control subjects and patients with pheochromatography with electrochemical detection. *Clin. Chim. Acta* **1980**, *103*, 109–116. [CrossRef]
14. Muskiet, F.A.J.; Stratingh, M.C.; Stob, G.J.; Wolthers, B.G. Simultaneous determination of the four major catecholamine metabolites and estimation of a serotonin metabolite in urine by capillary gas chromatography of their *tert*-butyldimethylsilyl derivatives. *Clin Chem* **1981**, *27*, 223–227. [CrossRef] [PubMed]
15. Robinson, C.; Hoult, J.R.S.; Waddell, K.A.; Blair, I.A.; Dollery, C.T. Total profiling by GC/NICIMS of the major cyclo-oxygenase products from antigen and leukotriene-challenged guinea-pig lung. *Biochem. Pharmacol.* **1984**, *33*, 395–400. [CrossRef]
16. Weichert, H.; Kolbe, A.; Kraus, A.; Wasternack, C.; Feussner, I. Metabolic profiling of oxylipins in germinating cucumber seedlings-lipoxygenase-dependent degradation of triacylglycerols and biosynthesis of volatile aldehydes. *Planta* **2002**, *215*, 612–619. [CrossRef]
17. Ruocco, N.; Costantini, S.; Zupo, V.; Lauritano, C.; Caramiello, D.; Ianora, A.; Budillon, A.; Romano, G.; Nuzzo, G.; D'Ippolito, G.; et al. Toxigenic effects of two benthic diatoms upon grazing activity of the sea urchin: Morphological, metabolomic and de novo transcriptomic analysis. *Sci. Rep.* **2018**, *8*, 1–13. [CrossRef]
18. Miralto, A.; Barone, G.; Romano, G.; Poulet, S.A.; Ianora, A.; Russo, G.L.; Buttino, I.; Mazzarella, G.; Laablr, M.; Cabrini, M.; et al. The insidious effect of diatoms on copepod reproduction. *Nature* **1999**, *402*, 173–176. [CrossRef]
19. Ruocco, N.; Varrella, S.; Romano, G.; Ianora, A.; Bentley, M.G.; Somma, D.; Leonardi, A.; Mellone, S.; Zuppa, A.; Costantini, M. Diatom-derived oxylipins induce cell death in sea urchin embryos activating caspase-8 and caspase 3/7. *Aquat. Toxicol.* **2016**, *176*, 128–140. [CrossRef]
20. Sansone, C.; Braca, A.; Ercolesi, E.; Romano, G.; Palumbo, A.; Casotti, R.; Francone, M.; Ianora, A. Diatom-derived polyunsaturated aldehydes activate cell death in human cancer cell lines but not normal cells. *PLoS ONE* **2014**, *9*, e0101220. [CrossRef]

21. González-Domínguez, R.; Sayago, A.; Fernández-Recamales, Á. Metabolomics in Alzheimer's disease: The need of complementary analytical platforms for the identification of biomarkers to unravel the underlying pathology. *J. Chromatogr. B* **2017**, *1071*, 75–92. [CrossRef] [PubMed]
22. Dwivedi, P.; Schultz, A.J.; Hill, H.H. Metabolic profiling of human blood by high-resolution ion mobility mass spectrometry (IM-MS). *Int. J. Mass Spectrom.* **2010**, *298*, 78–90. [CrossRef] [PubMed]
23. Segers, K.; Declerck, S.; Mangelings, D.; Heyden, Y.V.; Eeckhaut, A. Van Analytical techniques for metabolomic studies: A review. *Bioanalysis* **2019**, *11*, 2297–2318. [CrossRef] [PubMed]
24. Cevallos-Cevallos, J.M.; Reyes-De-Corcuera, J.I.; Etxeberria, E.; Danyluk, M.D.; Rodrick, G.E. Metabolomic analysis in food science: A review. *Trends Food Sci. Technol.* **2009**, *20*, 557–566. [CrossRef]
25. Bayona, L.M.; de Voogd, N.J.; Choi, Y.H. Metabolomics on the study of marine organisms. *Metabolomics* **2022**, *18*, 17. [CrossRef]
26. Kim, H.K.; Choi, Y.H.; Verpoorte, R. NMR-based metabolomic analysis of plants. *Nat. Protoc.* **2010**, *5*, 536–549. [CrossRef]
27. Houssen, W.E.; Jaspars, M. Isolation of marine natural products. In *Natural Products Isolation*; Humana Press: Totowa, NJ, USA, 2006; pp. 353–390. ISBN 9781617796241.
28. Cutignano, A.; Nuzzo, G.; Ianora, A.; Luongo, E.; Romano, G.; Gallo, C.; Sansone, C.; Aprea, S.; Mancini, F.; D'Oro, U.; et al. Development and application of a novel SPE-method for bioassay-guided fractionation of marine extracts. *Mar. Drugs* **2015**, *13*, 5736–5749. [CrossRef]
29. Ivanišević, J.; Thomas, O.P.; Lejeusne, C.; Chevaldonné, P.; Pérez, T. Metabolic fingerprinting as an indicator of biodiversity: Towards understanding inter-specific relationships among Homoscleromorpha sponges. *Metabolomics* **2011**, *7*, 289–304. [CrossRef]
30. Anderson, P.E.; Mahle, D.A.; Doom, T.E.; Reo, N.V.; DelRaso, N.J.; Raymer, M.L. Dynamic adaptive binning: An improved quantification technique for NMR spectroscopic data. *Metabolomics* **2011**, *7*, 179–190. [CrossRef]
31. Wu, W.; Daszykowski, M.; Walczak, B.; Sweatman, B.C.; Connor, S.C.; Haselden, J.N.; Crowther, D.J.; Gill, R.W.; Lutz, M.W. Peak alignment of urine NMR spectra using fuzzy warping. *J. Chem. Inf. Model.* **2006**, *46*, 863–875. [CrossRef]
32. Forshed, J.; Schuppe-Koistinen, I.; Jacobsson, S.P. Peak alignment of NMR signals by means of a genetic algorithm. *Anal. Chim. Acta* **2003**, *487*, 189–199. [CrossRef]
33. Izquierdo-García, J.L.; Villa, P.; Kyriazis, A.; Del Puerto-Nevado, L.; Pérez-Rial, S.; Rodriguez, I.; Hernandez, N.; Ruiz-Cabello, J. Descriptive review of current NMR-based metabolomic data analysis packages. *Prog. Nucl. Magn. Reson. Spectrosc.* **2011**, *59*, 263–270. [CrossRef] [PubMed]
34. Röst, H.L.; Sachsenberg, T.; Aiche, S.; Bielow, C.; Weisser, H.; Aicheler, F.; Andreotti, S.; Ehrlich, H.C.; Gutenbrunner, P.; Kenar, E.; et al. OpenMS: A flexible open-source software platform for mass spectrometry data analysis. *Nat. Methods* **2016**, *13*, 741–748. [CrossRef] [PubMed]
35. Pluskal, T.; Castillo, S.; Villar-Briones, A.; Orešič, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinform.* **2010**, *11*, 395. [CrossRef] [PubMed]
36. Smith, C.A.; Want, E.J.; O'Maille, G.; Abagyan, R.; Siuzdak, G. XCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal. Chem.* **2006**, *78*, 779–787. [CrossRef]
37. Tsugawa, H.; Cajka, T.; Kind, T.; Ma, Y.; Higgins, B.; Ikeda, K.; Kanazawa, M.; Vandergheynst, J.; Fiehn, O.; Arita, M. MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat. Methods* **2015**, *12*, 523–526. [CrossRef]
38. Saccenti, E.; Hoefsloot, H.C.J.; Smilde, A.K.; Westerhuis, J.A.; Hendriks, M.M.W.B. Reflections on univariate and multivariate analysis of metabolomics data. *Metabolomics* **2014**, *10*, 361–374. [CrossRef]
39. Trygg, J.; Holmes, E.; Lundstedt, T. Chemometrics in metabonomics. *J. Proteome Res.* **2007**, *6*, 469–479. [CrossRef]
40. Marston, A. Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry* **2007**, *68*, 2786–2798. [CrossRef]
41. Soni, N.R. Improve GC separations with derivatization for selective response and detection in novel matrices. *J. Environ. Life Sci.* **2016**, *1*, 14–25.
42. Issaq, H.J.; Van, Q.N.; Waybright, T.J.; Muschik, G.M.; Veenstra, T.D. Analytical and statistical approaches to metabolomics research. *J. Sep. Sci.* **2009**, *32*, 2183–2199. [CrossRef] [PubMed]
43. Poole, C.F. Stationary phases for packed-column supercritical fluid chromatography. *J. Chromatogr. A* **2012**, *1250*, 157–171. [CrossRef] [PubMed]
44. Jandera, P. Stationary and mobile phases in hydrophilic interaction chromatography: A review. *Anal. Chim. Acta* **2011**, *692*, 1–25. [CrossRef] [PubMed]
45. Gu, H.; Pan, Z.; Xi, B.; Asiago, V.; Musselman, B.; Raftery, D. Principal component directed partial least squares analysis for combining nuclear magnetic resonance and mass spectrometry data in metabolomics: Application to the detection of breast cancer. *Anal. Chim. Acta* **2011**, *686*, 57–63. [CrossRef] [PubMed]
46. Lei, Z.; Huhman, D.V.; Sumner, L.W. Mass spectrometry strategies in metabolomics. *J. Biol. Chem.* **2011**, *286*, 25435–25442. [CrossRef]
47. Glish, G.L.; Vachet, R.W. The basics of mass spectrometry in the twenty-first century. *Nat. Rev. Drug Discov.* **2003**, *2*, 140–150. [CrossRef]
48. Villas-Bôas, S.G.; Mas, S.; Åkesson, M.; Smedsgaard, J.; Nielsen, J. Mass spectrometry in metabolome analysis. *Mass Spectrom. Rev.* **2005**, *24*, 613–646. [CrossRef]

49. Chaleckis, R.; Meister, I.; Zhang, P.; Wheelock, C.E. Challenges, progress and promises of metabolite annotation for LC–MS-based metabolomics. *Curr. Opin. Biotechnol.* **2019**, *55*, 44–50. [CrossRef]
50. Lu, X.; Zhao, X.; Bai, C.; Zhao, C.; Lu, G.; Xu, G. LC-MS-based metabonomics analysis. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2008**, *866*, 64–76. [CrossRef]
51. Wu, H.; Guo, J.; Chen, S.; Liu, X.; Zhou, Y.; Zhang, X.; Xu, X. Recent developments in qualitative and quantitative analysis of phytochemical constituents and their metabolites using liquid chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **2013**, *72*, 267–291. [CrossRef]
52. Want, E.J.; Nordström, A.; Morita, H.; Siuzdak, G. From exogenous to endogenous: The inevitable imprint of mass spectrometry in metabolomics. *J. Proteome Res.* **2007**, *6*, 459–468. [CrossRef] [PubMed]
53. Dettmer, K.; Aronov, P.A.; Hammock, B.D. Mass spectrometry-based metabolomics. *Mass Spectrom. Rev.* **2007**, *26*, 51–78. [CrossRef] [PubMed]
54. Miggiels, P.; Wouters, B.; van Westen, G.J.P.; Dubbelman, A.C.; Hankemeier, T. Novel technologies for metabolomics: More for less. *Trends Anal. Chem.* **2019**, *120*, 115323. [CrossRef]
55. Leenders, J.; Frédérich, M.; De Tullio, P. Nuclear magnetic resonance: A key metabolomics platform in the drug discovery process. *Drug Discov. Today Technol.* **2015**, *13*, 39–46. [CrossRef]
56. Weckwerth, W. Metabolomics in Systems Biology. *Annu. Rev. Plant Biol.* **2003**, *54*, 669–689. [CrossRef] [PubMed]
57. Emwas, A.H.; Roy, R.; McKay, R.T.; Tenori, L.; Saccenti, E.; Nagana Gowda, G.A.; Raftery, D.; Alahmari, F.; Jaremko, L.; Jaremko, M.; et al. NMR spectroscopy for metabolomics research. *Metabolites* **2019**, *9*, 123. [CrossRef] [PubMed]
58. Batool, F.; Emwas, A.H.; Gao, X.; Munawar, M.A.; Chotana, G.A. Synthesis and Suzuki Cross-Coupling Reactions of 2,6-Bis(trifluoromethyl)pyridine-4-boronic Acid Pinacol Ester. *Synthesis* **2017**, *49*, 1327–1334. [CrossRef]
59. Elbaz, A.M.; Gani, A.; Hourani, N.; Emwas, A.H.; Sarathy, S.M.; Roberts, W.L. TG/DTG, FT-ICR mass spectrometry, and NMR spectroscopy study of heavy fuel oil. *Energy and Fuels* **2015**, *29*, 7825–7835. [CrossRef]
60. Abdul Jameel, A.G.; Oudenhoven, V.; Van Emwas, A.H.; Sarathy, S.M. Predicting octane number using nuclear magnetic resonance spectroscopy and artificial neural networks. *Energy and Fuels* **2018**, *32*, 6309–6329. [CrossRef]
61. Beckonert, O.; Keun, H.C.; Ebbels, T.M.D.; Bundy, J.; Holmes, E.; Lindon, J.C.; Nicholson, J.K. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat. Protoc.* **2007**, *2*, 2692–2703. [CrossRef]
62. Reo, N.V. NMR-based metabolomics. *Drug Chem. Toxicol.* **2002**, *25*, 375–382. [CrossRef] [PubMed]
63. Godwa, G.A.N.; Raftery, D. Recent advances in NMR-based metabolomics. *Anal. Chem.* **2017**, *89*, 490–510. [CrossRef]
64. Evilia, R.F. Quantitative NMR spectroscopy. *Anal. Lett.* **2001**, *34*, 2227–2236. [CrossRef]
65. Nicholson, J.K.; Wilson, I.D. High resolution proton magnetic resonance spectroscopy of biological fluids. *Prog. Nucl. Magn. Reson. Spectrosc.* **1989**, *21*, 449–501. [CrossRef]
66. Beger, R. A review of applications of metabolomics in cancer. *Metabolites* **2013**, *3*, 552–574. [CrossRef]
67. Warburg, O.; Wind, F.; Negelein, E. The metabolism of tumors in the body. *J. Gen. Physiol.* **1931**, *8*, 519. [CrossRef]
68. Serkova, N.J.; Niemann, C.U. Pattern recognition and biomarker ^{1}H-NMR-based metabolomics. *Expert Rev. Mol. Diagn.* **2006**, *6*, 717–731. [CrossRef] [PubMed]
69. Kim, J.W.; Dang, C.V. Cancer's molecular sweet tooth and the warburg effect. *Cancer Res.* **2006**, *66*, 8927–8930. [CrossRef]
70. Pedersen, P.L. Warburg, me and Hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. *J. Bioenerg. Biomembr.* **2007**, *39*, 211–222. [CrossRef]
71. Dunn, W.B. Current trends and future requirements for the mass spectrometric investigation of microbial, mammalian and plant metabolomes. *Phys. Biol.* **2008**, *5*, 011001. [CrossRef]
72. Armitage, E.G.; Barbas, C. Metabolomics in cancer biomarker discovery: Current trends and future perspectives. *J. Pharm. Biomed. Anal.* **2014**, *87*, 1–11. [CrossRef] [PubMed]
73. Reverter, M.; Rohde, S.; Parchemin, C.; Tapissier-Bontemps, N.; Schupp, P.J. Metabolomics and marine biotechnology: Coupling metabolite profiling and organism biology for the discovery of new compounds. *Front. Mar. Sci.* **2020**, *7*, 613471. [CrossRef]
74. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2012**, *29*, 144–222. [CrossRef] [PubMed]
75. Romano, G.; Costantini, M.; Sansone, C.; Lauritano, C.; Ruocco, N.; Ianora, A. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. Res.* **2017**, *128*, 58–69. [CrossRef]
76. Sagar, S.; Kaur, M.; Minneman, K.P. Antiviral lead compounds from marine sponges. *Mar. Drugs* **2010**, *8*, 2619–2638. [CrossRef]
77. Hirata, Y.; Uemura, D. Halichondrins—Antitumor polyether macrolides from a marine sponge. *Pure Appl. Chem.* **1986**, *58*, 701–710. [CrossRef]
78. Menis, J.; Twelves, C. Eribulin (Halaven): A new, effective treatment for women with heavily pretreated metastatic breast cancer. *Breast Cancer Targets Ther.* **2011**, *3*, 101–111. [CrossRef]
79. Minouni, V.; Ulmann, L.; Pasquet, V.; Mathieu, M.; Picot, L.; Bougaran, G.; Cadoret, J.P.; Morant-Manceau, A.; Schoefs, B. The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Curr. Pharm. Biotechnol.* **2012**, *13*, 2733–2750. [CrossRef]

80. Atkin, S.L.; Backett, S.T.; Mackenzie, G. Topical Formulations Containing Sporopollenin. US Patent 20080311213 A1, 22 July 2014.
81. Balskus, E.P.; Walsh, C.T. The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. *Science* **2010**, *329*, 1653–1656. [CrossRef]
82. Dionisio-Se Se, M.L. Aquatic microalgae as potential sources of UV-screening compounds. *Philipp. J. Sci.* **2010**, *139*, 5–19.
83. Priyadarshani, L.; Rath, B. Commercial and industrial applications of microalgae—A review. *J. Alagl Biomass Util.* **2012**, *3*, 89–100.
84. Lordan, S.; Paul Ross, R.; Stanton, C. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. *Mar. Drugs* **2011**, *9*, 1056–1100. [CrossRef] [PubMed]
85. Gierhart, D.L.; Fox, J.A. Protection against Sunburn and Skin Problems with Orally-Ingested High Dosage Zeaxanthin. US Patent 8088363 B2, 3 January 2012.
86. Guerin, M.; Huntley, M.E.; Olaizola, M. *Haemotococcus astaxanthin*: Applications for human health and nutrition. *Trends Biotechnol.* **2003**, *21*, 210–216. [CrossRef]
87. Tominaga, K.; Hongo, N.; Karato, M.; Yamashita, E. Cosmetic benefits od astaxanthin on humans sujects. *Acta Biochim. Pol.* **2012**, *59*, 43–47. [CrossRef] [PubMed]
88. Walker, T.L.; Purton, S.; Becker, D.K.; Collet, C. Microalgae as bioreactors. *Plant Cell Rep.* **2005**, *24*, 629–641. [CrossRef]
89. Storey, M.A.; Andreassend, S.K.; Bracegirdle, J.; Brown, A.; Keyzers, R.A.; Ackerley, D.F.; Northcote, P.T.; Owen, J.G. Metagenomic exploration of the marine sponge *Mycale hentscheli* uncovers multiple polyketide-producing bacterial symbionts. *MBio* **2020**, *11*, e02997-19. [CrossRef]
90. Esposito, R.; Ruocco, N.; Viel, T.; Federico, S.; Zupo, V.; Costantini, M. Sponges and their symbionts as a source of valuable compounds in cosmeceutical field. *Mar. Drugs* **2021**, *19*, 444. [CrossRef]
91. Mioso, R.; Marante, F.J.T.; Bezerra, R.D.S.; Borges, F.V.P.; Santos, B.V.D.O.; De Laguna, I.H.B. Cytotoxic compounds derived from marine sponges. A review (2010–2012). *Molecules* **2017**, *22*, 8. [CrossRef]
92. Khan, S.; Al-Fadhli, A.A.; Tilvi, S. Discovery of cytotoxic natural products from Red Sea sponges: Structure and synthesis. *Eur. J. Med. Chem.* **2021**, *220*, 113491. [CrossRef] [PubMed]
93. Abdelhameed, R.F.A.; Habib, E.S.; Eltahawy, N.A.; Hassanean, H.A.; Ibrahim, A.K.; Mohammed, A.F.; Fayez, S.; Hayallah, A.M.; Yamada, K.; Behery, F.A.; et al. New cytotoxic natural products from the red sea sponge *Stylissacarteri*. *Mar. Drugs* **2020**, *18*, 241. [CrossRef] [PubMed]
94. El-Hawary, S.S.; Sayed, A.M.; Mohammed, R.; Hassan, H.M.; Rateb, M.E.; Amin, E.; Mohammed, T.A.; El-Mesery, M.; Muhsinah, A.B.; Alsayari, A.; et al. Bioactive brominated oxindole alkaloids from the red sea sponge *Callyspongia siphonella*. *Mar. Drugs* **2019**, *17*, 465. [CrossRef]
95. Einarsdottir, E.; Magnusdottir, M.; Astarita, G.; Köck, M.; Ögmundsdottir, H.M.; Thorsteinsdottir, M.; Rapp, H.T.; Omarsdottir, S.; Paglia, G. Metabolic profiling as a screening tool for cytotoxic compounds: Identification of 3-alkyl pyridine alkaloids from sponges collected at a shallow water hydrothermal vent site North of Iceland. *Mar. Drugs* **2017**, *15*, 52. [CrossRef] [PubMed]
96. Paglia, G.; Kliman, M.; Claude, E.; Geromanos, S.; Astarita, G. Applications of ion-mobility mass spectrometry for lipid analysis. *Anal. Bioanal. Chem.* **2015**, *407*, 4995–5007. [CrossRef] [PubMed]
97. Musa, A.; Elmaidomy, A.H.; Sayed, A.M.; Alzarea, S.I.; Al-Sanea, M.M.; Mostafa, E.M.; Hendawy, O.M.; Abdelgawad, M.A.; Ayoussif, K.; Refaat, H.; et al. Cytotoxic potential, metabolic profiling, and liposomes of *Coscinoderma* sp. crude extract supported by in silico analysis. *Int. J. Nanomedicine* **2021**, *16*, 3861. [CrossRef]
98. Olsen, E.K.; Søderholm, K.L.; Isaksson, J.; Andersen, J.H.; Hansen, E. Metabolomic profiling reveals the N-Acyl-Taurine Geodiataurine in extracts from the marine sponge *Geodia macandrewii* (Bowerbank). *J. Nat. Prod.* **2016**, *79*, 1285–1291. [CrossRef]
99. Li, F.; Peifer, C.; Janussen, D.; Tasdemir, D. New discorhabdin alkaloids from the antarctic deep-sea sponge *Latrunculia biformis*. *Mar. Drugs* **2019**, *17*, 439. [CrossRef]
100. Hasin, O.; Shoham, S.; Kashman, Y.; Ilan, M.; Carmeli, S. Theonellamides J and K and 5-*cis*-Apoa-theopalauamide, bicyclic glycopeptides of the Red Sea sponge *Theonella swinhoei*. *Mar. Drugs* **2022**, *20*, 31. [CrossRef]
101. Mohanty, I.; Podell, S.; Biggs, J.S.; Garg, N.; Allen, E.E.; Agarwal, V. Multi-omic profiling of Melophlus sponges reveals diverse metabolomic and microbiome architectures that are non-overlapping with ecological neighbors. *Mar. Drugs* **2020**, *18*, 124. [CrossRef]
102. Piel, J. Metabolites from symbiotic bacteria. *Nat. Prod. Rep.* **2009**, *26*, 338–362. [CrossRef] [PubMed]
103. Esposito, R.; Federico, S.; Bertolino, M.; Zupo, V.; Costantini, M. Marine Demospongiae: A challenging treasure of bioactive compounds. *Mar. Drugs* **2022**, *20*, 244. [CrossRef]
104. Wang, G. Diversity and biotechnological potential of the sponge-associated microbial consortia. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 545–551. [CrossRef] [PubMed]
105. Cheng, C.; Othman, E.M.; Stopper, H.; Edrada-Ebel, R.A.; Hentschel, U.; Abdelmohsen, U.R. Isolation of Petrocidin A, a new cytotoxic cyclic dipeptide from the marine sponge-derived bacterium *Streptomyces* sp. SBT348. *Mar. Drugs* **2017**, *15*, 383. [CrossRef] [PubMed]
106. Ibrahim, A.; Attia, E.; Hajjar, D.; Anany, M.; Desoukey, S.; Fouad, M.; Kamel, M.; Wajant, H.; Gulder, T.; Abdelmohsen, U. New cytotoxic cyclic peptide from the marine sponge-associated *Nocardiopsis* sp. UR67. *Mar. Drugs* **2018**, *16*, 290. [CrossRef] [PubMed]
107. Shady, N.H.; Tawfike, A.F.; Yahia, R.; Fouad, M.A.; Brachmann, A.O.; Piel, J.; Abdelmohsen, U.R.; Kamel, M.S. Cytotoxic activity of actinomycetes *Nocardia* sp. and *Nocardiopsis* sp. associated with marine sponge *Amphimedon* sp. *Nat. Prod. Res.* **2021**, *36*, 2917–2922. [CrossRef]

108. Hifnawy, M.S.; Hassan, H.M.; Mohammed, R.; Fouda, M.M.; Sayed, A.M.; Hamed, A.A.; AbouZid, S.F.; Rateb, M.E.; Alhadrami, H.A.; Abdelmohsen, U.R. Induction of antibacterial metabolites by co-cultivation of two Red-Sea-sponge-associated actinomycetes *Micromonospora* sp. UR56 and *Actinokinespora* sp. EG49. *Mar. Drugs* **2020**, *18*, 243. [CrossRef]
109. Guiry, M.D.; Guiry, G.M.; Morrisono, L.; Rindi, F.; Mathieson, A.C.; Miranda, S.V.; Parker, B.C.; Langangen, A.; John, D.M.; Barbara, I.; et al. AlgaeBase: An on-line resource for algae. *Cryptogam. Algol.* **2014**, *35*, 105–115. [CrossRef]
110. Ibanez, E.; Herrero, M.; Mendiola, J.A.; Castro-Puyana, M. Extraction and characterization of bioactive compounds with health benefits from marine resources: Macro and micro algae, cyanobacteria, and invertebrates. In *Marine Bioactive Compounds: Souzrces, Characterization and Application*; Springer Science & Business Media: Berlin, Germany, 2012; pp. 55–98. ISBN 9781461412472.
111. Lauritano, C.; Andersen, J.H.; Hansen, E.; Albrigtsen, M.; Escalera, L.; Esposito, F.; Helland, K.; Hanssen, K.; Romano, G.; Ianora, A. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. *Front. Mar. Sci.* **2016**, *3*, 68. [CrossRef]
112. Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2019**, *36*, 122. [CrossRef]
113. Lahaye, M.; Robic, A. Structure and function properties of Ulvan, a polysaccharide from green seaweeds. *Biomacromolecules* **2007**, *8*, 1765–1774. [CrossRef] [PubMed]
114. Thanh, T.T.T.; Quach, T.M.T.; Nguyen, T.N.; Vu Luong, D.; Bui, M.L.; Tran, T.T. Van Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. *Int. J. Biol. Macromol.* **2016**, *93*, 695–702. [CrossRef] [PubMed]
115. Mofeed, J.; Deyab, M.; El, A.; Sabry, N.; Ward, F. In vitro anticancer activity of five marine seaweeds extract from Egypt against human breast and colon cancer cell lines. *Res. Sq.* **2021**, *11*, 583–589.
116. Geisen, U.; Zenthoefer, M.; Peipp, M.; Kerber, J.; Plenge, J.; Managò, A.; Fuhrmann, M.; Geyer, R.; Hennig, S.; Adam, D.; et al. Molecular mechanisms by which a *Fucus vesiculosus* extract mediates cell cycle inhibition and cell death in pancreatic cancer cells. *Mar. Drugs* **2015**, *13*, 4470–4491. [CrossRef] [PubMed]
117. Zenthoefer, M.; Geisen, U.; Hofmann-Peiker, K.; Fuhrmann, M.; Kerber, J.; Kirchhöfer, R.; Hennig, S.; Peipp, M.; Geyer, R.; Piker, L.; et al. Isolation of polyphenols with anticancer activity from the Baltic Sea brown seaweed *Fucus vesiculosus* using bioassay-guided fractionation. *J. Appl. Phycol.* **2017**, *29*, 2021–2037. [CrossRef]
118. Martin, L.J. Fucoxanthin and its metabolite fucoxanthinol in cancer prevention and treatment. *Mar. Drugs* **2015**, *13*, 4784–4798. [CrossRef]
119. Rokkaku, T.; Kimura, R.; Ishikawa, C.; Yasumoto, T.; Senba, M.; Kanaya, F.; Mori, N. Anticancer effects of marine carotenoids, fucoxanthin and its deacetylated product, fucoxanthinol, on osteosarcoma. *Int. J. Oncol.* **2013**, *43*, 1176–1186. [CrossRef]
120. Shao, P.; Chen, X.; Sun, P. In vitro antioxidant and antitumor activities of different sulfated polysaccharides isolated from three algae. *Int. J. Biol. Macromol.* **2013**, *62*, 155–161. [CrossRef]
121. Abreu, A.C.; Molina-Miras, A.; Aguilera-Saéz, L.M.; López-Rosales, L.; Cerón-Garciá, M.D.C.; Sánchez-Mirón, A.; Olmo-Garciá, L.; Carrasco-Pancorbo, A.; Garciá-Camacho, F.; Molina-Grima, E.; et al. Production of amphidinols and other bioproducts of interest by the marine microalga *Amphidinium carterae* unraveled by nuclear magnetic resonance metabolomics approach coupled to multivariate data analysis. *J. Agric. Food Chem.* **2019**, *67*, 9667–9682. [CrossRef]
122. AtaseverArslan, B.; Yılancıoğlu, K.; KuşoğluGültekin, S.; Albayrak, İ. Chemical constituent of *Isochrysis galbana* microalgae extract and its cytotoxic activities on leukemic cell lines. *İstanbul J. Pharm.* **2022**, *52*, 64–68. [CrossRef]
123. Karakaş, C.Y.; TekarslanŞahin, H.; İnan, B.; Özçimen, D.; Erginer, Y. In vitro cytotoxic activity of microalgal extracts loaded nano–micro particles produced via electrospraying and microemulsion methods. *Biotechnol. Prog.* **2019**, *35*, e2876. [CrossRef] [PubMed]
124. Hussein, H.A.; Mohamad, H.; Ghazaly, M.M.; Laith, A.A.; Abdullah, M.A. Cytotoxic effects of *Tetraselmis suecica* chloroform extracts with silver nanoparticle co-application on MCF-7, 4 T1, and Vero cell lines. *J. Appl. Phycol.* **2020**, *32*, 127–143. [CrossRef]
125. Hussein, H.A.; Kassim, M.N.I.; Maulidiani, M.; Abas, F.; Abdullah, M.A. Cytotoxicity and 1H-NMR metabolomics analyses of microalgal extracts for synergistic application with Tamoxifen on breast cancer cells with reduced toxicity against Vero cells. *Heliyon* **2022**, *8*, e09192. [CrossRef] [PubMed]
126. Fayyad, R.J.; Ali, A.N.M.; Dwaish, A.S.; Al-Abboodi, A.K.A. Anticancer activity of *Spirulina platensis* methanolic extracts against L20B and MCF7 human cancer cell lines. *Plant Arch.* **2019**, *19*, 1419–1426.
127. El-Baz, F.K.; Hussein, R.A.; Mahmoud, K.; Abdo, S.M. Cytotoxic activity of carotenoid rich fractions from *Haematococcus pluvialis* and *Dunaliella salina* microalgae and the identification of the phytoconstituents using LC-DAD/ESI-MS. *Phyther. Res.* **2018**, *32*, 298–304. [CrossRef] [PubMed]
128. Savio, S.; Farrotti, S.; Paris, D.; Arnaìz, E.; Díaz, I.; Bolado, S.; Muñoz, R.; Rodolfo, C.; Congestri, R. Value-added co-products from biomass of the diatoms *Staurosirella pinnata* and *Phaeodactylum tricornutum*. *Algal Res.* **2020**, *47*, 101830. [CrossRef]
129. Costantini, S.; Guerriero, E.; Teta, R.; Capone, F.; Caso, A.; Sorice, A.; Romano, G.; Ianora, A.; Ruocco, N.; Budillon, A.; et al. Evaluating the effects of an organic extract from the mediterranean sponge *Geodia cydonium* on human breast cancer cell lines. *Int. J. Mol. Sci.* **2017**, *18*, 2112. [CrossRef]
130. Heavisides, E.; Rouger, C.; Reichel, A.F.; Ulrich, C.; Wenzel-Storjohann, A.; Sebens, S.; Tasdemir, D. Seasonal variations in the metabolome and bioactivity profile of *Fucus vesiculosus* extracted by an optimised, pressurised liquid extraction protocol. *Mar. Drugs* **2018**, *16*, 503. [CrossRef]

131. Ale, M.T.; Maruyama, H.; Tamauchi, H.; Mikkelsen, J.D.; Meyer, A.S. Fucoidan from *Sargassum sp.* and *Fucus vesiculosus* reduces cell viability of lung carcinoma and melanoma cells in vitro and activates natural killer cells in mice in vivo. *Int. J. Biol. Macromol.* **2011**, *49*, 331–336. [CrossRef]
132. Zaragozá, M.C.; López, D.; Sáiz, M.P.; Poquet, M.; Pérez, J.; Puig-Parellada, P.; Màrmol, F.; Simonetti, P.; Gardana, C.; Lerat, Y.; et al. Toxicity and antioxidant activity in vitro and in vivo of two *Fucus vesiculosus* extracts. *J. Agric. Food Chem.* **2008**, *56*, 7773–7780. [CrossRef]

marine drugs

MDPI

Review

Multiple Roles of Diatom-Derived Oxylipins within Marine Environments and Their Potential Biotechnological Applications

Nadia Ruocco [1], Luisa Albarano [1,2,†], Roberta Esposito [1,2,†], Valerio Zupo [1,*], Maria Costantini [1,*] and Adrianna Ianora [1]

[1] Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; nadia.ruocco@gmail.com (N.R.); luisa.albarano@szn.it (L.A.); roberta.esposito@szn.it (R.E.); ianora@szn.it (A.I.)

[2] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Napoli, Italy

* Correspondence: valzupo@szn.it (V.Z.); maria.costantini@szn.it (M.C.); Tel.: +39-081-5833503 (V.Z.); +39-081-583-3315 (M.C.); Fax: +39-081-764-1355 (M.C.)

† These authors contributed equally to this work.

Received: 2 June 2020; Accepted: 24 June 2020; Published: 30 June 2020

Abstract: The chemical ecology of marine diatoms has been the subject of several studies in the last decades, due to the discovery of oxylipins with multiple simultaneous functions including roles in chemical defence (antipredator, allelopathic and antibacterial compounds) and/or cell-to-cell signalling. Diatoms represent a fundamental compartment of marine ecosystems because they contribute to about 45% of global primary production even if they represent only 1% of the Earth's photosynthetic biomass. The discovery that they produce several toxic metabolites deriving from the oxidation of polyunsaturated fatty acids, known as oxylipins, has changed our perspectives about secondary metabolites shaping plant–plant and plant–animal interactions in the oceans. More recently, their possible biotechnological potential has been evaluated, with promising results on their potential as anticancer compounds. Here, we focus on some recent findings in this field obtained in the last decade, investigating the role of diatom oxylipins in cell-to-cell communication and their negative impact on marine biota. Moreover, we also explore and discuss the possible biotechnological applications of diatom oxylipins.

Keywords: biotechnology; chemical ecology; diatoms; oxylipins

1. Introduction

Diatoms are unicellular photosynthetic eukaryotes (class *Bacillariophyceae*), the primary production of which is the major driving force for incorporating organic carbon into the oceans thereby playing a central role in the biological carbon pump from the surface to deep sea [1,2]. Diatoms account for the highest number of plant species living in both marine and freshwater ecosystems and, due to a large variety of structural features, an estimation of the right number of species appears to be extremely difficult [3–5]. The chemical ecology of diatoms has been widely discussed, since the discovery of secondary metabolites, which negatively affect the reproduction of marine invertebrates. Known as the paradox of diatom–copepod interactions in pelagic food webs, since diatoms can both provide a source of energy for copepod larval growth and reduce their fecundity and/or hatching success [6–8].

This biological model is new and has no other equivalent in marine plant–herbivore systems, since most negative plant–animal interactions are generally related to repellent or poisoning processes, but never to reproductive failure (reviewed by Ianora and Miralto [9]). Moreover, when environmental

conditions (sunlight intensity or nutrient concentrations) are optimal for the massive production of diatoms (algal blooms), the negative influence of toxic compounds may severely impact target consumers [10–14].

The enzymatic cascade leading to oxylipin production involves lipoxygenase (LOX)/hydroperoxide lyase (HPL) enzymes, which convert polyunsaturated fatty acids (PUFAs) into fatty acid hydroperoxides that are, in turn, converted into a plethora of compounds through mechanisms that are still largely unknown [15–20]. Oxylipins are a large family of compounds comprising polyunsaturated aldehydes (PUAs), firstly identified from *Thalassiosira rotula* [7], and other fatty acid derivatives with hydroxy-, keto-, oxo- and hydroxy-epoxy units, generically named non-volatile oxylipins [21] and recently reported as linear oxygenated fatty acids [22]. Several studies have suggested that, in addition to PUAs and oxygenated fatty acids, fatty acid hydroperoxides can trigger impacts on marine biota because these primary LOX products are reactive oxygen species (ROS), inducing DNA and protein damage that contribute to cell ageing [19]. Despite the negative impact observed in marine invertebrates, some volatile oxylipins were also proposed as odour compounds, attracting herbivores towards their food, thus suggesting that the function of oxylipins could change depending on the ecological context of diatom–invertebrate interactions [23–25].

Lipoxygenases (LOXs) constitute a family of dioxygenases that catalyse the oxygenation of free and esterified polyunsaturated fatty acids containing a (1Z,4Z)-penta-1,4-diene system to produce the corresponding hydroperoxy derivatives [25]. LOXs are expressed in plants [26] and in the animal kingdom [27,28], but have not been found in bacteria and yeast [29].

Chemical analyses of mono-algal cultures has revealed strictly LOX species-specificity [30], where the most common pathway shared by different genera of diatoms rely on a 15*S*-LOX activity, and a minor group of oxylipins are the products of 5-LOX, 8-LOX, 9*S*-LOX, 11-LOX, 12-LOX and 14-LOX [19,20,22,30–34] activity, depending on the specific regiochemistry of carbon oxidation. Oxylipin quantification and variation in time and space has been evaluated in field studies [35–41]. A recent survey demonstrated that oxylipin pathways in diatoms were mostly based on the oxygenation of hexadecatrienoic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, and, within phytoplankton communities, these secondary metabolites largely derived from diatoms [42]. Moreover, daily fluctuations of PUAs were more correlated to the cellular physiological state of diatoms than exclusively to the taxonomical composition of phytoplankton communities [43].

Interestingly, terrestrial plants also produce oxylipins in response to pathogen infections but differently from those described in marine diatoms. LOX substrates mostly consist of linoleic acid, α-Linolenic acid and hexadecatrienoic acid [44–47]. In analogy to plants, the production of oxylipins in diatoms was considered as a chemical defence against grazers. In fact, diatom-based diets or treatments with pure molecules induced a detrimental effect on gamete viability, embryogenesis and larval fitness of marine invertebrates, such as polychaetes, echinoderms, ascidians, crustaceans and molluscs [48].

Diatoms also produced a variety of bioactive secondary metabolites acting as chemical signals within phytoplankton communities [49–52]. Since PUAs were demonstrated to inhibit cell growth in diatom cultures and associated bacteria [53–58], a possible role as allelochemicals/infochemicals regulating the ecological success of diatom populations was proposed [59–62]. A few studies additionally demonstrated that PUAs exerted antiproliferative activities on human cancer cell lines promoting the activation of apoptotic pathways [7,63]. These findings have thus suggested that oxylipins could be used as a suitable source of new anticancer therapies.

2. Detrimental Impact of Oxylipins on Marine Invertebrates

In the last decade, several studies have explored the negative impact of oxylipins, mainly using the pure molecules of commercially available PUAs, 2*E*,4*E*-heptadienal (HD), 2*E*,4*E*-octadienal (OD), 2*E*,4*E*,7*Z*-octatrienal (OT), 2*E*,4*E*-decadienal (DD) and 2*E*,4*E*,7*Z*-decatrienal (DT), and four oxygenated fatty acids with hydroxy functionalities, called hydroxyacids, 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoic acid (5-HEPE), 9-hydroxy-5*Z*,7*E*,11*Z*,14*Z*,17*Z*-eicosapentaenoic

acid (9-HEPE), 11-hydroxy-5*Z*,8*Z*,12*E*,14*Z*,17*Z*-eicosapentaenoic acid (11-HEPE) and 15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*,17*Z*-eicosapentaenoic acid (15-HEPE). The chemical structures are reported in Figure 1.

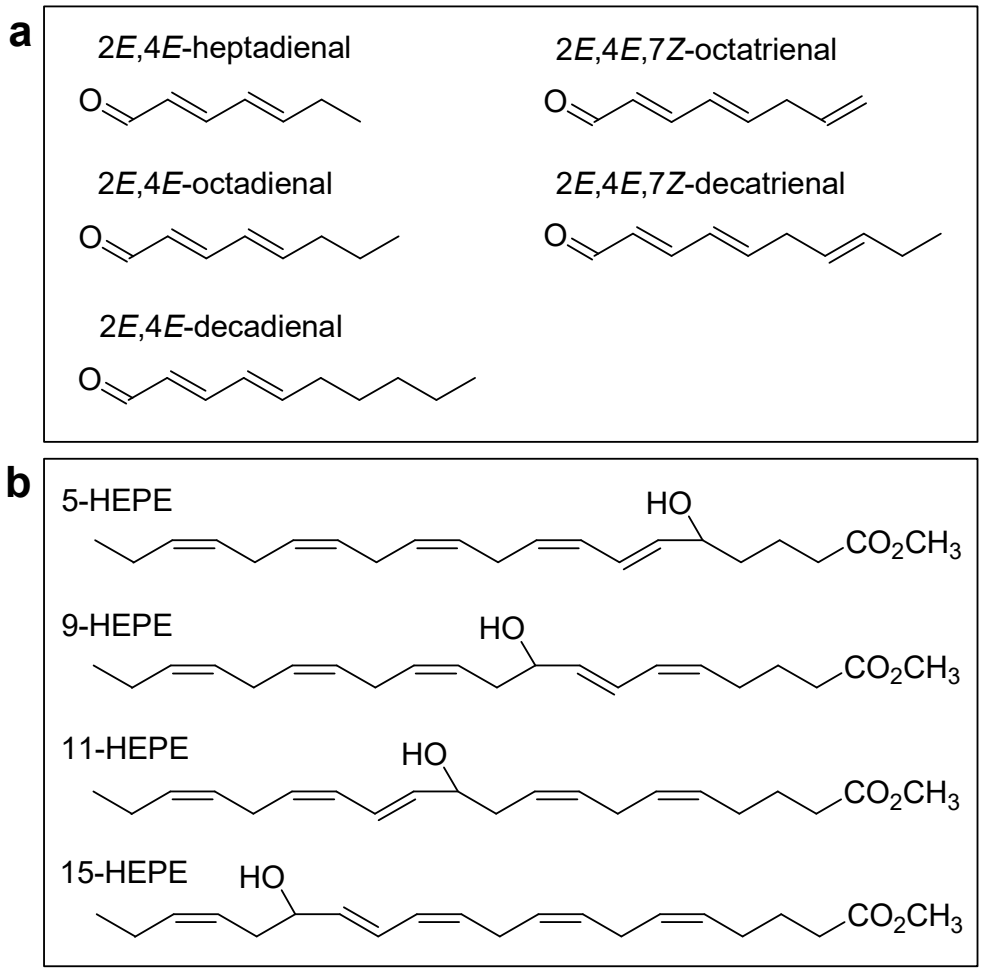

Figure 1. Chemical structures of commercially available PUAs (**a**) and HEPEs (**b**) used in experiments evaluating harmful effects of oxylipins on invertebrate reproduction and survival. Oxylipins were designed using ChemDraw Pro v8.0 software.

All these studies were performed on the reproduction of sea urchins, tunicates and copepods, which are common diatom feeders in both benthic and planktonic environments.

The greatest novelty of these studies has been the introduction of genomic approaches to explain the effects of oxylipins on gene expression patterns in these organisms. Comparisons of these results indicate that some common pathways are activated in response to grazing of diatom-producing oxylipins by these marine invertebrates belonging to different phyla, as shown in Figure 2.

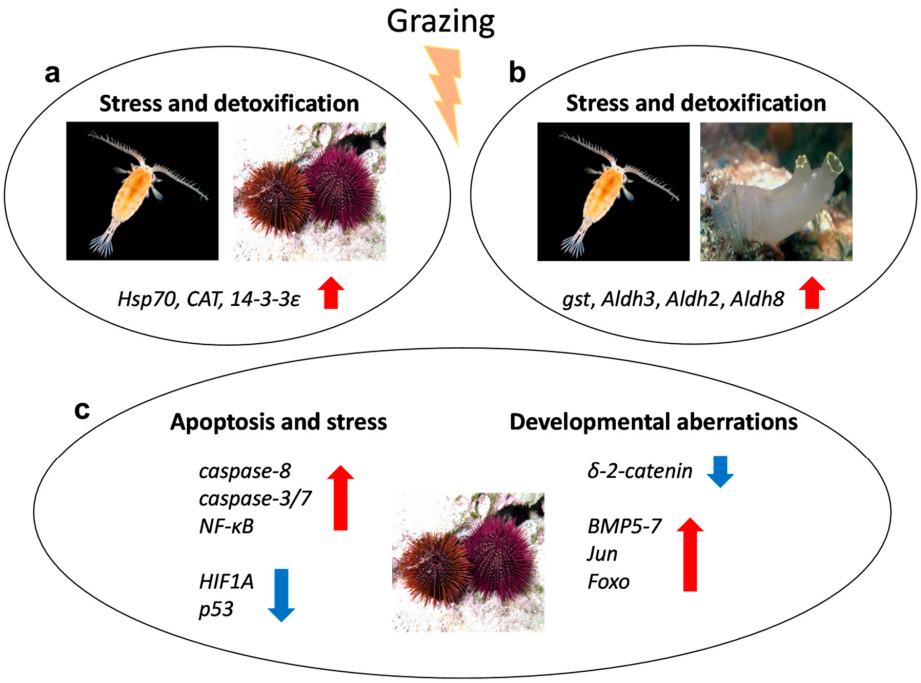

Figure 2. Molecular response of marine invertebrates to diatom's oxylipins; possible common molecular pathway between copepods vs. sea urchins (**a**) and copepods vs. tunicates (**b**), together with some of the mostly strongly affected genes in sea urchins (**c**). Red arrows indicate upregulation of gene expression; blue arrows indicate downregulation of genes. Photos of copepods, sea urchins and tunicates were retrieved from the website https://www.marinespecies.org/.

2.1. Sea Urchins

Overall, the experimental evaluation of the effects of oxylipins on sea urchins were mainly conducted through in vitro tests with the pure molecules of PUAs and HEPEs (Table 1).

Table 1. Species, oxylipins or diatom diets, concentrations tested and morphological and molecular effects (highlighting the most representative results) reported in the literature on sea urchins during 2010–2020. Abbreviations: DD, 2*E*,4*E*-decadienal; HD, 2*E*,4*E*-heptadienal; OD, 2*E*,4*E*-octadienal; OT, 2*E*,4*E*,7*Z*-octatrienal; 5-HEPE, 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoic acid; 9-HEPE, 9-hydroxy-5*Z*,7*E*,11*Z*,14*Z*,17*Z*-eicosapentaenoic acid; 11-HEPE, 11-hydroxy-5*Z*,8*Z*,12*E*,14*Z*,17*Z*-eicosapentaenoic acid; 15-HEPE, 15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*,17*Z*-eicosapentaenoic acid.

Species	Oxylipins (μM)/Diatom	Morphological Effects	Molecular Effects	Reference
P. lividus	DD, HD, OD and OT (0.658–32)	Cleavage inhibition; Malformed plutei with decadienal	Not detected	[64]
P. lividus	DD (0.002–0.03)	Increase of endogenous NO levels and consequently apoptosis induction	Upregulation of *hsp70* and *caspase-8*; downregulation of *NOS*	[65]
P. lividus	DD (0.001–0.0023)	Differentially expressed genes with dose dependent effect	Upregulation of *hsp70*, *hsp56*, *hsp60*, *hat*, *BP10*, *14-3-3ε*, *p38 MAPK*, *GS* and *MTase*; downregulation of *sox9*, *uni*, *SM30*, *Nec* and *SM50*	[66]
P. lividus	DD (0.5–2.5), HD (1.0–6.0) and OD (2.0–9.0)	Dose-dependent malformations of sea urchin plutei	Upregulation of *hsp70*, *hat*; downregulation of *SM50*, *Wnt6*, *MT4* and *MT6*	[67]

Table 1. *Cont.*

Species	Oxylipins (μM)/Diatom	Morphological Effects	Molecular Effects	Reference
S. droebachiensis *E. acutus*	*C. socialis*, *S. marinoi*, *C. furcellatus*, *A. longicornis*, *T. gravida* and *P. glacialis* (20 and 50 μg/L)	Blocks of first mitotic division after 4 hpf with *S. marinoi*	Not detected	[68]
E. mathaei	DD (0.4–1.2), HD (0.5–1.5) and OD (0.6–1.7)	Dose-dependent malformations of sea urchin plutei	Not detected	[69]
P. lividus	5-, 9-, 11-, 15-HEPE (100), DD (3.3), HD (9.0) and OD (11.0)	Impairment at blastula and pluteus stage with PUAs and HEPEs	Activation of *caspase-8* and *caspase-3/7*	[70]
P. lividus	DD (1.0–2.3), HD (2.0–6.0) and OD (2.5–8.0)	Not detected	Upregulation of *MTase* and *p38 MAPK*; downregulation of *MT6*, *CAT Alix* and *SM50*	[71]
P. lividus	5- and 15-HEPE (6–30)	Dose-dependent malformations of sea urchin plutei	Upregulation of *hsp70*, *hsp56*, *14-3-3ε*, *Blimp* and *MT5*; downregulation of *HIF1A* and *SM50*	[72]
P. lividus	DD (1.6), HD (3.0) and OD (4.5)	Not detected	Upregulation of *Jun* and *Foxo*	[73]
P. lividus	Mixture of 5-, 9-, 11-, 15-HEPEs (1.0–7.0)	Synergic effect of HEPEs	Downregulation of *MTase*, *p38 MAPK* and *Alix*	[74]
P. lividus	*N. shiloi* (1.8) and *C. closterium* (1.6)	Malformed plutei	Upregulation of *Blimp*, *hsp70*, hsp60, *GS*, *cytb*, *14-3-3ε*, *Nec*, *p19*, *jun*, *Blimp*, *Wnt6*, *nodal*, *FoxG*, *Foxo*, *OneCut*, *MT*, *CAT*, *MDR1*; downregulation of *MTase*, *p53*, *HIF1A*, *SM30*, *BMP5/7*, *uni*, *FOXA*, *GFI1*, *δ-2-catenin*, *VEGF* and *MT8*	[75]
P. lividus	Mixture of DD (0.5), HD (1.0) and OD (1.5)	Synergic effect of PUAs	Downregulation of *cytb*, *caspase-8*, *Alix*, *δ-2-catenin*, *tcf4*, *GFI1*, *OneCut*, *TAK1* and *MT7*	[76]
P. lividus	*C. scutellum* (1.5) and *Diploneis* sp. (1.6)	Malformed plutei with *Diploneis* sp.	Upregulation of *p53*, *GS*, *Alix*, *Wnt5*, *NF-kB*, *ERCC3*, *p16*, *MT*, *CAT* and *MDR1*; downregulation of *δ-2-catenin*, *hsp70*, *hsp60*, *tcf4* and *MT8*	[77]
P. lividus	Mixture of PUAs (DD 0.3, HD 0.7 and OD 1) and HEPEs (1.6)	Higher morphological effects than those detected with individual oxylipins and PUAs/HEPEs mixtures	Upregulation of *ADMP2*, *Delta*, *Goosecoid*, *KIF19*, *jun* and *CAT*; downregulation of *ARF1*, *GS*, *HIF1A* and *sox9*	[78]

Firstly, Romano et al. [64] demonstrated that, when the eggs of the sea urchin *Paracentrotus lividus* were incubated with four different PUAs (DD, OD, OT and HD), a severe dose-dependent block of the first cleavage was induced, with decadienal exerting the greatest activity. Moreover, at lower concentrations (1.32–5.26 μM), decadienal induced an increase in the number of malformed and delayed embryos at 48 h post-fertilisation (hpf, pluteus stage), with a high number of positive embryos to the Tdt-mediated dUTP nick end labelling (TUNEL) assay, indicating imminent death in embryos and larvae [64]. Subsequently, treatments with DD on sea urchin embryos exhibited increasing levels in the production of endogenous nitric oxide (NO) in a dose-dependent manner [65]. At high DD concentrations (>2.5 μg/mL), NO levels led to the activation of apoptotic events, whereas, at low concentrations (0.25 μg/mL), NO protected sea urchin embryos against teratogenesis by upregulating *hsp70* gene expression. However, at the pluteus stage, NO was not able to exert its positive role, since *hsp70* and *nitric oxide synthase* levels decreased and *caspase-8* gene, that normally initiates apoptotic signalling, was found upregulated [65]. The effects of two PUAs, HD and OD, together with the

already studied DD, were investigated on embryo development of *P. lividus* [67]. The authors reported that PUAs caused similar malformations affecting the apex and the arms in a dose-dependent manner, with DD inducing the strongest effects, acting in a very narrow range of concentrations (0.5–2.5 μM) in comparison to HD and OD (1.0–6.0 and 2.0–9.0 μM, respectively). The same authors conducted post-recovery experiments revealing that treated embryos were able to recover depending on both PUA concentrations and washing time [67]. The same PUAs (DD, HD and OD) were also tested on embryo development of the Pacific sea urchin *Echinometra mathae* [69]. PUA toxicity scale (HD>OD>DD), evaluated at the *morula* and *pluteus* stages, was in contrast with previous results [67] and explained as an inverse correlation between PUA toxicity and carbon chain length. Specifically, the authors showed that HD induced 97% of abnormal plutei, while OD and DD induced lower percentages (72% and 28%, respectively) at 0.125 mg/mL. This result revealed a possible species-specific sensitivity, rendering sea urchins of different genera and/or living in different habitats differentially responsive to environmental toxins [69].

As mentioned above, several studies were also conducted on HEPEs, another class of oxylipins belonging to the non-volatile oxygenated fatty acids. Varrella et al. [72] tested the impact of 5- and 15-HEPEs on the sea urchin *P. lividus* for the first time. Experimental data showed that both HEPEs were not able to block the first cleavage, and, compared to the effects already observed for PUAs [67], HEPEs were found to be less active even if they induced the same types of malformations in embryos and larvae. However, HEPEs caused a developmental delay still detectable at a concentration of 7 μM, which prevailed at 30 μM, where treated embryos were all at the early pluteus stage instead of pluteus stage [72]. Conversely to PUAs [67], post-recovery experiments indicated that embryos were unable to undergo normal development when eggs were washed in seawater without HEPEs after HEPE treatment [72]. Moreover, to further explore the apoptogenic capabilities of oxylipins [65], the activation of *caspase 3/7* and *caspase 8* genes was followed in sea urchin embryos treated with two PUAs (HD and OD) and four HEPEs (5-, 9-, 11- and 15-HEPE) [70]. In particular, both classes of compounds induced apoptosis, mostly at 9 and 24 hpf, detected by the luminometric assay and real time qPCR. Microscope observations showed that embryos subjected to PUA treatments were dead at 48 hpf, whereas HEPEs induced a developmental delay at both blastula and *pluteus* stages, confirming that PUAs greatly impacted sea urchin embryo development [70].

Several molecular approaches were also applied in order to explore the gene pathways activated by PUAs and HEPEs. Marrone et al. [66] revealed that the expression of sixteen genes, involved in stress response, skeletogenesis and development/differentiation processes, was significantly affected by DD, in a dose-dependent manner. In this study, the authors suggested that these genes are part of the "defensome": genes and proteins integrated in a functional network able to protect an organism against natural toxins and xenobiotics [66]. The expression of these sixteen and other genes was also altered by HD and OD [67]. These targeted genes, examined by interactomic analysis (Ingenuity Pathway Analysis, IPA) were found functionally correlated to four *HUB* genes, *NF-κB*, *p53*, *δ-2-catenin* and *HIF1A*, which, in turn, were affected by PUA exposure [71]. In a similar study, IPA analysis was applied to further explore the molecular pathway involved in the response to PUAs in *P. lividus*. In particular, an additional twelve genes (*FOXA*, *FoxG*, *GFI-1*, *nodal*, *JNK*, *OneCut/Hnf6*, *TAK1*, *tcf4*, *TCF7*, *VEGF*, *Foxo* and *Jun*), linked to those isolated in previous studies [67,71], were also shown to modify their expression in sea urchin embryos treated with PUAs [73]. Molecular analyses using the primer pairs for the same genes analysed by Varrella et al. [67,71] and Ruocco et al. [73] revealed that 5- and 15-HEPEs had very few common molecular targets, with 5-HEPE switching on the highest number of genes, mainly at the early and swimming blastula stages [72].

Since marine organisms are normally exposed to LOX products as a whole, very recent studies were conducted to evaluate the potentially negative effect of PUAs and HEPEs mixtures on the sea urchin *P. lividus* [74,76,78]. Specifically, Ruocco et al. [76] showed that, by decreasing PUAs concentrations to one third of those used in individual tests (reported in Varrella et al. [67]), both binary and ternary mixtures were able to induce malformations in a synergic way, with the highest percentage of malformed

plutei achieved in the case of 0.5 μM DD plus 1.0 μM HD at 48 hpf. A similar study [74] was done with combinations of the four HEPEs already tested separately in a previous study [72]. In particular, Albarano and co-workers observed several malformations that were much more severe compared to those reported in individual tests, revealing, also in this case, a synergic effect of these natural toxins. From the molecular viewpoint, these mixtures induced an additive effect when compared to experiments with single compounds [67,72], since a greater number of genes were affected [74,76]. Interestingly, PUA mixtures affected gene expression mainly at 48 hpf [76], while HEPEs were most effective in early developmental stages (particularly at 5 hpf) [74], confirming the inability of sea urchin embryos to recover after HEPE treatment [72].

The effects of oxylipin combinations on sea urchin embryos were further clarified by testing PUAs plus HEPEs mixtures [78]. Morphological observations revealed that these mixtures induced a stronger effect, compared to single compounds, with a dose-dependent developmental delay. Differently to individual tests, the high capability of PUAs to cause abnormalities was almost completely reverted by the presence of HEPEs in the same mixture. In fact, even if PUAs in individual tests resulted stronger than HEPEs, when in mixtures PUAs + HEPEs the effects of HEPEs diluted those of PUAs. In fact, in the first 48 hpf, oxylipin mixtures only induced developmental delay in sea urchin embryos and no malformed embryos were detected [78]. Moreover, IPA analysis led to the isolation of twelve new genes that were functionally correlated to eleven genes already identified in previous studies [67,71,73]. Real time qPCR analyses revealed that almost all of the genes, belonging to stress and developmental processes were significantly altered (>2-fold) [78]. Taken together, all these results strongly indicate that the delay observed in the early development of sea urchins exposed to oxylipin mixtures may be due to HEPEs, which act in an irreversible way, targeting many genes involved in skeletogenesis and development/differentiation processes already at the blastula stage.

All of the above studies reported the effects of in vitro tests on sea urchin eggs with commercially available pure molecules, but data from in vivo exposure to diatom-producing oxylipins are quite scarce. Gudimova et al. [68] conducted several tests incubating the eggs of the sea urchins *Strongylocentrotus droebachiensis* and *Echinus acutus* with the diatoms *Chaetoceros socialis*, *Skeletonema marinoi*, *Chaetocerus furcellatus*, *Attheya longicornis*, *Thalassiosira gravida* and *Porosira glacialis*. Specifically, to define the effects on sea urchin embryo development and survival, they used two diatom concentrations corresponding to the highest and lowest levels found during the spring bloom. At low (20 μg/L) and high (50 μg/L) concentrations, *S. marinoi* was the diatom causing the strongest impairment in the first cleavage of eggs of *S. droebachiensis* after 4 h of exposure and cell death in both sea urchins after 24 h of treatment [68]. The stronger impact of *S. marinoi* could be explained by its capability to release PUAs from cells before reaching the decline phase [79,80]. Feeding experiments with the same diatom species on *S. droebachiensis* plutei showed that the 4-arm plutei solely ingested *A. longicornis* species, the least harmful diatom in egg exposure experiments, while the other species triggered a high mortality rate (100% in the case of *T. gravida*). Regarding the six-arm plutei, no mortalities were recorded, revealing that probably the early stages of development were more sensitive to diatom toxins [68].

Very recently, feeding experiments were also conducted on adult *P. lividus* using four benthic diatom species in order to explore their negative impact on sea urchins compared to planktonic diatom species [75,77]. In particular, one-month of feeding on *Nanofrustulum shiloi*, *Cylindrotheca closterium* and *Diploneis* sp. induced malformations in sea urchin plutei spawned from diatom-fed individuals, with *N. shiloi* being the most toxic diet (55% of malformed plutei). The fourth species, *Cocconeis scutellum*, did not induce any effect on sea urchin offspring with a percentage of abnormal plutei very similar to controls (about 10%). De novo transcriptome approaches also revealed that benthic species affected several molecular pathways with very few common targets [75,77]. The highest activity detected in *N. shiloi* species may be explained by chemical analyses [22]. In fact, *N. shiloi* revealed a high content of both oxygenated fatty acids and PUAs, while *C. closterium* was less rich in oxylipins and produced mainly non-volatile oxygenated fatty acids. Interestingly, *Diploneis* sp., inducing about 40%

of malformed plutei, exhibited several unknown compounds, probably related to a LOX-independent fatty acids metabolism [22]. According to feeding experiments, in which no negative effects were detected [77], a total absence of oxylipins was found in *C. scutellum* [22]. This result supports those by Zupo et al. [81] that post-larval feeding with *C. scutellum* induces only positive effects such as post-larvae settlement (about 63%) and survival.

Lipoxygenase activity has been reported in the sea urchin *Strongylocentrotus purpuratus*, leading to the formation of four hydroxyeicosanoids in homogenates of eggs, (11R)-hydroxy-5,8,12,14-ZZEZ-eicosatetraenoic acid and (12R)-hydroxy-5,8,10,14-ZZEZ eicosatetraenoic acid (from arachidonic acid) and the corresponding (11R)- and (12R)-hydroxy analogues of eicosapentaenoic acid [29,82,83]. No data are available on the sea urchin *P. lividus*. To date, gene sequencing confirms the presence of lipoxygenases in the sea urchin *S. purpuratus* and in other marine invertebrates but their mechanism of action is still unknown. Furthermore, the presence of lipoxygenases in the genome of the sea urchin does not imply that these genes are actually expressed and that they have the same function as in diatoms. In contrast, given the wide variety of ecological roles of lipoxygenases in various organisms (from terrestrial plants to marine animals and microalgae), it is likely that lipoxygenase activity reported for some sea urchins has a physiologic role that is quite different from that of marine diatoms.

2.2. Marine Copepods

As opposed to the literature on sea urchin–oxylipin interactions, few studies have reported on the effects of exposure to pure molecules in copepods. The effects of diatom-derived oxylipins were mostly evaluated by feeding adults and/or larvae with specific diatom species (mostly the PUA-producing *S. marinoi*) for which a LOX activity was already described (Table 2).

Diets of two bloom-forming algae, the diatom *S. marinoi* and the dinoflagellate *Scrippsiella hangoei*, were evaluated on egg production in the copepod *Acartia bifilosa*. Copepods produced the highest number of eggs with the *S. hangoei* diet, whereas *S. marinoi* was the most effective in impairing copepod reproduction [91]. The effect of three *S. marinoi* strains producing different quantities of PUAs were assessed in three common planktonic copepods *Acartia tonsa, Pseudocalanus elongatus* and *Temora longicornis* [84]. The hatching success of *A. tonsa* was almost the same for all diets until Day 6, after which a significant decrease (less than 30% of hatched nauplii) was observed with strain GF04-9B. A reduction in the number of hatched nauplii was also observed in *P. elongatus* and *T. longicornis* fed with the GF04-9B strain. Since the most toxic strain was not the richest in terms of PUAs production, no significant correlation was found between the impairment of embryo development and the abundance of PUAs [84].

Monoalgal and mixed diets of *Prorocentrum minimum* (control diet), *S. marinoi* (positive control) and *T. rotula* were also evaluated on the development and sex differentiation of the copepod *Temora stylifera* [86]. Mortality rates were higher in *Temora stylifera* fed with *T. rotula* compared to *P. minimum* plus *T. rotula*, suggesting that a beneficial food was able to dilute the negative effect of a toxic diatom. On the contrary, no significant differences were recorded between *S. marinoi* and mixed diets (*S. marinoi* plus *P. minimum*), in both maternal and larval diets. In particular, offspring generated by females fed with *P. minimum*/*S. marinoi*, and successively raised on *P. minimum*/*S. marinoi* or mixed diets (*P. minimum* plus *S. marinoi*), arrested their development within a few days. Furthermore, larval feeding strongly affected the final sex ratio of *T. stylifera* in both *S. marinoi* and *T. rotula* diets [86]. Maternal and larval diets were also tested in the copepod *Paracartia latisetosa* analysing the effect of *S. marinoi* ingestion compared to the control *P. minimum* [96]. Feeding of both adults and offspring on *S. marinoi* induced the lowest egg production and viability, as well as a strong delay in embryo development. Moreover, mixed diets revealed that nauplii were more sensitive to the PUA-producing diatom *S. marinoi*. In fact, development to adulthood (up to eleven days) was observed when nauplii were reared on *P. minimum* and spawned from females fed on *S. marinoi*, whereas, in the opposite condition, a blockage of naupliar development was observed [96].

Table 2. Species, oxylipins or diatom diets, concentrations tested and morphological and molecular effects reported in the last ten years on copepods. Abbreviations: DD, 2*E*,4*E*-decadienal; HD, 2*E*,4*E*-heptadienal; 15-HEPE, 15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*,17*Z*-eicosapentaenoic acid; PUAs, polyunsaturated aldehydes.

Species	Oxylipins/Diatom	Morphological Effects	Molecular Effects	Reference
A. tonsa *P. elongates* *T. longicornis*	*S. marinoi* GF04-9B (264 μm^3), GF04-1G (622 μm^3) and GF04-7J (141 μm^3)	Reduction of egg production and hatching success	Not detected	[84]
T. stylifera	*S. marinoi* and *S. pseudocostatum* (60*10^3 cells/mL), *T. rotula* CCMP1647 and CCMP1018 (8*10^3 cells/mL)	Reduction of egg production and viability, naupliar and female survival	Not detected	[85]
T. stylifera	*T. rotula* (2036 μm^3) and *S. marinoi* (196 μm^3)	Increase of mortality	Not detected	[86]
T. stylifera	15-HEPE, DD and HD (1.0 to 20 μg/mL)	Reduction of egg production, naupliar and female survival	Not detected	[87]
C. finmarchicus	*S. marinoi* G4 (400 cells/mL and 1000 cells/mL)	No effect on hatching success and naupliar survival	Not detected	[88]
C. helgolandicus	*S. marinoi* (45,000–60,000 cells/mL)	Not detected	Downregulation of *ATUB* and *BTUB*	[89]
C. helgolandicus	*S. marinoi* (45,000–60,000 cells/mL) and *C. socialis* (48,000–58,000 cells/mL)	Not detected	Upregulation of *CYP4*; downregulation of *GST*, *GSH-S*, *SOD*, *ALDH6*, *ALDH2*, *CARP*, *CAS*, *IAP*, *ATUB*	[90]
A. bifilosa	*S. marinoi* (41.6 μgC/cell)	Reduction of egg production	Not detected	[91]
C. helgolandicus	*S. marinoi* (45,000–6,0000 cells/mL)	Not detected	Upregulation of *HSP70*, *GST*, *SOD*, *ALDH3*, *ALDH8*, *ALDH9*, *BTUB*	[92]
T. stylifera	DD (0.5 to 2 μg/mL)	Reduction of egg production, hatching success and increase of mortality	Not detected	[24]
C. helgolandicus	*S. marinoi* (45,000 cells/mL)	Not detected	Upregulation of *HSP70*, *cyclin B1*, *glicoprotein 93*, *chaperonin-subunit ETA*, *diphosphate kinase*, *finger protein 121*,*14-3-3-protein*, *superoxide dismutase*; downregulation of *proteasome subunit*	[93]
P. annandalei	DD (0.75 to 4.5 μM)	Dose-dependent reduction of female survival and nauplii production	Not detected	[94]

Table 2. *Cont.*

Species	Oxylipins/Diatom	Morphological Effects	Molecular Effects	Reference
A. clausi *C. helgolandicus*	PUAs (0.97 µg/mg protein in 2004 and 1.2 µg/mg protein in 2005) and oxygenated fatty acids (3.6 µg/mg protein in 2004 and 14 µg/mg protein in 2005)	Reduction of egg production and hatching success	Not detected	[40]
C. sinicus	*S. marinoi* (45,000 cells/mL)	Not detected	Upregulation of *ALDH2*, *ALDH8*, *ALDH9*, *SOD*, *GSH-S*, *GST*, *CAS*, *CARP*; downregulation of *HSP70*	[95]
P. latisetosa	*S. marinoi* (10^4 to 10^6 cells/mL)	Reduction of egg production, hatching success and incomplete naupliar development	Not detected	[96]
C. helgolandicus	oxygenated fatty acids (0.001 to 1389.13 ng/mg)	Reduction of egg production and hatching success	Upregulation of *ALDH8*, *ALDH7*, *ALDH6*, *CAT*, *GST*, *HSP40*, *HSP70*; downregulation of *CAS*, *CARP*, *BTUB*, *ATUB*, *ALDH3*, *SOD*, *CYP*	[41]
T. japonicus *A. pacifica* *P. annandalei*	*C. muelleri* and *N. closterium f. minutissima* (0.35 to 17.00 µgC/mL for *T. japonicus* and 0.35 to 8.5 µgC/mL for *A. pacifica* and *P. annandalei*)	Incomplete naupliar development	Not detected	[97]

Three PUAs-producing diatom species were tested on the copepod *T. stylifera* by measuring egg production and hatching success [85]. In particular, these authors analysed the effects of *S. marinoi*, *Thalassiosira rotula* Strain CCMP 1647 (TR1), *T. rotula* Strain CCMP 1018 (TR2) and a species that did not exhibit PUAs activity, *Skeletonema pseudocostatum*. All diatoms reduced egg production rates and hatching success compared to diets of the dinoflagellate *P. minimum* (control diet). Surprisingly, *S. pseudocostatum*, the non-PUA producing species, together with *S. marinoi* induced the strongest toxicity. Moreover, 88% of nauplii that hatched from copepods fed with *S. pseudocostatum*, were TUNEL-positive after 48 h, revealing that some apoptotic events had occurred. The effects of this diatom were attributed to other oxylipins belonging to oxygenated fatty acids (15*S*-HEPE, 13,14-HEpETE and 15-oxoacid) reported in the same study [85]. *T. stylifera* was also fed with two diatoms, the PUAs-producing *S. marinoi* and *P. delicatissima* [87], in which an oxygenated fatty acid (15*S*-HEPE) was previously described [32]. Both diets affected egg production rates (less than 10%) and viability, which declined more dramatically with a diet of *S. marinoi*. Female survival was slightly reduced after 15 days of feeding (93.7% with SKE and 94.7% with *P. delicatissima*), reaching more significant values at the end of the experiment (75% with *S. marinoi*). Several TUNEL-positive regions were also observed in nauplii hatched after *T. styifera* feeding with both diatoms, revealing that, probably, 15*S*-HEPE could also trigger some apoptotic events and negatively affect the reproductive capability of copepods [87]. Comparative studies of diatom blooms in 2004 and 2005 corroborated this hypothesis [40]. In particular, the hatching rate in both *Acartia clausi* and *Calanus helgolandicus* decreased from 2004 to 2005 (~80% in 2004 and ~60% in 2005), when the abundance of oxygenated fatty acids was significantly higher [40].

More recently, diets with two diatom-producing oxygenated fatty acids, *Chaetoceros muelleri* and *Nitzschia closterium f. minutissima* [22,90,98], were tested on the planktonic copepods *Acartia pacifica* and *Pseudodiaptomus annandalei* and the benthic species *Tigriopus japonicus* [97]. These studies showed that the effect of diatoms was species-specific, since a diet with *N. closterium f. minutissima* was found to be particularly unsuitable only for the copepod *A. pacifica*. In fact, at all concentrations tested, this copepod was not able to complete naupliar development, whereas *P. annandalei* nauplii normally developed to adults. Furthermore, all diatom species analysed did not have a significant impact on the development of the benthic copepod *Tigriopus japonicus* [97]. A species-specific interaction could explain the contrasting results of some mesocosm experiments, showing no effects of the PUA-producing diatom *S. marinoi* on the reproduction of the copepod *Calanus finmarchicus* [88]. In particular, diets with low and high concentrations of *S. marinoi* supplied with nitrates, phosphates and silicates did not affect hatching success and the survival of nauplii [88].

The first molecular studies on copepods investigated the effects of diets of the diatom *S. marinoi* on the copepod *C. helgolandicus* compared to control diets with the dinoflagellate *P. minimum* and the green alga *Rhodomonas baltica*, which does not produce oxylipins [89]. The expression levels of two mitochondrial subunits were found significantly altered with *S. marinoi* diets, with the downregulation of α- and β-tubulin. Conversely, *P. minimum* and *R. baltica* diets induced no significant changes in the expression of α- and β-tubulin [89]. A similar study showed that a monoalgal diet of *S. marinoi* was sufficient to downregulate a pool of genes involved in stress response, defence system and detoxification in the copepod *C. helgolandicus* [90]. These data were compared to a diet of *C. socialis* that produces low amounts of oxylipins that did not affect the expression levels of genes under analysis. The same authors performed a comparative study between two populations of *C. helgolandicus* from the Swedish Western Coast and the Mediterranean Sea [92]. *S. marinoi* diets altered the expression of detoxification enzymes and proteins involved in apoptosis and cell cycle progression, with the Mediterranean population being more susceptible after 24 and 48 h of feeding [92]. The molecular effects of *S. marinoi* diets on a different *Calanus* species (*C. sinicus*) were also analysed, using *R. baltica* diet as a control [95]. Although a significant downregulation of genes involved in defence and detoxification systems was detected after five days of feeding, this copepod species was more resistant than the congeneric species *C. helgolandicus*. Field studies evaluated the impact of spring blooms in Goro and Rimini stations (Adriatic Sea) on the reproductive success

of the copepod *C. helgolandicus* [41]. Interestingly, they showed that the area with the lowest egg production and hatching success corresponded with high oxylipin abundance. Furthermore, copepods collected in both sites had a significant upregulation of stress-related genes, such as heat shock proteins, catalase, S-transferase glutathione and aldehyde dehydrogenase, compared to laboratory conditions in which copepods were fed with the dinoflagellate *P. minimum* [41]. Molecular studies were also performed by Carotenuto et al. [93], who generated two Expressed Sequence Tags (ESTs) libraries of the copepod *C. helgolandicus* fed on both *S. marinoi* and the control *R. baltica*, using suppression subtractive hybridisation (SSH). Comparison of SSH libraries revealed that some biological processes, such as response to stimuli, signal transduction and protein folding were over-expressed in copepods fed with *S. marinoi*. These results were also validated by real time qPCR [93].

Feeding investigations were also combined to in vitro exposure of ripe females with DD, HD and 15*S*-HEPE, at a concentration range of 1.0–20 µg/mL [87]. All compounds induced a similar dose-dependent reduction in hatching success in *T. stylifera* nauplii, with the two PUAs inducing stronger effects compared to 15*S*-HEPE. This result was also confirmed using the TUNEL assay, showing that apoptotic tissues were visible in treatments with 15*S*-HEPE at a concentration ten times greater than PUAs [87]. A similar study tested various concentrations of DD (0.5–12 µg/mL) on adults of the copepod *T. stylifera* [24]. In particular, although egg production rates indicated a dose-dependent increase, hatching time and success were significantly altered with only 54% of hatched nauplii at 2 µg/mL. Among nauplii, the majority displayed apoptotic features, detected using the TUNEL-assay. In addition, DD at a concentration greater than 3 µg/mL induced a higher mortality in males and females compared to the controls. Moreover, odour choice experiments revealed that some unknown mechanisms stimulate copepods towards DD, acting as chemical signals [24]. Dhanker et al. [94] also studied the effects of exposure to several concentrations of DD (0.75, 1.5, 3 and 4.5 mM) on the copepod *Pseudodiaptomus annandalei*. As a result, DD significantly reduced female survival and naupliar production in a dose-dependent manner. Furthermore, this PUA induced high mortalities in nauplii and significantly delayed development times [94].

2.3. Miscellaneous

A few studies have explored the possible negative effects of oxylipins on other organisms in the last ten years (Table 3).

Table 3. Species, oxylipins or diatom diets, concentrations tested and morphological and molecular effects reported in other marine invertebrates from 2010 to 2020. Abbreviations: DD, 2*E*,4*E*-decadienal; HD, 2*E*,4*E*-heptadienal; OD, 2*E*,4*E*-octadienal.

Species	Oxylipins (µM)/Extract	Morphological Effects	Molecular Effects	Reference
N. virens	DD (up to 50)	Dose- and time-dependent effects on reproductive and cycle-life	Not detected	[99]
C. intestinalis	DD (0.8–8.9)	Delay or block of metamorphosis and decrease of endogenous NO levels	Upregulation of *gclm* and *ggt*	[100]
C. intestinalis	DD (2–3.3)	Dose-dependent malformations and delay of larvae	Upregulation of *gclm*, *gst*; downregulation of *hox1*, *hox12*, *cdx*	[101]
Microzooplankton	Mixtures of HD (0.005–0.02) and OD (0.0005–0.002)	Dose-dependent delay of growth	Not detected	[102]
O. dioica	DD (0.33–16.42), *S. marinoi* and *C. affinis* extracts (5–100)	Dose-dependent aberrations of chordate embryos	Upregulation of *gclm*, *Aldh3*, *Aldh2*, *Aldh8*	[103]
R. canadum	DD (0, 0.1, 0.3, 0.5, 0.7, 1.0)	Decreasing of larval survival and juvenile growth	Not detected	[104]

In 2011, the effects of different DD concentrations were evaluated on the reproductive success and life cycle of the polychaete *Nereis virens* [99]. In particular, the authors showed that DD treatments were able to cause a strong decrease in fertilisation rate and larval viability, together with a significant impairment of sperm motility in a dose- and time-dependent manner.

Moreover, Comet assays revealed visible DNA damage in treated sperm, with results even higher when compared to those induced with copper sulphate [99].

The tunicate *Ciona intestinalis* was used to test the effects of increasing DD concentrations on post-hatched embryos at three different times after hatching (early, middle and late larval stages) [100]. At lower concentrations (0.8 µM), the authors observed a significant delay in settlement time and metamorphosis after 24 h of treatment when DD was added to middle and late larval stages. At higher concentrations (8.9 µM), metamorphosis was completely blocked. Moreover, the 2,3-diaminonaphthalene (DAN) assay showed a decrease in endogenous NO that was confirmed by molecular experiments, revealing that middle larvae (20–21 hpf) treated with DD displayed no significant variation in the expression of the *NO synthase* (*NOS*) gene. NO levels were also shown to be finely regulated by several genes involved in redox homeostasis, such as the *glutamate–cysteine ligase regulatory subunit* (*gclm*) and *gamma-glutamyl transpeptidase* (*ggt*) genes, which were found significantly upregulated in treated larvae [100]. Since the ERK pathway is known to promote metamorphosis, real time qPCR was also applied to evaluate the expression of genes implicated in the ERK pathway. In particular, relative expression analysis indicated that DD was able to inhibit metamorphosis by inducing a strong upregulation of a specific *map kinase phosphatases* (*mkp1*), whose activity is able to block ERK signalling [100]. In a similar study, *C. intestinalis* oocytes were treated with increasing concentrations of DD to follow embryo development [102]. Morphological analyses indicated a dose-dependent developmental delay and aberrations mainly affecting the larval tail. Furthermore, a reduction in hatching capabilities was recorded, with a very low percentage of hatched larvae (less than 20%) at the highest concentration tested. From the molecular point of view, the authors showed that DD exposure was able to target many genes involved in developmental and stress response processes [101]. DD effects were also evaluated on the tunicate *Oikopleura dioica* [103]. In particular, the authors showed that *O. dioica* embryos, deriving from eggs treated with different concentrations of DD (0.25–2.0 µg/mL), evolved dose-dependent aberrations, affecting morphogenesis, midline convergence and tail elongation processes. At higher DD concentrations (>2.5 µg/mL), embryo abnormalities were more severe, with a complete blockage of first cleavage. Moreover, the authors validated these data by whole-mount in situ hybridisation, showing that DD treatments were able to cause a systematic delay in the expression of many developmental genes [103]. Finally, tests with crude extracts of oxylipin-producing diatoms, *S. marinoi* and *Chaetoceros affinis*, on the eggs of *O. dioica* showed that natural PUAs and/or oxygenated fatty acids were able to induce the same aberrations as those observed with DD treatments [103].

Since diatoms constitute a great source of nutrients for small organisms living in the zooplankton, Lavrentyev et al. [102] tested HD and OD mixtures at different concentrations on several microzooplankton species, to define the impact of these natural toxicants on their development. Specifically, PUAs treatments induced variable developmental delays in a dose- and species-dependent manner. In fact, the results showed a negative effect on some ciliates and dinoflagellates, whereas other species were not affected or their response was activated only at the highest concentrations [102].

Very recently, the impact of a simulated marine warming environment in combination to DD exposure was evaluated on larval fitness of the fish cobia *Rachycentron canadum* [104]. Survival slightly decreased (16%) after exposure to high temperature (29 °C) and 0.5 µM DD, reaching higher values when these disturbances were combined. In fact, when PUA-treated larvae were exposed to high temperatures, the percentage of viable larvae was reduced to about 60%, revealing a synergistic effect [104].

3. Oxylipins as Cell Signalling Molecules in Diatom Communities

As mentioned above, oxylipins may act as toxic compounds regulating population dynamics at the end of blooms when stress conditions increase and nutrient availability is quite limited [59,62,105]. It has been hypothesised that an accurate mechanism of bloom regulation could be activated [59]. In fact, 2*E*,4*E*-decadienal was found to trigger programmed cell death (PCD) in diatom cells by inducing the release of intracellular calcium and a consequent increase in NO levels. Surprisingly, when treatments were applied at sub-lethal concentrations (660 nM), diatoms became resistant to higher successive doses (13.2 μM), without the activation of PCD and a total unresponsive calcium cascade [59]. In addition to oxylipins, the end of bloom events was also associated to bacterial or viral infections controlling phytoplankton dynamics [106,107]. Recently, a chemical defence role against some bacterial species that leads to cell lysis in microalgal blooms was proposed. In particular, when a diatom-producing HEPEs, *Chaetoceros didymus*, was co-cultured with an algicidal bacteria, *Kordia algicida*, a significant decrease in bacterial growth and cell lysis was detected. Chemical analyses of culture media confirmed a huge production of HEPEs, particularly 15-HEPE, which might be considered the main HEPE responsible for *C. didymus* protection during bloom events [108]. Interestingly, a surprising plasticity of PUAs production was found. Laboratory cultures with low silicate concentrations revealed high PUA levels in the medium, which corresponded to a poor degree of cell wall silicification, thus suggesting that a probable switch between chemical and mechanical defence was finely regulated [109].

The first evidence indicating that PUAs were produced not only after wound-activation but also to regulate bloom events and cell-to-cell communication was published by Casotti et al. [53]. Moreover, Vidoudez and Pohnert [79] observed that HD and OD concentrations increased in culture media of *S. marinoi* by Day 21. Moreover, the addition of these PUAs during different stages of growth revealed a strong decrease in cell numbers when added at the stationary and declining phases, possibly indicating that PUAs act as intra-population signals able to regulate bloom events.

Many studies have also corroborated the hypothesis of a possible role of oxylipins as allelochemicals. For instance, a negative effect of DD was observed in cell growth and viability of the diatom *Thalassiosira weissflogii*; incubation with decadienal decreased the growth rate in a dose- and time-dependent manner, with dead cells displaying the typical characteristics of apoptotic events, including cell shrinkage and DNA damage [53]. Allelopathy was also shown when the three PUAs, DD, HD and OD were tested on the prymnesiophyte *Isochrysis galbana*, the chlorophyte *Tetraselmis suecica* and the diatom *S. marinoi* [54]. Flow cytometry experiments revealed that PUAs altered the morphology of all species, with *S. marinoi* being the most resistant to oxylipins toxicity [54]. The same PUAs, in single and mixture experiments, were tested on *S. marinoi* and *Phaeodactylum tricornutum* diatom cultures [57]. In particular, the diatom *S. marinoi*, exerted a reduction in NO levels with a parallel ROS increase when treated with HD and OD, two compounds produced by this diatom species. Since NO levels increased significantly in *P. tricornutum* exposed to DD, a species-specific response was proposed, in which *S. marinoi* perceived HD and OD as intra-population signals, while *P. tricornutum* recognised them as allelochemicals [57]. The same authors further demonstrated that, in addition to free radical species, PUAs response involved the generation of O_2^- and superoxide dismutase (SOD) activity, which was confirmed by measuring the accumulation H_2O_2 [110]. The allelopathic potential of diatom oxylipins was also investigated in the invasive dinoflagellate *Ostreopsis* cf. *ovata* [111,112]. The exposure to DD, HD and OD induced a growth inhibition and cell abnormalities, with higher effects triggered by the long-chained aldehyde DD, comparing to the shorter PUAs [111].

Some mechanisms of auto-allelopathy have also been recorded under low-nutrient conditions, which normally occur at the end of blooms [113]. Recently, auto-allelopathic interactions were observed in treatments with the hydroxyacid 15-HEPE purified from the medium of a *S. costatum* strain [114]. In particular, when 15-HEPE was administered to a culture of the dinoflagellate *Alexandrium minutum*, no inhibitory effect was detected, while a strong decrease in growth rate was measured in *S. costatum* cultures.

Diatom-derived oxylipins were also involved in the regulation of bacteria–phytoplankton community dynamics [51], influencing cell growth and species composition that, in some cases, were hypothesised to be implicated in combination with additional molecules involved in diatom–bacteria interactions [115]. The PUAs DD, HD and OD were tested on 33 marine bacterial strains, including several species isolated from a bloom of the PUA-producing diatom *S. marinoi* [55]. Since a visible resistance of bloom species was detected, PUAs were confirmed to be fundamental in shaping associated bacterial communities, particularly at the end of bloom events when senescence and declining nutrient concentrations favour an increase in the production of PUAs [55]. The same PUAs were confirmed to promote the growth of PUAs-resistant species when tested on a natural bacterial community. This result is of significant ecological relevance, since resistance to PUAs toxicity could provide a precious advantage to bacterial communities, by increasing the possibility of using the organic matter released by diatoms [56]. The mechanism of action of PUAs entry into bacterial cells was later described as the strong accumulation on cytoplasmic membranes due to their hydrophobic properties [116].

PUAs also play a critical role in sinking processes and particulate organic carbon (POC) exportation from swallow to deeper waters [58]. In particular, incubation of PUAs at low concentrations (1–10 μM) was found to induce the remineralisation of organic matter and the growth of POC associated bacteria (about 50% greater than control), together with a significant change in bacterial community structure. On the contrary, at higher concentrations (100 μM), bacterial cell abundance and metabolism was significantly lower. These results led to the conclusion that, on inter-annual timescales, PUAs decrease the efficiency of POC export from surface to deeper waters and, consequently, induce the retention in shallow waters of phosphorus and other nutrients, which are, in turn, available to primary producers [58]. The influence of PUAs on carbon export in marine environments was further explored in a mesocosm experiment [117]. Transparent exopolymeric particles that spontaneously form through abiotic processes were found to be critical for particle aggregation and organic carbon flux from shallow to deeper zones. The addition of a mix of three PUAs (DD, HD and OD) during the exponential phase of an artificial bloom of *T. rotula*, significantly increased the quantity of dissolved organic carbon (DOC) and the abundance of exopolymeric particles with respect to the control. Since exopolymeric particles levels and size significantly increased at the end of the bloom, PUAs were confirmed to enhance the export of organic carbon, altering food web structure and the consequent size and distribution of available food particles [117]. Contrary to the results of Edwards et al. [58], the abundance of free bacteria was almost the same at the end of the experiment, suggesting that PUAs did not influence the bacterial community [117]. Recently, this latter observation was confirmed, since treatments with heptadienal and octadienal on two strains of *S. marinoi*, a PUA- and a non-PUA producer, showed no significant differences in bacterial communities between the two cultures [118]. Overall, these contrasting results reinforce the idea, previously suggested by Paul et al. [115], that PUAs could act in a more complex manner, where additional chemical mediators are also involved.

4. Biotechnological Applications of Oxylipins

Thus far, few studies have focused on the possible biotechnological applications of oxylipins, including anti-cancer, anti-bacterial, anti-fungal and anti-parasitic activities (Table 4). The first study to suggest the possible anti-cancer activity of oxylipins was published by Miralto et al. [7]. In particular, MTT (thiazolyl blue) and TUNEL assays revealed antiproliferative and apoptotic activities of the diatom-derived PUAs, DD and 2*E*,4E/*Z*,7*Z*-decatrienal (DT), in human colon adenocarcinoma cell lines Caco2 [7].

Table 4. Biotechnological applications of oxylipins, reporting oxylipins or diatoms analysed, target cells and/or organism and the activity detected. Abbreviations: DD, 2*E*,4*E*-decadienal; DT, 2*E*,4E/*Z*,7*Z*-decatrienal; HD, 2*E*,4*E*-heptadienal; OD, 2*E*,4*E*-octadienal.

Oxylipins/Diatom	Target Cells/Organism	Activity	Reference
DD and DT	Human colon adenocarcinoma (Caco2)	Anticancer	[7]
DD	*B. proboscidea* and *T. heterouncinata*	Anti-parasitic	[119]
DD	*S. salar*	Anti-parasitic	[120]
C. scutellum parva	Breast carcinoma (BT20)	Anticancer	[121]
DD, HD, OD	Adenocarcinoma (A549 and COLO 205)	Anticancer	[63]
C. debaryana	TNF-α, IL-1β, IL-6 and IL-17	Anti-inflammatory	[122]
S. marinoi	Human melanoma (A2058) and *S. aureus*	Anti-cancer and anti-bacterial	[123]
S. costatum, *C. pseudocurvisetus*	*M. tuberculosis* and *M. bovis*	Anti-tuberculosis	[124]

Afterwards, other studies have tried to explore these interesting findings. The extracts and fractions from *Cocconeis scutellum parva*, an oxylipin-producing diatom [125], were tested on several cancer cell lines [121]. In particular, an EPA-enriched fraction from the diethyl-ether extract was the most active against breast carcinoma (BT20) cells, triggering up to 89.2% apoptosis. Furthermore, a dose-dependent decrease of BT20 cell viability was associated to the activation of caspases-8 and caspase-3 and the blockage of cell cycle progression from S to G2-M phases [121]. Several concentrations of three synthetic PUAs, DD, HD and OD, were also tested on two adenocarcinoma cell lines (A549 and COLO 205) [63]. MTT assays indicated that DD had the highest anti-proliferative activity on these two cancer cell lines but was not active on the normal lung/brunch epithelial BEAS-2B cell line. Moreover, immunoblotting analyses showed that all PUAs were able to activate the apoptotic extrinsic pathway mediated by Tumour Necrosis Factor Receptor 1 (TNFR1) and Fas Associated Death Domain (FADD). These results were confirmed by molecular approaches. In fact, DD and HD induced a significant upregulation of a pool of genes involved in apoptosis such as, *TNFRSF1A* and *TNFRSF1B* (coding for the two receptors TNFR1 and TNFR2), *FADD*, *caspase-3* and *AIFM1*. On the contrary, OD showed a lower activity, since no variation in gene expression was recorded. Finally, the apoptotic events were also evaluated by flow cytometry techniques, revealing, once again, a lower anticancer activity in OD treatments [63]. An ambiguous result was achieved when 32 microalgae species were screened for anti-inflammatory, antitumor, antibacterial, antidiabetic and antioxidant activities [123]. In fact, of two clones of the diatom *S. marinoi* tested, only one had anti-cancer activity against human melanoma cells (A2058), depending on nutrient conditions. Moreover, since the antibacterial activity on *Staphylococcus aureus* was found in both clones but only in nitrogen-starvation conditions, the authors suggested that probably oxylipins are not responsible for such activity [123]. The same authors found an anti-tuberculosis activity from the extracts of the PUA-producing diatom *S. costatum* together with *Chaetoceros pseudocurvisetus*. In particular, these algae were found to be active against *Mycobacterium tuberculosis* and *M. bovis* only in phosphate-starvation culturing condition [124].

Possible nutraceutical applications of diatom-derived oxylipins were also proposed [122]. In particular, the effects of an oxylipin-containing lyophilised (OLM) biomass from the freshwater alga *Chlamydomonas debaryana* were evaluated on a recurrent 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis mice model. The oral administration of OLM lyophilised induced anti-inflammatory activities with a significant decrease of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-17), iNOS, COX-2 and NF-κB, together with the increase of PPAR-γ levels [122].

In some cases, oxylipins were also used as possible anti-parasitic agents useful to treat diseases that commonly occur in aquaculture practices. For instance, Simon et al. [119] tested the effects of different decadienal concentrations on the survival and growth of two polychaetae larvae, *Boccardia proboscidea* and *Terebrasabella heterouncinata*, that normally infest breeding of the abalone *Haliotis midae*. Specifically,

they observed a dose- and time-dependent negative impact on larval development and survival of both polychaetes with a higher sensitivity of *T. heterouncinata* [119]. In a different study, infected salmon (*Salmo salar*) with the parasite *Caligus rogercresseyi* were treated with DD, using this aldehyde as a food supply [120]. In particular, no significant toxicity was observed in histopathological sections of salmon injected with increasing concentrations of decadienal in brain, intestine, skin, liver and muscle tissue. Moreover, DD feeding at non-toxic concentrations was able to impair the reproductive capability of *C. rogercresseyi* by decreasing the number of mature females and eggs [120].

Another key issue is the influence of symbiotic bacteria on the ecology, physiology and the biotechnological potential of diatoms. Diatoms and bacteria have co-occurred in common habitats for more than 200 million years [126]. As a result, hundreds of genes found in certain diatoms species, have been acquired from bacteria [127,128]. During evolution, diatoms have established a species-specific relationship with bacteria due to a strong cooperation that favours one another. In fact, the oxygen coming from photosynthesis is used for bacterial degradation of organic matter, while bacteria release CO_2 through remineralisation processes to facilitate the complete photosynthetic cycle [129]. Interestingly, a functional carbon flux between diatoms and bacteria has been observed since some bacterial species have been found as intermediate providers for the biosynthesis of bioactive metabolites [130]. Overall, co-occurring bacteria promote the growth of diatom cells, influencing their metabolism and improving their biotechnological potential [131–136].

5. Concluding Remarks

The ecological role of diatom-derived oxylipins is extremely complex, since it consists of multiple functions affecting population dynamics of aquatic environments.

As reported above, some oxylipins are toxic compounds produced by diatoms that negatively affect the reproductive success of several marine invertebrate consumers. In fact, oxylipins, particularly PUAs, act as chemical deterrents against grazers, interfering with the reproductive success of some marine invertebrates, starting from gamete viability, fertilisation processes, embryogenesis until larval fitness. Most studies in the last ten years mainly investigated their effects on sea urchins and copepods. Given the importance of diatom blooms in marine environments and the ecological implications, it would be interesting to extend these studies to other marine invertebrates, in order to better understand the mechanisms of response to oxylipins.

Moreover, in addition to their synthesis upon wound-activation, these oxygenated fatty acid derivatives can also be actively produced by intact diatoms cells through mechanisms that are still unknown. The role of these small chemical mediators is to regulate cell–cell communication within the same species and among different species, influencing the structure and the composition of phytoplankton communities (Figure 3).

Few studies have evaluated the possible biotechnological applications of oxylipins; this is probably due to a low chemical stability that makes them quite difficult to manipulate in laboratory conditions. Nevertheless, interesting bioactivities have been reported, ranging from anticancer to antibacterial capabilities. Overall, the ecological role of diatom oxylipins and their potential pharmacological applications deserve further investigations.

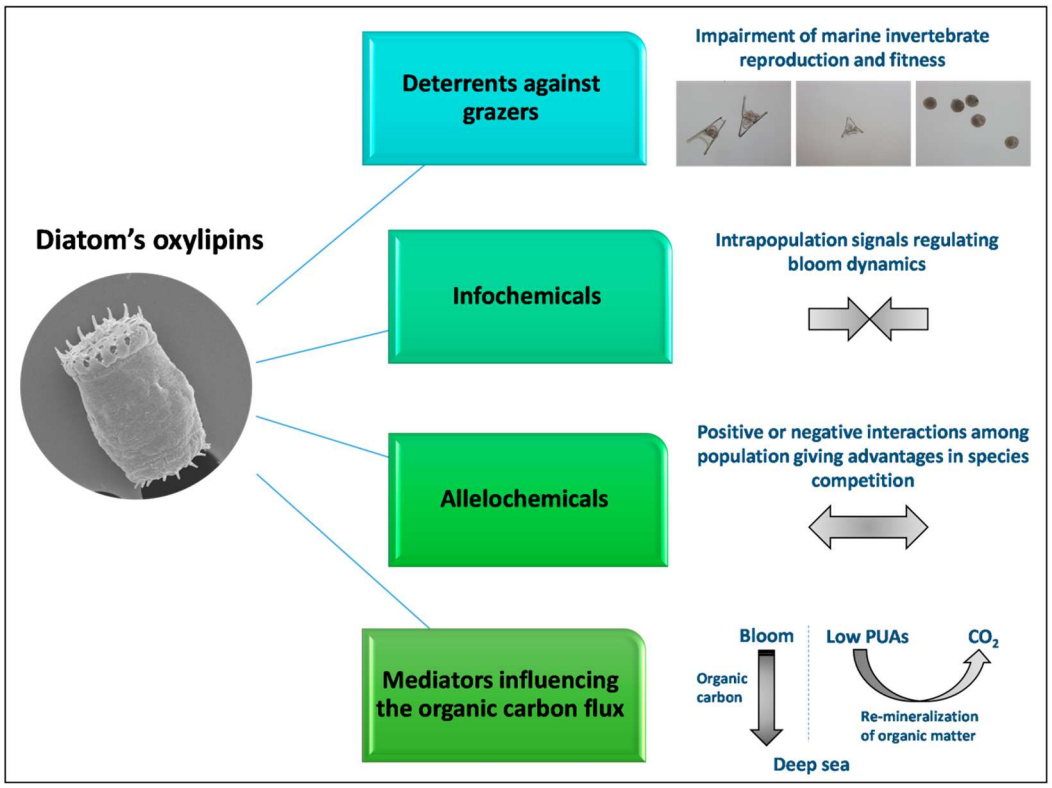

Figure 3. Oxylipins in aquatic environments can act as deterrents against grazers, info-chemicals, allelochemicals and mediators that influence carbon recycling.

Author Contributions: M.C., N.R., L.A. and R.E. conceived and designed the scheme of the review; N.R., L.A. and R.E. performed the bibliographic research and prepared the original draft, including tables and figures; N.R. coordinated L.A. and R.E. in the preparation of the original draft; and M.C., V.Z. and A.I. reviewed, edited and supervised the manuscript. All authors read and agree to the final version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Nadia Ruocco was supported by a research grant "Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374). Luisa Albarano was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship co-funded by the Stazione Zoologica Anton Dohrn and University of Naples Federico II. Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Falkowski, P.G.; Barber, R.T.; Smetacek, V. Biogeochemical controls and feedbacks on ocean primary production. *Science* **1998**, *281*, 200–206. [CrossRef] [PubMed]
2. Smetacek, V. A watery arms race. *Nature* **2001**, *411*, 745. [CrossRef]
3. Hasle, G.R.; Syvertsen, E.E. Identifying marine phytoplankton. In *Marine Diatoms*; Tomas, C.R., Ed.; Academic Press: San Diego, CA, USA, 1997; pp. 5–385.
4. Mann, D.G. The species concept in diatoms: Evidence for morphologically distinct, sympatric gamodemes in four epipelic species. *Plant Syst. Evol.* **2005**, *164*, 215–237. [CrossRef]
5. Jain, S. Marine diatoms. In *Fundamentals of Invertebrate Palaeontology*; Springer: New Delhi, India, 2020; pp. 131–142.
6. Ban, S.; Burns, C.; Castel, J.; Chaudron, Y.; Christou, E.; Escribano, R.; Umani, S.F.; Gasparini, S.; Ruiz, F.G.; Hoffmeyer, M.; et al. The paradox of diatom-copepod interactions. *Mar. Ecol. Prog. Ser.* **1997**, *157*, 287–293. [CrossRef]

7. Miralto, A.; Barone, G.; Romano, G.; Poulet, S.A.; Ianora, A.; Russo, G.L.; Buttino, I.; Mazzarella, G.; Laabir, M.; Cabrini, M.; et al. The insidious effect of diatoms on copepod reproduction. *Nature* **1999**, *402*, 173–176. [CrossRef]
8. Ianora, A.; Poulet, S.A.; Miralto, A. The effects of diatoms on copepod reproduction: A review. *Phycologia* **2003**, *42*, 351–363. [CrossRef]
9. Ianora, A.; Miralto, A. Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: A review. *Ecotoxicology* **2010**, *19*, 493–511. [CrossRef] [PubMed]
10. Landsberg, J.H. The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* **2002**, *10*, 113–390. [CrossRef]
11. Galland, G.R.; Pennebaker, S.L. A benthic diatom bloom in the Gulf of California, Mexico. *BioInvasions Rec.* **2012**, *1*, 65–69. [CrossRef]
12. Ahn, I.Y.; Moon, H.W.; Jeon, M.; Kang, S.H. First record of massive blooming of benthic diatoms and their association with megabenthic filter feeders on the shallow seafloor of an Antarctic Fjord: Does glacier melting fuel the bloom? *Ocean Sci. J.* **2016**, *51*, 273–279. [CrossRef]
13. Bothwell, M.L.; Taylor, B.W. Blooms of benthic diatoms in phosphorus-poor streams. *Front. Ecol. Environ.* **2017**, *15*, 110–111. [CrossRef]
14. Zohdi, E.; Abbaspour, M. Harmful algal blooms (red tide): A review of causes, impacts and approaches to monitoring and prediction. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 1789–1806. [CrossRef]
15. Cutignano, A.; D'Ippolito, G.; Romano, G.; Lamari, N.; Cimino, G.; Febbraio, F.; Nucci, R.; Fontana, A. Chloroplastic glycolipids fuel aldehyde biosynthesis in the marine diatom *Thalassiosira rotula*. *ChemBioChem* **2006**, *7*, 450–456. [CrossRef] [PubMed]
16. D'Ippolito, G.; Romano, G.; Caruso, T.; Spinella, A.; Cimino, G.; Fontana, A. Production of octadienal in the marine diatom *Skeletonema costatum*. *Org. Lett.* **2003**, *5*, 885–887. [CrossRef] [PubMed]
17. D'Ippolito, G.; Tucci, S.; Cutignano, A.; Romano, G.; Cimino, G.; Miralto, A.; Fontana, A. The role of complex lipids in the synthesis of bioactive aldehydes of the marine diatom *Skeletonema costatum*. *Biochim. Biophys. Acta* **2004**, *1686*, 100–107. [CrossRef] [PubMed]
18. D'Ippolito, G.; Cutignano, A.; Tucci, S.; Romano, G.; Cimino, G.; Fontana, A. Biosynthetic intermediates and stereochemical aspects of aldehyde biosynthesis in the marine diatom *Thalassiosira rotula*. *Phytochemistry* **2006**, *67*, 314–322. [CrossRef] [PubMed]
19. Fontana, A.; Cutignano, A.; Romano, G.; Lamari, N.; Gallucci, M.; Cimino, G.; Miralto, A.; Ianora, A. LOX-induced lipid peroxidation mechanism responsible for the detrimental effect of marine diatoms on zooplankton grazers. *ChemBioChem* **2007**, *8*, 1810–1818. [CrossRef] [PubMed]
20. Fontana, A.; D'Ippolito, G.; Cutignano, A.; Miralto, A.; Ianora, A.; Romano, G.; Cimino, G. Chemistry of oxylipin pathways in marine diatoms. *Pure Appl. Chem.* **2007**, *79*, 481–490. [CrossRef]
21. D'Ippolito, G.; Nuzzo, G.; Sardo, A.; Manzo, E.; Gallo, C.; Fontana, A. Lipoxygenases and lipoxygenase products in marine diatoms. *Methods Enzymol.* **2018**, *605*, 69–100. [CrossRef]
22. Ruocco, N.; Nuzzo, G.; D'Ippolito, G.; Manzo, E.; Sardo, A.; Ianora, A.; Romano, G.; Iuliano, A.; Zupo, V.; Costantini, M.; et al. Lipoxygenase pathways in diatoms: Occurrence and correlation with grazer toxicity in four benthic diatoms. *Mar. Drugs* **2020**, *18*, 66. [CrossRef]
23. Fink, P.; Von Elert, E.; Jüttner, F. Oxylipins from freshwater diatoms act as attractants for a benthic herbivore. *Arch. Hydrobiol.* **2006**, *167*, 561–574. [CrossRef]
24. Kâ, S.; Carotenuto, Y.; Romano, G.; Hwang, J.S.; Buttino, I.; Ianora, A. Impact of the diatom-derived polyunsaturated aldehyde 2-trans,4-trans decadienal on the feeding, survivorship and reproductive success of the calanoid copepod *Temora stylifera*. *Mar. Environ. Res.* **2014**, *93*, 31–37. [CrossRef] [PubMed]
25. Kühn, H.; Thiele, B.J. The diversity of the lipoxygenase family. Many sequence data but little information on biological significance. *FEBS Lett.* **1999**, *449*, 7–11. [CrossRef]
26. Veldink, G.; Hilbers, M.; Nieuwenhuizen, W.; Vliegenthart, J. Plant lipoxygenase: Stucture and mechanism. In *Eicosanoids and Related Compounds in Plants and Animals*; Rowley, A., Kühn, H., Schewe, T., Eds.; Portland Press: London, UK, 1998; pp. 69–96.
27. Ueda, N.; Suzuki, H.; Yamamoto, S. Mammalian lipoxygenases: Structure, function and evolutionary aspects. In *Eicosanoids and Related Compounds in Plants and Animals*; Rowley, A., Kühn, H., Schewe, T., Eds.; Portland Press: London, UK, 1998; pp. 47–68.

28. Kühn, H.; Banthiyaa, S.; Van Leyen, K. Mammalian lipoxygenases and their biological relevance. *Biochim. Biophys. Acta* **2015**, *1851*, 308–330. [CrossRef]
29. Kühn, H. Structural basis for the positional specificity of lipoxygenases. *Prostaglandins Other Lipid Mediat.* **2000**, *62*, 255–270. [CrossRef]
30. Lamari, N.; Ruggiero, M.V.; D'Ippolito, G.; Kooistra, W.H.C.F.; Fontana, A.; Montresor, M. Specificity of lipoxygenase pathways supports species delineation in the marine diatom genus *Pseudo-nitzschia*. *PLoS ONE* **2013**, *8*, e73281. [CrossRef]
31. D'Ippolito, G.; Cutignano, A.; Briante, R.; Febbraio, F.; Cimino, G.; Fontana, A. New C16 fatty-acid-based oxylipin pathway in the marine diatom *Thalassiosira rotula*. *Org. Biomol. Chem.* **2005**, *3*, 4065–4070. [CrossRef]
32. D'Ippolito, G.; Lamari, N.; Montresor, M.; Romano, G.; Cutignano, A.; Gerecht, A.; Cimino, G.; Fontana, A. 15S-Lipoxygenase metabolism in the marine diatom *Pseudo-nitzschia delicatissima*. *New Phytol.* **2009**, *183*, 1064–1071. [CrossRef]
33. Nanjappa, D.; D'Ippolito, G.; Gallo, C.; Zingone, A.; Fontana, A. Oxylipin diversity in the diatom family leptocylindraceae reveals DHA derivatives in marine diatoms. *Mar. Drugs* **2014**, *12*, 368–384. [CrossRef]
34. Rettner, J.; Werner, M.; Meyer, N.; Werz, O.; Pohnert, G. Survey of the C20 and C22 oxylipin family in marine diatoms. *Tetrahedron Lett.* **2018**, *59*, 828–831. [CrossRef]
35. Wichard, T.; Poulet, S.A.; Boulesteix, A.L.; Ledoux, J.B.; Lebreton, B.; Marchetti, J.; Pohnert, G. Influence of diatoms on copepod reproduction. II. Uncorrelated effects of diatom-derived α,β,γ,δ-unsaturated aldehydes and polyunsaturated fatty acids on *Calanus helgolandicus* in the field. *Prog. Oceanogr.* **2008**, *77*, 30–44. [CrossRef]
36. Vidoudez, C.; Casotti, R.; Bastianini, M.; Pohnert, G. Quantification of dissolved and particulate polyunsaturated aldehydes in the Adriatic Sea. *Mar. Drugs* **2011**, *9*, 500–513. [CrossRef]
37. Bartual, A.; Arandia-Gorostidi, N.; Cózar, A.; Morillo-Garciá, S.; Ortega, M.J.; Vidal, M.; Cabello, A.M.; Gonzaĺez-Gordillo, J.I.; Echevarría, F. Polyunsaturated aldehydes from large phytoplankton of the Atlantic Ocean surface (42°N to 33°S). *Mar. Drugs* **2014**, *12*, 682–699. [CrossRef]
38. Morillo-García, S.; Valcaŕcel-Pérez, N.; Cózar, A.; Ortega, M.J.; Maciás, D.; Ramiŕez-Romero, E.; García, C.M.; Echevarría, F.; Bartual, A. Potential polyunsaturated aldehydes in the Strait of Gibraltar under two tidal regimes. *Mar. Drugs* **2014**, *12*, 1438–1459. [CrossRef]
39. Ribalet, F.; Bastianini, M.; Vidoudez, C.; Acri, F.; Berges, J.; Ianora, A.; Miralto, A.; Pohnert, G.; Romano, G.; Wichard, T.; et al. Phytoplankton cell lysis associated with polyunsaturated aldehyde release in the northern Adriatic Sea. *PLoS ONE* **2014**, *9*, e85947. [CrossRef]
40. Ianora, A.; Bastianini, M.; Carotenuto, Y.; Casotti, R.; Roncalli, V.; Miralto, A.; Romano, G.; Gerecht, A.; Fontana, A.; Turner, J.T. Non-volatile oxylipins can render some diatom blooms more toxic for copepod reproduction. *Harmful Algae* **2015**, *44*, 1–7. [CrossRef]
41. Lauritano, C.; Romano, G.; Roncalli, V.; Amoresano, A.; Fontanarosa, C.; Bastianini, M.; Braga, F.; Carotenuto, Y.; Ianora, A. New oxylipins produced at the end of a diatom bloom and their effects on copepod reproductive success and gene expression levels. *Harmful Algae* **2016**, *55*, 221–229. [CrossRef] [PubMed]
42. Russo, E.; D'Ippolito, G.; Fontana, A.; Sarno, D.; D'Alelio, D.; Busseni, G.; Ianora, A.; von Elert, E.; Carotenuto, Y. Density-dependent oxylipin production in natural diatom communities: Possible implications for plankton dynamics. *ISME J.* **2020**, *14*, 164–177. [CrossRef] [PubMed]
43. Bartual, A.; Hernanz-Torrijos, M.; Sala, I.; Ortega, M.J.; González-García, C.; Bolado-Penagos, M.; López-Urrutia, A.; Romero-Martínez, L.; Lubián, L.M.; Bruno, M.; et al. Types and distribution of bioactive polyunsaturated aldehydes in a gradient from mesotrophic to oligotrophic waters in the Alborán Sea (Western Mediterranean). *Mar. Drugs* **2020**, *18*, 159. [CrossRef]
44. Blée, E. Impact of phyto-oxylipins in plant defense. *Trends Plant Sci.* **2002**, *7*, 315–322. [CrossRef]
45. Christensen, S.A.; Kolomiets, M.V. The lipid language of plant-fungal interactions. *Fungal Genet. Biol.* **2011**, *48*, 4–14. [CrossRef] [PubMed]
46. Wasternack, C.; Feussner, I. The oxylipin pathways: Biochemistry and function. *Annu. Rev. Plant Biol.* **2018**, *69*, 363–386. [CrossRef] [PubMed]
47. Genva, M.; Obounou Akong, F.; Andersson, M.X.; Deleu, M.; Lins, L.; Fauconnier, M.L. New insights into the biosynthesis of esterified oxylipins and their involvement in plant defense and developmental mechanisms. *Phytochem. Rev.* **2019**, *18*, 343–358. [CrossRef]

48. Caldwell, G.S. The influence of bioactive oxylipins from marine diatoms on invertebrate reproduction and development. *Mar. Drugs* **2009**, *7*, 367–400. [CrossRef]
49. Leflaive, J.; Ten-Hage, L. Chemical interactions in diatoms: Role of polyunsaturated aldehydes and precursors. *New Phytol.* **2009**, *184*, 794–805. [CrossRef]
50. Stonik, V.S.; Stonik, I. Low-molecular-weight metabolites from diatoms: Structures, biological roles and biosynthesis. *Mar. Drugs* **2015**, *13*, 3672–3709. [CrossRef]
51. Brown, E.R.; Cepeda, M.R.; Mascuch, S.J.; Poulson-Ellestad, K.L.; Kubanek, J. Chemical ecology of the marine plankton. *Nat. Prod. Rep.* **2019**, *36*, 1093–1116. [CrossRef]
52. Russo, E.; Ianora, A.; Carotenuto, Y. Re-shaping marine plankton communities: Effects of diatom oxylipins on copepods and beyond. *Mar. Biol.* **2019**, *166*, 9. [CrossRef]
53. Casotti, R.; Mazza, S.; Brunet, C.; Vantrepotte, V.; Ianora, A.; Miralto, A. Growth inhibition and toxicity of the diatom aldehyde 2-*trans*,4-*trans*-decadienal on *Thalassiosira weissflogii* (Bacillariophyceae). *J. Phycol.* **2005**, *41*, 7–20. [CrossRef]
54. Ribalet, F.; Berges, J.A.; Ianora, A.; Casotti, R. Growth inhibition of cultured marine phytoplankton by toxic algal-derived polyunsaturated aldehydes. *Aquat. Toxicol.* **2007**, *85*, 219–227. [CrossRef]
55. Ribalet, F.; Intertaglia, L.; Lebaron, P.; Casotti, R. Differential effect of three polyunsaturated aldehydes on marine bacterial isolates. *Aquat. Toxicol.* **2008**, *86*, 249–255. [CrossRef] [PubMed]
56. Balestra, C.; Alonso-Sáez, L.; Gasol, J.M.; Casotti, R. Group-specific effects on coastal bacterioplankton of polyunsaturated aldehydes produced by diatoms. *Aquat. Microb. Ecol.* **2011**, *63*, 123–131. [CrossRef]
57. Gallina, A.A.; Brunet, C.; Palumbo, A.; Casotti, R. The effect of polyunsaturated aldehydes on *Skeletonema marinoi* (Bacillariophyceae): The involvement of reactive oxygen species and nitric oxide. *Mar. Drugs* **2014**, *12*, 4165–4187. [CrossRef] [PubMed]
58. Edwards, B.R.; Bidle, K.D.; Van Mooy, B.A.S. Dose-dependent regulation of microbial activity on sinking particles by polyunsaturated aldehydes: Implications for the carbon cycle. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5909–5914. [CrossRef] [PubMed]
59. Vardi, A.; Formiggini, F.; Casotti, R.; De Martino, A.; Ribalet, F.; Miralto, A.; Bowler, C. A stress surveillance system based on calcium and nitric oxide in marine diatoms. *PLoS Biol.* **2006**, *4*, e60. [CrossRef] [PubMed]
60. Vardi, A. Cell signaling in marine diatoms. *Commun. Integr. Biol.* **2008**, *1*, 134–136. [CrossRef]
61. Bidle, K.D. The molecular ecophysiology of programmed cell death in marine phytoplankton. *Ann. Rev. Mar. Sci.* **2015**, *7*, 341–375. [CrossRef]
62. Bidle, K.D. Programmed cell death in unicellular phytoplankton. *Curr. Biol.* **2016**, *26*, R594–R607. [CrossRef]
63. Sansone, C.; Braca, A.; Ercolesi, E.; Romano, G.; Palumbo, A.; Casotti, R.; Francone, M.; Ianora, A. Diatom-derived polyunsaturated aldehydes activate cell death in human cancer cell lines but not normal cells. *PLoS ONE* **2014**, *9*, e101220. [CrossRef]
64. Romano, G.; Miralto, A.; Ianora, A. Teratogenic effects of diatom metabolites on sea urchin *Paracentrotus lividus* embryos. *Mar. Drugs* **2010**, *8*, 950–967. [CrossRef] [PubMed]
65. Romano, G.; Costantini, M.; Buttino, I.; Ianora, A.; Palumbo, A. Nitric oxide mediates the stress response induced by diatom aldehydes in the sea urchin *Paracentrotus lividus*. *PLoS ONE* **2011**, *6*, e25980. [CrossRef] [PubMed]
66. Marrone, V.; Piscopo, M.; Romano, G.; Ianora, A.; Palumbo, A.; Costantini, M. Defensome against toxic diatom aldehydes in the sea urchin *Paracentrotus lividus*. *PLoS ONE* **2012**, *7*, e31750. [CrossRef] [PubMed]
67. Varrella, S.; Romano, G.; Ianora, A.; Bentley, M.G.; Ruocco, N.; Costantini, M. Molecular response to toxic diatom-derived aldehydes in the sea urchin *Paracentrotus lividus*. *Mar. Drugs* **2014**, *12*, 2089–2113. [CrossRef]
68. Gudimova, E.; Eilertsen, H.C.; Jørgensen, T.; Hansen, E. In vivo exposure to northern diatoms arrests sea urchin embryonic development. *Toxicon* **2016**, *109*, 63–69. [CrossRef] [PubMed]
69. Sartori, D.; Gaion, A. Toxicity of polyunsaturated aldehydes of diatoms to Indo-Pacific bioindicator organism *Echinometra mathaei*. *Drug Chem. Toxicol.* **2016**, *39*, 124–128. [CrossRef] [PubMed]
70. Ruocco, N.; Varrella, S.; Romano, G.; Ianora, A.; Bentley, M.G.; Somma, D.; Leonardi, A.; Mellone, S.; Zuppa, A.; Costantini, M. Diatom-derived oxylipins induce cell death in sea urchin embryos activating *caspase-8* and *caspase 3/7*. *Aquat. Toxicol.* **2016**, *176*, 128–140. [CrossRef]
71. Varrella, S.; Romano, G.; Costantini, S.; Ruocco, N.; Ianora, A.; Bentley, M.G.; Costantini, M. Toxic diatom aldehydes affect defence gene networks in sea urchins. *PLoS ONE* **2016**, *11*, e0149734. [CrossRef]

72. Varrella, S.; Romano, G.; Ruocco, N.; Ianora, A.; Bentley, M.G.; Costantini, M. First morphological and molecular evidence of the negative impact of diatom-derived hydroxyacids on the sea urchin *Paracentrotus lividus*. *Toxicol. Sci.* **2016**, *151*, 419–433. [CrossRef]
73. Ruocco, N.; Maria Fedele, A.; Costantini, S.; Romano, G.; Ianora, A.; Costantini, M. New inter-correlated genes targeted by diatom-derived polyunsaturated aldehydes in the sea urchin *Paracentrotus lividus*. *Ecotoxicol. Environ. Saf.* **2017**, *142*, 355–362. [CrossRef]
74. Albarano, L.; Ruocco, N.; Ianora, A.; Libralato, G.; Manfra, L.; Costantini, M. Molecular and morphological toxicity of diatom-derived hydroxyacid mixtures to sea urchin *Paracentrotus lividus* embryos. *Mar. Drugs* **2019**, *17*, 144. [CrossRef]
75. Ruocco, N.; Costantini, S.; Zupo, V.; Lauritano, C.; Caramiello, D.; Ianora, A.; Budillon, A.; Romano, G.; Nuzzo, G.; D'Ippolito, G.; et al. Toxigenic effects of two benthic diatoms upon grazing activity of the sea urchin: Morphological, metabolomic and *de novo* transcriptomic analysis. *Sci. Rep.* **2018**, *8*, 5622. [CrossRef]
76. Ruocco, N.; Annunziata, C.; Ianora, A.; Libralato, G.; Manfra, L.; Costantini, S.; Costantini, M. Toxicity of diatom-derived polyunsaturated aldehyde mixtures on sea urchin *Paracentrotus lividus* development. *Sci. Rep.* **2019**, *9*, 517. [CrossRef] [PubMed]
77. Ruocco, N.; Cavaccini, V.; Caramiello, D.; Ianora, A.; Fontana, A. Noxious effects of the benthic diatoms *Cocconeis scutellum* and *Diploneis* sp. on sea urchin development: Morphological and *de novo* transcriptomic analysis. *Harmful Algae* **2019**, *86*, 64–73. [CrossRef]
78. Esposito, R.; Ruocco, N.; Albarano, L.; Ianora, A.; Manfra, L.; Libralato, G.; Costantini, M. Combined effects of diatom-derived oxylipins on the sea urchin *Paracentrotus lividus*. *Int. J. Mol. Sci.* **2020**, *21*, 719. [CrossRef] [PubMed]
79. Vidoudez, C.; Pohnert, G. Growth phase-specific release of polyunsaturated aldehydes by the diatom *Skeletonema marinoi*. *J. Plankton Res.* **2008**, *30*, 1305–1313. [CrossRef]
80. Ianora, A.; Bentley, M.G.; Caldwell, G.S.; Casotti, R.; Cembella, A.D.; Engström-Öst, J.; Halsband, C.; Sonnenschein, E.; Legrand, C.; Llewellyn, C.A.; et al. The relevance of marine chemical ecology to plankton and ecosystem function: An emerging field. *Mar. Drugs* **2011**, *9*, 1625–1648. [CrossRef]
81. Zupo, V.; Glaviano, F.; Caramiello, D.; Mutalipassi, M. Effect of five benthic diatoms on the survival and development of *Paracentrotus lividus* post-larvae in the laboratory. *Aquaculture* **2018**, *495*, 13–20. [CrossRef]
82. Hawkins, D.J.; Brash, A.R. Eggs of the sea urchin *Strongylocentrotus purpuratus* contain a prominent (11R) and (12R) lipoxygenase activity. *J. Biol. Chem.* **1987**, *262*, 7629–7634.
83. Hawkins, D.J.; Brash, A.R. Mechanism of biosynthesis of 11R- and 12R-hydroxyeicosatetraenoic acids by eggs of the sea urchin *Strongylocentrotus purpuratus*. *FEBS Lett.* **1989**, *247*, 9–12. [CrossRef]
84. Amin, R.M.; Koski, M.; Båmstedt, U.; Vidoudez, C. Strain-related physiological and behavioral effects of *Skeletonema marinoi* on three common planktonic copepods. *Mar. Biol.* **2011**, *158*, 1965–1980. [CrossRef]
85. Barreiro, A.; Carotenuto, Y.; Lamari, N.; Esposito, F.; Ippolito, G.D.; Fontana, A.; Romano, G.; Ianora, A.; Miralto, A.; Guisande, C. Diatom induction of reproductive failure in copepods: The effect of PUAs versus non volatile oxylipins. *J. Exp. Mar. Bio. Ecol.* **2011**, *401*, 13–19. [CrossRef]
86. Carotenuto, Y.; Ianora, A.; Miralto, A. Maternal and neonate diatom diets impair development and sex differentiation in the copepod *Temora stylifera*. *J. Exp. Mar. Bio. Ecol.* **2011**, *396*, 99–107. [CrossRef]
87. Ianora, A.; Romano, G.; Carotenuto, Y.; Esposito, F.; Roncalli, V.; Buttino, I.; Miralto, A. Impact of the diatom oxylipin 15S-HEPE on the reproductive success of the copepod *Temora stylifera*. *Hydrobiologia* **2011**, *666*, 265–275. [CrossRef]
88. Koski, M.; Yebra, L.; Dutz, J.; Jónasdóttir, S.H.; Vidoudez, C.; Jakobsen, H.H.; Pohnert, G.; Nejstgaard, J.C. The effect of egg versus seston quality on hatching success, naupliar metabolism and survival of *Calanus finmarchicus* in mesocosms dominated by *Phaeocystis* and diatoms. *Mar. Biol.* **2012**, *159*, 643–660. [CrossRef]
89. Lauritano, C.; Borra, M.; Carotenuto, Y.; Biffali, E.; Miralto, A.; Procaccini, G.; Ianora, A. First molecular evidence of diatom effects in the copepod *Calanus helgolandicus*. *J. Exp. Mar. Bio. Ecol.* **2011**, *404*, 79–86. [CrossRef]
90. Lauritano, C.; Borra, M.; Carotenuto, Y.; Biffali, E.; Miralto, A.; Procaccini, G.; Ianora, A. Molecular evidence of the toxic effects of diatom diets on gene expression patterns in copepods. *PLoS ONE* **2011**, *6*, e26850. [CrossRef] [PubMed]

91. Vehmaa, A.; Larsson, P.; Vidoudez, C.; Pohnert, G.; Reinikainen, M.; Engström-Öst, J. How will increased dinoflagellate:diatom ratios affect copepod egg production?—A case study from the Baltic Sea. *J. Exp. Mar. Bio. Ecol.* **2011**, *401*, 134–140. [CrossRef]
92. Lauritano, C.; Carotenuto, Y.; Miralto, A.; Procaccini, G.; Ianora, A. Copepod population-specific response to a toxic diatom diet. *PLoS ONE* **2012**, *7*, e47262. [CrossRef]
93. Carotenuto, Y.; Dattolo, E.; Lauritano, C.; Pisano, F.; Sanges, R.; Miralto, A.; Procaccini, G.; Ianora, A. Insights into the transcriptome of the marine copepod *Calanus helgolandicus* feeding on the oxylipin-producing diatom *Skeletonema marinoi*. *Harmful Algae* **2014**, *31*, 153–162. [CrossRef]
94. Dhanker, R.; Molinero, J.C.; Kumar, R.; Tseng, L.C.; Ianora, A.; Hwang, J.S. Responses of the estuarine copepod *Pseudodiaptomus annandalei* to diatom polyunsaturated aldehydes: Reproduction, survival and postembryonic development. *Harmful Algae* **2015**, *43*, 74–81. [CrossRef]
95. Lauritano, C.; Carotenuto, Y.; Vitiello, V.; Buttino, I.; Romano, G.; Hwang, J.S.; Ianora, A. Effects of the oxylipin-producing diatom *Skeletonema marinoi* on gene expression levels of the calanoid copepod *Calanus sinicus*. *Mar. Genomics* **2015**, *24*, 89–94. [CrossRef]
96. Brugnano, C.; Granata, A.; Guglielmo, L.; Minutoli, R.; Zagami, G.; Ianora, A. The deleterious effect of diatoms on the biomass and growth of early stages of their copepod grazers. *J. Exp. Mar. Bio. Ecol.* **2016**, *476*, 41–49. [CrossRef]
97. Chen, Z.; Wang, G.; Zeng, C.; Wu, L. Comparative study on the effects of two diatoms as diets on planktonic calanoid and benthic harpacticoid copepods. *J. Exp. Zool. Part A Ecol. Integr. Physiol.* **2018**, *329*, 140–148. [CrossRef]
98. Azizan, A.; Bustamam, M.S.A.; Maulidiani, M.; Shaari, K.; Ismail, I.S.; Nagao, N.; Abas, F. Metabolite profiling of the microalgal diatom *Chaetoceros calcitrans* and correlation with antioxidant and nitric oxide inhibitory activities via 1H NMR-based metabolomics. *Mar. Drugs* **2018**, *16*, 154. [CrossRef]
99. Caldwell, G.S.; Lewis, C.; Pickavance, G.; Taylor, R.L.; Bentley, M.G. Exposure to copper and a cytotoxic polyunsaturated aldehyde induces reproductive failure in the marine polychaete *Nereis virens* (Sars). *Aquat. Toxicol.* **2011**, *104*, 126–134. [CrossRef] [PubMed]
100. Castellano, I.; Ercolesi, E.; Romano, G.; Ianora, A.; Palumbo, A. The diatom-derived aldehyde decadienal affects life cycle transition in the ascidian *Ciona intestinalis* through nitric oxide/ERK signalling. *Open Biol.* **2015**, *5*, 140182. [CrossRef] [PubMed]
101. Lettieri, A.; Esposito, R.; Ianora, A.; Spagnuolo, A. *Ciona intestinalis* as a marine model system to study some key developmental genes targeted by the diatom-derived aldehyde decadienal. *Mar. Drugs* **2015**, *13*, 1451–1465. [CrossRef]
102. Lavrentyev, P.J.; Franzè, G.; Pierson, J.J.; Stoecker, D.K. The effect of dissolved polyunsaturated aldehydes on microzooplankton growth rates in the Chesapeake Bay and Atlantic coastal waters. *Mar. Drugs* **2015**, *13*, 2834–2856. [CrossRef]
103. Torres-Águila, N.P.; Martí-Solans, J.; Ferrández-Roldán, A.; Almazán, A.; Roncalli, V.; D'Aniello, S.; Romano, G.; Palumbo, A.; Albalat, R.; Cañestro, C. Diatom bloom-derived biotoxins cause aberrant development and gene expression in the appendicularian chordate *Oikopleura dioica*. *Commun. Biol.* **2018**, *1*, 121. [CrossRef]
104. Le, M.H.; Dinh, K.V.; Nguyen, M.V.; Rønnestad, I. Combined effects of a simulated marine heatwave and an algal toxin on a tropical marine aquaculture fish cobia (*Rachycentron canadum*). *Aquac. Res.* **2020**, *51*, 2535–2544. [CrossRef]
105. Vardi, A.; Bidle, K.D.; Kwityn, C.; Hirsh, D.J.; Thompson, S.M.; Callow, J.A.; Falkowski, P.; Bowler, C. A diatom gene regulating nitric-oxide signaling and susceptibility to diatom-derived aldehydes. *Curr. Biol.* **2008**, *18*, 895–899. [CrossRef] [PubMed]
106. Llewellyn, C.A.; Tarran, G.A.; Galliene, C.P.; Cummings, D.G.; De Menezes, A.; Rees, A.P.; Dixon, J.L.; Widdicombe, C.E.; Fileman, E.S.; Wilson, W.H. Microbial dynamics during the decline of a spring diatom bloom in the Northeast Atlantic. *J. Plankton Res.* **2008**, *30*, 261–273. [CrossRef]
107. Vardi, A.; Van Mooy, B.A.S.; Fredricks, H.F.; Popendorf, K.J.; Ossolinski, J.E.; Haramaty, L.; Bidle, K.D. Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton. *Science* **2009**, *326*, 861–865. [CrossRef] [PubMed]
108. Meyer, N.; Rettner, J.; Werner, M.; Werz, O.; Pohnert, G. Algal oxylipins mediate the resistance of diatoms against algicidal bacteria. *Mar. Drugs* **2018**, *16*, 486. [CrossRef] [PubMed]

109. Ribalet, F.; Vidoudez, C.; Cassin, D.; Pohnert, G.; Ianora, A.; Miralto, A.; Casotti, R. High plasticity in the production of diatom-derived polyunsaturated aldehydes under nutrient limitation: Physiological and ecological implications. *Protist* **2009**, *160*, 444–451. [CrossRef]
110. Gallina, A.A.; Palumbo, A.; Casotti, R. Oxidative pathways in response to polyunsaturated aldehydes in the marine diatom *Skeletonema marinoi* (Bacillariophyceae). *J. Phycol.* **2016**, *52*, 590–598. [CrossRef]
111. Pichierri, S.; Pezzolesi, L.; Vanucci, S.; Totti, C.; Pistocchi, R. Inhibitory effect of polyunsaturated aldehydes (PUAs) on the growth of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*. *Aquat. Toxicol.* **2016**, *179*, 125–133. [CrossRef] [PubMed]
112. Ternon, E.; Pavaux, A.S.; Marro, S.; Thomas, O.P.; Lemée, R. Allelopathic interactions between the benthic toxic dinoflagellate *Ostreopsis* cf. *ovata and a co-occurring diatom*. *Harmful Algae* **2018**, *75*, 35–44. [CrossRef] [PubMed]
113. Wang, J.; Zhang, Y.; Li, H.; Cao, J. Competitive interaction between diatom *Skeletonema costatum* and dinoflagellate *Prorocentrum donghaiense* in laboratory culture. *J. Plankton Res.* **2013**, *35*, 367–378. [CrossRef]
114. Wang, R.; Wang, J.; Xue, Q.; Sha, X.; Tan, L.; Guo, X. Allelopathic interactions between *Skeletonema costatum* and *Alexandrium minutum*. *Chem. Ecol.* **2017**, *33*, 485–498. [CrossRef]
115. Paul, C.; Reunamo, A.; Lindehoff, E.; Bergkvist, J.; Mausz, M.A.; Larsson, H.; Richter, H.; Wängberg, S.Å.; Leskinen, P.; Båmstedt, U.; et al. Diatom derived polyunsaturated aldehydes do not structure the planktonic microbial community in a mesocosm study. *Mar. Drugs* **2012**, *10*, 775–792. [CrossRef]
116. Pepi, M.; Heipieper, H.J.; Balestra, C.; Borra, M.; Biffali, E.; Casotti, R. Toxicity of diatom polyunsaturated aldehydes to marine bacterial isolates reveals their mode of action. *Chemosphere* **2017**, *177*, 258–265. [CrossRef]
117. Bartual, A.; Vicente-Cera, I.; Flecha, S.; Prieto, L. Effect of dissolved polyunsaturated aldehydes on the size distribution of transparent exopolymeric particles in an experimental diatom bloom. *Mar. Biol.* **2017**, *164*. [CrossRef]
118. Eastabrook, C.L.; Whitworth, P.; Robinson, G.; Caldwell, G.S. Diatom-derived polyunsaturated aldehydes are unlikely to influence the microbiota composition of laboratory-cultured diatoms. *Life* **2020**, *10*, 29. [CrossRef]
119. Simon, C.A.; Bentley, M.G.; Caldwell, G.S. 2,4-Decadienal: Exploring a novel approach for the control of polychaete pests on cultured abalone. *Aquaculture* **2010**, *310*, 52–60. [CrossRef]
120. Troncoso, J.; González, J.; Pino, J.; Ruohonen, K.; El-Mowafi, A.; González, J.; Yany, G.; Saavedra, J.; Córdova, A. Effect of polyunsatured aldehyde (A3) as an antiparasitary ingredient of *Caligus rogercresseyi* in the feed of Atlantic salmon, *Salmo salar* José. *Lat. Am. J. Aquat. Res.* **2011**, *39*, 439–448. [CrossRef]
121. Nappo, M.; Berkov, S.; Massucco, C.; Di Maria, V.; Bastida, J.; Codina, C.; Avila, C.; Messina, P.; Zupo, V.; Zupo, S. Apoptotic activity of the marine diatom *Cocconeis scutellum* and eicosapentaenoic acid in BT20 cells. *Pharm. Biol.* **2012**, *50*, 529–535. [CrossRef]
122. Ávila-Román, J.; Talero, E.; De los Reyes, C.; Zubía, E.; Motilva, V.; García-Mauriño, S. Cytotoxic activity of microalgal-derived oxylipins against human cancer cell lines and their impact on ATP levels. *Nat. Prod. Commun.* **2016**, *11*, 1871–1875. [CrossRef]
123. Lauritano, C.; Andersen, J.H.; Hansen, E.; Albrigtsen, M.; Romano, G.; Ianora, A. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. *Front. Mar. Sci.* **2016**, *3*, 68. [CrossRef]
124. Lauritano, C.; Martín, J.; De La Cruz, M.; Reyes, F.; Romano, G.; Ianora, A. First identification of marine diatoms with anti-tuberculosis activity. *Sci. Rep.* **2018**, *8*, 2284. [CrossRef] [PubMed]
125. Jüttner, F.; Messina, P.; Patalano, C.; Zupo, V. Odour compounds of the diatom *Cocconeis scutellum*: Effects on benthic herbivores living on *Posidonia oceanica*. *Mar. Ecol. Prog. Ser.* **2010**, *400*, 63–73. [CrossRef]
126. Amin, S.A.; Parker, M.S.; Armbrust, E.V. Interactions between diatoms and bacteria. *Microbiol. Mol. Biol. Rev.* **2012**, *76*, 667–684. [CrossRef] [PubMed]
127. Armbrust, E.V.; Berges, J.A.; Bowler, C.; Green, B.R.; Martinez, D.; Putnam, N.H.; Zhou, S.; Allen, A.E.; Apt, K.E.; Bechner, M.; et al. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. *Science* **2004**, *306*, 79–86. [CrossRef] [PubMed]
128. Bowler, C.; Allen, A.E.; Badger, J.H.; Grimwood, J.; Jabbari, K.; Kuo, A.; Maheswari, U.; Martens, C.; Maumus, F.; Otillar, R.P.; et al. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* **2008**, *456*, 239–244. [CrossRef] [PubMed]
129. Cho, B.C.; Azam, F. Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* **1988**, *332*, 441–443. [CrossRef]

130. Bates, S.S.; Gaudet, J.; Kaczmarska, I.; Ehrman, J.M. Interaction between bacteria and the domoic-acid-producing diatom *Pseudo-nitzschia multiseries* (Hasle) Hasle; Can bacteria produce domoic acid autonomously? *Harmful Algae* **2004**, *3*, 11–20. [CrossRef]
131. Subashchandrabose, S.R.; Ramakrishnan, B.; Megharaj, M.; Venkateswarlu, K.; Naidu, R. Consortia of cyanobacteria/microalgae and bacteria: Biotechnological potential. *Biotechnol. Adv.* **2011**, *29*, 896–907. [CrossRef]
132. Paul, C.; Mausz, M.A.; Pohnert, G. A co-culturing/metabolomics approach to investigate chemically mediated interactions of planktonic organisms reveals influence of bacteria on diatom metabolism. *Metabolomics* **2013**, *9*, 349–359. [CrossRef]
133. Kouzuma, A.; Watanabe, K. Exploring the potential of algae/bacteria interactions. *Curr. Opin. Biotechnol.* **2015**, *33*, 125–129. [CrossRef]
134. Lépinay, A.; Turpin, V.; Mondeguer, F.; Grandet-Marchant, Q.; Capiaux, H.; Baron, R.; Lebeau, T. First insight on interactions between bacteria and the marine diatom *Haslea ostrearia*: Algal growth and metabolomic fingerprinting. *Algal Res.* **2018**, *31*, 395–405. [CrossRef]
135. Moore, E.R.; Davie-Martin, C.L.; Giovannoni, S.J.; Halsey, K.H. Pelagibacter metabolism of diatom-derived volatile organic compounds imposes an energetic tax on photosynthetic carbon fixation. *Environ. Microbiol.* **2020**, *22*, 1720–1733. [CrossRef] [PubMed]
136. Vuong, T.T.; Kwon, B.R.; Eom, J.I.; Shin, B.K.; Kim, S.M. Interaction between marine bacterium *Stappia* sp. K01 and diatom *Phaeodactylum tricornutum* through extracellular fatty acids. *J. Appl. Phycol.* **2020**, *32*, 71–82. [CrossRef]

foods

MDPI

Review

Bioactive Compounds of Nutraceutical Value from Fishery and Aquaculture Discards

Mirko Mutalipassi [1,†], Roberta Esposito [2,3,†], Nadia Ruocco [2,†], Thomas Viel [1], Maria Costantini [2,*] and Valerio Zupo [1,*]

1 Stazione Zoologica Anton Dohrn, Department of Marine Biotechnology, Villa Dohrn, Punta San Pietro, 80077 Naples, Italy; mirko.mutalipassi@szn.it (M.M.); thomas.viel7@gmail.com (T.V.)
2 Stazione Zoologica Anton Dohrn, Department of Marine Biotechnology, Villa Comunale, 80121 Naples, Italy; roberta.esposito@szn.it (R.E.); nadia.ruocco@szn.it (N.R.)
3 Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Naples, Italy
* Correspondence: maria.costantini@szn.it (M.C.); valerio.zupo@szn.it (V.Z.)
† These authors contributed equally to this work.

Abstract: Seafood by-products, produced by a range of different organisms, such as fishes, shellfishes, squids, and bivalves, are usually discarded as wastes, despite their possible use for innovative formulations of functional foods. Considering that "wastes" of industrial processing represent up to 75% of the whole organisms, the loss of profit may be coupled with the loss of ecological sustainability, due to the scarce recycling of natural resources. Fish head, viscera, skin, bones, scales, as well as exoskeletons, pens, ink, and clam shells can be considered as useful wastes, in various weight percentages, according to the considered species and taxa. Besides several protein sources, still underexploited, the most interesting applications of fisheries and aquaculture by-products are foreseen in the biotechnological field. In fact, by-products obtained from marine sources may supply bioactive molecules, such as collagen, peptides, polyunsaturated fatty acids, antioxidant compounds, and chitin, as well as catalysts in biodiesel synthesis. In addition, those sources can be processed via chemical procedures, enzymatic and fermentation technologies, and chemical modifications, to obtain compounds with antioxidant, anti-microbial, anti-cancer, anti-hypertensive, anti-diabetic, and anti-coagulant effects. Here, we review the main discards from fishery and aquaculture practices and analyse several bioactive compounds isolated from seafood by-products. In particular, we focus on the possible valorisation of seafood and their by-products, which represent a source of biomolecules, useful for the sustainable production of high-value nutraceutical compounds in our circular economy era.

Keywords: wastes; seafood; aquaculture; fishery; functional foods; bioactive compounds; biotechnology; sustainability

Citation: Mutalipassi, M.; Esposito, R.; Ruocco, N.; Viel, T.; Costantini, M.; Zupo, V. Bioactive Compounds of Nutraceutical Value from Fishery and Aquaculture Discards. *Foods* **2021**, *10*, 1495. https://doi.org/10.3390/foods10071495

Academic Editors: Ermelinda Prato and Francesca Biandolino

Received: 13 May 2021
Accepted: 25 June 2021
Published: 28 June 2021

1. Introduction

1.1. Fishery/Aquaculture Practices and Targeted Organisms

Global seafood production in the year 2016 was assessed to be about 171 million tons (Figure 1) [1]. Fishery activities and aquaculture generate a wide array of different wastes. First of all, plastic wastes are heavily produced due to abandoned, lost or discarded fishing gear bilges, as well as other wastes from vessel operations. In parallel, fisheries bycatch discards are produced through low-selective fishing gears, not equipped to exclude non-targeted organisms. These latter methods may catch significant amounts of finfish species, juveniles, benthic animals, marine mammals, marine birds and vulnerable or endangered species, which are often immediately discarded. Moreover, unmarketable organisms due to small size, as well as damaged and inedible specimens, cannot be retained due to management or quota restrictions [2].

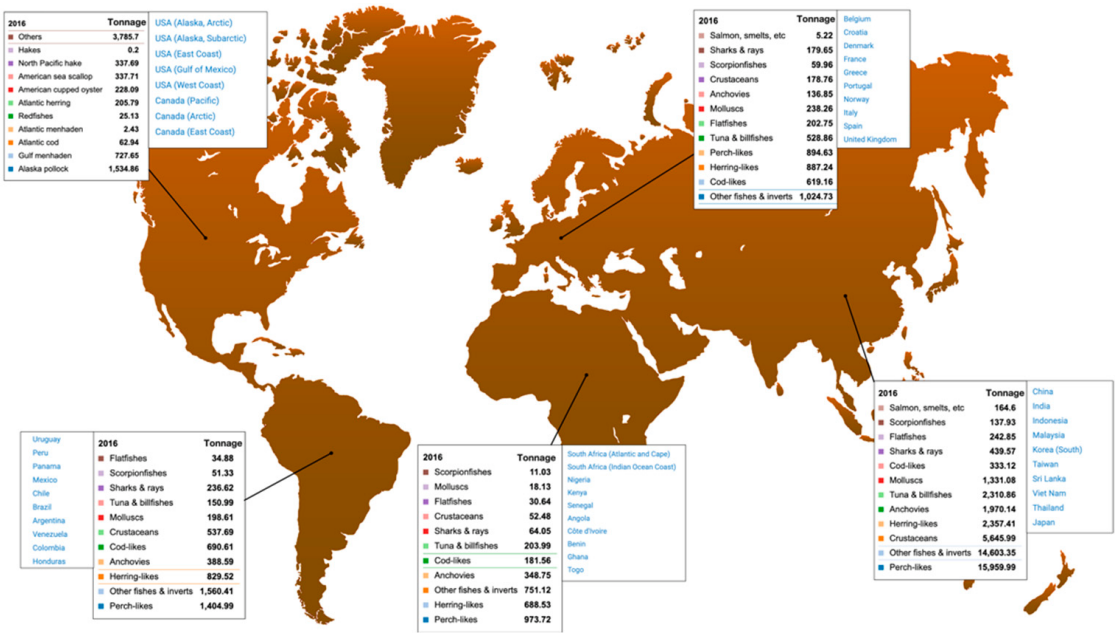

Figure 1. Global seafood production (updated in 2016) according to Pauly et al. [1].

Within fisheries management, discarding is currently one of the most important issues, both from the economic and the environmental point of view [3]. The Food and Agriculture Organization (FAO) Fisheries Glossary describes it as "that proportion of the total organic material of animal origin in the catch which is thrown away or dumped at sea, for whatever reason. It does not include plant materials and postharvest wastes such as offal. The discards may be dead or alive". In the United States, during the period 2009–2013, about 47% of the edible seafood was not used for human consumption, representing a large percentage of harvest discarded as by-catch during commercial fishing [4]. A comparable situation was found in Europe, where the ratio between seafood consumed and discarded as waste is estimated to be 1:1 [5]. Scientists and fishery managers underlined the importance of reducing these wastes, minimizing the ecological impacts of fishery [6–12]. In addition, a strong diversification of marine harvest was recommended, to reduce the fishing pressure on current target species by using those that are not considered interesting for commercial purposes [12].

Active bottom-contact gears (e.g., bottom trawls) are widespread large-scale fishing techniques, generally known to produce the highest discarding as compared to any other fishing gear which in many countries are becoming a real concern [2]. Thus, shrimp fisheries, particularly in tropical waters, had the highest total amount and proportion of discards with a weighted average rate of 62% [2,13–15]. Shrimp trawling produces the highest level of discard/catch rations if compared with other fishing techniques with values ranging from 3:1 to 15:1, according to target species, seasons and areas [16]. Along the Indian coasts, fishery bycatch is mainly composed of high proportions of juveniles and sub-adult individuals of commercially important species. It was estimated that in 2008 the annual economic loss due to juveniles in fishing, by trawlers, purse seiners, ring seiners and mini-trawlers together, was about US$ 19.445 million yr^{-1} [17]. If compared to shrimp fisheries, finfish trawling has a relatively low discard rate, contributing to a substantial total amount of discards worldwide. In addition, tuna and high migratory species contribute to total bycatch with up to 28.5% of the weighted average discard rate. In contrast, small-scale and artisanal fisheries exhibit very low or negligible discards, although in some areas, for example in the Mediterranean, the total amount of discards can still be very substantial due to a huge presence of artisanal fleets [18,19].

The taxonomic composition of discards varies among different areas and techniques according to the natural biodiversity patterns of fishing areas, target taxa, fisheries types, and

gears. In the Atlantic Ocean, redfish (Sebastidae, 19%), hake (Merlucciidae, 18%), American plaice (Pleuronectidae, 13%) and rays (Rajidae, 12%) are the dominating species [2]. These organisms are potentially marketable but, when taken by fishing gears, are not retained and utilized, either because of poor product quality or small size and/or due to deliberate high-grading of catches (especially in areas outside of management intervention). In the northeast Atlantic Ocean, discarded species are quite different ranging from haddock (Gadidae, 19%), redfish (16%), Atlantic cod (Gadidae, 11%) and hake (6%). Data from eastern central Atlantic area (west Africa, FAO area 34) show a lack of resolution but it is possible to identify some taxonomically distinct entities, such as cephalopods (Cephalopoda, 4%), followed by small pelagic species (European anchovy, Engraulidae) and the European pilchard (Clupeidae). An important number of discards are "miscellaneous marine organisms", both for pelagic and coastal fisheries, which account for up to 24% of the total discarded animals that are generally non-marketable species, such as starfish (Asteroidea, 6%) and sharks (e.g., *Prionace glauca*, Carcharhinidae) [2].

In addition to other bycatch species, jellyfish are interesting cases of study [20], since they are usually avoided by fishermen for their capability to interfere with fishing activities [21]. The occurrence of high-density gelatinous organisms during blooms are correlated with both favourable conditions in marine environments and intrinsic biological characteristics of each group [22]. In Brazil, jellyfish are identified as the organisms caught in the highest frequency and number, especially during spring and summer as in the case of the hydromedusa *Rhacostoma atlanticum*. Potential uses of jellyfish for commercial purposes are still in early stages although some studies were conducted to capture and process these organisms for human consumption. In fact, in some Asian countries, jellyfish are considered a delicacy and their commercial market is increasing [20]. In addition to this potential utilization, jellyfish are extremely rich in secondary metabolites that could find interesting applications in biotechnological fields [23]. Recently, a commercial product named Prevagen®, a dietary supplement containing apoaequorin, a protein extracted from the jellyfish Aequorea victoria, has been demonstrated to bind calcium in the brain, improving the electrical signals between nerve cells and contributing to prevent dementia and Alzheimer's disease [24,25].

1.2. Most Useful Discards from Several Seafood Taxa

Seafood wastes are used as raw material for silage, fish meal, and fertilizer or as a component of aqua- and poultry feeds [26–29] thank to the high content of proteins, polyunsaturated fatty acids (PUFA), and other nutrients having various health benefits including carotenoids, minerals, vitamins, squalene, glycosaminoglycans. For this reason, despite the low value traditionally assigned to fishery by-products, there is a growing interest in the potential use of these wastes as functional ingredients, nutraceuticals, and pharmaceuticals in a wide range of applications [30–37]. On one side, this approach offers a significant benefit from an economical point of view, providing additional income from a material that, in some cases, has a disposal cost. On the other side, the valorization of seafood wastes, including bycatch and byproduct, can strongly reduce environmental pollution [38]. Since fishery and aquaculture wastes are rich in high-quality nutrients, there is a great potential in the marine bioprocess industry to convert and use a large fraction of these valuable products. For instance, seafood represent a rich source of proteins varying in functional and biological properties [26,39], available in high concentration in fish heads, backbones, tails etc. These proteins, as well as other biomolecules retrieved in seafood and especially in shellfish, can be easily extracted using new technologies developed for biotechnological purposes, as in the case of the chitosan produced from exoskeletons of the shrimp *Pleoticus muelleri* [40,41]. These advances include (a) macromolecules biotransformation via enzymes or microorganisms, (b) subcritical and supercritical extractions for the isolation of target products, (c) ultra-filtration, (d) microwave and (e) ultrasound-assisted recovery processes and membrane separation [37,39,42,43]. For these reasons, cheap and

energy-efficient enzymatic techniques are emerging in food processing, based on the use of proteases, glycoside hydrolases, lipases, and transglutaminases [44,45].

The pre-processing operations of seafood, from fisheries and aquaculture, generate miscellaneous wastes, depending on the raw material and the desired final products in diverse markets [46,47]. Seafood processing wastes include beheading, de-shelling, skinning, gutting, removal of fins and scales, filleting, washing, etc. This waste can represent up to 40% of the total seafood. This material can be wasted as solid discards, offal, or by-product [33]. The percentage of waste materials can vary according to the processed organism, as in the case of finfish, generating up to 50% of waste material that comprises entrails, heads, skeletal frames, skin, scales, and viscera. The same wastes are produced during tuna canning operations but the process, in this case, results in a higher percentage of solid wastes (about 70%).

Crustacean wastes and byproducts can reach 75% of the shellfish, as in the case of lobster processing industries, which are composed of cephalothorax, carapace, tail, and shell [30–33,48]. Shellfish wastes are largely insoluble and very resistant to natural biodegradation, which might lead to health and environmental concerns. However, the constituents of shells, generally 30% protein and 30% chitin, make them interesting for further processing. A major problem with shrimp biomaterial valorization is the high perishability of the material that, in a tropical climate, is rapidly deteriorated by bacterial activities. Various technologies have been developed to use shrimp wastes, replace standard and hazardous chemical methods and extract bioactive compounds [49]. Shellfish are rich in carotenoids, which are lipophilic compounds responsible for yellow and red colours in nature [50] and, in particular, astaxanthin is commercially exploited due to its role as antioxidant, and in aquaculture as a feed additive for enhancing flesh colouration (i.e., the pink colour) of farmed salmonids which is generally desired by consumers [37,51,52].

In addition to fishes and crustaceans, marine algae wastes can be potentially exploited. In fact, from a nutritional point of view, edible seaweeds are rich in minerals and vitamins, being recognized as an ideal source of iodine as well as one of the few plant sources of vitamin B12 [53]. Various seaweeds have been historically harvested for human consumption and *Ulva* (Chlorophyta), *Porphyra* (Rhodophyta), *Undaria*, *Laminaria*, *Himanthalia*, and *Saccharina* (Phaeophyceae) are common ingredients of many Asian recipes [54], improving the quality of various food products [55]. Macroalgae wastes are not sufficient to satisfy worldwide demand and several algal species are intensively cultivated, especially in integrated aquacultures [56,57].

1.3. Aim of the Review

In the present review, we considered the studies describing the seafood-derived compounds with potential use in nutraceutical field (see Tables 1–7). Several classes of compounds were chosen, including collagen, gelatin, minerals, proteins, lipids, carotenoids, polysaccharides, and phenols. For each of them, we outlined which taxa, organism or, tissue, might be the most interesting source. When available, we highlighted the bioactivity of these compounds, demonstrated through in vitro or in vivo tests, such as antioxidant, anti-hypertensive, anti-diabetes and so on. We finally reported some examples of commercialized products containing seafood-derived compounds already used for treating human diseases. The biotechnological significance of aquaculture, fishing, and industrial by-products was thus investigated and debated, together with future expectations and challenges.

2. Collagen and Gelatin

Collagen type I, II, and IV have been particularly extracted from skin, bones, scales and cartilages [58], through a sustainable approach respecting the European zero-waste strategy. In fact, fish, echinoderms and jellyfish discards are suitable sources of high-quality collagen that has been efficiently extracted and processed by acid, alkaline, and enzymatic treatments coupled to mechanical methods, such as pH adjustments, homogenization,

and sonication [59]. Type I collagen was extracted from the tissue of sea urchins [60,61], octopus [62], starfish [63], jellyfish [63], and several species of fish [64–66]. The main amino acids found within collagen and gelatin molecules are glycine (Gly), alanine (Ala), proline (Pro) and hydroxyproline, with basically a Gly-X-Y triplet as a repeating unit. Amino acids composition may differ depending on the environmental conditions (e.g., temperature), type of tissue, and extraction methods [67].

Nowadays, collagen displayed a wide range of applications in the health-related sectors, namely in cosmetics, the pharmaceutical industry and medical care (including plastic surgery, orthopaedics, ophthalmology and dentistry) [68]. Despite the high potentialities of collagen and gelatin for developing medical products and new therapeutic strategies, so far, only a few examples of commercially available drugs have been recorded [69]. Concerning non-health sectors, a noteworthy use of collagen is related to the food sector (food processing and nutraceuticals), but most often as gelatin, i.e., in its denatured form [36]. Indeed, collagen has become a functional ingredient towards the "healthy foods" development. Generally, collagen production decreases with age and bad diet [70] and consequently food supplements are intended to uphold the skin, hair, nails, and body tissues of the users [71]. Fish gelatin can be obtained by hydrolyzing and denaturing collagen [72] through a pre-treatment step, which is necessary before the extraction for improving the extraction efficiency. The pre-treatment consists of acid or alkaline hydrolysis, a method chosen according to the source material [72], and gelatin can be prepared by acid or water extraction [73]. The properties of gelatin are influenced by two main factors: the characteristics of the initial collagen and the extraction process [73,74]. Gelatin is widely used as an ingredient to improve the elasticity, texture and stability of foods but it may also give rise to biologically active peptides by protease hydrolysis. These metabolites have a potential activity as inhibitors of angiotensin I converting enzyme (ACE) or as antioxidants. For instance, gelatin obtained by acid extraction from four different species of local marine fish caught off the coast of Langkawi Island, Malaysia, such as "kerapu" (*Epinephelus sexfasciatus*), "jenahak" (*Lutjianus argentimaculatus*), "kembung" (*Rastrelliger kanagurta*), and "kerisi" (*Pristipomodes typus*) contained essential amino acids, with glycine being the most predominant (Table 1) [75]. Gelatin extracted from tunafish and giant squid tunics (*Dosidicus gigas*) demonstrated antioxidant activity after hydrolysis with trypsin, α-chymotrypsin or pepsin [76]. This work confirmed the high antioxidant capacity of whole and fractionated alcalase hydrolysates of gelatin from giant squids. This capacity was noticeably higher than that obtained from tuna fish, under the same hydrolysis conditions [76]. In a similar study, the antioxidant activities of gelatins extracted from frozen inner and outer tunics of the jumbo flying squid (*Dosidicus gigas*), tuna (*Thunnus* spp.) and halibut (*Hypoglossus* spp.) skins were evaluated by FRAP (ferric reducing antioxidant power) and ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assays [77]. In particular, gelatin extracted from squid showed a greater antioxidant activity due to the reduction of iron and an enhancement of the removal of free radicals [77]. Peptides purified from skin gelatin of the Pacific cod (*Gadus macrocephalus*), in particular papain hydrolysate, showed potent antioxidant activity [78]. Furthermore, the peptides obtained from the purification of papain hydrolysate showed a potential inhibitory effect of the ACE enzyme [78]. The gelatin extracted from the skin of the Chum Salmon (*Oncorhynchus keta*) and its hydrolysate had protective effects against UV irradiation-induced skin photoaging [79]. In particular, gelatin hydrolysate of the salmon skin could be used in the nutraceutical and cosmeceutical industries to reduce oxidative stress, maintain the homeostasis of the collagen matrix [80], and strengthen the immune system [81].

Ultrasound treatment for the extraction of collagen from the skin of the sea bass *Lateolabrax japonicus* has been developed by several authors [82]. In addition, collagen extracted by giant edible jellyfish, *Nemopilema nomurai*, stimulated the production of immunoglobulins and cytokines by human hybridoma cells and human peripheral blood lymphocytes without inducing allergic complications [83].

Table 1. Gelatin and collagen from seafood wastes, together with taxa, species and tissue from which they were isolated, and their biotechnological application. N/A refers to "not applicable".

Taxa	Species	Tissue	Product	Biological Activity	Reference
Fish	*Epinephelus sexfasciatu*, *Lutjianus argentimaculatus*, *Rastrelliger kanagurta*, *Pristipomodes typus*	N/A	Gelatin	N/A	[75]
Bivalve	*Dosidicus gigas*	Tunics	Gelatin	Antioxidant	[76]
Bivalve and fish	*Dosidicus giga*, *Thunnus* spp., *Hypoglossus* spp.	Inner and outer tunics	Gelatin	Antioxidant	[77]
Fish	*Gadus macrocephalus*	Skin	Gelatin	Antioxidant	[78]
Fish	*Lateolabrax japonicus*	Skin	Collagen	N/A	[82]
Fish	*Oncorhynchus keta*	Skin	Gelatin	Antioxidant	[79]
Jellyfish	*Nemopilema nomurai*	Body	Collagen	Antioxidant	[83]

3. Mineral Salts

These compounds are divided into the major minerals (macro-minerals), such as calcium, sodium and potassium, and trace minerals (micro-minerals), including iron, copper, zinc, and manganese (Table 2) [84].

Among them, calcium is a fundamental element for the physiology of vertebrates, to build and maintain strong bones, as well as for many other tissues and biochemical processes [85]. Calcium extracted from food wastes showed good potentialities to make calcium-enriched bread for treating patients with osteoporosis deficiencies [86]. As demonstrated by Jung et al. [87], the skeletons discarded from industrial processing of hoki (*Johnius belengerii*) and its digested products could be used as nutraceuticals with potential calcium-binding activity. Milk tablets supplemented with nano-powdered eggshell (NPES) or nano-powdered oyster shell (NPOS), NPES with zinc (Zn-NPES), and NPOS with activated zinc (Zn-NPOS) were found suitable tools for calcium supply since did not show any significant differences in pH, consistency, colour, humidity when compared to control milk tablets [88]. Moreover, bread enriched with oyster shells showed higher protein, ash and fiber contents than control bread. In addition, the eggshell and oyster bread had significantly higher levels of calcium, iron, zinc, and phosphorus than the control. The fortification of bread with natural sources of calcium, such as skimmed milk powder and waste products, like oyster shell and eggshell powders, was demonstrated to improve the rheological characteristics of dough and the quality and nutritional properties compared to the control bread [89]. Although some studies investigated the high value of calcium from seafood by-products as food supplement, it must be considered that solubilization and ionization processes are necessary for its real adsorption. This latter issue raises not a few limits to a concrete application of marine calcium minerals for human consumption [90].

Iron is another mineral involved in many biochemical processes such as oxygen transport, energy production and cell proliferation [91] and for these reasons is one of the most important trace minerals in human physiology. However, nearly one-fifth of the population in the world reports some nutrition issues due to iron deficiency [92,93]. Therefore, iron can be administered through the diet in salts, metal chelating agents and iron-chelating peptides. For instance, the skin of the Alaska pollock hydrolyzed with trypsin generates iron-chelating peptides of high stability and adsorption features useful as iron vehicles in therapeutic food supplements [94].

Marine seaweeds contain 10–100 times more minerals than traditional vegetables with iron, copper, calcium, and magnesium present in higher concentrations [95]. Moreover, seaweeds can be considered as the best inexpensive food to fulfil the iodine requirements of humans and, more generally, they can be used as mineral food supplement [96], as well as, for their beneficial effect on hypercholesterolemia and arterial hypertension [97]. Due to their nutritional value, algae are commonly used as dietary supplements, as in the case of algae belonging to the *Spirulina* and *Chlorella* genera [55]. For example, *Laminaria japonica*

is known to store marine minerals in a highly concentrated form, and it has been used to produce algae-based ingredients for skin protection against UV damage [98].

Table 2. Minerals and peptides carrying minerals from seafood wastes, together with taxa, species and tissue from which they were isolated, and their biotechnological application.

Taxa	Species	Tissue	Product	Biological Activity/ Nutraceutical Application	Reference
Fish	*Johnius belengerii*, *Thunnus thynnus*	Skeletons	Phosphopeptide	Potential calcium-binding activity/food supply	[87]
Bivalve	N/A	Shell	Calcium	Food supplement	[88]
Bivalve	N/A	Shell	Calcium, iron, zinc and phosphorus	Food supplement	[89]
Fish	*Gadus chalcogrammus*	Skin	Peptides	Iron-chelating/food supply	[94]
Brown alga	*Laminaria japonica*	NA	Minerals	Antioxidant	[98]

4. Protein and Protein Hydrolysates

Despite the availability on the market of various molecules with anti-hypertensive activity, clinical tests indicate that side effects, such as cough and angioedema, are extremely common [99]. A potential solution is to replace the synthetic inhibitors, normally used in therapeutic formulation, with natural peptides retrieved in food proteins [100]. Interestingly, marine bioactive peptides possess various biological functions, including the inhibition of ACE, antioxidant, immunomodulatory, anti-microbial, and anti-coagulant activities (Table 3) [101,102]. Fish protein hydrolysates, for their amino acid composition and easily digestible proteins, are considered to have excellent quality, from a nutritional point of view. Nevertheless, due to the unpleasant fishy smell and flavour, they were mostly used for animal nutrition [103,104]. Recent studies provided evidence that marine bioactive peptides from several marine organisms act as potential antioxidant inhibiting lipid peroxidation and removing reactive oxygen species [105,106]. Interestingly, free radicals scavenging activity has been addressed to the hydrophobic amino acids (e.g., alanine, phenylalanine, isoleucine, leucine, valine and glycine and proline, methionine, tyrosine, histidine, lysine and cysteine) that may improve the efficiency of antioxidant peptides. In fact, these amino acids could act as proton donors or electron and/or as lipid radical scavengers [107]. For instance, His-Gly-Pro-Leu-Gly-Pro-Leu (797 Da) peptides extracted from fish Hoki (*Johnius belengerii*) skin gelatin had antioxidant activity, tested in a linoleic acid peroxidation system and radical-scavenging potency. In addition, antioxidative enzyme levels in cultured human hepatoma cells increased in the presence of this peptide, suggesting that it was involved in maintaining the redox balance in the cell environment [108]. Alkalin-pretreated cobia (*Rachycentron canadum*) skin was extracted in a retort for 30 min to obtain a retorted skin gelatin hydrolysate (RSGH). Cobia RSGH and its derivatives showed a strong antioxidant activity by inhibiting lipid peroxidation. It is well-known that lipid peroxidation occurring in food products deteriorates food quality, resulting in rancidity, unacceptable taste, and shorter shelf-life. The RSGH retarded lipid deterioration and may be used as a natural antioxidant for food products. In fact, these peptides can be used as antioxidants in functional foods and supplements [109]. In another study, antioxidant activity of fish protein hydrolysates obtained from cod backbones (*Gadus morhua*) was evaluated using liposomes and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Moreover, the DPPH scavenging activity showed that the anti-oxidative activity of hydrolysates could be due to the ability to scavenge lipid radicals [110]. The protein hydrolysates obtained through different enzymatic treatments from sardine heads and/or entrails (*Sardinella aurita*) [111,112], Alaska pollack (*Theragra chalcogramma*) skin [113], and from the muscles of the sea bream (*Nemipterus japonicus*) and other fish (*Exocoetus volitans*) showed antioxidant activity. Moreover, the trypsin protein hydrolysates of both fishes showed maximum free radical scavenging potential and lipid peroxidation inhibi-

tion. Furthermore, these peptides showed a significant anti-proliferative effect on Hep G2 (human hepatocellular liver carcinoma) cell line [114]. A peptide was isolated from the Black pomfret (*Parastromateus niger*) viscera, showing a peculiar antioxidant activity, able to inhibit lipid peroxidation and oxidative damage [115]. A similar activity was also identified in Horse mackerel visceral protein hydrolysate from the fish *Magalaspis cordyla*. In particular, this peptide inhibited lipid peroxidation avoiding oxidative damage in living systems [116]. In vitro assays reported antioxidant activities in two peptides isolated from skin protein hydrolysates of the horse mackerel (*Magalaspis cordyla*) and the croaker (*Otolithes ruber*) [117,118], and eight hydrolysates from cuttlefish (*Sepia officinalis*) by-products (skin and viscera) obtained through treatment with various gastrointestinal proteases (chymotrypsin, trypsin, and crude alkaline enzyme and bacterial proteases) [119]. As reported for the antioxidant properties, ACE inhibitory activity was also attributed to the differences in chain length and amino acids sequences of peptides, as well as, to their hydrophobicity [120]. For instance, an ACE inhibitory Gly-Leu-Pro-Leu-Asn-Leu-Pro (M.W. 770 Da) hydrophobic peptide isolated from salmon skin (*Oncorhynchus keta*) was found to reduce systolic blood pressure after oral administration in rats, suggesting a possible use of this peptide as a functional food with anti-hypertensive effect [121]. Moreover, the jellyfish aqueous/hydroalcoholic extracts and the hydrolyzed peptides resulting from pepsin and collagenase digestions obtained from three Mediterranean species of jellyfish (*Aurelia* sp., *Cotylorhiza tuberculate*, and *Rhizostoma pulmo*) showed evident antioxidant activity, with suitable application in nutraceutical, cosmeceutical, and pharmacological fields [122]. Antioxidant and ACE inhibitory molecules can be found in other jellyfishes, as in the case of *Rhopilema esculentus* Kishinouye, where a Ser-Tyr dipeptide abundant in the gonads of this species, had DPPH, hydroxyl and superoxide radical scavenging effects with IC_{50} 84.623 μM, 1177.632 μM, 456.663 μM, respectively [123,124].

Table 3. Proteins and protein hydrolysates from seafood wastes, together with the taxa, species and tissue from which they were isolated, and their biotechnological application.

Taxa	Species	Tissue	Product	Biological Activity	Reference
Fish	*Johnius belengeri*	Skin	Peptides	Antioxidant	[108]
Fish	*Rachycentron canadum*	Skin	Gelatin derivate	Antioxidant	[109]
Fish	*Gadus morhua*	Backbones	Protein hydrolysates	Antioxidant	[110]
Fish	*Sardinella aurita*	Heads and/or entrails	Protein hydrolysates	Antioxidant	[111,112]
Fish	*Theragra chalcogramma*	Skin	Peptides	Antioxidant	[113]
Fish	*Nemipterus japonicus*	Muscles	Hydrolysates and peptide fractions	Antioxidant	[114]
Fish	*Exocoetus volitans*	Muscles	Hydrolysates and peptide fractions	Antioxidant and anti-tumor	[114]
Fish	*Parastromateus niger*	Viscera	Peptides	Antioxidant	[115]
Fish	*Magalaspis cordyla*	Viscera	Peptides	Antioxidant	[116]
Fish	*Magalaspis cordyla*	Skin	Peptides	Antioxidant	[117]
Fish	*Otolithes ruber*	Skin	Peptides	Antioxidant	[118]
Bivalve	*Sepia officinalis*	Skin and viscera	Protein hydrolysates	Antioxidant	[119]
Fish	*Oncorhynchus keta*	Skin	Peptides	Anti-hypertensive	[121]
Jellyfish	*Aurelia* sp., *Cotylorhiza tuberculate*, *Rhizostoma pulmo*	Body	Hydrolyzed peptides	Antioxidant	[122]
Jellyfish	*Rhopilema esculentus*, *Kishinouye*	Gonads	Protein hydrolysates	Antioxidant	[123,124]
Red alga	*Porphyra* spp.	Leaf	Peptides	Anti-diabetic	[125]
Red alga	*Porphyra yezoensis*	Leaf	Peptides	Anti-thrombotic	[126]

As mentioned before, macroalgae can be also used for the development of nutraceutical products. Particularly interesting is the case of two peptides (Gly-Gly-Ser-Lys and Glu-Leu-Ser) identified from proteolytic enzymes hydrolysates isolated from the red seaweed laver (*Porphyra* species), that significantly inhibited α-amylase activity at 1 mg/mL

by colorimetric method. Since this enzymatic activity can reduce blood glucose levels, the potential application of seaweed hydrolysates in diabetes treatments has been proposed [125]. Similarly, a novel peptide isolated from Nori hydrolysate inhibited the clotting factors involved in the intrinsic pathway of coagulation and, for this reason, it could be used as a functional food in the prevention of thrombosis [126].

5. Lipids

Lipids belong to a fundamental group of nutrients for humankind since they contribute the structure of the biological membranes [127], and act both as energy storage and key signalling molecules [128]. Their components are fatty acids (FAs), which could be classified into saturated (SFAs—without double bonds), monosaturated (MUFAs—with one double bond), and PUFAs (with two or up to six double bonds) [129]. Nowadays, essential FAs are considered to be functional foods and nutraceuticals with many benefits for human health, including the potential of reducing the risk of cardiovascular diseases, cancer, osteoporosis, diabetes [129], inflammation and neurocognitive function [130], and autoimmune diseases [131].

Among lipids, lecithin is a sticky fatty substance mainly composed of phospholipid mixtures with a small percentage of glycerides, neutral lipids, and other suspended matter. Lecithin was used for its emulsifying properties in nutraceutical (i.e., lecithin nanovesicles as supplementary food) [132], pharmaceutical (i.e., hypercholesterolemia, neurologic disorders and liver ailments) [133], and cosmetic sectors (i.e., beauty lotions and cosmetic oil) (Table 4) [134]. Marine lecithin was mostly isolated and characterized from squid (*Todarodes pacificus*) viscera residues de-oiled by supercritical carbon dioxide (SC-CO_2) extraction. In particular, the main phospholipids of lecithin from squid viscera were phosphatidylcholine and phosphatidylethanolamine [135,136]. Some differences in the composition of the phospholipids of squid viscera arose [137] and can be probably explained by variations in habitat, food intake, fishing seasons of sampled organisms, and isolation/quantification processes. However, in other studies, the composition of these phospholipids from squid viscera was almost the opposite [137].

Table 4. Lipids from seafood wastes, together with taxa, species and tissue from which they were isolated, and their biotechnological application.

Taxa	Species	Tissue	Product	Biological Activity	Reference
Cephalopod	*Todarodes pacificus*	Viscera residues	Lecithin	Emulsifying properties	[135,136]
Crustacean	*Jasus edwardsii*	Liver	PUFA	Anti-inflammatory, anti-hypertensive, anti-diabetic	[138]
Crustacean	*Pandalus borealis*	Head, shell and tail	EPA and DHA	Anti-inflammatory, anti-hypertensive, anti-diabetic	[139]
Fish	*Rastrelliger kanagurta*	Ground skin	EPA and DHA	Anti-inflammatory, anti-hypertensive, anti-diabetic	[140]
Fish	*Thunnus tonggol*	Head	DHA, omega-3 and -6 FAs	Anti-inflammatory, anti-hypertensive, anti-diabetic	[141]
Fish	*Salmo salar*	Belly part, trimmed muscle, frame bone and skin	Oil	Free radical scavenging	[142]
Bivalve	*Perna canaliculus*	Body	PUFA/Lyprinol®	Anti-inflammatory and anti-arthritis	[143,144]

Lipids extracted from the liver of the Australian lobster (*Jasus edwardsii*), using SC-CO_2, contained high concentrations of PUFA and low levels of contaminants such as lead, arsenic, mercury and cadmium [138]. So, lipids extracted from the liver of lobsters may be useful in the prevention and treatment of several disorders and diseases including

coronary heart disease, rheumatoid arthritis, asthma, cancers, diabetes. A deep red oil rich in omega-3 PUFAs, especially EPA and DHA, can be also obtained from the by-products (head, shell and tail) of the Northern shrimp (*Pandalus borealis*) [139] and from the ground skin of the Indian mackerel (*Rastrelliger kanagurta*). In particular, oil extracted from Indian mackerel had the highest recoveries of PUFAs [140]. When ethanol was added as a co-solvent of SC-CO_2, higher recovery of PUFAs, especially DHA, omega-3, and omega-6, from fish by-products (e.g., head of the longtail tuna *Thunnus tonggol*) was obtained [141]. Interestingly, oils extracted from various salmon by-products (belly part, trimmed muscle, frame bone and skin) with different techniques (hexane extraction, SC-CO_2 and pressed oil) did not show any differences in fatty acid composition. However, significant variation was detected in free radical scavenging activity, since oils extracted by SC-CO_2 exhibited greater antioxidant properties than those extracted by hexane [142].

A good example of commercialised product is Lyprinol®, a lipid fraction of the freeze-dried extract from the farmed green-lipped mussel *Perna canaliculus*. Due to its anti-inflammatory activities, the drug is currently sold to reduce the inflammatory processes related to arthritis [143,144].

Considering lipids from waste sources, it is important to underline the importance of squalene as a bioactive molecule. Squalene is a natural lipid belonging to the terpenoid family, which partly originates from endogenous cholesterol synthesis and partly from dietary sources, especially in populations consuming large amounts of olive oil or shark liver, olives, wheat germ, and rice bran [145]. Squalene is considered an excellent emollient and moisturizer for the skin, also having antioxidant and anti-cancer properties, to relieve skin irritations and/or tumors [146,147].

A few words should also be addressed to vitamin E, which is a lipid-soluble antioxidant occurring both in plants and animals for the protection of biological membranes against lipid peroxidation. Four homologue pairs (α-, β-, γ-, δ-tocopherols and -tocotrienols) have been described, with the α form being the most active [148]. Marine tocopherols have been firstly isolated from salmon eggs and subsequently from tissues and organs of several fishes, making them a considerable source of these beneficial molecules [149].

6. Carotenoids

Salmons, crustaceans, and shrimps processing wastes could be an important source of carotenoids (e.g., astaxanthin; Table 5) [150–152]. On one side, the intake of carotenoids from food or supplementation should be carefully considered due to the lack of specific biosynthetic pathways in humans [153]. On the other side, carotenoids are interesting bioactive molecules able to mitigate the damaging effect of oxidative stress [154]. In particular, a positive link between higher dietary intake, tissue concentrations of carotenoids, and lower risk of chronic diseases was found [155]. Moreover, the antioxidant activity of astaxanthin modulates the biological functions related to lipid peroxidation, having beneficial effects on chronic diseases, such as cardiovascular diseases, macular degeneration, and cancer [150]. In the specific case of astaxanthin, [156] its oral administration of 1 mg/kg/day for 14 days significantly reduced hepatic metastasis in rats, suggesting an important role in enhancing the immunological response through the inhibition of stress-induced lipid peroxidation. Astaxanthin and its esters displayed a strong antioxidant activity with increasing extract concentration [157]. Furthermore, astaxanthin had an anti-proliferative effect on human laryngeal carcinoma cells (Hep 2 cells), as demonstrated by Sila et al. [157]. Interestingly, to improve the stability of astaxanthin obtained from shrimp shells (*Litopenaeus vannamei*), encapsulation in alginate-chitosan beads was attempted for allowing its real use as a functional ingredient [158].

The content of fucoxanthin, a carotenoid extracted from the macroalga *Undaria pinnatifida*, is also used to control eutrophication and aids the sustainable development of aquaculture [159]. It was lower than the fucoxanthin content found in Japanese commercial wakame products, probably because of the different places and months of collection.

Fucoxanthin extracted from fresh samples exerted a potent antioxidant activity that was higher than the commercial algae due to the loss of phenols and fucoxanthin during processing [160]. Similarly, fucoxanthin extracted from the species *Sargassum wightii* Greville showed antioxidant activity in vitro and inhibition of ACE, with potential application as a food ingredient to overcome hypertension [161].

Table 5. Carotenoids from seafood wastes, together with taxa, species and tissue from which they were isolated, and their biotechnological application.

Taxa	Species	Tissue	Product	Biological Activity	Reference
Crustacean	*Litopenaeus vannamei*	Shell	Astaxanthin	Antioxidant	[158]
Brown alga	*Undaria pinnatifida*	Leaf	Fucoxanthin	Antioxidant	[159]
Brown alga	*Sargassum wightii*	Leaf	Fucoxanthin	Antioxidant and ACE inhibition	[161]

7. Polysaccharides

Crustaceans, shrimps, and crabs are the main sources of chitin from the sea [162]. The most important derivative of chitin is chitosan, obtained through partial deacetylation of chitin under alkaline conditions or enzymatic hydrolysis in the presence of a chitin deacetylase [162]. Several studies proved that chitosan and its derivatives have antioxidant, anti-microbial and anti-viral activities [163–166]. Chitin, chitosan, and their derivatives also act as inhibitors of ACE, an enzyme associated with hypertension. Chemical methods for obtaining chitin from marine wastes (shrimp shells and crabs) provided demineralization and deproteinization, with the use of strong acids or base. Meanwhile, biological methods utilize enzymatic treatment by protease and microbial fermentation [167].

Interesting studies demonstrated the availability of chitin and chitosan in various marine organisms, as in the case of the eggs of the snail *Rapana venosa* and the exoskeleton of the marine crab *Eriphia verrucosa* [168] or the scales of the Red snapper fish (*Lutjanus* sp.) [169], containing chitosan available for biotechnological, agricultural and industrial purposes (Table 6). Moreover, chiton shells were also found to contain a higher abundance of chitin and chitosan than commercial products [170], and *Sepia prashadi* cuttlebone, which proved to store a great content of these compounds, as compared to other sources (e.g., crab shells) [171]. Sulfated polysaccharides extracted from the macroalgae *Gracilaria caudata* and *Gracilaria debilis* by enzymatic and water extraction, respectively, showed antioxidant activity in a concentration-dependent manner [172,173]. The antioxidant capability of these polysaccharides was evaluated in vitro by ferrous ion chelating ability and total antioxidant capacity and in vivo using an oxidative stress rat model induced by 2,2′-azobis (2-methylpropionamidine) dihydrochloride (ABAP) [173].

Carbohydrate complexes, named glycosaminoglycans (GAGs, e.g., chondroitin sulphate, dermatan sulphate, hyaluronic acid), are another class of polysaccharides with interesting bioactivities including, anti-viral, anti-metastatic, anti-inflammatory and anti-coagulant ones, plus a great potentiality in tissue engineering. The therapeutic properties normally depend on the amount and pattern of sulfate groups along the disaccharide chain. GAGs have been extracted from numerous marine organisms that may represent a seafood waste of fishery, aquaculture and industrial processes such as, sea urchins, sea cucumbers, sea squirts and shrimps [174–177]. GAGs extracted from the mussel *P. canaliculus*, were found to exert an important role as anti-arthritic agents. Studies were conducted on the commercial product Biolane™, which contains GAGs plus matrix metalloprotease (MMP's), a family of enzymes necessary for normal tissue re-modelling. The main results revealed that this marine-derived mixture exhibited a wide range of beneficial activities, such as inhibition of pro-inflammatory prostaglandins (PGEs), cyclooxygenase-2 (COX-2), together with anti-platelet aggregation, and fibrinolytic potencies [178]. The cold extract from the same mussel species, extremely rich in glycosaminoglycans, was clinically proven to reduce joint pain and enhance joint mobility. It was commercialized as GlycOmega-PLUS™ [179].

Alginate is a polysaccharide found in the intercellular matrix of brown algae extremely rich in sodium, calcium, magnesium, strontium, and barium ions. Alginate is widely used in industry for its ability to retain water and for its gelling, viscosifier and stabilizing properties [180]. However, in addition to its use in the textile industry as a printing paste, alginate could find several applications in nutraceutical field. Alginate extracted through different techniques (water, acid, alkalase, and cellulase) from the alga *Sargassum angustifolium* showed antioxidant activity in a dose-dependent manner [181]. The brown seaweed cell wall and some marine invertebrates contain also a group of fucose-rich sulfated heteropolysaccharide compound, named fucoidan, which was consumed as dietary fiber in many Asian countries [182]. The structure of the fucoidan varies among species, but usually it contains L-fucose and sulfate, along with small quantities of D-galactose, D-mannose, D-xylose, and uronic acid. These fucoidans revealed various significant biological activities, such as antioxidant, anti-inflammatory, anti-allergic, anti-tumour, anti-obesity, anti-coagulant, anti-viral, anti-hepatopathy, anti-uropathy, and anti-renalpathy effects [183]. For instance, fucoidan extracted from the brown alga *Sargassum polycystum* showed antioxidant activity at 1000 μg/mL and anti-proliferative activity with IC_{50} of 50 μg/mL against the human breast cancer cell line [184]. In addition, fucoidan isolated from *Sargassum wightii* was found to regulate postprandial hyperglycemia in diabetic patients acting as α-D-glucosidase inhibitor in a dose-dependent manner [185].

Table 6. Polysaccharides from seafood waste together taxa, species and tissue from which they were isolated, and their biotechnological application.

Taxa	Species	Tissue	Product	Biological Activity	Reference
Mollusc	*Rapana venosa*	Eggs	Chitin and chitosan	Antioxidant, anti-microbial, anti-viral and anti-hypertension	[168]
Crustacean	*Eriphia verrucosa*	Exoskeleton	Chitin and chitosan	Antioxidant, anti-microbial, anti-viral and anti-hypertension	[168]
Fish	*Lutjanus* sp.	Scales	Chitin and chitosan	Antioxidant, anti-microbial, anti-viral and anti-hypertension	[169]
Mollusc	Several species of chiton	Shell	Chitin and chitosan	Antioxidant, anti-microbial, anti-viral and anti-hypertension	[170]
Cephalopod	*Sepia prashadi*	Cuttlebone	Chitin and chitosan	Antioxidant, anti-microbial, anti-viral and anti-hypertension	[171]
Red alga	*Gracilaria caudata*, *Gracilaria debilis*	Leaf	Sulfated polysaccharides	Antioxidant	[172,173]
Bivalve	*Perna canaliculus*	Body	Glycosaminoglycans/ Biolane™	Anti-inflammatory	[178]
Bivalve	*Perna canaliculus*	Body	Glycosaminoglycans/ GlycOmega-PLUS™	Anti-arthritic	[179]
Brown alga	*Sargassum angustifolium*	Leaf	Alginate	Antioxidant	[181]
Brown alga	*Sargassum angustifolium*	Leaf	Fucoidan	Antioxidant, anti-inflammatory, anti-allergic, anti-tumor, anti-obesity, anti-viral	[183]
Brown alga	*Sargassum polycystum*	Leaf	Fucoidan	Antioxidant	[184]
Brown alga	*Sargassum wightii*	Leaf	Fucoidan	Anti-diabetic	[185]

8. Phenols

As part of both animal and human diet, the nutraceutical properties assigned to phenolic compounds are almost endless including protective effects against cardiovascular disease, neurodegeneration, and cancer [186]. Macroalgae phenolic compounds, particularly phlorotannins, gained particular attention due to their specific bioactivities, including antioxidant, anti-proliferative, or anti-diabetic, despite the high abundance of polysaccharides on the macroalgae matrix made the isolation and characterization quite difficult [186]. Few examples of phenolic bioactive compounds have been reported in the literature (Table 7). For instance, a 2,5-dihydroxybenzoic acid isolated from the macroal-

gae *Laminaria digitata* and *Undaria pinnatifida* displayed a potent α-amylase inhibitory activity [187], whereas the polyphenol-rich extract isolated from the seaweed *Sargassum vachellianum* showed a good free radical scavenging ability, anti-microbial activity and effectively absorbed the UVB and UVA rays [188]. In contrast, another marine polyphenol (Dieckol), isolated from the brown alga *Ecklonia cava*, showed sleep-enhancing effects by increasing the amount of non-rapid eye movement sleep and decreasing wakefulness during the same hours. These results implied that Dieckol can be used as a promising herbal sleep aid with minimal side effects, unlike the existing hypnotics [189]. These macroalgae represent a considerable source of waste products since they are used in intensive aquaculture and management of the eutrophication phenomenon [159,190].

Table 7. Phenols from seafood wastes, together with taxa, species and tissue from which they were isolated, and their biotechnological application.

Taxa	Species	Tissue	Product	Biological Activity	Reference
Brown alga	*Laminaria digitata, Undaria pinnatifida*	Leaf	2,5-dihydroxybenzoic acid	Anti-diabetic (α-amylase inhibition)	[187]
Brown alga	*Sargassum vachellianum*	Leaf	Polyphenol-rich extract	Free radical scavenging, antimicrobial activity and anti-UV	[188]
Brown alga	*Ecklonia cava*	Leaf	Dieckol	Sleep-enhancing	[189]

9. Industrial Status and Trends

In the last ten years, 620 scientific contributions related to marine biotechnology have been published, with a particular focus on the pharmacological and food industry [191]. More than 1000 novel compounds have been, annually, described with a focus on seafood wastes, and several examples of nutraceutical products are already sold in the market [192]. Several companies, such as Aquapreneur (www.aquapreneur.com, accessed date 15 March 2021), Sederma (http://www.sederma.fr, accessed date 15 March 2021), NutraIngredients (www.Nutraingredients.com, accessed date 15 March 2021), SpecialChem (http://www.specialchem4cosmetics.com, accessed date 5 April 2021), Fortitech (fortitechpremixes.com, accessed date 5 April 2021), Copalis (http://www.copalis.fr/, accessed date 5 April 2021), and so on, are currently working on new marine ingredients made of collagen, various peptides, GAGs, oils, calcium supplements, and classes of compounds described above (see Sections 2–8).

Food supplements containing tripeptides, dipeptides, and also free amino acids from fish gelatin and collagen are already available commercially for the preservation of bones and tendon integrity [193]. Moreover, a huge production of fish oil as nutraceutical products was also performed all over the world. Oils contain elevated levels of long-chain omega-3 PUFAs, exhibiting beneficial activities [194]. For instance, Lovaza®, which contains ethyl esters of EPA (20:5) and DHA (22:6), is now available in the market to treat diabetes and cardiovascular diseases (https://www.drugs.com/pro/lovaza.html, accessed date 15 March 2021). Concerning chitosan and its derivatives, at present, several companies are involved in the production of medical and food products with nutraceutical purposes. Common examples are Seatone® and Lyprinol® obtained from mussels that are now sold as functional foods in anti-arthritic and anti-inflammatory treatments [195]. Concluding, it must be considered that additional compounds are still the subject of clinical trials, as in the case of the fish hydrolysate Gabolysat [196], and the mussel and fish neurotoxin Tetrodotoxin (TTX), which is investigated for its analgesic properties [197].

10. Conclusions and Future Perspectives

This review is aimed at an environmentally friendly and sustainable use of marine resources, to foresee possible economic benefits for the sector. The data reported show that seafood by-products contain a range of valuable biomolecules, fully appreciating what is usually considered a "waste" and exploiting them to improve human wellness and health.

In ancient times, hunting, fishing and gathering were three fundamental practices for food supply. Nowadays, humans still relieve on marine natural resources as one of the main ingredients for human consumption. Since oceans occupy more than 70% of the Earth's surface, their high biodiversity makes them a target for searching raw resources, including natural and bioactive compounds. With the increase of the global human population, marine organisms play a role not only as a supply of high-quality food, but also as a source of various compounds for pharmaceuticals, cosmetics, and nutraceutical industries. In fact, several bioactive molecules were isolated from the sea, with beneficial properties including antioxidant, anti-microbial, anti-diabetic, anti-proliferative, anti-obesity, anti-Alzheimer, anti-fibrotic, neuroprotection, sleep-enhancing, lipid-lowering, wound healing, and skin protection activities. Exploiting marine resources in a sustainable way, to satisfy the food requirement of the growing human population, puts high pressure on the natural resources of the planet. Hence, more rational exploitation of the available natural assets should be adopted. It is imperative to develop functional foods from marine products, and in particular from fishery discards, since they are widely available and they can prevent or cure various diseases. Ineffective use of the marine raw materials and the common use of non-selective fishing gears generate a loss of up to 50% of the marine captures, that are discarded in the sea, and up to 80% of the seafood raw material, that is not processed and discarded. A huge amount of wastes generated during seafood industrial processing can be properly handled to obtain raw materials. This management requires a green revolution in industrial processing with the integration of standard processing methods with environmentally friendly and cost-effective ones, to achieve sustainable production with a low ecological footprint. In addition, seafood discards are considered hazardous to the environment and can create a serious waste disposal problem. Here, we reported data demonstrating the opportunity to effectively reuse wastes and by-products from aquaculture and fisheries, which would potentially go to waste, rather than being used to produce high-quality nutraceuticals for human consumption.

The identification of functional ingredients and their nutraceutical application is a growing field. Interesting compounds, such as bioactive peptides, polysaccharides, polyunsaturated fats, carotenoids, polyphenolic compounds, minerals, collagen, gelatin, saponins, phycobiliproteins, and phytosterols, are in fact abundant in fish bycatch and food industrial scraps. The major advantages of extracting waste-derived nutraceuticals, besides their low costs, are found in the easy availability of raw materials, high recovery rates, interesting functional properties of the isolated substances, and the aforementioned potential applications.

In addition, the use of waste compounds opens new perspectives in integrative aquaculture. For example, with the potential use of edible seaweeds in phyto-depuration techniques applicable for multispecies culture systems, species can be sold as food or food complements after the production cycle. Indeed, the use of wastes in nutraceuticals has led to various answers to common issues, such as recycling, increasing of profits by industries, reduction of human footprint activities, and sustainability of marine sources.

However, many aspects should be faced, as in the case of the relationships between processing procedure and the functionality of final products. Further studies are required to evaluate the best procedures to assure the stability of marine bioactive molecules during the processing and storage, as well as the uniformity of the bioactive contents according to natural variability over the fishing areas, the seasons, and the production processes themselves. These latter issues are still limiting the successful exploitation of seafood by-products for the food industry, and further research is needed to bypass them and allow the effective production of compounds for human wellbeing.

Author Contributions: Conceptualization of the review, M.C. and V.Z.; bibliographic research and original draft preparation, M.M., R.E. and N.R.; preparation of figure and tables, T.V.; review and editing of the text, M.C. and V.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374).

Acknowledgments: Mirko Mutalipassi and Nadia Ruocco were supported by the research grants on "Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374). Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. R. Messina kindly revised the English text.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pauly, D.; Zeller, D.; Palomares, M.L.D. Sea around Us. Concepts, Design and Data (seaaroundus.org). Available online: http://www.seaaroundus.org/data/#/spatial-catch (accessed on 15 March 2021).
2. Zeller, D.; Cashion, T.; Palomares, M.; Pauly, D. Global marine fisheries discards: A synthesis of reconstructed data. *Fish Fish.* **2018**, *19*, 30–39. [CrossRef]
3. Catchpole, T.L.; Gray, T.S. Reducing discards of fish at sea: A review of European pilot projects. *J. Environ. Manag.* **2010**, *91*, 717–723. [CrossRef]
4. Love, D.C.; Fry, J.P.; Milli, M.C.; Neff, R. Wasted seafood in the United States: Quantifying loss from production to consumption and moving toward solutions. *Glob. Environ. Chang.* **2015**, *35*, 116–124. [CrossRef]
5. Karadeniz, F.; Kim, S.-K. *Trends in the Use of Seafood Processing By-Products in Europe*; Springer Science and Business Media LLC.: Berlin, Germany, 2013; Volume 9781461495, pp. 11–19.
6. Boopendranath, M.R. Waste minimisation in fishing operations. *Fish. Technol.* **2012**, *49*, 109–119.
7. Suuronen, P.; Chopin, F.; Glass, C.; Løkkeborg, S.; Matsushita, Y.; Queirolo, D.; Rihan, D. Low impact and fuel efficient fishing—Looking beyond the horizon. *Fish. Res.* **2012**, *119–120*, 135–146. [CrossRef]
8. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef] [PubMed]
9. Macfadyen, G.; Huntington, T.; Cappell, R. *Abandoned, Lost or Otherwise Discarded Fishing Gear*; FAO: Rome, Italy, 2009.
10. Thompson, R.C.; La Belle, B.E.; Bouwman, H.; Neretin, L. Marine Debris as a Global Environmental Problem: Introducing a Solutions-Based Framework Focused on Plastic. A STAP Information Document. Available online: https://www.thegef.org/sites/default/files/publications/STAP_MarineDebris_-_website_1.pdf (accessed on 5 April 2021).
11. Link, J.; Segal, B.; Casarini, L.M. Abandoned, lost or otherwise discarded fishing gear in Brazil: A review. *Perspect. Ecol. Conserv.* **2019**, *17*, 1–8. [CrossRef]
12. Bellido, J.M.; Santos, M.B.; Pennino, M.G.; Valeiras, X.; Pierce, G. Fishery discards and bycatch: Solutions for an ecosystem approach to fisheries management? *Hydrobiology* **2011**, *670*, 317–333. [CrossRef]
13. Binns, C.W. Discards in the world's marine fisheries: An update. *FAO Fish. Tech. Pap.* **2011**, *16*, 177–178.
14. Gilman, E.; Roda, A.P.; Huntington, T.; Kennelly, S.J.; Suuronen, P.; Chaloupka, M.; Medley, P.A.H. Benchmarking global fisheries discards. *Sci. Rep.* **2020**, *10*, 1–8. [CrossRef]
15. Pauly, D.; Zeller, D. Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nat. Commun.* **2016**, *7*, 10244. [CrossRef]
16. Environmental Justice Foundation Squandering the Seas: How Shrimp Trawling is Threatening Ecological Integrity and Food Security around the World: A Report. 2003. Available online: https://www.eldis.org/document/A12837 (accessed on 15 April 2021).
17. Najmudeen, T.; Sathiadhas, R. Economic impact of juvenile fishing in a tropical multi-gear multi-species fishery. *Fish. Res.* **2008**, *92*, 322–332. [CrossRef]
18. Nunoo, F.; Boateng, J.O.; Ahulu, A.M.; Agyekum, K.A.; Sumaila, U.R. When trash fish is treasure: The case of Ghana in West Africa. *Fish. Res.* **2009**, *96*, 167–172. [CrossRef]
19. Stergiou, K.I.; Machias, A.; Somarakis, S.; Kapantagakis, A. Can we define target species in Mediterranean trawl fisheries? *Fish. Res.* **2003**, *59*, 431–435. [CrossRef]
20. Bazi, C.C.; Pessatti, M.; Junior, C.R. Utilization of the jellyfish occuring in the bycatch for human consumption in the south of Brazil. *Panam. J. Aquat. Sci.* **2019**, *14*, 13–23.
21. Nagata, R.M.; Haddad, M.A.; Nogueira, M. The nuisance of medusae (*Cnidaria, Medusozoa*) to shrimp trawls in central part of southern Brazilian Bight, from the perspective of artisanal fishermen. *Panam. J. Aquat. Sci.* **2009**, *4*, 312–325.
22. Lucas, C.H.; Dawson, M.N. *What Are Jellyfishes and Thaliaceans and Why Do They Bloom*; Springer Science and Business Media LLC.: Berlin, Germany, 2014; pp. 9–44.
23. Ohta, N.; Sato, M.; Ushida, K.; Kokubo, M.; Baba, T.; Taniguchi, K.; Urai, M.; Kihira, K.; Mochida, J. Jellyfish mucin may have potential disease-modifying effects on osteoarthritis. *BMC Biotechnol.* **2009**, *9*, 98. [CrossRef] [PubMed]
24. Moran, D.L.; Underwood, M.Y.; Gabourie, T.A.; Lerner, K.C. Effects of a Supplement Containing Apoaequorin on Verbal Learning in Older Adults in the Community. *Adv. Mind Body Med.* **2016**, *30*, 4–11.
25. Moran, D.L.; Tetteh, A.O.; Goodman, R.E.; Underwood, M.Y. Safety assessment of the calcium-binding protein, apoaequorin, expressed by Escherichia coli. *Regul. Toxicol. Pharmacol.* **2014**, *69*, 243–249. [CrossRef] [PubMed]

26. Esteban, M.; García, A.; Ramos, P.; Márquez, M. Evaluation of fruit–vegetable and fish wastes as alternative feedstuffs in pig diets. *Waste Manag.* **2007**, *27*, 193–200. [CrossRef]
27. Bhaskar, N.; Suresh, P.; Sakhare, P.; Sachindra, N. Shrimp biowaste fermentation with Pediococcus acidolactici CFR2182: Optimization of fermentation conditions by response surface methodology and effect of optimized conditions on deproteination/demineralization and carotenoid recovery. *Enzym. Microb. Technol.* **2007**, *40*, 1427–1434. [CrossRef]
28. De Arruda, L.F.; Borghesi, R.; Oetterer, M. Use of fish waste as silage: A review. *Braz. Arch. Biol. Technol.* **2007**, *50*, 879–886. [CrossRef]
29. Vázquez, J.; Nogueira, M.; Durán, A.; Prieto, M.A.; Rodríguez-Amado, I.; Rial, D.; González, M.; Murado, M. Preparation of marine silage of swordfish, ray and shark visceral waste by lactic acid bacteria. *J. Food Eng.* **2011**, *103*, 442–448. [CrossRef]
30. Ms, R.V.B.; Ae, R.V.G. Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review. *J. Microb. Biochem. Technol.* **2013**, *5*, 107–129. [CrossRef]
31. Venugopal, V.; Lele, S.S. Nutraceuticals and bioactive compounds from seafood processing waste. In *Springer Handbook of Marine Biotechnology*; Kim, S.K., Ed.; Springer: Berlin, Germany, 2015; pp. 1405–1425, ISBN 9783642539718.
32. Nazzaro, F.; Fratianni, F.; Ombra, M.N.; D'Acierno, A.; Coppola, R. Recovery of biomolecules of high benefit from food waste. *Curr. Opin. Food Sci.* **2018**, *22*, 43–54. [CrossRef]
33. Venugopal, V. *Seafood Processing: Adding Value through Quick Freezing, Retortable Packaging, and Cook-Chilling*; CRC Press: Boca Raton, FL, USA, 2006; ISBN 9781420027396.
34. Ferraro, V.; Cruz, I.B.; Jorge, R.F.; Malcata, F.X.; Pintado, M.M.; Castro, P. Valorisation of natural extracts from marine source focused on marine by-products: A review. *Food Res. Int.* **2010**, *43*, 2221–2233. [CrossRef]
35. Olsen, R.L.; Toppe, J.; Karunasagar, I. Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. *Trends Food Sci. Technol.* **2014**, *36*, 144–151. [CrossRef]
36. Gates, K.W. Marine Products for Healthcare: Functional and Bioactive Nutraceutical Compounds from the Ocean, Vazhiyil Venugopal. *J. Aquat. Food Prod. Technol.* **2010**, *19*, 48–54. [CrossRef]
37. Mao, X.; Guo, N.; Sun, J.; Xue, C. Comprehensive utilization of shrimp waste based on biotechnological methods: A review. *J. Clean. Prod.* **2017**, *143*, 814–823. [CrossRef]
38. Sasidharan, A.; Venugopal, V. Proteins and Co-products from Seafood Processing Discards: Their Recovery, Functional Properties and Applications. *Waste Biomass Valorization* **2020**, *11*, 5647–5663. [CrossRef]
39. Nguyen, T.T.; Barber, A.R.; Corbin, K.; Zhang, W. Lobster processing by-products as valuable bioresource of marine functional ingredients, nutraceuticals, and pharmaceuticals. *Bioresour. Bioprocess.* **2017**, *4*, 1–19. [CrossRef]
40. Fasciglione, G.; Goñi, M.; Yommi, A.; Perez-Bravo, J.; Ortueta, R.; Scampini, A.; Buffa, L.; Andreu, A.; Creus, C. Revaluation of waste from fishing industry through generation of chitosan coatings to improve quality and extend shelf-life of minimally processed lettuce. *Postharvest Biol. Technol.* **2020**, *170*, 111310. [CrossRef]
41. Bonecco, M.B.; Martínez Sáenz, M.G.; Buffa, L.M. Chitosan, from residue to industry. In *Advances in Physicochemical Properties of Biopolymers*; Masuell, M., Renard, D., Eds.; Bentham Science Publishers: Sharjah, United Arab Emirates, 2017; pp. 224–256, ISBN 978-1-68108-545-6.
42. Kim, S.-K.; Senevirathne, M. Membrane Bioreactor Technology for the Development of Functional Materials from Sea-Food Processing Wastes and Their Potential Health Benefits. *Membranes* **2011**, *1*, 327–344. [CrossRef]
43. Freitas, A.C.; Rodrigues, D.; Rocha-Santos, T.A.; Gomes, A.M.; Duarte, A.C. Marine biotechnology advances towards applications in new functional foods. *Biotechnol. Adv.* **2012**, *30*, 1506–1515. [CrossRef]
44. Zhang, Y.; He, S.; Simpson, B.K. Enzymes in food bioprocessing—Novel food enzymes, applications, and related techniques. *Curr. Opin. Food Sci.* **2018**, *19*, 30–35. [CrossRef]
45. Menon, V.V. Enzymes from Seafood Processing Waste and Their Applications in Seafood Processing. *Adv. Food Nutr. Res.* **2016**, *78*, 47–69. [CrossRef]
46. Gustavsson, J.; Cederberg, C.; Sonesson, U.; van Otterdijk, R.; Meybeck, A. Global Food Losses and Food Waste: Extent, Causes and Prevention. Available online: http://www.fao.org/fileadmin/user_upload/ags/publications/GFL_web.pdf (accessed on 2 February 2021).
47. Al Khawli, F.; Martí-Quijal, F.J.; Ferrer, E.; Ruiz, M.-J.; Berrada, H.; Gavahian, M.; Barba, F.J.; de la Fuente, B. Aquaculture and its by-products as a source of nutrients and bioactive compounds. *Adv. Food Nutr. Res.* **2020**, *92*, 1–33. [CrossRef]
48. Hamed, I.; Ozogul, F.; Ozogul, Y.; Regenstein, J.M. Marine Bioactive Compounds and Their Health Benefits: A Review. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 446–465. [CrossRef]
49. Kandra, P.; Challa, M.M.; Jyothi, H.K.P. Efficient use of shrimp waste: Present and future trends. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 17–29. [CrossRef]
50. Wade, N.M.; Gabaudan, J.; Glencross, B.D. A review of carotenoid utilisation and function in crustacean aquaculture. *Rev. Aquac.* **2015**, *9*, 141–156. [CrossRef]
51. Higuera-Ciapara, I.; Félix-Valenzuela, L.; Goycoolea, F.M. Astaxanthin: A Review of its Chemistry and Applications. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 185–196. [CrossRef] [PubMed]
52. Lim, K.C.; Yusoff, F.M.; Shariff, M.; Kamarudin, M.S. Astaxanthin as feed supplement in aquatic animals. *Rev. Aquac.* **2018**, *10*, 738–773. [CrossRef]

53. Kumar, C.S.; Ganesan, P.; Suresh, P.V.; Bhaskar, N. Seaweeds as a source of nutritionally beneficial compounds—A review. *J. Food Sci. Technol.* **2008**, *45*, 1–13.
54. Carvalho, L.G.; Pereira, L. Review of marine algae as source of bioactive metabolites. In *Marine Algae: Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology*; Pereira, L., Neto, J.M., Eds.; CRC Press: Boca Raton, FL, USA, 2014; pp. 195–227, ISBN 9781466581814.
55. Ścieszka, S.; Klewicka, E. Algae in food: A general review. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 3538–3547. [CrossRef] [PubMed]
56. FAO. *The State of World Fisheries and Aquaculture*; FAO: Rome, Italy, 2020.
57. Ferdouse, F.; Holdt, S.L.; Smith, R.; Murúa, P.; Yang, Z. The global status of seaweed production, trade and utilization. *FAO Globefish Res. Program.* **2018**, *124*, 120.
58. Silva, T.H.; Moreira-Silva, J.; Marques, A.L.P.; Domingues, A.; Bayon, Y.; Reis, R.L. Marine Origin Collagens and Its Potential Applications. *Mar. Drugs* **2014**, *12*, 5881–5901. [CrossRef]
59. Coppola, D.; Oliviero, M.; Vitale, G.A.; Lauritano, C.; D'Ambra, I.; Iannace, S.; De Pascale, D. Marine Collagen from Alternative and Sustainable Sources: Extraction, Processing and Applications. *Mar. Drugs* **2020**, *18*, 214. [CrossRef]
60. Omura, Y.; Urano, N.; Kimura, S. Occurrence of fibrillar collagen with structure of (α1)2α2 in the test of sea urchin Asthenosoma ijimai. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **1996**, *115*, 63–68. [CrossRef]
61. Nagai, T.; Suzuki, N. Partial characterization of collagen from purple sea urchin (*Anthocidaris crassispina*) test. *Int. J. Food Sci. Technol.* **2000**, *35*, 497–501. [CrossRef]
62. Kimura, S.; Takema, Y.; Kubota, M. Octopus skin collagen. Isolation and characterization of collagen comprising two distinct alpha chains. *J. Biol. Chem.* **1981**, *256*, 13230–13234. [CrossRef]
63. Kimura, S.; Miura, S.; Park, Y.-H. Collagen as the Major Edible Component of Jellyfish (*Stomolophus nomural*). *J. Food Sci.* **1983**, *48*, 1758–1760. [CrossRef]
64. Kimura, S.; Zhu, X.-P.; Matsui, R.; Shijoh, M.; Takamizawa, S. Characterization of Fish Muscle Type I Collagen. *J. Food Sci.* **1988**, *53*, 1315–1318. [CrossRef]
65. Nagai, T.; Araki, Y.; Suzuki, N. Collagen of the skin of ocellate puffer fish (*Takifugu rubripes*). *Food Chem.* **2002**, *78*, 173–177. [CrossRef]
66. Meyers, M.A.; Lin, Y.S.; Olevsky, E.A.; Chen, P.-Y. Battle in the Amazon: Arapaima versus Piranha. *Adv. Eng. Mater.* **2012**, *14*, B279–B288. [CrossRef]
67. Regenstein, J.M.; Zhou, P. Collagen and gelatin from marine by-products. In *Maximising the Value of Marine By-Products*; Shahidi, F., Ed.; Woodhead Publishing: Shaxton, UK, 2007; pp. 279–303, ISBN 9781845690137.
68. Salvatore, L.; Gallo, N.; Natali, M.L.; Campa, L.; Lunetti, P.; Madaghiele, M.; Blasi, F.S.; Corallo, A.; Capobianco, L.; Sannino, A. Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare. *Mater. Sci. Eng. C* **2020**, *113*, 110963. [CrossRef] [PubMed]
69. Lim, Y.-S.; Ok, Y.-J.; Hwang, S.-Y.; Kwak, J.-Y.; Yoon, S. Marine Collagen as A Promising Biomaterial for Biomedical Applications. *Mar. Drugs* **2019**, *17*, 467. [CrossRef]
70. Hashim, P.; Mohd Ridzwan, M.S.; Bakar, J.; Mat Hashim, D. Collagen in food and beverage industries. *Int. Food Res. J.* **2015**, *22*, 1–8.
71. King'ori, A.M. A Review of the Uses of Poultry Eggshells and Shell Membranes. *Int. J. Poult. Sci.* **2011**, *10*, 908–912. [CrossRef]
72. Benjakul, S.; Kittiphattanabawon, P.; Regenstein, J.M. Fish Gelatin. In *Food Biochemistry and Food Processing*; Wiley: Hoboken, NJ, USA, 2012; pp. 388–405.
73. Karim, A.; Bhat, R. Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocoll.* **2009**, *23*, 563–576. [CrossRef]
74. Boran, G.; Regenstein, J.M. Fish Gelatin. In *Advances in Food and Nutrition Research*; Elsevier BV: Amsterdam, The Netherlands, 2010; Volume 60, pp. 119–143.
75. Irwandi, J.; Faridayanti, S.; Mohamed, E.S.M.; Hamzah, M.S.; Torla, H.H.; Man, Y.B.C. Extraction and characterization of gelatin from different marine fish species in Malaysia. *Int. Food Res. J.* **2009**, *16*, 381–389.
76. Gómez-Guillén, M.; Me, L.-C.; Giménez, B.; Montero, P. Antioxidant and antimicrobial peptide fractions from squid and tuna skin gelatin. In *Sea By-Products as a Real Material: New Ways of Application*; Le Bihan, E., Ed.; Kerala: Madrid, Spain, 2010; Volume 661, pp. 89–115, ISBN 9788178954851.
77. Aleman, A.; Giménez, B.; Montero, P.; Gómez-Guillén, M.C. Antioxidant activity of several marine skin gelatins. *LWT* **2011**, *44*, 407–413. [CrossRef]
78. Ngo, D.-H.; Ryu, B.; Vo, T.-S.; Himaya, S.; Wijesekara, I.; Kim, S.-K. Free radical scavenging and angiotensin-I converting enzyme inhibitory peptides from Pacific cod (*Gadus macrocephalus*) skin gelatin. *Int. J. Biol. Macromol.* **2011**, *49*, 1110–1116. [CrossRef]
79. Chen, T.; Hou, H.; Lu, J.; Zhang, K.; Li, B. Protective effect of gelatin and gelatin hydrolysate from salmon skin on UV irradiation-induced photoaging of mice skin. *J. Ocean. Univ. China* **2016**, *15*, 711–718. [CrossRef]
80. Liang, J.; Pei, X.; Zhang, Z.; Wang, N.; Wang, J.; Li, Y. The Protective Effects of Long-Term Oral Administration of Marine Collagen Hydrolysate from Chum Salmon on Collagen Matrix Homeostasis in the Chronological Aged Skin of Sprague-Dawley Male Rats. *J. Food Sci.* **2010**, *75*, H230–H238. [CrossRef]
81. Fan, J.; Zhuang, Y.; Li, B. Effects of Collagen and Collagen Hydrolysate from Jellyfish Umbrella on Histological and Immunity Changes of Mice Photoaging. *Nutrient* **2013**, *5*, 223–233. [CrossRef] [PubMed]

82. Kim, S.K.; Venkatesan, J. Bycatch utilization in Asia. In *Seafood Processing By-Products: Trends and Applications*; Kim, S., Ed.; Springer-Verlag: New York, NY, USA, 2014; pp. 1–597, ISBN 9781461495901.
83. Morishige, H.; Sugahara, T.; Nishimoto, S.; Muranaka, A.; Ohno, F.; Shiraishi, R.; Doi, M. Immunostimulatory effects of collagen from jellyfish in vivo. *Cytotechnology* **2011**, *63*, 481–492. [CrossRef] [PubMed]
84. Gharibzahedi, S.M.T.; Jafari, S.M. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends Food Sci. Technol.* **2017**, *62*, 119–132. [CrossRef]
85. Trailokya, A.; Srivastava, A.; Bhole, M.; Zalte, N. Calcium and calcium salts. *J. Assoc. Physicians India* **2017**, *65*, 100–103.
86. Das, J.K.; Salam, R.A.; Kumar, R.; Bhutta, Z.A. Micronutrient fortification of food and its impact on woman and child health: A systematic review. *Syst. Rev.* **2013**, *2*, 67. [CrossRef] [PubMed]
87. Jung, W.-K.; Park, P.-J.; Byun, H.-G.; Moon, S.-H.; Kim, S.-K. Preparation of hoki (*Johnius belengerii*) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. *Food Chem.* **2005**, *91*, 333–340. [CrossRef]
88. Lee, Y.-K.; Kim, A.Y.; Min, S.-G.; Kwak, H.-S. Characteristics of milk tablets supplemented with nanopowdered eggshell or oyster shell. *Int. J. Dairy Technol.* **2016**, *69*, 337–345. [CrossRef]
89. Alsuhaibani, A.M.A. Rheological and Nutritional Properties and Sensory Evaluation of Bread Fortified with Natural Sources of Calcium. *J. Food Qual.* **2018**, *2018*, 1–7. [CrossRef]
90. Kim, S.-K.; Ravichandran, Y.D.; Kong, C.-S. Applications of Calcium and its Supplement derived from Marine Organisms. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 469–474. [CrossRef]
91. Gkouvatsos, K.; Papanikolaou, G.; Pantopoulos, K. Regulation of iron transport and the role of transferrin. *Biochim. Biophys. Acta Gen. Subj.* **2012**, *1820*, 188–202. [CrossRef] [PubMed]
92. Lee, S.-H.; Bin Song, K. Purification of an iron-binding nona-peptide from hydrolysates of porcine blood plasma protein. *Process. Biochem.* **2009**, *44*, 378–381. [CrossRef]
93. Yun, S.; Zhang, T.; Li, M.; Chen, B.; Zhao, G. Proanthocyanidins Inhibit Iron Absorption from Soybean (Glycine max) Seed Ferritin in Rats with Iron Deficiency Anemia. *Plant. Foods Hum. Nutr.* **2011**, *66*, 212–217. [CrossRef]
94. Guo, L.; Hou, H.; Li, B.; Zhang, Z.; Wang, S.; Zhao, X. Preparation, isolation and identification of iron-chelating peptides derived from Alaska pollock skin. *Process. Biochem.* **2013**, *48*, 988–993. [CrossRef]
95. Tanna, B.; Mishra, A. Metabolites Unravel Nutraceutical Potential of Edible Seaweeds: An Emerging Source of Functional Food. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 1613–1624. [CrossRef]
96. Rajapakse, N.; Kim, S.-K. Nutritional and Digestive Health Benefits of Seaweed. *Adv. Food Nutr. Res.* **2011**, *64*, 17–28. [CrossRef]
97. Ren, D.; Noda, H.; Amano, H.; Nishino, T.; Nishizawa, K. Study on Antihypertensive and Antihyperlipidemic Effects of Marine Algae. *Fish. Sci.* **1994**, *60*, 83–88. [CrossRef]
98. Martins, A.; Vieira, H.; Gaspar, H.; Santos, S. Marketed Marine Natural Products in the Pharmaceutical and Cosmeceutical Industries: Tips for Success. *Mar. Drugs* **2014**, *12*, 1066–1101. [CrossRef]
99. Dicpinigaitis, P.V. Angiotensin-Converting Enzyme Inhibitor-Induced Cough. *Chest* **2006**, *129*, 169S–173S. [CrossRef]
100. Cotton, J.; Hayashi, M.A.F.; Cuniasse, P.; Vazeux, G.; Ianzer, D.; De Camargo, A.C.M.; Dive, V. Selective Inhibition of the C-Domain of Angiotensin I Converting Enzyme by Bradykinin Potentiating Peptides. *Biochemistry* **2002**, *41*, 6065–6071. [CrossRef] [PubMed]
101. Kim, S.-K.; Mendis, E. Bioactive compounds from marine processing byproducts—A review. *Food Res. Int.* **2006**, *39*, 383–393. [CrossRef]
102. Khora, S.S. Marine fish-derived bioactive peptides and proteins for human therapeutics. *Int. J. Pharm. Pharm. Sci.* **2013**, *5*, 31–37.
103. Chalamaiah, M.; Kumar, B.D.; Hemalatha, R.; Jyothirmayi, T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chem.* **2012**, *135*, 3020–3038. [CrossRef]
104. Khalili Tilami, S.; Sampels, S. Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals. *Rev. Fish. Sci. Aquac.* **2018**, *26*, 243–253. [CrossRef]
105. Kim, S.-K.; Wijesekara, I. Development and biological activities of marine-derived bioactive peptides: A review. *J. Funct. Foods* **2010**, *2*, 1–9. [CrossRef]
106. Ngo, D.-H.; Vo, T.-S.; Kim, S.-K. Biological Activities of Marine Bioactive Peptides. In *Marine Proteins and Peptides*; Wiley: Hoboken, NJ, USA, 2013; pp. 509–521.
107. Zamora-Sillero, J.; Gharsallaoui, A.; Prentice, C. Peptides from Fish By-product Protein Hydrolysates and Its Functional Properties: An Overview. *Mar. Biotechnol.* **2018**, *20*, 118–130. [CrossRef] [PubMed]
108. Mendis, E.; Rajapakse, A.N.; Kim, S.-K. Antioxidant Properties of a Radical-Scavenging Peptide Purified from Enzymatically Prepared Fish Skin Gelatin Hydrolysate. *J. Agric. Food Chem.* **2005**, *53*, 581–587. [CrossRef] [PubMed]
109. Yang, J.-I.; Ho, H.-Y.; Chu, Y.-J.; Chow, C.-J. Characteristic and antioxidant activity of retorted gelatin hydrolysates from cobia (*Rachycentron canadum*) skin. *Food Chem.* **2008**, *110*, 128–136. [CrossRef]
110. Šližytė, R.; Mozuraitytė, R.; Martínez-Alvarez, O.; Falch, E.; Fouchereau-Peron, M.; Rustad, T. Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (*Gadus morhua*) backbones. *Process. Biochem.* **2009**, *44*, 668–677. [CrossRef]
111. Barkia, A.; Bougatef, A.; Ben Khaled, H.; Nasri, M. Antioxidant activities of sardinelle heads and/or viscera protein hydrolysates prepared by enzymatic treatment. *J. Food Biochem.* **2010**, *34*, 303–320. [CrossRef]

112. Bougatef, A.; Nedjar-Arroume, N.; Manni, L.; Ravallec, R.; Barkia, A.; Guillochon, D.; Nasri, M. Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinellaaurita*) by-products proteins. *Food Chem.* **2010**, *118*, 559–565. [CrossRef]
113. Jia, J.; Zhou, Y.; Lu, J.; Chen, A.; Li, Y.; Zheng, G. Enzymatic hydrolysis of Alaska pollack (*Theragra chalcogramma*) skin and antioxidant activity of the resulting hydrolysate. *J. Sci. Food Agric.* **2010**, *90*, 635–640. [CrossRef] [PubMed]
114. Naqash, S.Y.; Nazeer, R.A. Antioxidant Activity of Hydrolysates and Peptide Fractions of Nemipterus japonicus and Exocoetus volitans Muscle. *J. Aquat. Food Prod. Technol.* **2010**, *19*, 180–192. [CrossRef]
115. Ganesh, R.J.; Nazeer, R.A.; Kumar, N.S.S. Purification and identification of antioxidant peptide from black pomfret, Parastromateus niger (Bloch, 1975) viscera protein hydrolysate. *Food Sci. Biotechnol.* **2011**, *20*, 1087–1094. [CrossRef]
116. Kumar, N.S.; Nazeer, R.; Jaiganesh, R. Purification and biochemical characterization of antioxidant peptide from horse mackerel (*Magalaspis cordyla*) viscera protein. *Peptides* **2011**, *32*, 1496–1501. [CrossRef]
117. Kumar, N.S.S.; Nazeer, R.A.; Jaiganesh, R. Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*). *Amino Acids* **2011**, *42*, 1641–1649. [CrossRef]
118. Nazeer, R.; Kumar, N.S.; Ganesh, R.J. In vitro and in vivo studies on the antioxidant activity of fish peptide isolated from the croaker (*Otolithes ruber*) muscle protein hydrolysate. *Peptides* **2012**, *35*, 261–268. [CrossRef] [PubMed]
119. Ktari, N.; Fakhfakh, N.; Balti, R.; Ben Khaled, H.; Nasri, M.; Bougatef, A. Effect of Degree of Hydrolysis and Protease Type on the Antioxidant Activity of Protein Hydrolysates from Cuttlefish (*Sepia officinalis*) By-Products. *J. Aquat. Food Prod. Technol.* **2013**, *22*, 436–448. [CrossRef]
120. Lassoued, I.; Mora, L.; Nasri, R.; Jridi, M.; Toldrá, F.; Aristoy, M.-C.; Barkia, A.; Nasri, M. Characterization and comparative assessment of antioxidant and ACE inhibitory activities of thornback ray gelatin hydrolysates. *J. Funct. Foods* **2015**, *13*, 225–238. [CrossRef]
121. Lee, J.K.; Jeon, J.-K.; Byun, H.-G. Antihypertensive effect of novel angiotensin I converting enzyme inhibitory peptide from chum salmon (*Oncorhynchus keta*) skin in spontaneously hypertensive rats. *J. Funct. Foods* **2014**, *7*, 381–389. [CrossRef]
122. Leone, A.; Lecci, R.M.; Durante, M.; Meli, F.; Piraino, S. The Bright Side of Gelatinous Blooms: Nutraceutical Value and Antioxidant Properties of Three Mediterranean Jellyfish (*Scyphozoa*). *Mar. Drugs* **2015**, *13*, 4654–4681. [CrossRef]
123. Zhang, Q.; Song, C.; Zhao, J.; Shi, X.; Sun, M.; Liu, J.; Fu, Y.; Jin, W.; Zhu, B. Separation and Characterization of Antioxidative and Angiotensin Converting Enzyme Inhibitory Peptide from Jellyfish Gonad Hydrolysate. *Molecules* **2018**, *23*, 94. [CrossRef] [PubMed]
124. Zhuang, Y.L.; Sun, L.P.; Zhao, X.; Hou, H.; Li, B.F. Investigation of gelatin polypeptides of jellyfish (*Rhopilema esculentum*) for their antioxidant activity in vitro. *Food Technol. Biotechnol.* **2010**, *48*, 222–228.
125. Admassu, H.; Gasmalla, M.A.A.; Yang, R.; Zhao, W. Identification of Bioactive Peptides with α-Amylase Inhibitory Potential from Enzymatic Protein Hydrolysates of Red Seaweed (*Porphyra* spp.). *J. Agric. Food Chem.* **2018**, *66*, 4872–4882. [CrossRef]
126. Indumathi, P.; Mehta, A. A novel anticoagulant peptide from the Nori hydrolysate. *J. Funct. Foods* **2016**, *20*, 606–617. [CrossRef]
127. Cullis, P.R.; Kruijff, B. Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta Rev. Biomembr.* **1979**, *559*, 399–420. [CrossRef]
128. Adamovich, Y.; Aviram, R.; Asher, G. The emerging roles of lipids in circadian control. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2015**, *1851*, 1017–1025. [CrossRef]
129. Mišurcová, L.; Ambrožová, J.; Samek, D. Seaweed Lipids as Nutraceuticals. *Adv. Food Nutr. Res.* **2011**, *64*, 339–355. [CrossRef] [PubMed]
130. Calder, P. Very long-chain n-3 fatty acids and human health: Fact, fiction and the future. *Proc. Nutr. Soc.* **2018**, *77*, 52–72. [CrossRef]
131. Li, X.; Bi, X.; Wang, S.; Zhang, Z.; Li, F.; Zhao, A.Z. Therapeutic Potential of ω-3 Polyunsaturated Fatty Acids in Human Autoimmune Diseases. *Front. Immunol.* **2019**, *10*, 2241. [CrossRef]
132. Chotard, É.; Mohammadi, F.; Julien, P.; Berthiaume, L.; Rudkowska, I.; Bertrand, N. Drinkable lecithin nanovesicles to study the biological effects of individual hydrophobic macronutrients and food preferences. *Food Chem.* **2020**, *322*, 126736. [CrossRef]
133. Higgins, J.P.; Flicker, L. Lecithin for dementia and cognitive impairment. *Cochrane Database Syst. Rev.* **2000**, CD001015. [CrossRef]
134. Baker, C. Lecithin in cosmetics. In *Lecithin: Sources, Manufacture & Uses*; Szuhaj, B.F., Ed.; American Oil Chemists' Society: Champaign, IL, USA, 1989; pp. 253–260.
135. Uddin, M.S.; Ahn, H.-M.; Kishimura, H.; Chun, B.-S. Production of valued materials from squid viscera by subcritical water hydrolysis. *J. Environ. Biol.* **2010**, *31*, 675–679. [PubMed]
136. Uddin, S.; Kishimura, H.; Chun, B.-S. Isolation and Characterization of Lecithin from Squid (*Todarodes pacificus*) Viscera Deoiled by Supercritical Carbon Dioxide Extraction. *J. Food Sci.* **2011**, *76*, C350–C354. [CrossRef] [PubMed]
137. Cho, S.-Y.; Joo, D.-S.; Choi, H.-G.; Nara, E.; Miyashita, K. Oxidative stability of lipids from squid tissues. *Fish. Sci.* **2001**, *67*, 738–743. [CrossRef]
138. Nguyen, T.T.; Zhang, W.; Barber, A.R.; Su, P.; He, S. Significant Enrichment of Polyunsaturated Fatty Acids (PUFAs) in the Lipids Extracted by Supercritical CO2from the Livers of Australian Rock Lobsters (*Jasus edwardsii*). *J. Agric. Food Chem.* **2015**, *63*, 4621–4628. [CrossRef] [PubMed]

139. Amiguet, V.T.; Kramp, K.L.; Mao, J.; McRae, C.; Goulah, A.; Kimpe, L.E.; Blais, J.; Arnason, J.T. Supercritical carbon dioxide extraction of polyunsaturated fatty acids from Northern shrimp (*Pandalus borealis Kreyer*) processing by-products. *Food Chem.* **2012**, *130*, 853–858. [CrossRef]
140. Sahena, F.; Zaidul, I.; Jinap, S.; Jahurul, M.; Khatib, A.; Norulaini, N. Extraction of fish oil from the skin of Indian mackerel using supercritical fluids. *J. Food Eng.* **2010**, *99*, 63–69. [CrossRef]
141. Ferdosh, S.; Sarker, Z.I.; Ab Rahman, N.N.N.; Akanda, J.H.; Ghafoor, K.; Ab Kadir, M.O. Simultaneous Extraction and Fractionation of Fish Oil from Tuna By-Product Using Supercritical Carbon Dioxide (SC-CO2). *J. Aquat. Food Prod. Technol.* **2014**, *25*, 230–239. [CrossRef]
142. Haq, M.; Ahmed, R.; Cho, Y.-J.; Chun, B.-S. Quality Properties and Bio-potentiality of Edible Oils from Atlantic Salmon By-products Extracted by Supercritial Carbon Dioxide and Conventional Methods. *Waste Biomass Valorization* **2016**, *8*, 1953–1967. [CrossRef]
143. Bierer, T.L.; Bui, L.M. Improvement of Arthritic Signs in Dogs Fed Green-Lipped Mussel (*Perna canaliculus*). *J. Nutr.* **2002**, *132*, 1634S–1636S. [CrossRef] [PubMed]
144. Whitehouse, M.W.; Macrides, T.A.; Kalafatis, N.; Betts, W.H.; Haynes, D.R.; Broadbent, J. Anti-inflammatory activity of a lipid fraction (lyprinol) from the NZ green-lipped mussel. *Inflammopharmacology* **1997**, *5*, 237–246. [CrossRef] [PubMed]
145. Reddy, L.H.; Couvreur, P. Squalene: A natural triterpene for use in disease management and therapy. *Adv. Drug Deliv. Rev.* **2009**, *61*, 1412–1426. [CrossRef]
146. Huang, Z.-R.; Lin, Y.-K.; Fang, J.-Y. Biological and Pharmacological Activities of Squalene and Related Compounds: Potential Uses in Cosmetic Dermatology. *Molecules* **2009**, *14*, 540–554. [CrossRef]
147. Kim, S.-K.; Karadeniz, F. Biological Importance and Applications of Squalene and Squalane. In *Advances in Food and Nutrition Research*; Elsevier BV: Amsterdam, The Netherlands, 2012; Volume 65, pp. 223–233.
148. Burton, G.W.; Ingold, K.U. Autoxidation of biological molecules. Antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. *J. Am. Chem. Soc.* **1981**, *103*, 6472–6477. [CrossRef]
149. Fujisawa, A.; Dunlap, W.C.; Yamamoto, Y. Vitamin E protection in the biochemical adaptation of marine organisms to cold-water environments. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **2010**, *157*, 145–158. [CrossRef]
150. Seabra, L.M.J.; Pedrosa, L.F.C. Astaxanthin: Structural and functional aspects. *Rev. Nutr.* **2010**, *23*, 1041–1050. [CrossRef]
151. Armenta-Lopez, R.; Guerrero, I.L.; Huerta, S. Astaxanthin Extraction from Shrimp Waste by Lactic Fermentation and Enzymatic Hydrolysis of the Carotenoprotein Complex. *J. Food Sci.* **2002**, *67*, 1002–1006. [CrossRef]
152. Sachindra, N.; Mahendrakar, N. Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. *Bioresour. Technol.* **2005**, *96*, 1195–1200. [CrossRef]
153. Eggersdorfer, M.; Wyss, A. Carotenoids in human nutrition and health. *Arch. Biochem. Biophys.* **2018**, *652*, 18–26. [CrossRef] [PubMed]
154. Rao, A.V.; Rao, L.G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216. [CrossRef]
155. Bohn, T. Carotenoids, Chronic Disease Prevention and Dietary Recommendations. *Int. J. Vitam. Nutr. Res.* **2017**, *87*, 121–130. [CrossRef] [PubMed]
156. Kurihara, H.; Koda, H.; Asami, S.; Kiso, Y.; Tanaka, T. Contribution of the antioxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with restraint stress. *Life Sci.* **2002**, *70*, 2509–2520. [CrossRef]
157. Sila, A.; Ayed-Ajmi, Y.; Sayari, N.; Nasri, M.; Martinez-Alvarez, O.; Bougatef, A. Antioxidant and Anti-proliferative Activities of Astaxanthin Extracted from the Shell Waste of Deep-water Pink Shrimp (*Parapenaeus longirostris*). *Nat. Prod. J.* **2013**, *3*, 82–89. [CrossRef]
158. Taksima, T.; Limpawattana, M.; Klaypradit, W. Astaxanthin encapsulated in beads using ultrasonic atomizer and application in yogurt as evaluated by consumer sensory profile. *LWT* **2015**, *62*, 431–437. [CrossRef]
159. Neori, A.; Chopin, T.; Troell, M.; Buschmann, A.H.; Kraemer, G.P.; Halling, C.; Shpigel, M.; Yarish, C. Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* **2004**, *231*, 361–391. [CrossRef]
160. Fung, A.; Hamid, N.; Lu, J. Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*. *Food Chem.* **2013**, *136*, 1055–1062. [CrossRef]
161. Raji, V.; Loganathan, C.; Sadhasivam, G.; Kandasamy, S.; Poomani, K.; Thayumanavan, P. Purification of fucoxanthin from *Sargassum wightii Greville* and understanding the inhibition of angiotensin 1-converting enzyme: An in vitro and in silico studies. *Int. J. Biol. Macromol.* **2020**, *148*, 696–703. [CrossRef] [PubMed]
162. Rinaudo, M. Chitin and chitosan: Properties and applications. *Prog. Polym. Sci.* **2006**, *31*, 603–632. [CrossRef]
163. Jayakumar, R.; Nwe, N.; Tokura, S.; Tamura, H. Sulfated chitin and chitosan as novel biomaterials. *Int. J. Biol. Macromol.* **2007**, *40*, 175–181. [CrossRef] [PubMed]
164. Artan, M.; Karadeniz, F.; Karagozlu, M.Z.; Kim, M.-M.; Kim, S.-K. Anti-HIV-1 activity of low molecular weight sulfated chitooligosaccharides. *Carbohydr. Res.* **2010**, *345*, 656–662. [CrossRef] [PubMed]
165. Rúnarsson, Ö.V.; Holappa, J.; Malainer, C.; Steinsson, H.; Hjálmarsdóttir, M.; Nevalainen, T.; Másson, M. Antibacterial activity of N-quaternary chitosan derivatives: Synthesis, characterization and structure activity relationship (SAR) investigations. *Eur. Polym. J.* **2010**, *46*, 1251–1267. [CrossRef]

166. Li, R.; Guo, Z.; Jiang, P. Synthesis, characterization, and antifungal activity of novel quaternary chitosan derivatives. *Carbohydr. Res.* **2010**, *345*, 1896–1900. [CrossRef]
167. Wang, S.-L.; Liang, T.-W.; Yen, Y.-H. Bioconversion of chitin-containing wastes for the production of enzymes and bioactive materials. *Carbohydr. Polym.* **2011**, *84*, 732–742. [CrossRef]
168. Apetroaei, M.R.; Zgârian, R.G.; Manea, A.-M.; Rau, I.; Tihan, G.T.; Schroder, V. New source of chitosan from Black Sea marine organisms identification. *Mol. Cryst. Liq. Cryst.* **2016**, *628*, 102–109. [CrossRef]
169. Takarina, N.D.; Fanani, A.A. Characterization of chitin and chitosan synthesized from red snapper (*Lutjanus* sp.) scale's waste. In Proceedings of the 2nd International Symposium on Current Progress in Mathematics and Sciences, Bali, Indonesia, 1–2 November 2016. [CrossRef]
170. Rasti, H.; Parivar, K.; Baharara, J.; Iranshahi, M.; Namvar, F. Chitin from the Mollusc Chiton: Extraction, Characterization and Chitosan Preparation. *Iran. J. Pharm. Res.* **2017**, *16*, 366–379.
171. Jothi, N.; Nachiyar, R.K. Identification and isolation of chitin and chitosan from cuttle bone of *Sepia prashadi* Winckworth, *Glob. J. Biotechnol. Biochem.* **2013**, *8*, 1–7. [CrossRef]
172. Alencar, P.O.C.; Lima, G.C.; Barros, F.C.N.; Costa, L.E.; Ribeiro, C.V.P.; Sousa, W.M.; Sombra, V.G.; Abreu, C.M.W.; Abreu, E.S.; Pontes, E.O.; et al. A novel antioxidant sulfated polysaccharide from the algae *Gracilaria caudata*: In vitro and in vivo activities. *Food Hydrocoll.* **2019**, *90*, 28–34. [CrossRef]
173. Sudharsan, S.; Subhapradha, N.; Seedevi, P.; Shanmugam, V.; Madeswaran, P.; Shanmugam, A.; Srinivasan, A. Antioxidant and anticoagulant activity of sulfated polysaccharide from *Gracilaria debilis* (Forsskal). *Int. J. Biol. Macromol.* **2015**, *81*, 1031–1038. [CrossRef]
174. Zainudin, N.H.; Sirajudeen, K.N.S.; Ghazali, F.C. Marine sourced glycosaminoglycans "GAGs". *J. Adv. Lab. Res. Biol.* **2014**, *5*, 46–53.
175. Valcarcel, J.; Novoa-Carballal, R.; Perez-Martin, R.; Reis, R.L.; Vázquez, J.A. Glycosaminoglycans from marine sources as therapeutic agents. *Biotechnol. Adv.* **2017**, *35*, 711–725. [CrossRef]
176. Mycroft-West, C.; Yates, E.A.; Skidmore, M.A. Marine glycosaminoglycan-like carbohydrates as potential drug candidates for infectious disease. *Biochem. Soc. Trans.* **2018**, *46*, 919–929. [CrossRef]
177. Colliec-Jouault, S.; Zykwinska, A. Marine glycosaminoglycans (GAGs) and GAG-mimetics: Applications in medicine and tissue engineering. In *Extracellular Sugar-Based Biopolymers Matrices*; Cohen, E., Merzendorfer, H., Eds.; Springer: Cham, Switzerland, 2019; pp. 625–648.
178. Cheras, P.; Stevenson, L.; Myers, S. Vascular mechanisms in osteoarthritis: Rationale for treatment with a marine-based complementary medicine. *Osteoarthr. Cartil.* **2005**, *13*, S95.
179. Aroma New Zealand Ltd. Available online: www.aromanz.com (accessed on 31 March 2021).
180. Draget, K.I. Alginates. In *Handbook of Hydrocolloids*, 2nd ed.; Phillips, G.O., Williams, P., Eds.; Woodhead Publishing: Shaxton, UK, 2009; pp. 807–828, ISBN 9781845695873.
181. Borazjani, N.J.; Tabarsa, M.; You, S.; Rezaei, M. Effects of extraction methods on molecular characteristics, antioxidant properties and immunomodulation of alginates from Sargassum angustifolium. *Int. J. Biol. Macromol.* **2017**, *101*, 703–711. [CrossRef]
182. Marudhupandi, T.; Kumar, T.T.A.; Lakshmanasenthil, S.; Suja, G.; Vinothkumar, T. In vitro anticancer activity of fucoidan from Turbinaria conoides against A549 cell lines. *Int. J. Biol. Macromol.* **2015**, *72*, 919–923. [CrossRef]
183. Vo, T.-S.; Kim, S.-K. Fucoidans as a natural bioactive ingredient for functional foods. *J. Funct. Foods* **2013**, *5*, 16–27. [CrossRef]
184. Palanisamy, S.; Vinosha, M.; Marudhupandi, T.; Rajasekar, P.; Prabhu, N.M. Isolation of fucoidan from *Sargassum polycystum* brown algae: Structural characterization, in vitro antioxidant and anticancer activity. *Int. J. Biol. Macromol.* **2017**, *102*, 405–412. [CrossRef]
185. Kumar, T.V.; Lakshmanasenthil, S.; Geetharamani, D.; Marudhupandi, T.; Suja, G.; Suganya, P. Fucoidan—A α-d-glucosidase inhibitor from *Sargassum wightii* with relevance to type 2 diabetes mellitus therapy. *Int. J. Biol. Macromol.* **2015**, *72*, 1044–1047. [CrossRef]
186. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid. Redox Signal.* **2013**, *18*, 1818–1892. [CrossRef]
187. Zaharudin, N.; Salmeán, A.A.; Dragsted, L.O. Inhibitory effects of edible seaweeds, polyphenolics and alginates on the activities of porcine pancreatic α-amylase. *Food Chem.* **2018**, *245*, 1196–1203. [CrossRef]
188. Jesumani, V.; Du, H.; Pei, P.; Aslam, M.; Huang, N. Comparative study on skin protection activity of polyphenol-rich extract and polysaccharide-rich extract from *Sargassum vachellianum*. *PLoS ONE* **2020**, *15*, e0227308. [CrossRef]
189. Yoon, M.; Kim, J.-S.; Seo, S.; Lee, K.; Um, M.Y.; Lee, J.; Jung, J.; Cho, S. Dieckol, a Major Marine Polyphenol, Enhances Non-Rapid Eye Movement Sleep in Mice via the GABAA-Benzodiazepine Receptor. *Front. Pharmacol.* **2020**, *11*, 1–10. [CrossRef]
190. Mai, H.; Fotedar, R.; Fewtrell, J. Evaluation of Sargassum sp. as a nutrient-sink in an integrated seaweed-prawn (ISP) culture system. *Aquaculture* **2010**, *310*, 91–98. [CrossRef]
191. Daniotti, S.; Re, I. Marine Biotechnology: Challenges and Development Market Trends for the Enhancement of Biotic Resources in Industrial Pharmaceutical and Food Applications. A Statistical Analysis of Scientific Literature and Business Models. *Mar. Drugs* **2021**, *19*, 61. [CrossRef]

192. Venugopal, V. Nutrients and nutraceuticals from seafood. In *Bioactive Molecules in Food*; Mérillon, J.M., Ramawat, K.G., Eds.; Springer International Publishing: New York, NY, USA, 2018; pp. 1–45.
193. Liu, D.; Nikoo, M.; Boran, G.; Zhou, P.; Regenstein, J.M. Collagen and Gelatin. *Annu. Rev. Food Sci. Technol.* **2015**, *6*, 527–557. [CrossRef]
194. Adam, O.; Beringer, C.; Kless, T.; Lemmen, C.; Adam, A.; Wiseman, M.; Adam, P.; Klimmek, R.; Forth, W. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatol. Int.* **2003**, *23*, 27–36. [CrossRef]
195. Grienke, U.; Silke, J.; Tasdemir, D. Bioactive compounds from marine mussels and their effects on human health. *Food Chem.* **2014**, *142*, 48–60. [CrossRef]
196. Cheung, R.C.F.; Ng, T.B.; Wong, J.H. Marine Peptides: Bioactivities and Applications. *Mar. Drugs* **2015**, *13*, 4006–4043. [CrossRef] [PubMed]
197. Kushnarev, M.; Pirvulescu, I.P.; Candido, K.D.; Knezevic, N.N. Neuropathic pain: Preclinical and early clinical progress with voltage-gated sodium channel blockers. *Expert Opin. Investig. Drugs* **2020**, *29*, 259–271. [CrossRef] [PubMed]

Marine Genomics 63 (2022) 100951

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

First certain record of Demospongiae class (Porifera) alien species from the Mediterranean Sea

Marco Bertolino [a,*], Gabriele Costa [a], Nadia Ruocco [b,1], Roberta Esposito [b,c,a,1], Sergio De Matteo [d], Giacomo Zagami [d], Maria Costantini [b,*]

[a] Department of Earth, Environmental and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genova, Italy.
[b] Stazione Zoologica Anton Dohrn, Department of Marine Biotechnology, Villa Comunale, 80121 Napoli, Italy
[c] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Napoli, Italy
[d] Department of Biological, Chemical, Pharmaceutical and Environmental Sciences, University of Messina, 98100 Messina, Italy

ARTICLE INFO

Keywords:
Sponge
Haliclona
Non-indigenous species
Molecular analysis
Sicily (Italy)

ABSTRACT

In this paper, we identify some sponge specimens collected in the Faro Lake in Sicily, and belonging to *Haliclona* (*Halicoclona*) by using morphological analysis accompanied by molecular analysis through amplification of several molecular markers (18S and 28S rRNA, CO1 and ITS).

The samples are identified as. *H.* (*Halichoclona*) *vansoesti* de Weerdt, de Kluijver & Gómez, 1999, a species native to the Caribbean, and therefore this is the first record of an alien species of the Demospongiae class (Porifera) from the Mediterranean Sea. This presence can be ascribed as results of global change (mainly global warming) that are affecting marine environment.

1. Introduction

The equilibrium of the Mediterranean Sea ecosystems is progressively threatened by climate change and by the increasingly harmful anthropogenic pressures (Cerrano et al., 2000; Lejeusne et al., 2010; Bianchi et al., 2012; Di Camillo and Cerrano, 2015; Montefalcone et al., 2018; Costa et al., 2019). These negative factors are also complicit in the introduction into this semi-closed basin of alien species that undermine the Mediterranean biodiversity and the balance of ecosystems (Occhipinti-Ambrogi, 2007). The Mediterranean, with over 800, is also the sea with the largest number of reported alien species (Galil et al., 2017; Ulman et al., 2017; Zenetos and Galanidi, 2020) and this is attributed to the shipping, which facilitates the spreading of alien organisms around the Mediterranean via biofouling and ballast waters, at the opening and enlargement of the Suez Canal, to the aquaculture and aquariology (Katsanevakis et al., 2014; Galil et al., 2015; Ulman et al., 2019). As for the Porifera, only one species, *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, described from the Western Atlantic, Brazil, is considered alien for the Mediterranean Sea. Up to now *P. magna* has been present throughout the Mediterranean Sea, with numerous reports in most of the coasts (Ulman et al., 2017; Zammit et al., 2009; Sghaier et al., 2019; Bensari et al., 2020; Longo et al., 2012; Guardiola et al., 2012; Cvitković et al., 2013; Bertolino et al., 2014; Marra et al., 2016; Topaloğlu et al., 2016; Gerovasileiou et al., 2017; Bachetarzi et al., 2019) since its first record was in the Ionian Sea (Longo et al., 2004; Longo et al., 2007). Other sponges are considered as probable Lessepsian species (Tsurnamal, 1969; Ilan et al., 2004; Vacelet et al., 2007; Ammar and Fadel, 2017; Evcen et al., 2020; Burton, 1936), but the data and studies are based on assumptions not including a detailed description of the samples and therefore doubtful. The coasts of the Mediterranean Sea have undergone strong urbanization in recent decades and above all coastal lagoons, such as the Faro Lake (Messina, Sicily-Italy), that are exploited for tourism, fishing, aquaculture and other human activities (Anthony et al., 2009). Although the Faro Lake is part of the "La Laguna di Capo Peloro" Nature Reserve, it is subject to a strong anthropogenic pressure, for the most part due to bivalve farming (Manganaro et al., 2009). Furthermore, despite the fact that the Faro Lake has the characteristics of a coastal lagoon, Capillo et al., 2018 confirmed that this environment is stable, but with fragile balances. This fragile equilibrium may be even more undermined by the introduction of alien species such as Protozoa (Saccà and Giuffrè, 2013), sponges (e. g. *P. magna* first reported in this lake by Marra et al., 2016), Polychaeta (Cosentino and

* Corresponding authors.
E-mail addresses: marco.bertolino@unige.it (M. Bertolino), maria.costantini@szn.it (M. Costantini).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.margen.2022.100951
Received 27 January 2022; Received in revised form 23 March 2022; Accepted 23 March 2022
Available online 5 April 2022

Giacobbe, 2011; Giangrande et al., 2012), Copepoda (Sabia et al., 2014; Pansera et al., 2014; Zagami et al., 2019), and "opisthobranch" (Vitale et al., 2016).

The aim of this work was to resolve the cryptogenic nature of a sponge belonging to the subgenus *Haliclona* (*Halichoclona*) and recently found in the Faro Lake (Sicily). It was possible to identify this sponge as *H.* (*Halichoclona*) *vansoesti* de Weerdt et al., 1999, native to the Caribbean Sea by combining morphological and genetic analyses. This is the first report of an alien species of Demospongiae in the Mediterranean Sea.

2. Materials and methods

2.1. Study area

The natural reserve "Laguna di Capo Peloro", which consists of two connected basins, Ganzirri and Faro, is located in the northeast of Sicily (Italy) and has a mainly brackish habitat. The Faro Lake (38°16′07″N, 15°38′13″E), notwithstanding its small size with an area of 263.600 m^2, is the deepest coastal basin in Italy, reaching 29 m depth in the eastern part, while it does not exceed 3.5 m in the western (Fig. 1) part. Its bathymetric morphology and its particular meromictic regime, characterized by chemical-physical stratification of the deepest water column, induce waters below 10 m depth to be anoxic (Saccà et al., 2008). The lake is connected to the sea by two small channels, one towards the Tyrrhenian Sea and the other towards the Strait of Messina (Fig. 1). Despite the small size of these channels, the waters of Faro Lake are continually renewed by marine inflows through strong tidal currents, in contrast to the lower hydrodynamics of the lake (Bottari et al., 2005; Vitale et al., 2016). These inflows of water from the sea lead to continuous changes in the chemical and physical parameters in the first 10–12 m of water, for example the temperature in this first layer varies from 10° to 30°, while below the temperature remains constant at about 15° (Saccà et al., 2008). The greatest conditioning of this lake is however given by the waters coming from the Strait of Messina with its peculiar conditions, which determines an ecological change of the benthic communities of shallow waters, creating a situation defined as "Atlantic type" (Drew, 1974; Bianchi, 2004). The map of the area is provided in

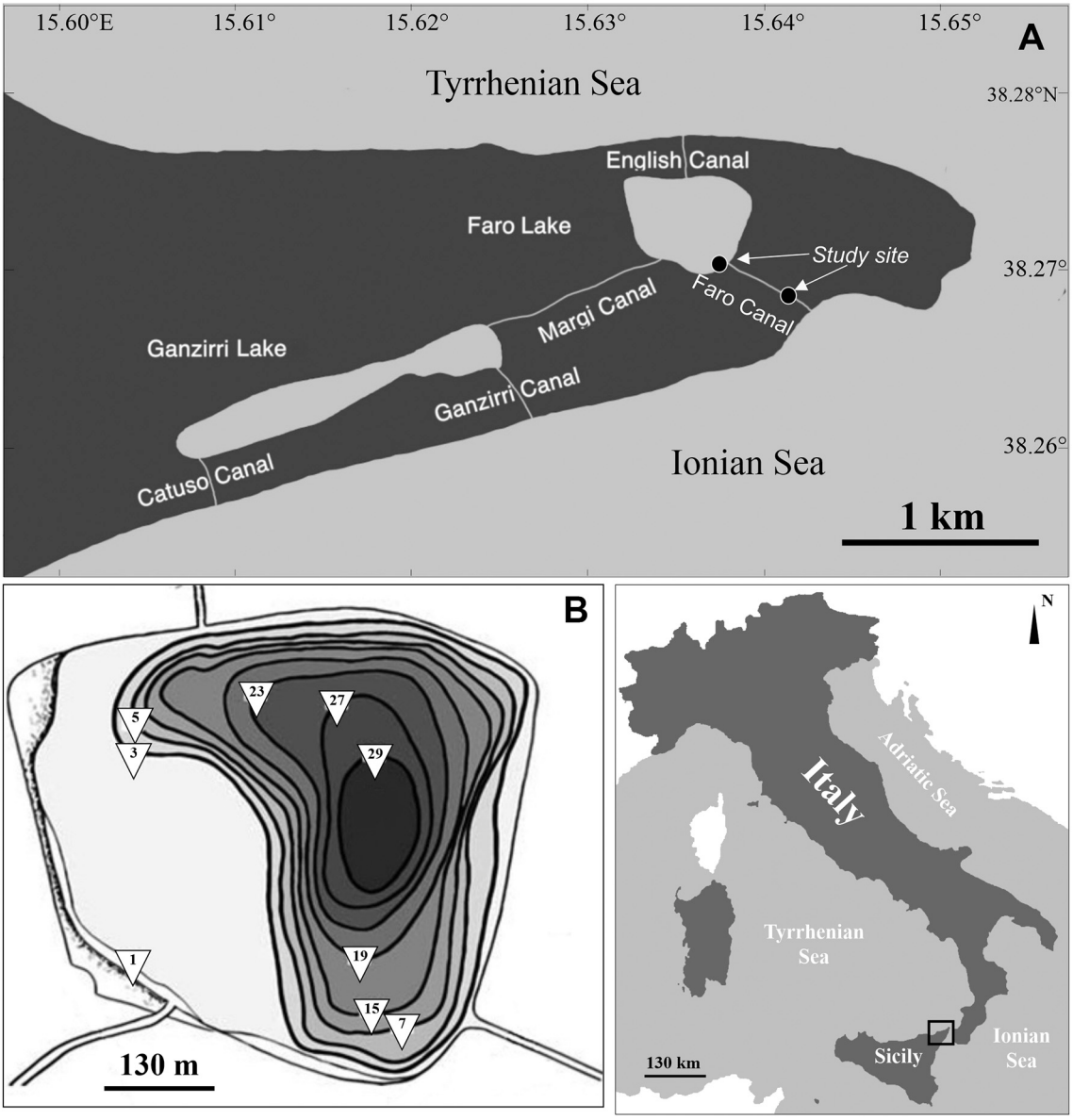

Fig. 1. Map of the study area – **A:** The natural reserve "Laguna di Capo Peloro" (Ganzirri and Faro Lakes); the black circles indicated the study sites of the Faro Lake; **B:** Faro Lake bathymetry; the triangles show the depths of the isobaths.

Fig. 1.

2.2. Morphological characterization

Four sponge samples (S22a, S22b, S22c and S22d) were collected at 2–3 m depth from the lake and the canal that goes towards the Strait of Messina (Fig. 1), by SCUBA diving in October 2019. Collected samples were photographed under and out of the water, immediately washed at least three times with filter-sterilized natural seawater and preserved in 70% ethanol and processed by standard methods for sponge identification (Rützler, 1978). Taxonomic decisions were taken according to the Systema Porifera (Hooper and Van Soest, 2002) implemented by the Demosponge revision of Morrow and Cardenas (2015) and the World Porifera Database (WPD) (de Voogd et al., 2022). Length and width of at least 30 spicules per type were measured for each species / specimen collected. Minimum, mean (in parentheses) and maximum values of spicule dimensions are reported. The most representative specimen was entrusted to the Museo Civico di Storia Naturale G. Doria of Genoa (collection acronym MSNG). Spicule slides and the other specimens examined are deposited in the sponge collection of the "Dipartimento di Scienze della Terra dell'Ambiente e della Vita" (DISTAV), Università degli Studi di Genova.

2.3. Molecular investigation

About 10 mg of tissue of four biological replicates was used for DNA extraction by QIAamp® DNA Micro kit (QIAGEN), according to the manufacturer's instructions. DNA quantity (ng/μL) was evaluated by a NanoDrop spectrophotometer. To identify the sponge species, primer pairs specific for the conserved regions CO1, 18S, 28S and ITS rRNA were used. PCR reactions were performed on C1000 Touch Thermal Cycler (BioRad) in a 30 μL reaction mixture including about 50–100 ng of genomic DNA, 6 μL of 5× Buffer GL (GeneSpin Srl, Milan, Italy), 3 μL of dNTPs (2 mM each), 1 μL of each forward and reverse primer (20 pmol/μL), 0.2 μL of Xtra Taq Polymerase (5 U/μL, GeneSpin Srl, Milan, Italy) as follows:

i. for 18S and 28S, a denaturation step at 95 °C for 2 min, 35 cycles denaturation step at 95 °C for 1 min, annealing step at 60 °C (A/B) (Schmitt et al., 2005), 57 °C (C2/D2) (Chombard et al., 1998), 55 °C (18S-AF/18S-BR, NL4F/NL4R) (Collins, 2002; Dohrmann et al., 2008), 52 °C (18S1/18S2) (Manuel et al., 2003) for 1 min and 72 °C of primer extension for 2 min], a final extension step at 72 °C for 10 min;

ii. ITS primers (RA2/ITS2.2) (Schmitt et al., 2005; Wörheide et al., 2002), a first denaturation at 95 °C for 2 min, [35 cycles denaturation step at 95 °C for 1 min, annealing step at 67 °C for 1 min and 72 °C of primer extension for 2 min, a final extension step at 72 °C for 10 min;

iii. CO1 primers (dgLCO1490/dgHCO2198) (Meyer et al., 2005), a first denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s and primer extension at 72 °C for 1 min.

PCR products were separated on 1.5% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) using a 100 bp DNA ladder (GeneSpin Srl, Milan, Italy) and purified by QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. PCR amplicons were then sequenced in both strands (~ 650 bases, 97.5% accuracy) through Applied Biosystems (Life Technologies) 3730 Analyzer (48 capillaries). The total 18S, 28S and CO1 region were aligned to GenBank using Basic Local Alignment Search Tool (BLAST) and then aligned with highly similar sequence using MultiAlin (http://multalin.toulouse.inra.fr/multalin/).

2.4. Phylogenetic analysis

The phylogenetic tree was constructed running MEGA X v11.0 software (Kumar et al., 2018). Sequences were aligned by CLUSTAL W (Thompson et al., 1994). The evolutionary history was inferred by using the Maximum Likelihood (ML) method and Tamura-Nei model (Tamura and Nei, 1993). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join (NJ) and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value.

The phylogenetic relationships for the Haplosclerida order (= Heteroscleromorpha sensu) (Redmond et al., 2011; Redmond et al., 2013) was performed by selecting some representative CO1, 18S and 28S rRNA sequences of several heteroscleromorphs downloaded from GenBank (Muricy et al., 2015).

3. Results

3.1. Systematics

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Subclass Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault, 2012
Order Haplosclerida Topsent, 1928
Family Chalinidae Gray, 1867
Genus *Haliclona* Grant, 1841.
Subgenus *Haliclona* (*Halichoclona*) de Laubenfels, 1932.
Haliclona (*Halichoclona*) *vansoesti* de Weerdt, de Kluijver & Gómez, 1999.

3.2. Diagnosis

Haliclona (*Halichoclona*) with a whitish translucent ectosome easily separable from the choanosome and cavernous structure; purplish to light pink salmon on the exterior, with beige inside; numerous large oscula at the extremities of long tubular projections; crumbly and fragile consistency. Megascleres oxeas. Microscleres absent.

3.3. Examined material

- The most representative sample: S22a – MSNG 62330; Faro Lake; 38°16′07″N, 15°38′13″E; depth 2–3 m; October 2019; on hard substrate;
- Other samples:

S22b; Faro Lake; 38°16′07″N, 15°38′13″E; depth 2–3 m; October 2019; on hard substrate;

S22c; Faro Lake; 38°16′07″N, 15°38′13″E; depth 2–3 m; October 2019; on hard substrate;

S22d; Faro Lake; 38°16′07″N, 15°38′13″E; depth 2–3 m; April 2020; on hard substrate.

3.4. Description

All the specimens have a massive irregular shape, anchored to hard substrate (rock), covering a surface that varies from 7.5 cm^2, in the smallest specimen, to 20 cm^2 in the largest one and a thickness ranging, 4 cm from the periphery, to 13 cm in the central body of the sponge. It has many conical or tubular projections up to 2 cm high, ending with circular ones or ovoid oscula up to 5 mm in diameter and numerous thin outgrowths branching off from the main sponge body. The specimens have a live colour from white to beige, purple to light violet while beige-cream in alcohol and in a dry state. Irregular, whitish translucent smooth surface, easily detachable from the choanosome. Brittle and fragile but hard consistency (Fig. 2A, B).

3.5. Skeleton

Ectosome. Easily detachable, formed by isotropic, predominantly paucispicular tangential reticulation of oxeas (Fig. 2C). Subectosomal

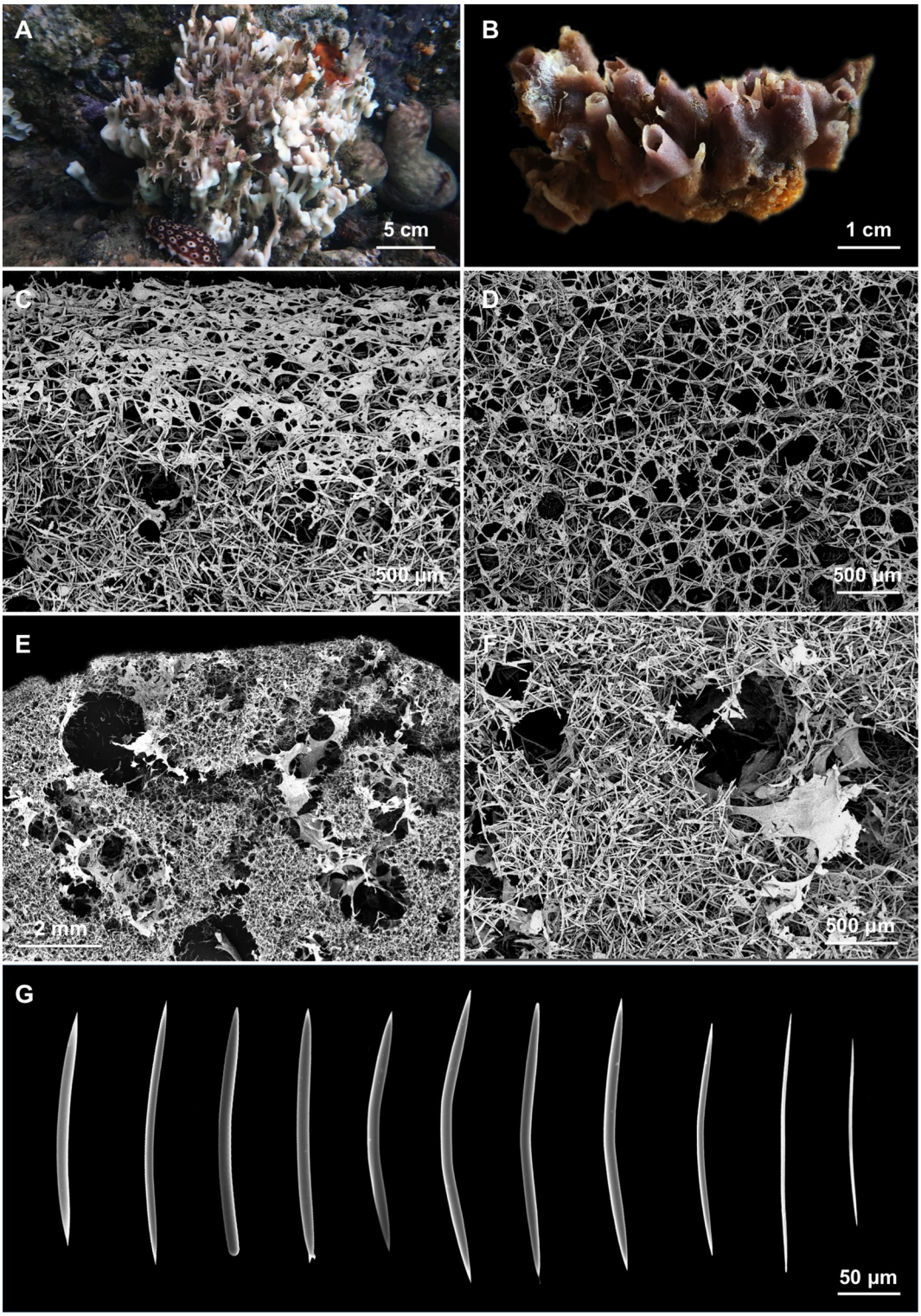

Fig. 2. *Haliclona* (*Halichoclona*) *vansoesti* - **A: live** sample MSNG 62330; **B:** freezed sample MSNG 62330; **C:** ectosomal skeleton; **D:** choanosomal skeleton; **E:** subectosomal and choanosomal lacunae; **F:** magnification of the choanosomal lacunae; **G:** spicules.

and choanosomal lacunae are common (Fig. 2E, F).

Choanosome. Structure very similar to that of the ectosome; isotropic disorganized skeleton, cavernous with lacunae, the tracts are often unispicular (Fig. 2D-F). Very little spongin present.

3.6. Spicules

Oxeas smooth, straight or fusiform, sometimes slightly central curved, with acerate or hastate tips (Fig. 2G);

S22a (MSNG 62330): 157.5 (202.3) 230 µm long and 2.5 (7.6) 12.5 µm thick;

S22b: 175 (205.7) 240 µm long and 2.5 (7.7) 10 µm thick;

S22c: 112.5 (197.5) 240 μm long and 2.5 (7.5) 12.5 μm thick;
S22d: 151.7 (196.9) 225.5 μm long and 2.6 (8.2) 10.4 μm thick.

3.7. Ecology

Recorded on hard substrate about 2–3 m depth.

3.8. Geographical distribution

The detailed distribution of *H.* (*Halichoclona*) *vansoesti* is reported in Table 1.

It was described, for the first time, for the Southern Caribbean Sea (de Weerdt et al., 1999); later recorded throughout the Caribbean Sea (de Weerdt, 2000; Valderrama and Zea, 2013; Rützler et al., 2014); in the southwest Atlantic Ocean in Palmas Island and several other islands

Table 1
Morphological characters, habitat and distribution of all records of *Haliclona* (*Halichoclona*) *vansoesti.*

Shape	Colour	Surface	Consistency	Skeleton	Spicules (μm)	Habitat and distribution	References
Massive irregular shape, 7.5–20 cm^2 large, 4–13 cm thickness; conical or tubular projections up to 2 cm high, ending with circular or ovoid oscula up to 5 mm in diameter.	Purple to light violet to beige alive. Surface whitish translucent. Beige-cream in alcohol and in dry state.	Irregular smooth	Brittle, fragile, hard	Ectosomal: easily detachable, isotropic, paucispicular reticulation of oxeas; lacunae are common; Choanosomal: isotropic disorganized, cavernous with lacunae, the tracts are often unispicular	Oxeas Smooth, fusiform, acerate, hastate 112.5 (197.5) 240 x 2.5 (7.5) 12.5	Rock, depth range 2–3 m Mediterranean Sea (Faro Lake)	This paper
Thick cushions with a loose, cavernous structure, to about 15 cm in diameter and 2–3 cm thick, with large, circular to elliptical oscula, 0.8–1 cm in diameter, on slightly raised elevations, with raised, transparent rims	Choanosome light purple, ectosome white, semi-transparent	Smooth, with irregularly raised 'roofs' over the acquiferous canals and slightly raised collars around small openings	Crisp, fragile, only slightly compressible	Ectosomal: delicate, tangential, subisotropic reticulation, extremely loosely lying on the choanosomal skeleton; Choanosomal: subisotropic reticulation, of a denser structure than the ectosome, but with many subectosomal and choanosomal spaces	Oxeas slightly curved, hastate 120 (175.9) 221.6 x 3.6 (7.2) 10.7	Reef environments, occupying crevices of hard substrata, depth range 2–52 m; Caribbean Sea (Curaçao, Jamaica, St. Vincent, Martinique)	de Weerdt et al., 1999 (First description) de Weerdt, 2000
Lobated, massively encrusting.	Light blue in vivo, almost white to cream-transparent when preserved in alcohol	Slightly raised oscula, approx. 5 mm in diameter, scattered over the surface	Firm, but brittle	–	Oxeas Hastate 166 (200 ± 12.8) 214 x 4.8 (9 ± 1.4) 11.9	Dead coral, calcareous terrace after cliff, 9 m depth; Caribbean Sea (Piscadera Baai, Curaçao)	Valderrama and Zea, 2013
Cavernous cushions, to 25 mm thick, extending over 10–80 cm^2 (and more) of coral-rock substratum; Oscula large and conspicuous, usually raised, more or less circular in outline, up to 12 mm in diameter	Light (neon-tone) blue, turning black during preservation in alcohol	Smooth and cavernous	Hard and brittle	Ectosomal: unispicular reticulation; Choanosomal: denser where interrupted by numerous cavities	Oxeas lightly curved tapering to sharp points 160 (219) 280 × 3 (6) 7	Fore reef crevice, bottom, 24 m; Caribbean Sea (Carrie Bow Cay – Belize)	Rützler et al., 2014
Thickly encrusting, very irregular, up to 25 cm wide by 1–3 cm thick, with abundant, short tube-shaped projections 2–30 mm high topped by circular oscules 2–10 mm in diameter. Oscular tubes are cylindrical or vulcaniform. Some specimens also present blind fistular projections	Light purple or violet to pink, lavender, light salmon or cream choanosome; translucent surface; white to cream after fixtation	Irregular, uneven, reticulate, very loosely connected to the choanosome, easily detachable	Friable and fragile, slightly compressible, rather inelastic	Ectosomal: easily detachable, slightly disorganized isotropic, predominantly paucispicular tangential reticulation of oxeas; Choanosomal: cavernous, relatively disorganized isotropic reticulation mostly unispicular	Oxeas Smooth, fusiform, slightly curved, acerate or hastate tips 139.1 (175.5 ± 11.1) 217.5 x 2.4 (7.3 ± 1.4) 12.5	Vertical rocky surfaces depth range 10–20 m; Island off Rio de Janeiro State (Brazil) South Atlantic Ocean (Palmas Island, Cagarra Islet, Cagarra Island, Rasa Island, Maricás Archipelago)	Muricy et al., 2015

off Rio de Janeiro (Brazil) (Muricy et al., 2015).

3.9. Taxonomic remarks

The specimens of this study, collected in the Faro Lake (Sicily, Mediterranean Sea), fit with the Atlantic tropical species *Haliclona* (*Halichoclona*) *vansoesti* on the basis of morphological and genetic data here presented. Ectosome whitish translucent and easily detachable from the choanosome, which is purplish; the growth form with chimney-shaped oscular conules, skeleton with many lacunae; shape and size of the spicules (oxeas). Our samples are also very similar to the other records in Caribbean and Brazil, both in the external morphology, and in the sizes and shapes of spicules (Table 1). As already noted by Muricy et al., 2015 for specimens from Brazil, there are small differences in the growth form between our specimens and those of the Atlantic, which can be considered as mere intraspecific variations. Conspecificity of the Mediterranean and the Atlantic specimens of *H.* (*Halichoclona*) *vansoesti* was confirmed through phylogenetic analysis of molecular data (see below).

3.10. Molecular identification and phylogenetic analysis

To BLASTn alignments on nucleotide collections on the three biological replicates confirmed the results achieved with the morphological analysis of spicules, displaying a high sequence similarity to *H.* (*H.*) *vansoesti* species. More specifically, CO1 and 18S rRNA primer pairs did not reveal the clearest data, whereas 28S rRNA was found the best molecular marker. CO1 primers (dgLCO1490/dgHCO2198, sequence length = 650 bp) showed, as first hit, the sponge genus *Niphatidae* (Accession Number: MT491525.1) with 98.6% of pairwise sequence similarity, while *H.* (*H.*) *vansoesti* species aligned with about 98% of sequence identity (Accession Number: KM203834.1). The 18S rRNA primer pair 18S-AF/18S-BR (sequence length = 1860 bp) showed the highest similarity (sequence identity = 99.2%) to *Haliclona* sp. (Accession Number: KP100454.1), and two *H.* (*H.*) *vansoesti* strains, with 98.5% (Accession Number: KC902323.1) and 98.4% (Accession Number: KM191356.1) of sequence similarity.

The most striking result was achieved with the 28S rRNA marker (NL4F/NL4R, sequence length = 1108 bp), since the PCR product aligned to *H.* (*H.*) *vansoesti* with ~97% of identity (Accession Number: KC869631.1) that clearly separated from the second one, displaying ~89% of similarity (Accession Number: KC869628.1).

The maximum likelihood phylogenetic tree based on the small subunit 28S rRNA gene (Fig. 3) showed that the *H.* (*H.*) *vansoesti* from the Faro Lake was very close to *Haliclona* (*Halichoclona*) *vansoesti* voucher P10x38 in the clade B (Redmond et al., 2011).

The maximum likelihood phylogenetic tree based on the small subunit 18S rRNA gene (Fig. 4), as well as for COI gene (Fig. 5), showed that the *H.* (*H.*) *vansoesti* from the Faro Lake, which was identified in this work, was very close to three samples of putative *H.* (*H.*) *vansoesti* from the State of Rio de Janeiro, SE Brazil (*H.* (*H.*) *vansoesti* voucher UERJ-POR10, *H.* (*H.*) *vansoesti* voucher UERJPOR30 and *H.* (*H.*) *vansoesti* voucher UERJPOR34) (Muricy et al., 2015), which in turn were very similar to *Haliclona* (*Halich.*) *vansoesti* from the Caribbean having been identified by de Weerdt et al., 1999, de Weerdt, 2000 and Valderrama and Zea, 2013. In the case on 18S rRNA gene the results supported the hypothesis that they belong to the same species. This maximum likelihood phylogenetic tree also included representative sequences of each of the five clades described in the molecular phylogenetic analysis of Demospongiae, including marine Haplosclerida (= Heteroscleromorpha) (Redmond et al., 2013).

With regards to COI gene, two monophyletic clades were described by Redmond et al., 2011 for this marker: clade A, composed mainly of *Haliclona* and *Callyspongia* species and clade B, formed by two *Haliclona*

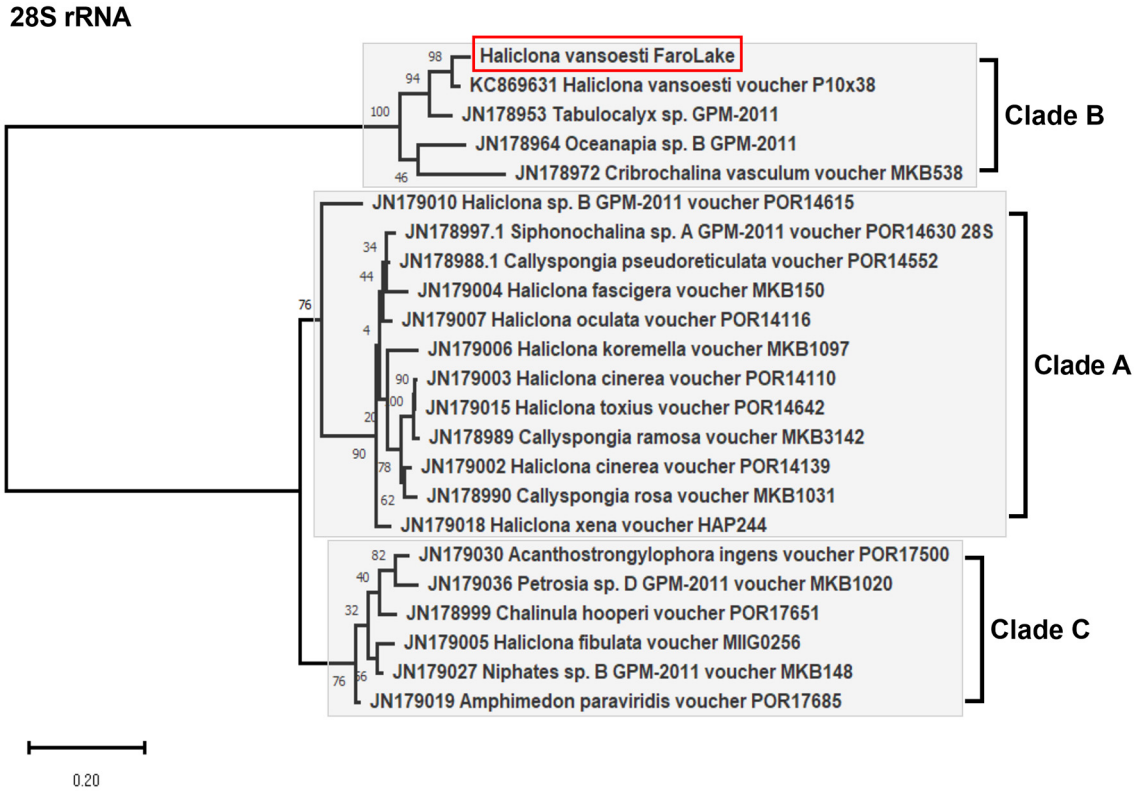

Fig. 3. Maximum likelihood phylogenetic tree of the Heteroscleromorpha based on sequences of the small subunit 28S rRNA gene, including *H.* (*H.*) *vansoesti* isolated in the Faro Lake in this study (highlighted with the red box). Only bootstrap values above 50% are shown. Clades A–C refer to Redmond et al., 2011. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

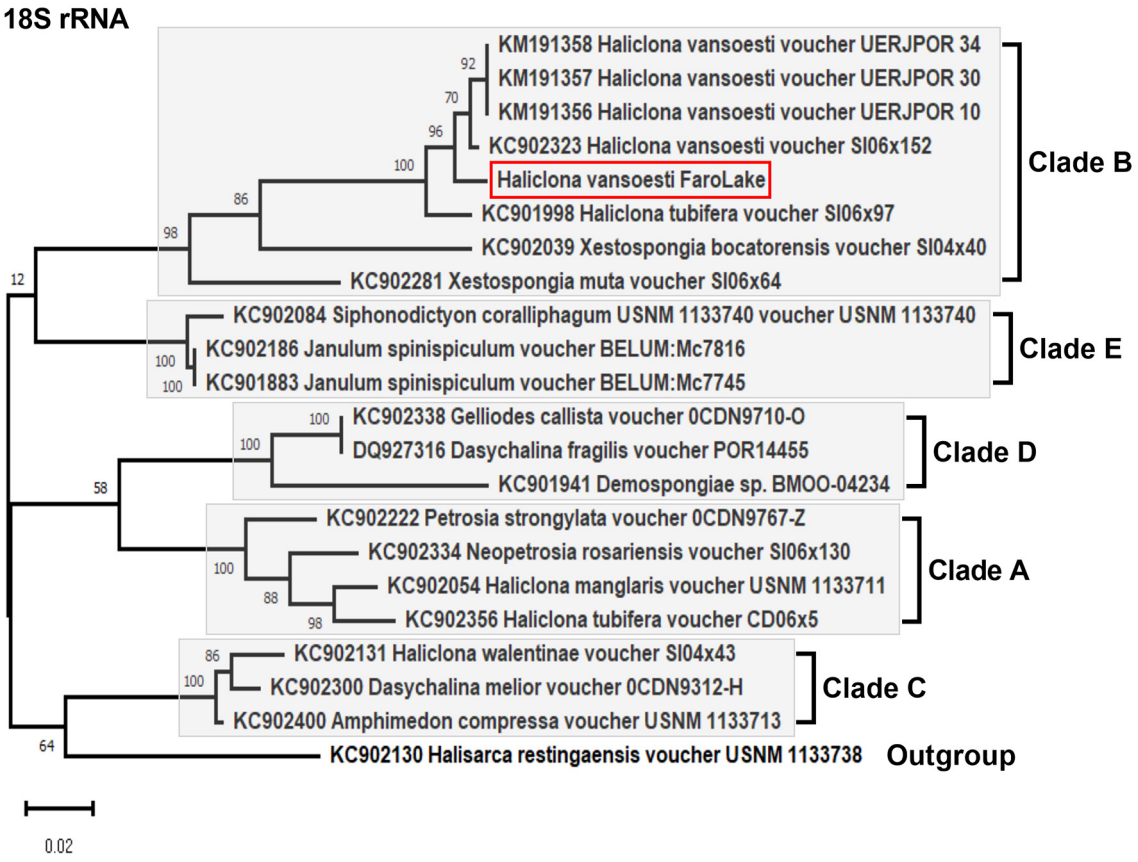

Fig. 4. Maximum likelihood phylogenetic tree of the Heteroscleromorpha based on sequences of the small subunit 18S rRNA gene, including *H.* (*H.*) *vansoesti* isolated in the Faro Lake in this study (highlighted with the red box). Only bootstrap values above 50% are shown. Clades A–E refer to Redmond et al., 2013. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and *Callyspongia* species and *Amphimedon queenslandica* Hooper & van Soest, 2006. In our analysis, the COI sequences for the putative *H.* (*Halich.*) *vansoesti* from SE Brazil form a monophyletic cluster with high bootstrap support outside these two clades, including the *Haliclona* species identified from the Faro Lake.

4. Discussion

Faro Lake is a stressed, confined, highly variable environment, with its abiotic parameters able to greatly fluctuate and linked to the seasonal variations, being very different from year to year. These ecological unpredictable features determine the presence of a highly tolerant sponge fauna subject to a high turnover. The Faro Lake reserve has undergone almost total cancellation of the natural landscape in recent decades to make room for uncontrolled overbuilding (Manganaro et al., 2011; Longo et al., 2016). Naturally, the repercussion in the benthic marine/lagoon environment is serious for local species, also increasing the introduction of numerous non-native species (Marra et al., 2016; Longo et al., 2016; Corriero et al., 2016) with continuous changes to ecological balances. Despite this, the sponge community of the Faro Lake has a rich diversity already investigated by several authors in recent decades (Marra et al., 2016; Longo et al., 2016; Labate and Arena, 1964). This list of sponges includes the alien species *Paraleucilla magna* (Calcarea class), first recorded in the Faro Lake by Marra et al. (2016) and the presence of this species is due to mussel farming systems, as in Taranto (Longo et al., 2007) and other areas of the Mediterranean (Bertolino et al., 2014).

After *P. magna*, *H.* (*Halichoclona*) *vansoesti* was sampled in the Faro Lake, it can be considered alien from our analyzes. This is the second report of a non-indigenous sponge and the first secure record for the Demospongiae class in the Mediterranean Sea.

H. (*Halichoclona*) *vansoesti* is a typical tropical water species. In fact, it was described for the first time in the Caribbean (de Weerdt et al., 1999), where it is present practically everywhere (de Weerdt, 2000; Valderrama and Zea, 2013; Rützler et al., 2014) and later for the coasts of Brazil (Muricy et al., 2015). The depth range is very wide, from 2 to 52 m, furthermore, de Weerdt et al., 1999, 2000 reported a large increase in the abundance of specimens with increasing depth in sheltered locations; our finding in the Faro Lake is in agreement with *H.* (*Halichoclona*) *vansoesti* preferences for sheltered locations.

It is not easy to evaluate the mechanism of introduction of this species from the Caribbean and Brazilian coasts to those of the Mediterranean Sea; however, the intense maritime traffic to which they are subjected and the increasing bivalve farms (*Crassostrea gigas*, *Ostrea edulis* and *Mytilus galloprovincialis*), often with organisms coming from the Atlantic Ocean (Uttieri et al., 2020), can be considered responsible for the discovery of this sponge in the Faro Lake. The presence of this sponge species in this lake could also be explained as a direct consequence of global change, mainly to climate change. In fact, the link between changing temperature conditions and the adaptation of non-indigenous species was extensively studied in the marine environment (Occhipinti-Ambrogi, 2007; Occhipinti-Ambrogi and Galil, 2010). This is a very important point considering that climate change is one of the three main drivers of biological invasions, together with transport and socio-economic changes, which will significantly affect the impacts of alien species on biodiversity in the future (Early et al., 2016; Essl et al., 2020). Finally, as reported in Raitsos et al., 2010 the speed of alien species invasion due to global warming seems to be much faster than temperature increase itself, representing an important issue for the biodiversity in the Mediterranean Sea.

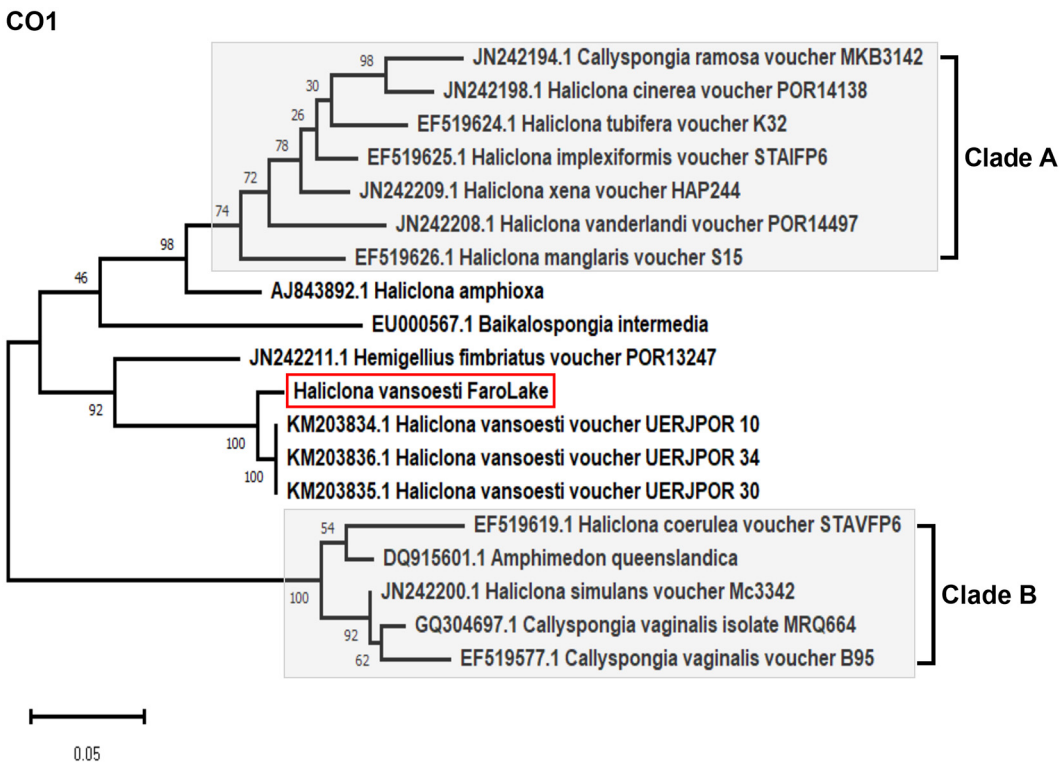

Fig. 5. Maximum likelihood phylogenetic tree of the Heteroscleromorpha based on sequences of the small subunit COI gene, including *H.* (*H.*) *vansoesti* isolated in the Faro Lake in this study (highlighted with the red box). Only bootstrap values above 50% are shown. Clades A–B refer to Redmond et al., 2011. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Credit author statement

Marco Bertolino, Gabriele Costa: morphological analysis of sponge samples. Nadia Ruocco, Roberta Esposito: molecular identification of sponge samples. Giacomo Zagami, Sergio De Matteo: collection of sponge samples and their identification. Marco Bertolino, Maria Costantini: data curation; formal analysis, investigation, conceptualization, funding acquisition, supervision, original draft writing - reviewing and editing.

Declaration of Competing Interest

The authors declare no competing interest.

Acknowledgments

Nadia Ruocco was supported by a research grant “Antitumor Drugs and Vaccines from the Sea (ADViSE)” project (PG/2018/0494374). Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. We also thank Davide Caramiello of the Marine Organisms Core Facility of the Stazione Zoologica for his technical support in sponge maintenance. English language editing was kindly provided by Rosanna Messina.

References

Ammar, I., Fadel, S., 2017. Update list of sponges of Latakia (Syria)-new record exotic species. J. Entomol. Zool. Stud. 5, 1041–1047.

Anthony, A., Atwood, J., August, P., Byron, C., Cobb, S., Foster, C., et al., 2009. Coastal lagoons and climate change: ecological and social ramifications in U.S. Atlantic and Gulf coast ecosystems. Ecol. Soc. 14, 8. https://doi.org/10.5751/ES-02719-140108.

Bachetarzi, R., Dilmi, S., Uriz, M.J., Vázquez-Luis, M., Deudero, S., Rebzani-Zahaf, C., 2019. The non-indigenous and invasive species *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004 (Porifera: Calcarea) in the algerian coast (South Western of Mediterranean Sea). Acta Adriat. 60, 41–46. https://doi.org/10.32582/aa.60.1.3.

Bensari, B., Bahbah, L., Lounaouci, A., Fahci, S.E., Bouda, A., Bachari, N.E.I., 2020. First records of non-indigenous species in port of Arzew (Algeria: South Western Mediterranean). Mediterr. Mar. Sci. 21, 393–399. https://doi.org/10.12681/mms.21927.

Bertolino, M., Calcinai, B., Cattaneo-Vietti, R., Cerrano, C., Lafratta, A., Pansini, M., et al., 2014. Stability of the sponge assemblage of Mediterranean coralligenous concretions along a millennial time span. Mar. Ecol. 35, 149–158. https://doi.org/10.1111/maec.12063.

Bianchi, C.N., 2004. Proposta di suddivisione dei mari italiani in settori biogeografici. Not della Soc Ital di Biol Mar. 46, 57–59.

Bianchi, C.N., Morri, C., Chiantore, M., Montefalcone, M., 2012. Mediterranean Sea biodiversity between the legacy from the past and a future of change. In: Stambler, N. (Ed.), Life in the Mediterranean Sea: A Look at Habitat Changes. Nova Science Publishers, New York, USA, pp. 1–55.

Bottari, A., Bottari, C., Carveni, P., Giacobbe, S., Spanò, N., 2005. Genesis and geomorphologic and ecological evolution of the Ganzirri salt marsh (Messina, Italy). Quat. Int. 140–141, 150–158.

Burton, M., 1936. The Fishery Grounds near Alexandria. IX. Sponges. Notes Mem No 8 Fish Res Dir Cairo, 17, pp. 1–28. https://doi.org/10.15468/9RJQLN.

Capillo, G., Silvestro, S., Sanfilippo, M., Fiorino, E., Giangrosso, G., Ferrantelli, V., et al., 2018. Assessment of electrolytes and metals profile of the Faro Lake (Capo Peloro Lagoon, Sicily, Italy) and its impact on Mytilus galloprovincialis. Chem. Biodivers. 15 https://doi.org/10.1002/cbdv.201800044.

Cerrano, C., Bavestrello, G., Bianchi, C.N., Cattaneo-Vietti, R., Bava, S., Morganti, C., et al., 2000. A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-Western Mediterranean), summer 1999. Ecol. Lett. 3, 284–293. https://doi.org/10.1046/j.1461-0248.2000.00152.x.

Chombard, C., Boury-Esnault, N., Tillier, S., 1998. Reassessment of homology of morphological characters in tetractinellid sponges based on molecular data. Syst. Biol. 47, 351–366.

Collins, A.G., 2002. Phylogeny of medusozoa and the evolution of cnidarian life cycles. J. Evol. Biol. 15, 418–432. https://doi.org/10.1046/j.1420-9101.2002.00403.x.

Corriero, G., Pierri, C., Accoroni, S., Alabiso, G., Bavestrello, G., Barbone, E., et al., 2016. Ecosystem vulnerability to alien and invasive species: a case study on marine habitats along the Italian coast. Aquat. Conserv. Mar. Freshwat. Ecosyst. 26, 392–409. https://doi.org/10.1002/aqc.2550.

Cosentino, A., Giacobbe, S., 2011. The new potential invader *Linopherus canariensis* (Polychaeta: Amphinomidae) in a Mediterranean coastal lake: colonization dynamics and morphological remarks. Mar. Pollut. Bull. 62, 236–245. https://doi.org/10.1016/j.marpolbul.2010.11.006.

Costa, G., Bavestrello, G., Micaroni, V., Pansini, M., Strano, F., Bertolino, M., 2019. Sponge community variation along the Apulian coasts (Otranto Strait) over a pluri-decennial time span. Does water warming drive a sponge diversity increasing in the

Mediterranean Sea? J. Mar. Biol. Assoc. United Kingdom. 99, 1519–1534. https://doi.org/10.1017/S0025315419000651.
Cvitković, I., Despalatović, M., Grubelić, I., Nikolić, V., Pleše, B., Žuljević, A., 2013. Occurrence of *Paraleucilla magna* (Porifera: Calcarea) in the eastern Adriatic Sea. Acta Adriat. 54, 93–99.
de Voogd, N.J., Alvarez, B., Boury-Esnault, N., Carballo, J.L., Cárdenas, P., Díaz, M.-C., Dohrmann, M., Downey, R., Hajdu, E., Hooper, J.N.A., Kelly, M., Klautau, M., Manconi, R., Morrow, C.C., Pisera, A.B., Ríos, P., Rützler, K., Schönberg, C., Vacelet, J., van Soest, R.W.M.. World Porifera Database. Accessed at. https://www.marinespecies.org/porifera.
de Weerdt, W.H., 2000. A monograph of the shallow-water Chalinidae (Porifera, Haplosclerida) of the Caribbean. Bull. Zool. Museum Univ. Amsterdam. 50, 1–67.
de Weerdt, W.H., de Kluijver, M.J., Gomez, R., 1999. Haliclona (Halichoclona) vansoesti n. sp., a new chalinid sponge species (Porifera, Demospongiae, Haplosclerida) from the Caribbean. Beaufortia. 49, 47–54.
Di Camillo, C.G., Cerrano, C., 2015. Mass mortality events in the NW Adriatic Sea: phase shift from slow- to fast-growing organisms. PLoS One 10, e0126689. https://doi.org/10.1371/journal.pone.0126689.
Dohrmann, M., Janussen, D., Reitner, J., Collins, A.G., Wörheide, G., 2008. Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). Syst. Biol. 57, 388–405. https://doi.org/10.1080/10635150802161088.
Drew, E.A., 1974. An ecological study of Laminaria ochroleuca Pyl. growing below 50 metres in the Straits of Messina. J. Exp. Mar. Biol. Ecol. 15, 11–24. https://doi.org/10.1016/0022-0981(74)90059-8.
Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., et al., 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. Nat. Commun. 7, 12485. https://doi.org/10.1038/ncomms12485.
Essl, F., Lenzner, B., Bacher, S., Bailey, S., Capinha, C., Daehler, C., et al., 2020. Drivers of future alien species impacts: an expert-based assessment. Glob. Chang. Biol. 26, 4880–4893. https://doi.org/10.1111/gcb.15199.
Evcen, A., Gözcelioğlu, B., Çinar, M., 2020. *Niphates toxifera* (Porifera, Demospongiae), a possible Lessepsian species now colonizing the coast of Turkey. J. Black. Sea. 26.
Galil, B.S., Boero, F., Fraschetti, S., Piraino, S., Campbell, M.L., Hewitt, C., Carlton, J., Cook, E., Jelmert, A., Macpherson, E., Marchini, A., Occhipinti-Ambrogi, A., Mckenzie, C., Minchin, D., Ojaveer, H., Olenin, S., Ruiz, G., 2015. The enlargement of the Suez Canal and introduction of non-indigenous species to the Mediterranean Sea. ASLO. 4 (2), 43–45. https://doi.org/10.1002/lob.10036.
Galil, B., Marchini, A., Occhipinti-Ambrogi, A., Ojaveer, H., 2017. The enlargement of the Suez Canal - Erythraean introductions and management challenges. Manag Biol Invasions. 8, 141–152.
Gerovasileiou, V., Akel, E., Akyol, O., Alongi, G., Azevedo, F., Babali, N., et al., 2017. New Mediterranean biodiversity records. Mediterr. Mar. Sci. 18, 355–384.
Giangrande, A., Cosentino, A., Presti, C.L., Licciano, M., 2012. Sabellidae (Annelida) from the Faro coastal lake (Messina, Ionian Sea), with the first record of the invasive species Branchiomma bairdi along the Italian coast. Med.Mar Sci 13 (2), 283–293.
Guardiola, M., Frotscher, J., Uriz, M.J., 2012. Genetic structure and differentiation at a short-time scale of the introduced calcarean sponge Paraleucilla magna to the western Mediterranean. Hydrobiologia. 687, 71–84. https://doi.org/10.1007/s10750-011-0948-1.
Hooper, J.N.A., Van Soest, R.W.M., 2002. Systema Porifera. A Guide to the Classification of Sponges. Systema Porifera. Springer, Boston, MA, pp. 1–7. https://doi.org/10.1007/978-1-4615-0747-5_1.
Ilan, M., Gugel, J., Van Soest, R.W.M., 2004. Taxonomy, reproduction and ecology of new and known Red Sea sponges. Sarsia. 89, 388–410. https://doi.org/10.1080/00364820410002659.
Katsanevakis, S., Coll, M., Piroddi, C., Steenbeek, J., Lasram, B.R.F., Zenetos, A., Cardoso, A.C., 2014. Invading the Mediterranean Sea: biodiversity patterns shaped by human activities. Front. Mar. Sci. 1, 32. https://doi.org/10.3389/fmars.2014.00032.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.
Labate, M., Arena, P., 1964. La fauna a poriferi dei laghi di Ganzirri e Faro (Messina). Arch. Zool. Ital. 49, 249–280.
Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C.F., Pérez, T., 2010. Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends Ecol. Evol. 25, 250–260. https://doi.org/10.1016/j.tree.2009.10.009.
Longo, C., Liaci, L., Manuel, M., Correiro, G., 2004. Note sui poriferi del Mar Grande e del Mar Piccolo di Taranto (Mar Ionio). Biol. Mar. Mediterr. 11, 440–443. https://doi.org/10.1017/S0025315407057748.
Longo, C., Mastrototaro, F., Corriero, G., 2007. Occurrence of *Paraleucilla magna* (Porifera: Calcarea) in the Mediterranean Sea. J. Mar. Biol. Assoc. United Kingdom. 87, 1749–1755. https://doi.org/10.1017/S0025315407057748.
Longo, C., Pontassuglia, C., Corriero, G., Gaino, E., 2012. Life-cycle traits of *Paraleucilla magna*, a calcareous sponge invasive in a coastal mediterranean basin. PLoS One 7, e42392. https://doi.org/10.1371/journal.pone.0042392.
Longo, C., Cardone, F., Mercurio, M., Nonnis Marzano, C., Pierri, C., Corriero, G., 2016. Spatial and temporal distributions of the sponge fauna in southern Italian lagoon systems. Mediterr. Mar. Sci. 17, 174–189. https://doi.org/10.12681/mms.1426.
Manganaro, A., Pulicanò, G., Reale, A., Sanfilippo, M., Sarà, G., 2009. Filtration pressure by bivalves affects the trophic conditions in Mediterranean shallow ecosystems. Chem. Ecol. 25, 467–478. https://doi.org/10.1080/02757540903325120.
Manganaro, A., Pulicanò, G., Sanfilippo, M., 2011. Temporal evolution of the area of capo Peloro (Sicily, Italy) from pristine site into urbanized area. Transitional Waters Bull. 5, 23–31. https://doi.org/10.1285/i1825229Xv5n1p23.
Manuel, M., Borchiellini, C., Alivon, E., Le Parco, Y., Vacelet, J., Boury-Esnault, N., 2003. Phylogeny and evolution of calcareous sponges: monophyly of calcinea and calcaronea, high level of morphological homoplasy, and the primitive nature of axial symmetry. Syst. Biol. 52, 311–333. https://doi.org/10.1080/10635150390196966.
Marra, M.V., Bertolino, M., Pansini, M., Giacobbe, S., Manconi, R., Pronzato, R., 2016. Long-term turnover of the sponge fauna in Faro Lake (north-East Sicily, Mediterranean Sea). Ital. J. Zool. 83, 579–588. https://doi.org/10.1080/11250003.2016.1251981.
Meyer, C.P., Geller, J.B., Paulay, G., 2005. Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. Evolution (N Y). 59, 113–125. https://doi.org/10.1111/j.0014-3820.2005.tb00899.x.
Montefalcone, M., De Falco, G., Nepote, E., Canessa, M., Bertolino, M., Bavestrello, G., et al., 2018. Thirty year ecosystem trajectories in a submerged marine cave under changing pressure regime. Mar. Environ. Res. 137, 98–110. https://doi.org/10.1016/j.marenvres.2018.02.022.
Morrow, C., Cardenas, P., 2015. Proposal for a revised classification of the Demospongiae (Porifera). Frontiers in Zoology 12 (1), 7.
Muricy, G., Esteves, E.L., Monteiro, L.C., Rodrigues, B.R., Albano, R.M., 2015. A new species of Haliclona (Demospongiae: Haplosclerida: Chalinidae) from southeastern Brazil and the first record of *Haliclona vansoesti* from the Brazilian coast. Zootaxa. 3925, 536–550. https://doi.org/10.11646/zootaxa.3925.4.3.
Occhipinti-Ambrogi, A., 2007. Global change and marine communities: alien species and climate change. Mar. Pollut. Bull. 55, 342–352. https://doi.org/10.1016/j.marpolbul.2006.11.014.
Occhipinti-Ambrogi, A., Galil, B., 2010. Marine alien species as an aspect of global change. Adv. Oceanogr. Limnol. 1, 199–218. https://doi.org/10.1080/19475721003743876.
Pansera, M., Granata, A., Guglielmo, L., Minutoli, R., Zagami, G., Brugnano, C., 2014. How does mesh-size selection reshape the description of zooplankton community structure in coastal lakes? Estuar. Coast. Shelf Sci. 151, 221–235. https://doi.org/10.1016/j.ecss.2014.10.015.
Raitsos, D.E., Beaugrand, G., Georgopoulos, D., Zenetos, A., Pancucci-Papadopoulou, A. M., Theocharis, A., et al., 2010. Global climate change amplifies the entry of tropical species into the eastern Mediterranean Sea. Limnol. Oceanogr. 55, 1478–1484. https://doi.org/10.4319/lo.2010.55.4.1478.
Redmond, N.E., Raleigh, J., van Soest, R.W.M., Kelly, M., Travers, S.A.A., Bradshaw, B., et al., 2011. Phylogenetic relationships of the marine Haplosclerida (Phylum Porifera) employing ribosomal (28s rRNA) and mitochondrial (cox1, nad1) gene sequence data. PLoS One 6, 24344. https://doi.org/10.1371/journal.pone.0024344.
Redmond, N.E., Morrow, C.C., Thacker, R.W., Diaz, M.C., Boury-Esnault, N., Cárdenas, P., et al., 2013. Phylogeny and systematics of demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. Integr. Comp. Biol. 388–415. https://doi.org/10.1093/icb/ict078.
Rützler, K., 1978. Sponges in coral reefs. Coral reefs: research methods. 299–313.
Rützler, K., Piantoni, C., Van Soest, R.W.M., Díaz, M.C., 2014. Diversity of sponges (Porifera) from cryptic habitats on the Belize barrier reef near Carrie Bow Cay. Zootaxa. 3805, 1–129. https://doi.org/10.11646/zootaxa.3805.1.1.
Sabia, L., Uttieri, M., Schmitt, F.G., Zagami, G., Zambianchi, E., Souissi, S., 2014. *Pseudodiaptomus marinus* Sato, 1913, a new invasive copepod in Lake Faro (Sicily): observations on the swimming behaviour and the sex-dependent responses to food. Zool. Stud. 53, 49. https://doi.org/10.1186/s40555-014-0049-8.
Saccà, A., Giuffrè, G., 2013. Biogeography and ecology of *Rhizodomus tagatzi*, a presumptive invasive tintinnid ciliate. J. Plankton Res. 35, 894–906. https://doi.org/10.1093/plankt/fbt036.
Saccà, A., Guglielmo, L., Bruni, V., 2008. Vertical and temporal microbial community patterns in a meromictic coastal lake influenced by the straits of Messina upwelling system. Hydrobiologia. 600, 89–104. https://doi.org/10.1007/s10750-007-9179-x.
Schmitt, S., Hentschel, U., Zea, S., Dandekar, T., Wolf, M., 2005. ITS-2 and 18S rRNA gene phylogeny of aplysinidae (verongida, demospongiae). J. Mol. Evol. 60, 327–336. https://doi.org/10.1007/s00239-004-0162-0.
Sghaier, Y.R., Zakhama-Sraieb, R., Ben, Hmida A., Charfi, F., 2019. An inventory of non-indigenous species (NIS) inside and outside three tourist marinas from the southern Mediterranean coast. J. Black Sea/Mediterranean Environ. 25 (1), 29–48.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. https://doi.org/10.1093/nar/22.22.4673.
Topaloğlu, B., Evcen, A., Çinar, M., 2016. Sponge fauna in the sea of Marmara. Turk. J. Fish. Aquat. Sci. 16, 051–059.
Tsurnamal, M., 1969. Sponges of red sea origin on the mediterranean coast of Israel. Isr J Zool. 18, 149–155. https://doi.org/10.1080/00212210.1969.10688280.
Ulman, A., Ferrario, J., Occhpinti-Ambrogi, A., Arvanitidis, C., Bandi, A., Bertolino, M., et al., 2017. A massive update of non-indigenous species records in Mediterranean marinas. PeerJ. e3954 https://doi.org/10.7717/peerj.3954.
Ulman, A., Ferrario, J., Forcada, A., Seebens, H., Arvanitidis, C., Occhipinti-Ambrogi, A., et al., 2019. Alien species spreading via biofouling on recreational vessels in the Mediterranean Sea. J. Appl. Ecol. 56, 2620–2629. https://doi.org/10.1111/1365-2664.13502.
Uttieri, M., Aguzzi, L., Aiese Cigliano, R., Amato, A., Bojanić, N., Brunetta, M., et al., 2020. WGEUROBUS–Working Group "Towards a EURopean OBservatory of the non-

indigenous calanoid copepod Pseudodiaptomus marinUS". Biol. Invasions 22 (3), 885–906.
Vacelet, J., Bitar, G., Carteron, S., Zibrowius, H., Perez, T., 2007. Five new sponge species (Porifera: Demospongiae) of subtropical or tropical affinities from the coast of Lebanon (eastern Mediterranean). J. Mar. Biol. Assoc. United Kingdom. 87, 1539–1552. https://doi.org/10.1017/S0025315407060687.
Valderrama, D., Zea, S., 2013. Annotated checklist of sponges (Porifera) from the southernmost Caribbean reefs (north-west gulf of Urabá), with description of new records for the Colombian Caribbean. Rev la Acad Colomb Ciencias Exactas Físicas y Nat. 37, 379–404.
Vitale, D., Giacobbe, S., Spinelli, A., De Matteo, S., Cervera, J.L., 2016. "Opisthobranch" (mollusks) inventory of the Faro Lake: a Sicilian biodiversity hot spot. Ital. J. Zool. 83 (4), 524–530. https://doi.org/10.1080/11250003.2016.1236843.
Wörheide, G., Degnan, B., Hooper, J., Reitner, J., 2002. Phylogeography and taxonomy of the indo-Pacific reef cave dwelling coralline demosponge *Astrosclera willeyana*: new data from nuclear internal transcribed spacer sequences. Proc. 9th Int. Coral. Reef. Symp. 1, 339–346. Available: http://www.imgp.gwdg.de/people/gwoerhe/pdfs/Worheide2002ProcCoralReefSymp-Astrosclera.pdf.
Zagami, G., Brugnano, C., Granata, A., Guglielmo, L., Minutoli, R., Aloise, A., 2019. Biogeographical distribution and ecology of the planktonic copepod Oithona davisae: rapid invasion in Lake Faro and Ganzirri (Central Mediterranean Sea). In: Uttieri, M. (Ed.), Trends in Copepod Studies. Nova Science Publishers, pp. 59–82.
Zammit, P.P., Longo, C., Schembri, P.J., 2009. Occurrence of *Paraleucilla magna* Klautau et al., 2004 (Porifera: Calcarea) in Malta. Mediterr. Mar. Sci. 10, 135–138. https://doi.org/10.12681/mms.114.
Zenetos, A., Galanidi, M., 2020. Mediterranean non indigenous species at the start of the 2020s: recent changes. Marine Biodiversity Records. 13, 10. https://doi.org/10.1186/s41200-020-00191-4.

scientific reports

Check for updates

OPEN

Microbial diversity in Mediterranean sponges as revealed by metataxonomic analysis

Nadia Ruocco[1,7], Roberta Esposito[1,2,7], Giacomo Zagami[3], Marco Bertolino[4], Sergio De Matteo[3], Michele Sonnessa[5], Federico Andreani[5], Stefania Crispi[1,6], Valerio Zupo[1] & Maria Costantini[1]✉

Although the Mediterranean Sea covers approximately a 0.7% of the world's ocean area, it represents a major reservoir of marine and coastal biodiversity. Among marine organisms, sponges (Porifera) are a key component of the deep-sea benthos, widely recognized as the dominant taxon in terms of species richness, spatial coverage, and biomass. Sponges are evolutionarily ancient, sessile filter-feeders that harbor a largely diverse microbial community within their internal mesohyl matrix. In the present work, we firstly aimed at exploring the biodiversity of marine sponges from four different areas of the Mediterranean: Faro Lake in Sicily and "Porto Paone", "Secca delle fumose", "Punta San Pancrazio" in the Gulf of Naples. Eight sponge species were collected from these sites and identified by morphological analysis and amplification of several conserved molecular markers (18S and 28S RNA ribosomal genes, mitochondrial cytochrome oxidase subunit 1 and internal transcribed spacer). In order to analyze the bacterial diversity of symbiotic communities among these different sampling sites, we also performed a metataxonomic analysis through an Illumina MiSeq platform, identifying more than 1500 bacterial taxa. Amplicon Sequence Variants (ASVs) analysis revealed a great variability of the host-specific microbial communities. Our data highlight the occurrence of dominant and locally enriched microbes in the Mediterranean, together with the biotechnological potential of these sponges and their associated bacteria as sources of bioactive natural compounds.

Several marine organisms, such as macro and microalgae, sponges and fishes have developed various defence mechanisms during their evolution, including the exploitation of a large variety of natural molecules. In addition to their ecological roles, these compounds display several biological activities, such as anticancer, anti-inflammatory, antioxidant, antimicrobial, antihypertensive, making them good candidates for biotechnological applications in the pharmaceutical, nutraceutical and cosmeceutical fields[1]. Marine sponges are multicellular, benthic and generally sessile organisms, spread throughout the seabed, from the tropics to the poles. In the 1950s, the interest in sponges was relighted thanks to the discovery of new bioactive nucleosides (spongotimidine and spongouridine) in the marine sponge *Tectitethya crypta* (i.e., *Tethya cripta*)[2]. These nucleosides were the basis for the synthesis of Ara-C, the first marine antitumor agent and antiviral drug[3]. It is important to consider that marine sponges are known for hosting microbial communities whose composition can be quite complex[4]. These symbiotic bacteria are usually involved in carbon and nitrogen fixation, nitrification, anaerobic metabolism, stabilization of the sponge skeleton, protection against UV. However, they are mainly responsible for the production of bioactive metabolites[5]. For example, it has been demonstrated that a Alphaproteobacteria symbiotic of the sponge *Dysidea avara* produce an inhibitor of angiogenesis 2-methylthio-1,4-naphthoquinone[6]. The structural classes of natural products commonly associated with microbial sources include non-ribosomal peptides (penicillin and

[1]Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy. [2]Department of Biology, University of Naples Federico II, Complesso Universitario Di Monte Sant'Angelo, Via Cinthia 21, 80126 Naples, Italy. [3]Dipartimento Di Scienze Biologiche, Chimiche, Farmaceutiche Ed Ambientali, Università Di Messina, 98100 Messina, Italy. [4]DISTAV, Università Degli Studi Di Genova, Corso Europa 26, 16132 Genoa, Italy. [5]Bio-Fab Research Srl, Via Mario Beltrami, 5, 00135 Rome, Italy. [6]Institute of Biosciences and BioResources Naples, National Research Council of Italy, Naples, Italy. [7]These authors contributed equally: Nadia Ruocco and Roberta Esposito. ✉email: maria.costantini@szn.it

Sample IDs	Sponge taxonomy	Sampling depth (m)	Sampling site	Coordinates	Substrate
O.per	*Oceanapia* cf. *perforata* (Sarà 1960)	2–3	Faro Lake	38°16'N, 15°38'E	Rocks, coralligenous concretions and caves
S.spi	*Sarcotragus spinosulus* (Schmidt 1862)	2–3	Faro Lake	38°16'N, 15°38'E	Hard substrates, coralligenous concretions and caves
E.dis	*Erylus discophorus* (Schmidt 1862)	2–3	Faro Lake	38°16'N, 15°38'E	Rocks, coralligenous concretions and caves
A.oro	*Agelas oroides* (Schmidt 1864)	7–9	Punta San Pancrazio	40°42′N, 13°57′E	Rocks, sand and coralligenous concretions
T.aur	*Tethya aurantium* (Pallas 1766)	15–17	Porto Paone	40°47′N, 14°9′E	Sand, mud, rocks, and coralligenous concretions
A.dam	*Axinella damicornis* (Esper 1794)	15–17	Porto Paone	40°47′N, 14°9′E	Rocks, mud, coralligenous concretions and caves
A.acu	*Acanthella acuta* (Schmidt 1862)	7–9	Punta San Pancrazio	40°42′N, 13°57′E	Rocks, sand and coralligenous concretions
G.cyd	*Geodia cydonium* (Jamenson 1811)	20	Secca delle Fumose, Parco Sommerso di Baia	40°49′N, 14°5′E	Roks, sand, mud, coralligenous concretions, *Posidonia* meadows and caves

Table 1. Sample IDs, taxonomy, sampling depth (m) and sites, geographical coordinates and type of substrate.

vancomycin), polyketides (erythromycin and tetracycline) and hybrid peptide polyketides (cyclosporin A and rapamycin). Some of these molecules are synthesized by non-ribosomal peptide synthases (NRPSs) and polyketide synthases (PKSs), which are encoded by genes clustered in the genome[7,8]. Several studies have highlighted the biotechnological potential of bacterial communities in marine sponges through the identification of PKSs and NRPSs genes, encoding for secondary metabolites[9–13].

Concerning the phylum Porifera, the Mediterranean is known to host a huge biodiversity, counting about 700 species[14] and more than half of these live in the coralligenous (a hard bottom of biogenic origin mainly produced by the accumulation of calcareous encrusting algae)[15–17]. Unfortunately, anthropogenic activities together with climate changes are strongly impacting the biodiversity of the Mediterranean[18,19] and, as a consequence, this facilitates the spreading of alien species[20]. Examples of these environmental events are *Paraleucilla magna*, a sponge firstly described in 2004 off the Brazilian waters and now widespread in many areas of the Mediterranean[21].

In the present work, we aim at deeper exploring the microbial communities associated with sponges in the Mediterranean Sea. Eight species of sponges were collected from four different areas of the Mediterranean, in Italy: Faro Lake (in Sicily) and "Porto Paone", "Secca delle fumose", "Punta San Pancrazio" (in the Gulf of Naples). Species characterization was performed by morphological observation of the skeleton and amplification of several conserved molecular markers (18S and 28S rRNA, ITS and CO1), with the only exception of *Geodia cydonium*, which has been previously characterized[22–24]. In order to analyse the biodiversity of symbiotic communities among different sampling sites, we performed a metataxonomic analysis of sponge samples through an Illumina MiSeq platform. More than 1500 bacterial isolates from eight samples were phylogenetically identified to understand if they were host-specific and/or site-specific. The ASVs analysis was then discussed to evaluate the biotechnological potential of sponges under investigation, in view of literature data.

Results

Morphological identification. All eight studied sponges belong to the class Demospongiae (Table 1). Seven species commonly live in the Mediterranean Sea, while *Oceanapia* cf. *perforate* (Sarà, 1960) is a rare species distributed in the western Mediterranean.

Molecular characterization. BLAST similarity search corresponded with the morphological identification achieved with two (S.spi and E.dis) of the three sponge samples collected in the Faro Lake (Sicily; see Tables S1-S2). In particular, molecular analyses confirmed S.spi as *Sarcotragus spinosulus* and E.dis as *Erylus discophorus*. ITS region displayed the best alignment for the identification of S.spi specimen with 98% of identity. Concerning the sample O.per, collected in the same site, the sequence of the species *Oceanapia* cf. *perforata*, identified by morphological analysis was not available in GenBank. Nevertheless, 18S and 28S rRNA primer pairs allowed a partial identification at the genus level, with high similarity to *Oceanapia isodictyiformis*, plus several hits annotated as *Oceanapia* sp. at low percentage of identity (Tables S1-S2).

In the case of sponges collected from "Porto Paone" in the Gulf of Naples, CO1 and 18S rRNA were the best molecular markers since they allowed the identification of sponges at the species level. Concerning T.aur sample, the species *Tethya aurantium* was identified (95% of identities) by CO1, whereas the sample A.dam, collected in the same site, was well identified as *Axinella damicornis* by 18S rRNA primers, producing a high percentage of sequence similarity (99%) (Tables S1-S2).

Molecular analysis confirmed the samples A.acu and A.oro, as *Acanthella acuta* and *Agelas oroides*, respectively, with 28S and 18S rRNA reporting the highest percentage of sequence similarity (100%) (Tables S1-S2). Alignments are reported in Figures S1-S7.

Diversity analysis. Alpha rarefaction on the observed features and three diversity indices (Chao1, Shannon and Simpson), were used to determine whether each sample was sequenced up to a sufficient depth. Rarefaction curves indicated that the majority of taxa was captured, since all samples under analysis reached a plateau (Fig. 1;

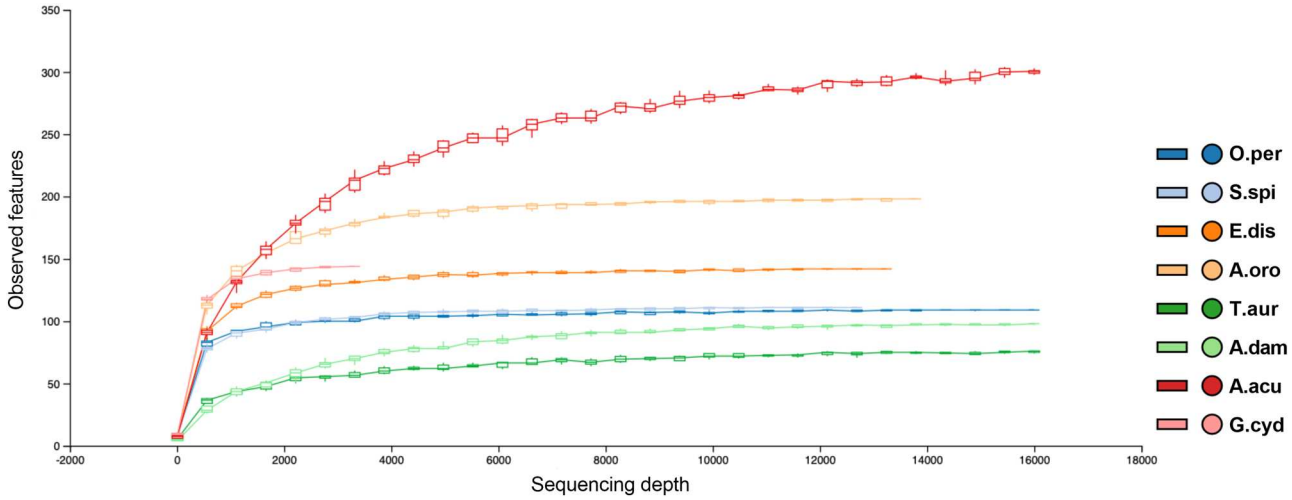

Figure 1. Alpha rarefaction of the observed features for each sponge sample under analysis. Sample IDs: O.per = *Oceanapia* cf. *perforata*, S.spi = *Sarcotragus spinosulus*, E.dis = *Erylus discophorus*, A.oro = *Agelas oroides*, T.aur = *Tethya aurantium*, A.dam = *Axinella damicornis* and A.acu = *Acanthella acuta*.

Figure S8). Alpha diversity within each sample, measured through diversity indices at the family, genus and species levels, revealed a considerable bacterial diversity for all sponges under analysis, particularly when the species were considered (Table S3). When the impact of the abiotic features (temperature, pH and salinity) was examined, no statistical differences were observed between the groups. Overall, PERMANOVA analysis revealed that the environmental features did not affect the species composition or abundance in the symbiotic community.

Further, a principal component analysis (PCA) was performed to reveal the bacterial species that greater contributed to the clustering of samples (Fig. 2). PCA showed seven components, with eigenvalues of 87.57, 21.42,19.12, 11.49, 10.82, 8.48, and 8.11. The first two components, including around 65.2% of the inertia of data, were used for further analyses, in order to detect the most interesting patterns. Firstly, PCA displayed three major groups, with 167 bacterial species that particularly influenced the clustering (Fig. 2).

Moreover, the sponge *A. acuta* (indicated as A.acu) clearly segregated, with several bacterial species (red vectors) contributing to the clustering (Fig. 2). Several taxa, such as Proteobacteria, Bacteroidia, Actinobacteria, Gracilibacteria, Cyanobacteria, Flavobacteria, Verrucomicrobiae, Campylobacteria, Planctomycetacia, Phycisphaerae, Nitrososphaeria, greatly affected the separation from the other sponges under analysis (Fig. 2). On the other hand, *G. cydonium*, *T. aurantium* and *A. damicornis* (reported as G.cyd, T.aur and A.dam, respectively), clustered into another group, where the bacteria belonging to the classes Anaerolineae, Alphaproteobacteria (Rhodobacteraceae, Hyphomicrobiaceae, Hyphomonadaceae, Kiloniellaceae, Magnetospiraceae families) and Gammaproteobacteria (Colwelliaceae and Vibrionaceae families) particularly contributed to the divergence (Fig. 2). The third cluster, corresponding to *O.* cf. *perforata*, *S. spinosulus*, *E. discophorus* and *A. oroides* (indicated as O.per, S.spi, E.dis and A.oro, respectively), was chacterized by an abundance of bacteria included into the phyla Proteobacteria, Chloroflexi, Tectomicrobia and Acidobacteria (Fig. 2).

Taxonomic profiling. ASVs analysis was performed on those reporting a confidence percentage ≥ of 75%. The full taxonomy of sponge samples was reported in Tables S4-S11. Among the sponges collected in the Faro Lake (Sicily) was found i. the largest number of features (142) in *E. discophorus*, especially Acidimicrobiia, Gemmatimonadetes, Nitrospira, Acidobacteria and Gammaproteobacteria (Fig. 3); ii. 111 ASVs in *S. spinosulus*, with a greater abundance of Dehalococcoidia, Anaerolineae, Acidimicrobiia, Dadabacteriia, Rhodothermia and Gammaproteobacteria (Fig. 3); iii. 109 ASVs in *Oceanapia* cf. *perforata*, where Verrucomicrobiae, Clostridium, Deltaproteobacteria, Nitrospirae, Dehalococcoidia, Anaerolineae, Thermoanaerobaculia, Acidimicrobiia, Gemmatimonadetes and Deltaproteobacteria were highly represented (Fig. 3).

In sponges *T. aurantium* and *A. damicornis*, both collected in the Porto Paone, in the Gulf of Naples, 76 and 98 ASVs were detected, respectively. The most abundant bacterial classes in *T. aurantium* were Alphaproteobacteria, Gammaproteobacteria, Acidimicrobiia and Dadabacteriia, whereas bacterial community of *A. damicornis* was dominated by Gammaproteobacteria, Nitrospira and Deltaproteobacteria (Fig. 3).

Among the sponges collected in Punta San Pancrazio (Ischia island, Bay of Naples), *A. acuta*, showed 316 features, with a few abundant classes (Gammaproteobacteria, Nitrospira, Alphaproteobacteria and Acidimicrobiia) and a long tail of extremely low ASVs (Fig. 4; Figure S9). In contrast, *A. oroides* mainly revealed five bacterial groups (Dehalococcoidia, Anaerolineae, Gammaproteobacteria, Dadabacteria and Deltaproteobacteria), with a total of 198 ASVs (Fig. 4; Figure S9).

G. cydonium, collected in "Parco Sommerso" of Baia (Bay of Naples), showed 144 ASVs with a significant abundance of Gammaproteobacteria, Poribacteria and Nitrospira (Fig. 3).

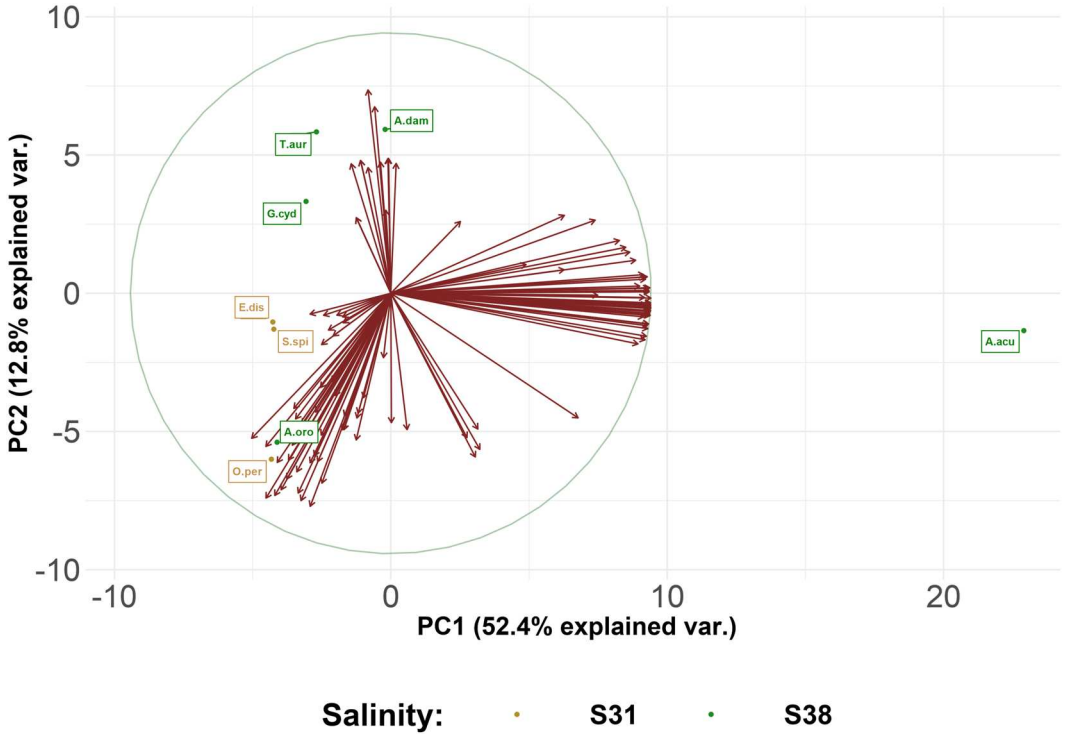

Figure 2. PCA analysis—Biplot of individuals (n = 8) and explanatory variables (n = 167) of two principal components (PC1 and PC2) of metataxonomic data. The majority of species diversity is explained by the first three PCs (76.7%), with PC1 and PC2 having the highest contribution (PC1 = 52.4% and PC2 = 12.8%). Biplot shows the PCA scores of the explanatory variables as vectors (in red) and individuals grouped for salinity class (S38 = Gulf of Naples, S31 = Strait of Messina). The circle represents the equilibrium of variables contribution. The importance of each variable is reflected by the magnitude of the corresponding values in the eigenvectors (higher magnitude-higher importance). Vectors pointing towards similar (small angle) and opposite directions (0 to 180 degrees) indicate positively or negatively correlated variables, and vectors at approximately right angles (90 to 270 degrees) suggest a low correlation. Sample IDs: O.per = *Oceanapia* cf. *perforata*, S.spi = *Sarcotragus spinosulus*, E.dis = *Erylus discophorus*, A.oro = *Agelas oroides*, T.aur = *Tethya aurantium*, A.dam = *Axinella damicornis* and A.acu = *Acanthella acuta*.

Specifically, the taxonomic composition revealed an abundance of Proteobacteria and Cloroflexi found in *O.* cf. *perforata* (4%, respectively), *S. spinosulus* (3% and 4%, respectively) and *A. oroides* (3% and 4% respectively) (Fig. 4; Figure S9).

Differently, a high percentage (17–20%) of Actinobacteria and Proteobacteria were detected in *E. discophorus*. In addition, *T. aurantium* revealed 14% of an unknown phylum, and low percentages (5%) of another phylum (Proteobacteria) (Fig. 4; Figure S9). The sponge *A. damicornis* revealed 12% of Gammaproteobacteria class, while a low percentage (1%) of Nitrospirae phylum. The sponge *A. acuta* revealed 16% of Proteobacteria phylum and 1% of Nitrospirae phylum. Interestingly, this sponge was the only species revealing a certain abundance of Archea belonging to the phylum Thaumarchaeota (Fig. 4; Figure S9). Concerning *G. cydonium*, the most abundant class was Dehalococcoidia with 29.1% and Gammaproteobacteria with 19.4% (Fig. 4; Figure S9).

As reported above, the sponges *O.* cf. *perforata*, *S. spinosulus, A. oroides* and *G. cydonium* revealed a similar composition in bacterial species distribution. In fact, a high abundance of Cloroflexi and Proteobacteria was observed in these species (Fig. 4; Figure S9).

The absolute abundance of each bacterial phylum retrieved from the eight sponge samples was reported in Table S12.

Discussion

In this study we analyzed the biodiversity of marine sponges in the Mediterranean, focusing on four sampling sites: one in the Messina Strait (North–East of Sicily) and three in the Gulf of Naples. This work represents an important step forward in the investigation of the Mediterranean, being considered as a biodiversity hotspot[25]. Long-term variations of biodiversity are significant signs of environmental change. Concerning the Mediterranean sea, data are available to compare possible variations in the species richness and faunal compositions, which are responsible of loss or turn-over of biodiversity[18,19,26]. Moreover, enclosed saline coastal basins, such as the case of the Faro Lake, represent good models of aquatic system to study temporal variation of sponge biodiversity[26].

Firstly, we identified seven sponges, complementing the traditional identification by morphological features using a molecular approach, based on DNA sequencing of 28S and 18S rDNA, ITS and CO1. Our results

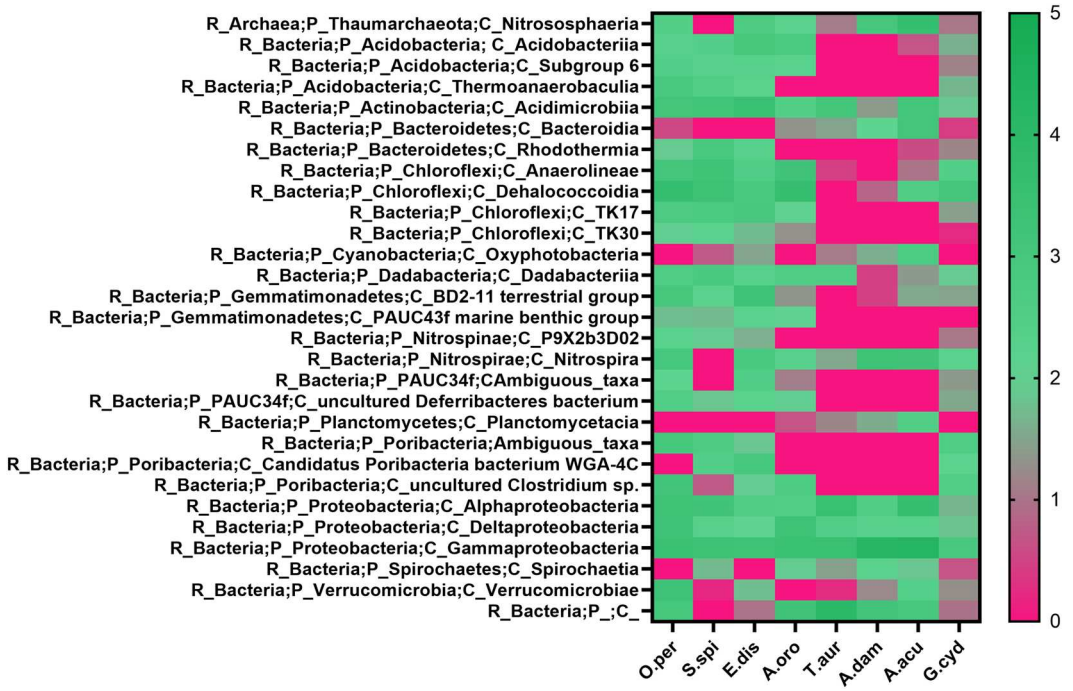

Figure 3. Heat-map comparing the abundance of the most representative bacterial classes identified from *O.* cf. *perforata* (O.per), *S. spinosulus* (S.spi), *E. discophorus* (E.dis), *A. oroides* (A.oro), *T. aurantium* (T.aur), *A. damicornis* (A.dam), *A. acuta* (A.acu) and *G. cydonium* (G.cyd). Color code: green = high number of features, pink = low number of features. Taxonomy code: R = regnum, P = phylum, C = class. Heat-map was performed using GraphPad Prism V. 9 (GraphPad Software, San Diego, CA, USA).

demonstrated that none of the molecular markers alone was able to define the sponges under analysis up to the lowest taxonomic level. Indeed, molecular markers were found to be suitable depending on the species of sponge to be classified. However, it must be considered that the barcoding analysis could be negatively affected by the lack of curated sequences collection.

Among these used molecular markers, 28S and 18S rRNA are characterized by sufficiently heterogeneous regions useful to address phylogeny at different levels[27,28]. Because of their rapid evolution, ITS regions are considered markers at high resolution[29]. The COI mitochondrial DNA locus, despite the high variability at the sequence level, it is easy to amplify for its conservation across multicellular animals and abundant in eukaryotic DNA[30,31]. In fact, it resulted to be the most successful molecular marker to discriminate sponges at various taxonomic levels[32,33]. According to these literature data, no single marker exists for all sponge species, having each marker its strength and limitations[34]. This difficulty is also linked to the incomplete sequences annotated in database, so limiting phylogeny-based molecular taxonomic approaches that are commonly used for species identification. For this reason, a multi-locus-based molecular approach is recommended for the reliability in the case of sponge identification[34]. This was in complete agreement with our experimental strategy for the identification of sponges under analysis.

An important finding achieved by this study regarded the fact that, among the three sponges collected at Faro Lake, only *E. discophorus* was recorded in 2013 during a survey on the long-term taxonomic composition and distribution of the shallow-water sponge fauna from this meromictic–anchialine coastal basin[26]. The other two species, *O.* cf. *perforata* and *S. spinosulus*, were not reported so far, suggesting them to be new colonizers of this lake. Recently, the significant number of first reports of species from several biogeographic regions found in the Faro Lake[35–38] is probably related to the import of bivalves from Atlantic and Mediterranean sites, for aquaculture activities. All three sponges usually live on rocks, coralligenous concretions and marine caves in the Mediterranean. In addition, *O.* cf. *perforata* is a rare species in the Mediterranean. Concerning the other sponges collected in the Gulf of Naples, *T. aurantium*, *A. damicornis*, *A. acuta* and *A. oroides*, represent typical species for the Mediterranean, as well as, *G. cydonium*.

Furthermore, through metataxonomic analysis, we also investigated the bacterial diversity among these Mediterranean sites. Recent advances in molecular ecology techniques, such as the sequencing of bacterial 16S rRNA gene, led to a clear picture of the taxonomic and functional composition of marine microbiota, including associated symbionts[39].

Our results showed that sponges under analysis host diverse bacterial communities. Surprisingly, sponges collected in the Faro Lake were characterized by a more diversified composition of phyla in comparison to those collected in the Gulf of Naples (Fig. 4; Figure S9). Moreover, *G. cydonium* revealed a little sequencing depth (Fig. 1), probably related to the uniqueness of the collecting site (Table 1), which has strictly influenced the symbiotic community by selecting a few species of well adapted bacteria. In fact, Secca delle Fumose represents a

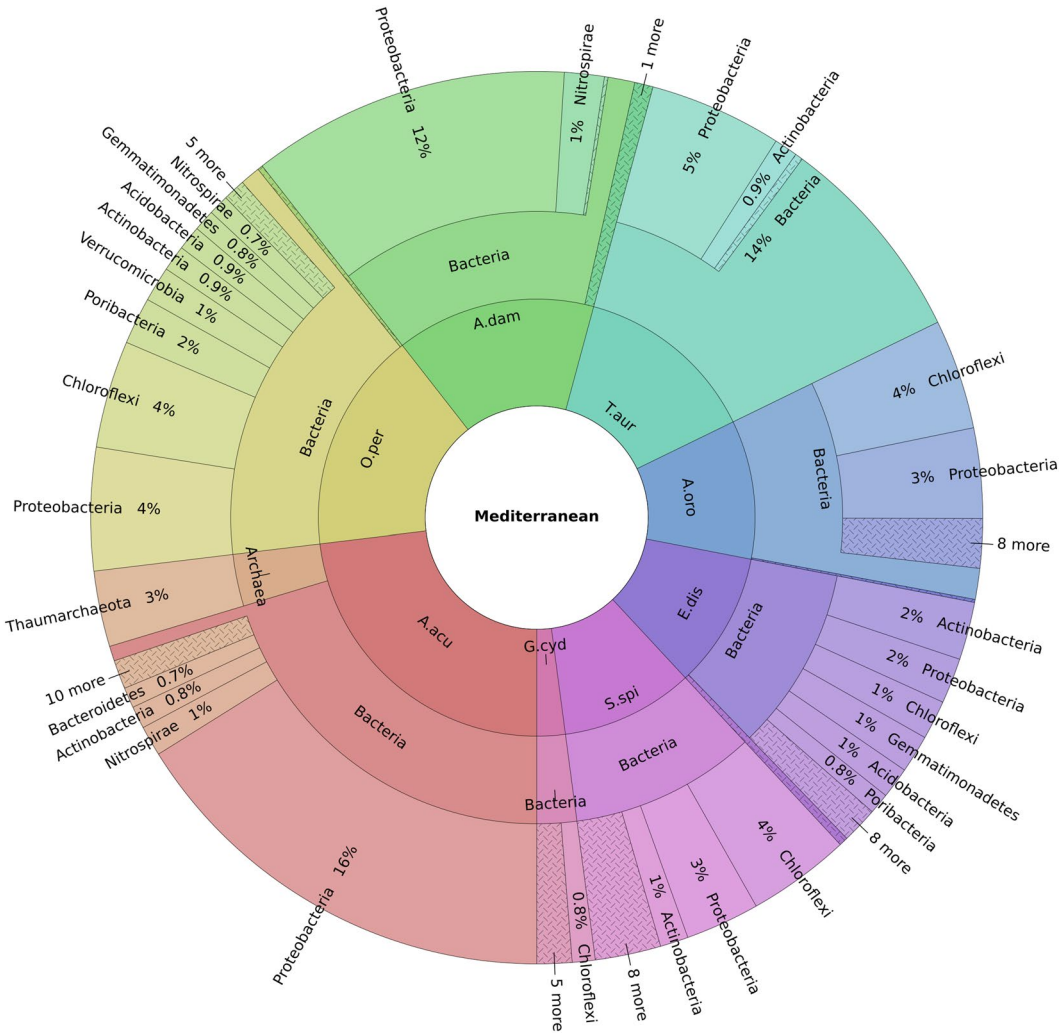

Figure 4. Krona Plot representing the most abundant phyla for each sponge under analysis. Sample code: T.aur = *T. aurantium*; A.dam = *A. damicornis*, A.acu = *A. acuta*, O.per = *O.* cf. *perforata*, S.spi = *S. spinosulus*, E.dis = *E. discophorus*, A.oro = *A. oroides* and G.cyd = *G. cydonium*.

good case study, due to the variations in seawater pH and gas-rich hydrothermal vents[40]. As reported in literature, extreme environments are well-known to inhabit a macro- and micro-biota with high biotechnological value[41–44].

Moreover, PCA analysis suggested interesting results for the sponges collected from Punta San Pancrazio (Ischia Island). In fact, *A. oroides* revealed considerable similarities to the sponges retrieved in the Strait of Messina, since they clustered in the same group (Fig. 2). On the other hand, *A. acuta* separated from the other sponges under analysis, revealing a completely different symbiotic community that needs to be taken into consideration (Fig. 2).

The phylogeny of sponges must also be considered in our analysis, because it probably influenced the community structure. In fact, *S. spinosolus*, *A. oroides*, *E. discophorus* belonging to Dictyoceratida, Agelasida, Tetractinellida orders, respectively, were recorded as High Microbial Abundance (HMA) species, while *T. aurantium*, *A. damicornis* and *A. acuta* were instead indicated as Low Microbial Abundance (LMA) species[45–47]. HMA sponges hosted a more diversified symbiont community than LMA, which were discovered to be extremely stable over seasonal and inter-annual scales[46]. The correlation of sponge taxonomy to the abundance and diversification of microbial communities was evident in the heat-map, since the HMA species displayed higher values of bacterial features (Fig. 3). Moreover, these considerations could justify the clustering obtained through the PCA analysis, where *T. aurantium* and *A. damicornis* separated from *S. spinosolus*, *A. oroides* and *E. discophorus* (Fig. 2).

Many studies reported about the sponge associated-bacteria as good candidates for the isolation of natural compounds, useful in biotechnological applications. This study represents a first evaluation of the biotechnological potential of the aforementioned sponges. For this reason, we will further discuss the known bioactivities of the most abundant bacterial phyla identified in the considered sponges, according to the available literature.

The symbiotic community of the five sponges from the Gulf of Naples, mainly in *T. aurantium*, *A. damicornis*, *A. acuta*, *A. oroides* and *G. cydonium* was dominated by Proteobacteria, classes Alphaproteobacteria, Deltaproteobacteria and Gammaproteobateria (Figs. 3, 4; Figure S9).

Alphaproteobacteria were commonly found in the Mediterranean, mainly in the sponges *Sarcotragus fasciculatus*, *Ircinia oros* and *Ircinia strobilina*[48,49]. Overall, proteobacteria are known to produce N-acyl homoserine lactone (AHL) signal molecules involved in bacterial quorum sensing[50]. In fact, several species belonging to Alpha- and Gamma-proteobacteria, isolated from the Mediterranean sponges *Halichondria panicea*, *Ircinia fasciculata*, *Axinella polypoides*, and *Acanthella* sp.[51] and from the Red sea sponge *Suberea mollis*[52] showed antimicrobial activities, making them suitable tools for pharmacological purposes[53–57].

The phylum Actinobacteria (class Acidimicrobiia) was the most abundant in *S. spinosulus* and *E. discophorus* (Figs. 3, 4; Figure S9), also found in *T. aurantium* and *A. acuta*. Actinobacteria are Gram positive, mostly aerobic, mycelial and primarily soil organisms, but recent studies revealed that some Actinobacteria taxa were also well-adapted to marine environments. Moreover, these bacteria were attracting interest as key producers of therapeutics, for their great potential in extracellular enzyme production, as well as in the synthesis of a variety of bioactive metabolites with antimicrobial and antifungal activity[11,58,59]. In fact, Actinobacteria together with the already discussed Proteobacteria, showed antagonistic activity against bacterial belonging to the genera *Bacillus*, *Pseudovibrio*, *Ruegeria*, *Staphylococcus aureus*, *Escherichia coli* K12, and fungi *Fusarium* sp. P25, *Trypanosoma brucei* TC 221, *Leishmania major*, *Aspergillus fumigatus*, *Candida glabrata* and *C. albicans*[13,52,60–71]. Furthermore, this group of bacteria, also isolated from *Suberites domuncula* and *Dysidea* sp., showed antimicrobial, antifungal and cytotoxic activity against different cell lines as HeLa cells and pheochromocytoma (PC12) cells[53–56].

Dehalococcoides and Anaerolineae (a class of the phylum Chloroflexi) seem to be peculiar species of both collection sites, being detected in *S. spinolosus*, *E. discophorous*, and *A. oroides* (Figs. 3, 4; Figure S9). This was an interesting finding, because both bacterial groups were isolated for the first time from Mediterranean sponges. In fact, *Anaerolineae* were found most abundant in *Aaptos suberitoides* and *Xestospongia testudinaria* collected in South East Misool, Raja Ampat, West Papua (Indonesia)[72,73]. No data were reported so far for marine biotechnology applications. In contrast, the anaerobic Dehalococcoides showed surprising capabilities to transform various chlorinated organic compounds via reductive dechlorination. For this reason, Dehalococcoides were extensively used for the restoration of environments contaminated by chlorinated organics, which are normally released through industrial and agricultural activities[74,75]. ASVs analysis showed a peculiar abundance of the phylum Verrucomicrobia (class Verrucomicrobiae) in the sponge *O.* cf. *perforata* (Figs. 3, 4; Figure S9). Little information was reported on the abundance and ecology of aquatic Verrucomicrobia, being prevalent in lakes characterized by nutrient abundance and phosphorus availability[76,77]. These bacteria play an important role in global carbon cycling, processing decaying organic materials and degrading various polysaccharides[78–81]. It was found that the sponge-symbiotic Verrucomicrobiae bacteria (e.g. *Petrosia ficiformis*) exhibited enrichment of the toxin-antitoxin (TA) system suggesting the hypothesis that these bacteria use these systems as a defense mechanism against antimicrobial activity deriving from the abundant microbial community co-inhabiting their host[77]. *Rubritalea squalenifaciens* (strain HOact23T; MBIC08254T) is a rare marine bacterium belonging to the phylum Verrucomicrobia, isolated from *Halichondria okadai* (collected in Japan), from which a novel acyl glyco-carotenoic acids, diapolycopenedioic acid xylosyl esters A, B, and C, were isolated as red pigments with a potent antioxidative activity[82].

Furthermore, Nitrospirae (class Nitrospira) was the most abundant bacterial phylum in the three sponges from the Gulf of Naples, *A. damicornis*, *G. cydonium* and *A. acuta*, as well as, in *O.* cf. *perforata* and *E. discophous* from the Faro Lake (Figs. 3, 4; Figure S9). The first described *Nitrospira* species was *N. marina*, isolated by Watson et al.[83] from water collected in the Gulf of Maine. In particular, *Nitrospira* spp. play pivotal roles in nitrification as anaerobic chemolithoautotrophic nitrite-oxidizing bacterium[84]. These bacteria also have been found in several sponge species such as *Theonella swinhoei* and *Geodia barretti*[85,86]. Concerning their biotechnological potential, very little information is available so far. A recent work, using BLASTp search against the Integrated Microbial Genomes (IMG) database, identified a *Pseudoalteromonas luteoviolacea* gene encoding for a L-amino acid oxidase (LAAO) with antimicrobial properties in a strain belonging to the phylum *Nitrospinae*[87].

Summarizing, our data pointed out the attention on the species biodiversity of the Mediterranean Sea and on 16S rRNA sequence datasets, which allowed to the detection of several signature resident microbial fauna. In addition, data reported on the biotechnological potential of the bacteria identified in the eight sponges under analysis, suggest the need for further validations through bioassay-guided fractionation to identify novel metabolites useful for the pharmaceutical, cosmeceutical and nutraceutical fields.

Methods

Sponge collection. The size of sponge samples ranged from 10 to 20 cm in diameter. Three sponge samples, O.per, S.spi and E.dis were collected at Faro Lake (Messina, Sicily; depth = 2–3 m; 38°16'N, 15°38'E, Fig. 5A; temperature 20 °C, pH 8.25, salinity 31 PSU) in October 2019.

The site Faro Lake (0.263 Km^2) is the deepest coastal lake in Italy located within the Natural Reserve of "Capo Peloro" (NE Sicily). The Faro Lake is characterized by a funnel-shape profile, with a steep sloping bottom reaching the maximum depth of 29 m in the central area and a wide nearshore shallow waters area. In the deepest part, the Faro Lake shows typical features of a meromictic temperate basin, with an oxygenated mixolimnion (the upper 15 m) and a lower anoxic and sulphidic monimolimnion[88]. Two channels, a northern and a north-eastern, connect the lake to the Tyrrhenian Sea and the Strait of Messina. Salinity ranges are from 26 to 36 PSU, temperature from 10 to 30 °C and pH ranges from 7.0–8.6[26]. Four samples were collected in the Gulf of Naples in September 2019 by scuba diving of Stazione Zoologica Anton Dohrn of Naples (temperature 23.9 °C, pH 8.3, salinity 38 PSU): two samples, reported as T.aur and A.dam, were collected at Porto Paone (depth = 15–17 m; 40°47′N, 14°9′E, Fig. 5B); A.acu and A.oro were retrieved from Punta San Pancrazio (depth = 7–9 m; 40°42′N, 13°57′E, Fig. 5C); G.cyd (*Geodia cydonium*) was harvested at Secca delle Fumose, Parco Sommerso di Baia

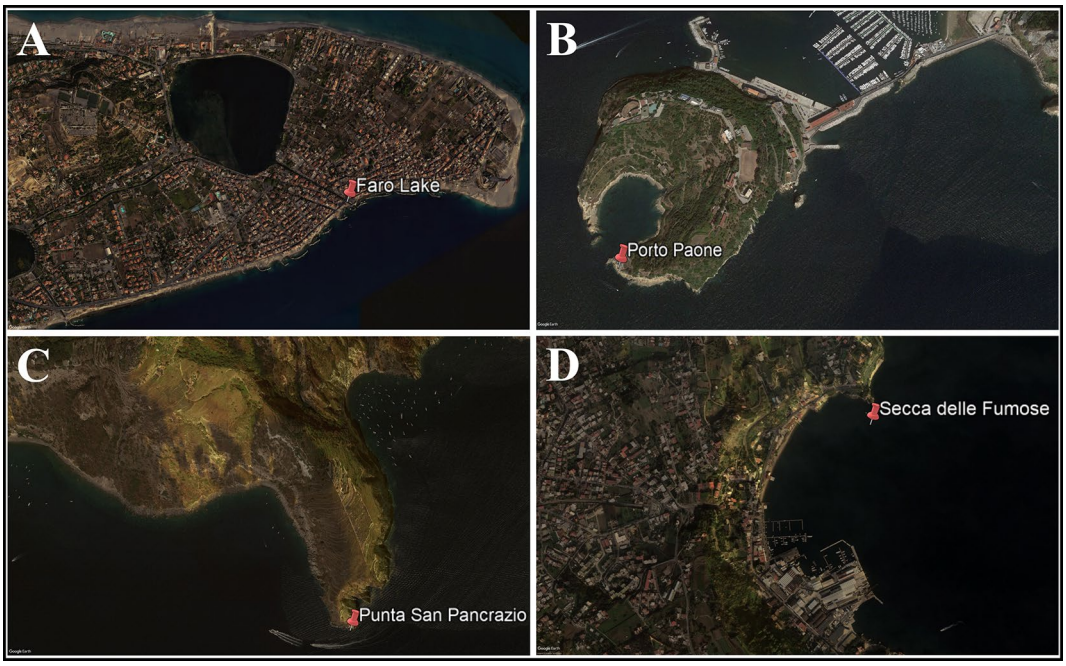

Figure 5. Sampling sites of sponge species collected in Faro Lake (Messina, **A**), Porto Paone (**B**), Punta San Pancrazio (**C**) and Secca delle fumose (**D**). Picture was created by Google Earth Software.

(depth = 20 m; 40°49′N, 14°5′E, Fig. 5D). All collecting sites were selected on the basis of some data reporting on the great biodiversity and, in some cases, the presence of alien species[26,89,90].

Collected samples were immediately washed at least three times with filter-sterilized natural seawater. A fragment of each specimen was preserved in 70% ethanol for taxonomic identification; another fragment was then placed into individual sterile tubes and kept in RNA*later*© at − 20 °C used for molecular analysis. Details on sampling were reported in Table 1.

Morphological analysis of the sponges. For the taxonomic analysis, the spicules of each sponge specimen, spicule complement and skeletal architecture, were examined under light microscopy following published protocols[91,92]. Taxonomic decisions were made according to the classification present in the World Porifera Database (WPD)[14]. The sponge samples were all identified at the species level.

DNA extraction and PCR amplification. About 10 mg of tissue was used for DNA extraction by *QIAamp® DNA Micro kit* (QIAGEN), according to the manufacturer's instructions. DNA quantity (ng/µL) was evaluated by a NanoDrop spectrophotometer. PCR reactions were performed on the C1000 Touch Thermal Cycler (BioRad) in a 30 µL reaction mixture final volume including about 50–100 ng of genomic DNA, 6 µL of 5X Buffer GL (GeneSpin Srl, Milan, Italy), 3 µL of dNTPs (2 mM each), 2 µL of each forward and reverse primer (25 pmol/µL), 0.2 µL of Xtra Taq Polymerase (5 U/µL, GeneSpin Srl, Milan, Italy) as follows (for primer sequences, see also Table S13):

i. for 18S and 28S, a denaturation step at 95 °C for 2 min, 35 cycles denaturation step at 95 °C for 1 min, annealing step at 60 °C (A/B[93,94]), 57 °C (C2/D2[95]), 55 °C (18S-AF/18S-BR, NL4F/NL4R[96,97]), 52 °C (18S1/18S2[98]) for 1 min and 72 °C of primer extension for 2 min, a final extension step at 72 °C for 10 min;
ii. ITS primers (RA2/ITS2.2[94,99]), a first denaturation at 95 °C for 2 min, 35 cycles denaturation step at 95 °C for 1 min, annealing step at 67 °C for 1 min and 72 °C of primer extension for 2 min, a final extension step at 72 °C for 10 min;
iii. CO1 primers (dgLCO1490/dgHCO2198[100]), a first denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s and primer extension at 72 °C for 1 min.

PCR products were separated on 1.5% agarose gel electrophoresis in TAE buffer (40 mM Tris–acetate, 1 mM EDTA, pH 8.0) using a 100 bp DNA ladder (GeneSpin Srl, Milan, Italy) and purified by *QIAquick Gel Extraction Kit* (Qiagen) according to the manufacturer's instructions. PCR amplicons were then sequenced in both strands through Applied Biosystems (Life Technologies) 3730 Analyzer (48 capillaries). Sequences produced were ~ 650 bases long in average with more than 97.5% accuracy, starting from PCR fragments. Each 18S, 28S and CO1 PCR products were aligned to GenBank using Basic Local Alignment Search Tool (BLAST) and then aligned with highly similar sequence using MultiAlin (http://multalin.toulouse.inra.fr/multalin/, see Figures S1-S7).

Metagenomic DNA extraction, Illumina MiSeq sequencing and diversity analysis. About 250 mg of tissue were weighted and used for DNA extraction by using *DNeasy® PowerSoil® Pro Kit* (QIAGEN), according to the manufacturer's instructions. DNA quantity (ng/μL) and quality (A260/280, A260/230) were evaluated by a NanoDrop spectrophotometer. DNA samples were separated by 0.8% agarose gel electrophoresis in TAE buffer (40 mM Tris–acetate, 1 mM EDTA, pH 8.0) to check DNA integrity. 30 ng/μL (final concentration) of sample was used for metataxonomic analysis performed by Bio-Fab Research (Roma, Italy). Illumina adapter overhang nucleotide sequences were added to the gene-specific primer pairs targeting the V3-V4 region (S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-2), with the following sequences:

Forward = 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3',

Reverse = 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'[101].

For 16S PCR amplification, 2.5 μL of microbial genomic DNA (5 ng/μL in 10 mM Tris pH 8.5), 5 μL of each Forward and Reverse primer and 12.5 μL of 2 × KAPA HiFi HotStart ReadyMix to a final volume of 25 μL were used. Thermocycler conditions were set as follows: initial denaturation at 95 °C for 3 min, 25 cycles of 95 °C (30 s), 55 °C (30 s), 72 °C (30 s), final extension at 72 °C for 5 min, hold at 4 °C.

After 16S amplification, a PCR clean-up was done to purify the V3-V4 amplicon from free primers and primer dimer species. This step was followed by another limited-cycle amplification step to add multiplexing indices and Illumina sequencing adapters by using a Nextera XT Index Kit. A second step of clean-up was further performed and then libraries were normalized and pooled by denoising processes (Table S14), and sequenced on Illumina MiSeq Platform with 2 × 300 bp paired-end reads. Taxonomy was assigned using "home made" Naive Bayesian Classifier trained on V3-V4 16S sequences of SILVA 132 database[102]. Frequencies per feature and per sample are shown in Figures S10-S11.

QIIME 2 (Quantitative Insights Into Microbial Ecology) platform[103] was used for microbiome analysis from raw DNA sequencing data. QIIME 2 analysis workflow was performed by demultiplexing, quality filtering, chimera removal, taxonomic assignment, and diversity analyses (alpha and beta).

Taxonomy BarPlot (Figure S9) was generated through a R version 4.1.1[104] using Cairo graphics library[105].

Species diversity was estimated by i. Chao 1 index[106], which is qualitative species-based method; ii. Shannon[107,108] and iii. Simpson[109] indices, which are quantitative species-based measures. All these indices were estimated at three taxa levels (Level 5 = Family, Level 6 = Genus, Level 7 = Species). For alpha and beta diversity, significant differences were assessed by Kruskal–Wallis test and pairwise PERMANOVA analysis, respectively. Moreover, Bray–Curtis and "un-, weighted" UniFrac metrics were used to calculate a distance matrix between each pair of samples, independently from the environmental variables.

Data availability

The full dataset of raw data was deposited in the SRA database (Submission ID: SUB8692761; BioProject ID: PRJNA683751).

Received: 17 April 2021; Accepted: 20 September 2021
Published online: 27 October 2021

References

1. Joseph, B. & Sujatha, S. Pharmacologically important natural products from marine sponges. *J. Nat. Prod.* **4**, 5–12 (2011).
2. Bergmann, W. & Feeney, R. J. Contributions to the study of marine products XXXII The nucleosides of sponges. *I. J. Org. Chem.* **16**, 981–987 (1951).
3. Munro, M. H. G., Luibrand, R. T. & Blunt, J. W. The search for antiviral and anticancer compounds from marine organisms. in *Bioorganic Marine Chemistry* (ed. Scheuer, P. J.) vol. 1 93–176 (Springer-Verlag, Berlin, Heidelberg, 1987).
4. Fuerst, J. A. Diversity and biotechnological potential of microorganisms associated with marine sponges. *Appl. Microbiol. Biotechnol.* **98**, 7331–7347 (2014).
5. Mehbub, M. F., Lei, J., Franco, C. & Zhang, W. Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Mar. Drugs* **12**, 4539–4577 (2014).
6. Piel, J. Metabolites from symbiotic bacteria. *Nat. Prod. Rep.* **26**, 338–362 (2009).
7. Piel, J. *et al.* Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 16222–16227 (2004).
8. Noro, J. C., Kalaitzis, J. A. & Neilan, B. A. Bioactive natural products from Papua New Guinea marine sponges. *Chem. Biodivers.* **9**, 2077–2095 (2012).
9. Schirmer, A. *et al.* Metagenomic analysis reveals diverse polyketide synthase gene clusters in microorganisms associated with the marine sponge *Discodermia dissoluta*. *Appl. Environ. Microbiol.* **71**, 4840–4849 (2005).
10. Siegl, A. & Hentschel, U. PKS and NRPS gene clusters from microbial symbiont cells of marine sponges by whole genome amplification. *Environ. Microbiol. Rep.* **2**, 507–513 (2010).
11. Graça, A. P. *et al.* Antimicrobial activity of heterotrophic bacterial communities from the marine sponge *Erylus discophorus* (Astrophorida, Geodiidae). *PLoS ONE* **8**, e78992 (2013).
12. Santos, O. C. S. *et al.* Investigation of biotechnological potential of sponge-associated bacteria collected in Brazilian coast. *Lett. Appl. Microbiol.* **60**, 140–147 (2015).
13. Su, P., Wang, D. X., Ding, S. X. & Zhao, J. Isolation and diversity of natural product biosynthetic genes of cultivable bacteria associated with marine sponge Mycale sp from the coast of Fujian. *China. Can. J. Microbiol.* **60**, 217–225 (2014).
14. Van Soest, R. W. M. *et al.* World Porifera Database. http://www.marinespecies.org/porifera/. (2020).
15. Bertolino, M. *et al.* Stability of the sponge assemblage of Mediterranean coralligenous concretions along a millennial time span. *Mar. Ecol.* **35**, 149–158 (2014).
16. Longo, C. *et al.* Sponges associated with coralligenous formations along the Apulian coasts. *Mar. Biodivers.* **48**, 2151–2163 (2018).
17. Costa, G. *et al.* Sponge community variation along the Apulian coasts (Otranto Strait) over a pluri-decennial time span Does water warming drive a sponge diversity increasing in the Mediterranean Sea?. *J. Mar. Biol. Assoc. United Kingdom* **99**, 1519–1534 (2019).
18. Bertolino, M. *et al.* Changes and stability of a Mediterranean hard bottom benthic community over 25 years. *J. Mar. Biol. Assoc. United Kingdom* **96**, 341–350 (2016).

19. Bertolino, M. *et al.* Have climate changes driven the diversity of a Mediterranean coralligenous sponge assemblage on a millennial timescale?. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **487**, 355–363 (2017).
20. Gerovasileiou, V. *et al.* New Mediterranean biodiversity records. *Mediterr. Mar. Sci.* **18**, 355–384 (2017).
21. Ulman, A. *et al.* A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ* **2017**, e3954 (2017).
22. Costantini, M. An analysis of sponge genomes. *Gene* **342**, 321–325 (2004).
23. Costantini, S. *et al.* Anti-inflammatory effects of a methanol extract from the marine sponge *Geodia cydonium* on the human breast cancer MCF-7 cell line. *Mediators Inflamm.* https://doi.org/10.1155/2015/204975 (2015).
24. Costantini, S. *et al.* Evaluating the effects of an organic extract from the mediterranean sponge *Geodia cydonium* on human breast cancer cell lines. *Int. J. Mol. Sci.* **18**, 2112 (2017).
25. Coll, M. *et al.* The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS ONE* **5**, e11842 (2010).
26. Marra, M. V. *et al.* Long-term turnover of the sponge fauna in Faro Lake (North-East Sicily, Mediterranean Sea). *Ital. J. Zool.* **83**, 579–588 (2016).
27. Cárdenas, P., Xavier, J. R., Reveillaud, J., Schander, C. & Rapp, H. T. Molecular phylogeny of the astrophorida (Porifera, Demospongiaep) reveals an unexpected high level of spicule homoplasy. *PLoS ONE* **6**, e18318 (2011).
28. Erpenbeck, D. *et al.* The phylogeny of halichondrid demosponges: past and present re-visited with DNA-barcoding data. *Org. Divers. Evol.* **12**, 57–70 (2012).
29. Abdul Wahab, M. A., Fromont, J., Whalan, S., Webster, N. & Andreakis, N. Combining morphometrics with molecular taxonomy: How different are similar foliose keratose sponges from the Australian tropics?. *Mol. Phylogenet. Evol.* **73**, 23–39 (2014).
30. Wörheide, G. Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge *Astrosclera willeyana* across the Indo-Pacific. *Mar. Biol.* **148**, 907–912 (2006).
31. Carella, M., Agell, G., Cárdenas, P. & Uriz, M. J. Phylogenetic reassessment of antarctic tetillidae (Demospongiae, Tetractinellida) reveals new genera and genetic similarity among morphologically distinct species. *PLoS ONE* **11**, 1–33 (2016).
32. Morrow, C. C. *et al.* Congruence between nuclear and mitochondrial genes in Demospongiae: a new hypothesis for relationships within the G4 clade (Porifera: Demospongiae). *Mol. Phylogenet. Evol.* **62**, 174–190 (2012).
33. Vargas, S. *et al.* Diversity in a cold hot-spot: DNA-barcoding reveals patterns of evolution among Antarctic demosponges (class demospongiae, phylum Porifera). *PLoS ONE* **10**, 1–17 (2015).
34. Yang, Q., Franco, C. M. M., Sorokin, S. J. & Zhang, W. Development of a multilocus-based approach for sponge (phylum Porifera) identification: refinement and limitations. *Sci. Rep.* **7**, 1–14 (2017).
35. Cosentino, A., Giacobbe, S. & Potoschi, A. The CSI of Faro coastal lake (Messina): a natural observatory for the incoming of marine alien species. *Biol. Mar. Mediterr.* **16**, 132–133 (2009).
36. Zagami, G., Costanzo, G. & Crescenti, N. First record in Mediterranean Sea and redescription of the bentho-planktonic calanoid copepod species *Pseudocyclops xiphophorus* Wells, 1967. *J. Mar. Syst.* **55**, 67–76 (2005).
37. Zagami, G. *et al.* Biogeographical distribution and ecology of the planktonic copepod Oithona davisae: rapid invasion in lakes Faro and Ganzirri (central Meditteranean Sea). in *Trends in copepod studies. Distribution, biology and ecology* (ed. Uttieri, M.) 1–55 (Nova Science Publishers, 2017).
38. Saccà, A. & Giuffrè, G. Biogeography and ecology of *Rhizodomus tagatzi*, a presumptive invasive tintinnid ciliate. *J. Plankton Res.* **35**, 894–906 (2013).
39. Cao, S. *et al.* Structure and function of the Arctic and Antarctic marine microbiota as revealed by metagenomics. *Microbiome* **8**, 47 (2020).
40. Donnarumma, L. *et al.* Environmental and benthic community patterns of the shallow hydrothermal area of Secca Delle Fumose (Baia, Naples, Italy). *Front. Mar. Sci.* **6**, 1–15 (2019).
41. Poli, A., Anzelmo, G. & Nicolaus, B. Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. *Mar. Drugs* **8**, 1779–1802 (2010).
42. Shukla, P. J., Nathani, N. M. & Dave, B. P. Marine bacterial exopolysaccharides [EPSs] from extreme environments and their biotechnological applications. *Int. J. Res. Biosci.* **6**, 20–32 (2017).
43. Patel, A., Matsakas, L., Rova, U. & Christakopoulos, P. A perspective on biotechnological applications of thermophilic microalgae and cyanobacteria. *Bioresour. Technol.* **278**, 424–434 (2019).
44. Schultz, J. & Rosado, A. S. Extreme environments: a source of biosurfactants for biotechnological applications. *Extremophiles* **24**, 189–206 (2020).
45. Gloeckner, V. *et al.* The HMA-LMA dichotomy revisited: An electron microscopical survey of 56 sponge species. *Biol. Bull.* **227**, 78–88 (2014).
46. Erwin, P. M., Coma, R., López-Sendino, P., Serrano, E. & Ribes, M. Stable symbionts across the HMA-LMA dichotomy: Low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts. *FEMS Microbiol. Ecol.* **91**, 1–11 (2015).
47. Moitinho-Silva, L. *et al.* The sponge microbiome project. *Gigascience* **6**, 1–13 (2017).
48. Hardoim, C. C. P. & Costa, R. Temporal dynamics of prokaryotic communities in the marine sponge *Sarcotragus spinosulus*. *Mol. Ecol.* **23**, 3097–3112 (2014).
49. Karimi, E. *et al.* Metagenomic binning reveals versatile nutrient cycling and distinct adaptive features in alphaproteobacterial symbionts of marine sponges. *FEMS Microbiol. Ecol.* **94**, 1–18 (2018).
50. Mohamed, N. M. *et al.* Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges. *Environ. Microbiol.* **10**, 75–86 (2008).
51. Thiel, V. & Imhoff, J. F. Phylogenetic identification of bacteria with antimicrobial activities isolated from Mediterranean sponges. *Biomol. Eng.* **20**, 421–423 (2003).
52. Bibi, F., Yasir, M., Al-Sofyani, A., Naseer, M. I. & Azhar, E. I. Antimicrobial activity of bacteria from marine sponge Suberea mollis and bioactive metabolites of Vibrio sp EA348. *Saudi J. Biol. Sci.* **27**, 1139–1147 (2020).
53. Thakur, A. N. *et al.* Antiangiogenic, antimicrobial, and cytotoxic potential of sponge-associated bacteria. *Mar. Biotechnol.* **7**, 245–252 (2005).
54. Taylor, M. W., Radax, R., Steger, D. & Wagner, M. Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. *Microbiol. Mol. Biol. Rev.* **71**, 295–347 (2007).
55. Thomas, T. R. A., Kavlekar, D. P. & LokaBharathi, P. A. Marine drugs from sponge-microbe association—A review. *Mar. Drugs* **8**, 1417–1468 (2010).
56. Brinkmann, C. M., Marker, A. & Kurtböke, D. I. An overview on marine sponge-symbiotic bacteria as unexhausted sources for natural product discovery. *Diversity* **9**, 40 (2017).
57. Haber, M. & Ilan, M. Diversity and antibacterial activity of bacteria cultured from Mediterranean Axinella spp sponges. *J. Appl. Microbiol.* **116**, 519–532 (2014).
58. Öner, Ö. *et al.* Cultivable sponge-associated Actinobacteria from coastal area of eastern Mediterranean Sea. *Adv. Microbiol.* **04**, 306–316 (2014).
59. Gonçalves, A. C. S. *et al.* Draft genome sequence of Vibrio sp strain Vb278, an antagonistic bacterium isolated from the marine sponge Sarcotragus spinosulus. *Genome Announc.* **3**, 2014–2015 (2015).
60. Cheng, C. *et al.* Biodiversity, anti-trypanosomal activity screening, and metabolomic profiling of actinomycetes isolated from Mediterranean sponges. *PLoS ONE* **10**, 1–21 (2015).

61. Graça, A. P. *et al.* The antimicrobial activity of heterotrophic bacteria isolated from the marine sponge *Erylus deficiens* (Astrophorida, Geodiidae). *Front. Microbiol.* **6**, 389 (2015).
62. Kuo, J. *et al.* Antimicrobial activity and diversity of bacteria associated with Taiwanese marine sponge *Theonella swinhoei*. *Ann. Microbiol.* **69**, 253–265 (2019).
63. Liu, T. *et al.* Diversity and antimicrobial potential of *Actinobacteria* isolated from diverse marine sponges along the Beibu Gulf of the South China Sea. *FEMS Microbiol. Ecol.* **95**, 1–10 (2019).
64. Hentschel, U. *et al.* Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. *FEMS Microbiol. Ecol.* **35**, 305–312 (2001).
65. Chelossi, E., Milanese, M., Milano, A., Pronzato, R. & Riccardi, G. Characterisation and antimicrobial activity of epibiotic bacteria from *Petrosia ficiformis* (Porifera, Demospongiae). *J. Exp. Mar. Bio. Ecol.* **309**, 21–33 (2004).
66. Kennedy, J. *et al.* Isolation and analysis of bacteria with antimicrobial activities from the marine sponge *Haliclona simulans* collected from irish waters. *Mar. Biotechnol.* **11**, 384–396 (2009).
67. Penesyan, A., Marshall-Jones, Z., Holmstrom, C., Kjelleberg, S. & Egan, S. Antimicrobial activity observed among cultured marine epiphytic bacteria reflects their potential as a source of new drugs. *FEMS Microbiol. Ecol.* **69**, 113–124 (2009).
68. Santos, O. C. S. *et al.* Isolation, characterization and phylogeny of sponge-associated bacteria with antimicrobial activities from Brazil. *Res. Microbiol.* **161**, 604–612 (2010).
69. Flemer, B. *et al.* Diversity and antimicrobial activities of microbes from two Irish marine sponges, *Suberites carnosus* and *Leucosolenia* sp. *J. Appl. Microbiol.* **112**, 289–301 (2012).
70. Margassery, L. M., Kennedy, J., O'Gara, F., Dobson, A. D. & Morrissey, J. P. Diversity and antibacterial activity of bacteria isolated from the coastal marine sponges *Amphilectus fucorum* and *Eurypon major*. *Lett. Appl. Microbiol.* **55**, 2–8 (2012).
71. Abdelmohsen, U. R. *et al.* Actinomycetes from Red Sea sponges: sources for chemical and phylogenetic diversity. *Mar. Drugs* **12**, 2771–2789 (2014).
72. Montalvo, N. F. & Hill, R. T. Sponge-associated bacteria are strictly maintained in two closely related but geographically distant sponge hosts. *Appl. Environ. Microbiol.* **77**, 7207–7216 (2011).
73. Cleary, D. F. R. *et al.* Compositional analysis of bacterial communities in seawater, sediment, and sponges in the Misool coral reef system. *Indonesia. Mar. Biodivers.* **48**, 1889–1901 (2018).
74. Bedard, D. L., Ritalahti, K. M. & Löffler, F. E. The *Dehalococcoides* population in sediment-free mixed cultures metabolically dechlorinates the commercial polychlorinated biphenyl mixture aroclor 1260. *Appl. Environ. Microbiol.* **73**, 2513–2521 (2007).
75. Taş, N., Van Eekert, M. H. A., De Vos, W. M. & Smidt, H. The little bacteria that can - Diversity, genomics and ecophysiology of 'Dehalococcoides' spp in contaminated environments. *Microb. Biotechnol.* **3**, 389–402 (2010).
76. Arnds, J., Knittel, K., Buck, U., Winkel, M. & Amann, R. Development of a 16S rRNA-targeted probe set for *Verrucomicrobia* and its application for fluorescence *in situ* hybridization in a humic lake. *Syst. Appl. Microbiol.* **33**, 139–148 (2010).
77. Sizikov, S. *et al.* Characterization of sponge-associated *Verrucomicrobia*: microcompartment-based sugar utilization and enhanced toxin–antitoxin modules as features of host-associated *Opitutales*. *Environ. Microbiol.* **22**, 4669–4688 (2020).
78. Cardman, Z. *et al. Verrucomicrobia* are candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. *Appl. Environ. Microbiol.* **80**, 3749–3756 (2014).
79. Cabello-Yeves, P. J. *et al.* Reconstruction of diverse verrucomicrobial genomes from metagenome datasets of freshwater reservoirs. *Front. Microbiol.* **8**, 2131 (2017).
80. He, S. *et al.* Ecophysiology of freshwater *Verrucomicrobia* inferred from metagenome-assembled genomes. *MSphere* **2**, e00277 (2017).
81. Sichert, A. *et al. Verrucomicrobia* use hundreds of enzymes to digest the algal polysaccharide fucoidan. *Nat. Microbiol.* **5**, 1026–1039 (2020).
82. Shindo, K. *et al.* Diapolycopenedioic acid xylosyl esters A, B, and C, novel antioxidative glyco-C_{30}-carotenoic acids produced by a new marine bacterium Rubritalea Squalenifaciens. *J. Antibiot. (Tokyo)* **61**, 185–191 (2008).
83. Watson, S. W., Bock, E., Valois, F. W., Waterbury, J. B. & Schlosser, U. Nitrospira marina gen. nov sp nov: a chemolithotrophic nitrite-oxidizing bacterium. *Arch. Microbiol.* **144**, 1–7 (1986).
84. Daims, H. & Wagner, M. Nitrospira. *Trends Microbiol.* **26**, 462–463 (2018).
85. Off, S., Alawi, M. & Spieck, E. Enrichment and physiological characterization of a novel nitrospira-like bacterium obtained from a marine sponge. *Appl. Environ. Microbiol.* **76**, 4640–4646 (2010).
86. Feng, G., Sun, W., Zhang, F., Karthik, L. & Li, Z. Inhabitancy of active *Nitrosopumilus*-like ammonia-oxidizing archaea and Nitrospira nitrite-oxidizing bacteria in the sponge *Theonella swinhoei*. *Sci. Rep.* **6**, 1–11 (2016).
87. Andreo-Vidal, A., Sanchez-Amat, A. & Campillo-Brocal, J. C. The *Pseudoalteromonas luteoviolacea* L-amino acid oxidase with antimicrobial activity is a flavoenzyme. *Mar. Drugs* **16**, 499 (2018).
88. Saccà, A., Guglielmo, L. & Bruni, V. Vertical and temporal microbial community patterns in a meromictic coastal lake influenced by the Straits of Messina upwelling system. *Hydrobiologia* **600**, 89–104 (2008).
89. Polese, G. *et al.* Meiofaunal assemblages of the bay of Nisida and the environmental status of the Phlegraean area (Naples, Southern Italy). *Mar. Biodivers.* **48**, 127–137 (2018).
90. Gambi, M. C., Tiberti, L. & Mannino, A. M. An update of marine alien species off the Ischia Island (Tyrrhenian Sea) with a closer look at neglected invasions of *Lophocladia lallemandii* (Rhodophyta). *Not. Sibm* **75**, 58–65 (2019).
91. Hooper, J. N. A. 'Sponguide'. Guide to sponge collection and identification. https://www.academia.edu/34258606/SPONGE_GUIDE_GUIDE_TO_SPONGE_COLLECTION_AND_IDENTIFICATION_Version_August_2000. (2000).
92. Rützler, K. Sponges in coral reefs. in *Coral reefs: Research methods, monographs on oceanographic methodology* (eds. Stoddart, D. R. & Johannes, R. E.) 299–313 (Paris: Unesco, 1978).
93. Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**, 491–499 (1988).
94. Schmitt, S., Hentschel, U., Zea, S., Dandekar, T. & Wolf, M. ITS-2 and 18S rRNA gene phylogeny of Aplysinidae (Verongida, Demospongiae). *J. Mol. Evol.* **60**, 327–336 (2005).
95. Chombard, C., Boury-Esnault, N. & Tillier, S. Reassessment of homology of morphological characters in Tetractinellid sponges based on molecular data. *Syst. Biol.* **47**, 351–366 (1998).
96. Collins, A. G. Phylogeny of medusozoa and the evolution of cnidarian life cycles. *J. Evol. Biol.* **15**, 418–432 (2002).
97. Dohrmann, M., Janussen, D., Reitner, J., Collins, A. G. & Wörheide, G. Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). *Syst. Biol.* **57**, 388–405 (2008).
98. Manuel, M. *et al.* Phylogeny and evolution of calcareous sponges: monophyly of calcinea and calcaronea, high level of morphological homoplasy, and the primitive nature of axial symmetry. *Syst. Biol.* **52**, 311–333 (2003).
99. Wörheide, G., Degnan, B., Hooper, J. & Reitner, J. Phylogeography and taxonomy of the Indo-Pacific reef cave dwelling coralline demosponge *Astrosclera willeyana*: new data from nuclear internal transcribed spacer sequences. *Proc. 9th Int. Coral Reef Symp.* **1**, 339–346 (2002).
100. Meyer, C. P., Geller, J. B. & Paulay, G. Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. *Evolution* **59**, 113–125 (2005).
101. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**, e1 (2013).

102. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **41**, 590–596 (2013).
103. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).
104. R Core Team. A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. (2020).
105. Urbanek, S. & Horner, J. Cairo: R Graphics device using Cairo graphics library for creating high-quality bitmap (PNG, JPEG, TIFF), vector (PDF, SVG, PostScript) and display (X11 and Win32) output. R package version 1.5–12.2. https://cran.r-project.org/package=Cairo (2020).
106. Chao, B. F. Interannual length-of-the-day variation with relation to the southern oscillation/El Nino. *Geophys. Res. Lett.* **11**, 541–544 (1984).
107. Shannon, C. E. A mathematical theory of communication. *Bell Syst. Tech. J.* **27**(379–423), 623–656 (1948).
108. Shannon, C. E. & Weaver, W. *The Mathematical Theory of Communication* (University of Illinois Press, 1949).
109. Simpson, E. H. Measurment of diversity. *Nature* **163**, 688 (1949).

Acknowledgements

We thank the "Parco Sommerso di Baia" in Naples for providing *Geodia cydonium* and the Fishing Service of Stazione Zoologica Anton Dohrn for its collection. We also thank Davide Caramiello from the Marine Organisms Core Facility of the Stazione Zoologica for his technical support in sponge maintenance.

Author contributions

N.R., R.E.: molecular identification of sponges, data curation; formal analysis. G.Z., S.D.M.: collection of sponge samples and their identification. M.B.: morphological analysis of sponges. M.S., F.A.: Metataxonomic analysis. S.C., V.Z.: writing—reviewing and editing. M.C.: Conceptualization, Funding acquisition, Supervision, Writing—original draft.

Funding

Nadia Ruocco was supported by a research grant "Antitumor Drugs and Vaccines from the Sea (ADViSE)" project (PG/2018/0494374). Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-00713-9.

Correspondence and requests for materials should be addressed to M.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

marine drugs

MDPI

Article

A Metataxonomic Approach Reveals Diversified Bacterial Communities in Antarctic Sponges

Nadia Ruocco [1,†], Roberta Esposito [1,2,†], Marco Bertolino [3], Gianluca Zazo [4], Michele Sonnessa [5], Federico Andreani [5], Daniela Coppola [1,6], Daniela Giordano [1,6], Genoveffa Nuzzo [7], Chiara Lauritano [1], Angelo Fontana [7], Adrianna Ianora [1], Cinzia Verde [1,6] and Maria Costantini [1,6,*]

1 Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; nadia.ruocco@szn.it (N.R.); roberta.esposito@szn.it (R.E.); daniela.coppola@ibbr.cnr.it (D.C.); daniela.giordano@ibbr.cnr.it (D.G.); chiara.lauritano@szn.it (C.L.); ianora@szn.it (A.I.); cinzia.verde@ibbr.cnr.it (C.V.)
2 Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Napoli, Italy
3 Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV), Università degli Studi di Genova, Corso Europa 26, 16132 Genova, Italy; marco.bertolino@unige.it
4 Department of Research Infrastructure for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; gianluca.zazo@szn.it
5 Bio-Fab Research srl, Via Mario Beltrami, 5, 00135 Roma, Italy; laboratori@biofabresearch.it (M.S.); michelesonnessa@biofabresearch.it (F.A.)
6 Institute of Biosciences and BioResources (IBBR), National Research Council (CNR), Via Pietro Castellino 111, 80131 Napoli, Italy
7 Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Via Campi Flegrei 34, 80078 Pozzuoli (Napoli), Italy; nuzzo.genoveffa@icb.cnr.it (G.N.); afontana@icb.cnr.it (A.F.)
* Correspondence: maria.costantini@szn.it
† These authors contributed equally to this work.

Abstract: Marine sponges commonly host a repertoire of bacterial-associated organisms, which significantly contribute to their health and survival by producing several anti-predatory molecules. Many of these compounds are produced by sponge-associated bacteria and represent an incredible source of novel bioactive metabolites with biotechnological relevance. Although most investigations are focused on tropical and temperate species, to date, few studies have described the composition of microbiota hosted by Antarctic sponges and the secondary metabolites that they produce. The investigation was conducted on four sponges collected from two different sites in the framework of the XXXIV Italian National Antarctic Research Program (PNRA) in November–December 2018. Collected species were characterized as *Mycale* (*Oxymycale*) *acerata*, *Haliclona* (*Rhizoniera*) *dancoi*, *Hemigellius pilosus* and *Microxina sarai* by morphological analysis of spicules and amplification of four molecular markers. Metataxonomic analysis of these four Antarctic sponges revealed a considerable abundance of Amplicon Sequence Variants (ASVs) belonging to the phyla Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia. In particular, *M.* (*Oxymycale*) *acerata*, displayed several genera of great interest, such as *Endozoicomonas*, *Rubritalea*, *Ulvibacter*, *Fulvivirga* and *Colwellia*. On the other hand, the sponges *H. pilosus* and *H.* (*Rhizoniera*) *dancoi* hosted bacteria belonging to the genera *Pseudhongella*, *Roseobacter* and *Bdellovibrio*, whereas *M. sarai* was the sole species showing some strains affiliated to the genus *Polaribacter*. Considering that most of the bacteria identified in the present study are known to produce valuable secondary metabolites, the four Antarctic sponges could be proposed as potential tools for the discovery of novel pharmacologically active compounds.

Keywords: Antarctica; Demospongiae; marine biotechnology; metataxonomics; microbiota

Citation: Ruocco, N.; Esposito, R.; Bertolino, M.; Zazo, G.; Sonnessa, M.; Andreani, F.; Coppola, D.; Giordano, D.; Nuzzo, G.; Lauritano, C.; et al. A Metataxonomic Approach Reveals Diversified Bacterial Communities in Antarctic Sponges. *Mar. Drugs* **2021**, *19*, 173. https://doi.org/10.3390/md19030173

Academic Editor: Bill J. Baker

Received: 29 January 2021
Accepted: 20 March 2021
Published: 22 March 2021

1. Introduction

The Antarctic region comprises ice shelves, waters and all the island territories in the Southern Ocean, covering about 10% of the total world ocean's area. The Antarctic

is characterized by low temperature and scarce availability of nutrients, together with a high seasonality in terms of light conditions. Due to the extreme environmental conditions, Antarctic fauna has developed several physiological and behavioural adaptations, leading to the evolution of unique characteristics [1]. For instance, a longer period of larval development or parental care has been observed in Antarctic invertebrates, including sponges [2–4]. Moreover, marine invertebrate communities living in this area have been subjected to a wide temporal and biogeographic isolation [5,6] dating back to about 140 million years ago when the Antarctic continent separated from Gondwana [7,8]. This event has promoted the development of specific traits, which make Antarctic organisms extremely diverse from those living in other southern hemisphere seas [9–11].

Sponges are sessile and filter-feeder organisms, belonging to the phylum Porifera, which represent, in terms of abundance and biomass, the major component of the Antarctic zoobenthos [12], with a total number of 400 known species [13]. Through their aquiferous system, they are able to capture several microorganisms (including bacteria, yeasts, microalgae) from surrounding water and harbour a huge microbial community within their body [14–16]. Sponges normally establish a strong interaction with their bacterial hosts due to several benefits that improve their fitness and survival, including nutritional supply, transport of waste products, and molecules that confer chemical and mechanical defence [17–19].

Although a good knowledge is available on sponge fauna, the Antarctic region covers an extraordinarily wide area that makes some zones almost unknown to the scientific community [20,21]. Until now, a few studies have investigated the composition of microbial communities living within Antarctic sponges [22–30]. Some of these studies have demonstrated that Antarctic sponges are mostly dominated by Proteobacteria and Bacteroidetes [23,26,29,31]. Interestingly, species composition has been found to be strictly specific, probably regulated by several bioactive molecules and quorum sensing [14,31–35]. Since several studies have revealed that symbiotic bacteria are able to produce bioactive metabolites (reviewed by Brinkmann et al. [36]), studying the species composition of microbiomes could shed light on the possible biotechnological applications of sponges. This scientific question becomes much more attractive when addressed to Antarctic species, which are still largely unknown, and might reserve a great potential considering that they have undergone incredible adaptations. Interactions between sponges and microorganisms may occur in many forms, representing these microorganisms' food sources, pathogens/parasites, or mutualistic symbionts [37–40]. Microbial associates can represent up to 40% of sponge tissue volume. Furthermore, the diversity in types of interactions may be matched by the phylogenetic diversity of microbes that occur within host sponges.

In the present work, we aimed to enlarge the yet scant knowledge on the bacterial communities inhabiting Antarctic sponges. In particular, we collected four sponges from two different sites of Tethys Bay (Victoria Land, Antarctica) in the framework of the XXXIV Italian National Antarctic Research Program (PNRA) expedition. Victoria Land (Tethys Bay) belongs to the Antarctic Specially Protected Area n. 161 [41] (ASPA 161; https://www.ats.aq/devAS/Meetings/Measure/688 accessed on 29 January 2021). Sponge species were characterized by morphological and molecular analysis. Metagenomic DNA extraction and Illumina MiSeq analysis were applied in order to identify the associated communities living within the analysed sponges. More than five hundred bacterial isolates were phylogenetically identified to establish whether the associated bacterial communities were host-specific. By relying on Amplicon Sequence Variants (ASVs) data, the biotechnological potential of sponge specimens was also considered.

2. Results

2.1. Species Identification

2.1.1. Morphological Analysis

The four sponge specimens belonged to the class Demospongiae and the following two orders: Haplosclerida with three species, *Hemigellius pilosus* (Kirkpatrick, 1907), *Microxina*

sarai (Calcinai and Pansini, 2000), and *Haliclona* (*Rhizoniera*) *dancoi* (Topsent, 1901), and Poecilosclerida with one species, *Mycale* (*Oxymycale*) *acerata* (Kirkpatrick, 1907) (Table 1).

Table 1. Sites, sample IDs, species identification, MNA code, geographic coordinates, sampling method in meters (m) and depth.

Site	Sample ID	Sponge Taxonomy	MNA code	Sampling Method	Sampling Depth (m)	Coordinates
1	B4	*Mycale* (*Oxymycale*) *acerata* (Kirkpatrick, 1907)	13264	SCUBA	26	74°42.067′S 164°02.518′E
2	C6	*Haliclona* (*Rhizoniera*) *dancoi* (Topsent, 1901)	13265	SCUBA	28	74°40.537′S 164°04.169′E
2	D4	*Hemigellius pilosus* (Kirkpatrick, 1907)	13266	SCUBA	28	74°40.537′S 164°04.169′E
2	D6	*Microxina sarai* (Calcinai & Pansini, 2000)	13267	SCUBA	28	74°40.537′S 164°04.169′E

2.1.2. Molecular Analysis

BLAST similarity search totally agreed with the morphological identification obtained for B4 and D4 samples. Molecular analysis confirmed B4 species as *M.* (*Oxymycale*) *acerata*, with CO1 primers that were the most specific (98% of pairwise identity) in comparison to 18S, 28S and ITS molecular markers. Similarly, CO1 also appeared to be the best molecular marker for the identification of the sponge D4, with a highest sequence similarity to *H. pilosus* (98% sequence identity). Regarding sample C6, molecular markers identified the genus corresponding to *Haliclona*, with the most striking result achieved using the 18S marker (92% similarity to *Haliclona sp.*). Unfortunately, it was not possible to identify this sponge at the species level, because there are no other available sequences on GenBank for *H.* (*Rhizoniera*) *dancoi*. Similarly, the results achieved with sample D6 were partially unclear, since several genera at low-sequence similarity were observed from BLAST outputs. In fact, the sequences of *M. sarai*, identified by spicule observations, are still not uploaded in GenBank (see Tables S1–S4; details on the alignments are reported in Figures S1–S3).

2.2. Metataxonomic Data Analysis

ASVs analysis was conducted considering those reporting a percentage of confidence $\geq$ 75%. The sponge *M.* (*Oxymycale*) *acerata* (B4) hosted the greatest abundance of bacterial taxa (250 ASVs), while *H. pilosus* (D4), *H.* (*Rhizoniera*) *dancoi* (C6) and *M. sarai* (D6) showed 47, 55 and 120 ASVs, respectively (Tables S5–S8). Overall, concerning the taxonomic profiling, sponge samples were all dominated by Gammaproteobacteria, Alphaproteobacteria and Bacteroidia (Figure 1).

In addition, *M.* (*Oxymycale*) *acerata*, *H. pilosus* and *H.* (*Rhizoniera*) *dancoi* revealed an abundance of both Deltaproteobacteria and Acidimicrobiia. Manhattan algorithm indicated that *M. sarai* clustered separately in comparison to the others, with *H. pilosus* and *H.* (*Rhizoniera*) *dancoi* resulting as the most similar in terms of species structure and abundance (Figure 1).

More specifically, a high relative frequency of Gammaproteobacteria and Bacteroidia were found in *M.* (*Oxymycale*) *acerata* (61.5% and 19%, respectively) and *M. sarai* (71% and 14%, respectively) (Figure 2; see also Figure S4).

On the contrary, a lower percentage (5–7%) of Alphaproteobacteria was detected in both species. In addition, *M.* (*Oxymycale*) *acerata* revealed 2–7% of bacteria belonging to Acidimicrobiia and Verrucomicrobiae classes, while lower percentages (~1%) of other bacterial phyla (Proteobacteria, Epsilonbacteraeota, Planctomycetes) together with 7% of an unknown phylum were recorded in *M. sarai* (Figure S4).

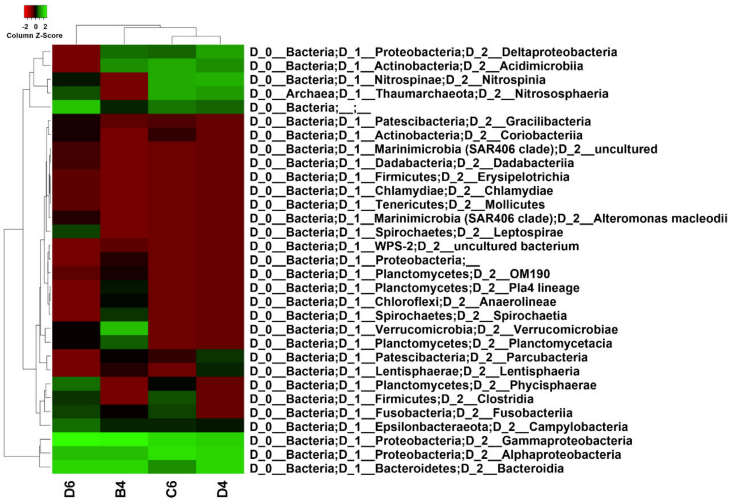

Figure 1. Heatmap of taxon relative abundance using taxonomic profiling, showing that sponge samples were all dominated by Gammaproteobacteria, Alphaproteobacteria and Bacteroidia. Sample code: B4 = *M.* (*Oxymycale*) *acerata*; D4 = *H. pilosus*, D6 = *M. sarai*, C6 = *H.* (*Rhizoniera*) *dancoi*. Scaling was done by column and clustering was performed using average linkage method and Manhattan distance measurement. Values were normalized as Log10.

Figure 2. Krona Plot at the class level. Gammaproteobacteria of the genus Endozoicomonas were identified from *M.* (*Oxymycale*) *acerata* as well as Gammaproteobacteria belonging to the genus *Colwellia* and some bacterial strains classified as *Fulvivirga* and *Ulvibacter*, two genera included into Bacteroidetes. Bacteria of the family Rhodobacteraceae (class Alphaproteobacteria) were identified in *H.* (*Rhizoniera*) *dancoi* and *H. pilosus*. *M. sarai* was the only species showing a relative abundance of Polaribacter, an additional species grouped into the Bacteroidetes phylum. Sample code: B4 = *M.* (*Oxymycale*) *acerata*; D4 = *H. pilosus*, D6 = *M. sarai*, C6 = *H.* (*Rhizoniera*) *dancoi*. "1 more" corresponds to Acidicrodobiia group, which is present in trace levels.

As reported above (Figure 1), the sponges *H.* (*Rhizoniera*) *dancoi* and *H. pilosus* revealed a similar composition in bacterial species distribution. In fact, a high abundance of Alphaproteobacteria (44% in *H.* (*Rhizoniera*) *dancoi* and 33.2% in *H. pilosus*) and Gammaproteobacteria (37% in *H.* (*Rhizoniera*) *dancoi* and 24% *H. pilosus*) was observed in both species. Moreover, lower percentages (0.5–5%) of additional taxa were recorded, including the Nitrospinia, Nitrososphaeria, Acidimicrobiia and Deltaproteobacteria groups. A huge difference was detected for bacteria belonging to the class Bacteroidia, since a higher relative abundance was found in *H. pilosus* (14%) in comparison to *H.* (*Rhizoniera*) *dancoi* (2%) (Figure S4).

3. Discussion

In the present study, we analyzed the species composition and abundance of the associated microbiota from four Antarctic sponges, *M.* (*Oxymycale*) *acerata*, *H.* (*Rhizoniera*) *dancoi*, *H. pilosus* and *M. sarai*, collected from Tethys Bay (Victoria Land, Antarctica). In particular, the associated community of *M.* (*Oxymycale*) *acerata*, collected from site 1 (Table 1), was similar to *H.* (*Rhizoniera*) *dancoi* and *H. pilosus* (Figure 1), retrieved from site 2 (Table 1). Interestingly, at this latter site, we collected the sponge *M. sarai*, whose species abundance was found statistically different by Manhattan clustering analysis (Figure 1).

As reported in most investigations focusing on sponge-associated bacteria [42–47], taxonomic profiling showed that Proteobacteria and Bacteroidetes dominated the four Antarctic sponges (Figure 2; Tables S5–S8). Previous studies identified these bacterial groups from *M.* (*Oxymycale*) *acerata* and other Antarctic species by metagenomic approaches [23,27,28,30–32,48]. These bacteria were frequently found to be the dominant bacterial phyla in marine ecosystems [49]. In particular, Proteobacteria showed different functions in host, including nitrogen fixation, and were involved in host defense mechanisms [50]. Furthermore, some bacteria were described as highly specialized hydrocarbon degrading microorganisms [51,52] and their wide distribution may be due to a strong positive interaction in environments where bacteria represent a fundamental source of nutrients, such as the case of Antarctica. This finding could be corroborated by results revealing that these bacteria are able to adapt to extreme environments, including polar habitats [53–56]. Concerning their biotechnological potential, genome-mining approaches reported several biosynthetic gene clusters (BGCs) encoding for bioactive molecules from marine Proteobacteria (reviewed by Buijs et al. [57]). However, there is no direct 100% correlation between the presence of a certain BGC, a bacterial genus and a bioactive metabolite. BGCs can be silent in certain conditions and, hence, methods should be developed to unlock their silent potential [58], to observe the production of a particular compound and induce the desired bioactivity. The most common approach known to discover new metabolites is the "OSMAC" (one strain many compounds) approach. The term OSMAC was coined for the first time by Zeeck and co-workers [59], indicating the ability of single strains to produce different metabolites when cultivated under different conditions. Examples are the use of different culturing strategies to trigger the production of secondary metabolites such as changing culturing conditions (e.g., nutrients or light exposure), mimicking environmental stressors and co-culturing with other species.

On the whole, several species belonging to Gammaproteobacteria and Alphaproteobacteria isolated from sponges and soils showed antibacterial, antiviral, antifungal and antiprotozoal activities that make them suitable tools in drug discovery research fields [36,43,60–63]. In particular, the Gammaproteobacteria of the genus *Endozoicomonas*, identified from *M.* (*Oxymycale*) *acerata* in the present work (Figure 2; Table S5), was found to induce antimicrobial activities [64,65].

Always *M.* (*Oxymycale*) *acerata* showed a relative abundance of a Gammaproteobacteria belonging to the genus *Colwellia* (Figure 2; Table S5), which is extremely interesting since it was recently proposed as a useful tool for the bioremediation of nitrogen pollutants [66]. Previous investigations also demonstrated that a sponge-associated *Colwellia* sp. produces

several extracellular polymeric substances (EPSs) with potential use in the production of cosmeceutical and nutraceutical ingredients [35,67].

M. (*Oxymycale*) *acerata* also revealed some bacterial strains classified as *Fulvivirga* and *Ulvibacter*, two genera included into Bacteroidetes, the second most abundant phylum found in the samples under analysis (Figure 2; Table S5). Genome-mining approaches coupled to chemical analyses revealed the presence of some amine acylated desferrioxamine siderophores from *Fulvivirga* sp. with anticancer properties [68]. Similarly, *Ulvibacter* species, already observed in Antarctic habitats [69], belong to the family Flavobacteriaceae, whose biotechnological applications are well-documented. In fact, several polysaccharide-digesting enzymes together with antibiotics and other bioactive compounds, such as quercetin (known for its antioxidant, anti-inflammatory, chemopreventive properties), were isolated [70,71].

The sequencing of 16S regions revealed that *M.* (*Oxymycale*) *acerata* was the sole species hosting a certain abundance of *Rubritalea* strains (phylum Verrucomicrobia) (Figure 2; Table S5). This bacterial group was already observed in other sponge species, from which some BGCs encoding for PKSs (polyketide synthases) were identified [42,72–77]. Verrucomicrobia, coupled with Planctomycetes and Chlamydiae, was classified in the PVC (Planctomycetes, Verrucomicrobia, and Chlamydiae) superphylum, which is known to include a wide number of species with biotechnological potential [78–80]. The finding of these bacteria within the symbiotic community of *M.* (*Oxymycale*) *acerata* may be extremely attractive since several bioactive molecules, such as carotenoids and squalene, were found in several bacteria belonging to the genus *Rubritalea* [72–74,81–84]. The potential capability to produce biotechnologically relevant compounds was also demonstrated by genomic analyses, revealing some genes involved in defense mechanisms mediated by toxin-antitoxin systems from sponge-associated verrucomicrobial strains [77].

ASV's data showed bacteria of the family Rhodobacteraceae (class Alphaproteobacteria) in *H.* (*Rhizoniera*) *dancoi* and *H. pilosus* (Figure 2; Tables S6 and S7). Several genera were recognized as a huge source of novel bioactives, especially *Pseudovibrio* species living in seawater and through symbiotic relationships with sponges, tunicates and corals [85,86]. For example, *H. pilosus* specifically hosted the genus *Roseobacter*, which was also studied for its antimicrobial properties [87,88]. The hydrocarbon-degrading Gammaproteobacteria of the genus *Pseudohongiella*, with potential use in the bioremediation of anthropogenic contaminants [89,90], were also revealed in *H.* (*Rhizoniera*) *dancoi* and *H. pilosus* (Figure 2; Tables S6 and S7).

Less abundant members living within *H. pilosus* and *H.* (*Rhizoniera*) *dancoi* belonged to the classes Nitrospinia (phylum Nitrospinae) and Nitrosophaeria (phylum Thaumarchaeota) (Figure 2; Tables S6 and S7). Recent investigations already reported low percentages of Nitrospinia from *H. pilosus*, *H.* (*Rhizoniera*) *dancoi* and other Antarctic species [26,29]. Concerning the capability to produce molecules with biotechnological potential, very little information is available so far. In a recent study, BLASTp search against the Integrated Microbial Genomes (IMG) database identified a *Pseudoalteromonas luteoviolacea* gene encoding for a L-amino acid oxidase (LAAO) with antimicrobial properties in the genome of a strain belonging to the phylum Nitrospinae [91].

The sponges under analysis had low percentages of Acidimicrobiia (phylum Actinobacteria), except for *M. sarai* (Tables S5–S8). According to our results, this bacterial class was recently reported from *H.* (*Rhizoniera*) *dancoi*, *H. pilosus* and other Antarctic sponges by metagenomic analysis [27,28]. Acidimicrobiia were widely observed in marine sponges, particularly from tropical species [63,92–97]. Similar to Proteobacteria, several studies demonstrated the great biotechnological potential of Actinobacteria, especially those belonging to the *Streptomyces* genus. In fact, several bioactive compounds with antimicrobial, antiviral, antiparasitic, antiprotozoal and antitumor effects have been described [98–107]. Moreover, genomic analyses revealed some BGCs encoding for secondary metabolites, such as PKS I and III, NRPS (nonribosomal peptides), terpene and bacteriocin gene clusters from a sponge-derived Actinobacteria showing antimicrobial activities [108–112].

ASV's analysis of the three sponges, *M.* (*Oxymycale*) *acerata*, *H. pilosus* and *H.* (*Rhizoniera*) *dancoi*, displayed Deltaproteobacteria (Figure 2; Tables S5–S7) belonging to the phylum Proteobacteria, that, as mentioned above, produce interesting bioactive metabolites [57]. For instance, *H. pilosus* exhibited some strains included into the genus *Bdellovibrio* (Figure 3; Table S7), which is an obligate predator of other Gram-negative bacteria that was proposed for possible biotechnological applications toward medicinal, agricultural and industrial fields [113–115].

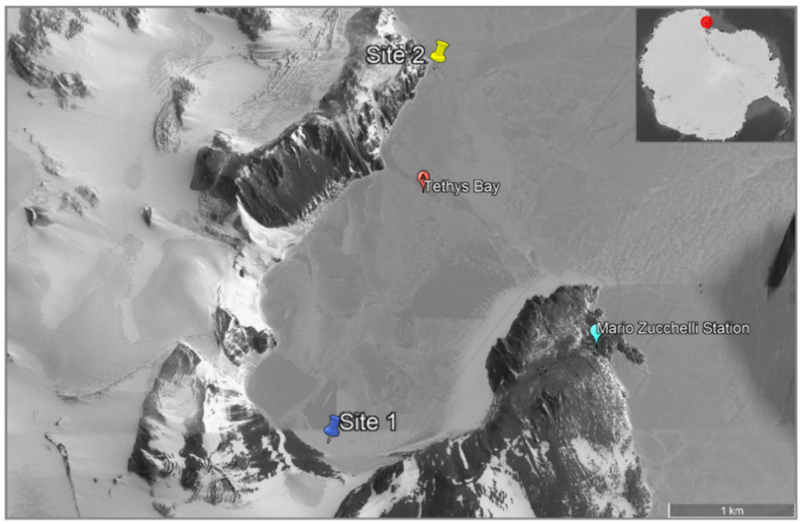

Figure 3. Map of Tethys Bay (Victoria Land, Antarctica). The collection sites were reported as blue (site 1) and yellow (site 2) icons. Scale bar = 1 km.

M. sarai was the only species showing a relative abundance of *Polaribacter*, an additional species grouped into the Bacteroidetes phylum (Figure 2; Table S8). Some data demonstrated that these cold-adapted bacteria produced interesting EPSs molecules with protective effects on human skin and anti-aging properties [116,117].

4. Materials and Methods

4.1. Sponge Collection

Four sponge samples, reported as B4, C6, D4 and D6, were collected by scuba divers in November–December 2018 at two sites of Tethys Bay: 1) B4 at 26 metres of depth (74°42.067′ S, 164°02.518′ E) and 2) C6, D4 and D6 at 28 metres of depth (74°40.537′ S, 164°04.169′ E) (Figure 3). Samples were immediately washed at least three times with filter-sterilized natural seawater to remove transient and loosely attached bacteria and/or debris [14,27]. Firstly, a small fragment of each sponge was preserved in 70% ethanol for taxonomic identification. Specimens were then placed into individual sterile tubes and kept in RNA*later*© at −20° C until transported to the Stazione Zoologica Anton Dohrn (Naples, Italy).

Sponge slides of spicules are deposited at the Italian National Antarctic Museum (MNA, Section of Genoa, Italy). The MNA voucher codes of the sponges investigated in the present work are reported in Table 1.

4.2. Morphological Analysis of Spicules

The taxonomic identification was conducted at the species level. Small fragments of each sponge were heat-dissolved in nitric acid, rinsed in water and dehydrated in ethanol. Then, spicules were mounted on slides for microscopic analyses, following standard methods [118]. The skeletal architecture was examined under light microscope and hand-cut sections of sponge portions were made as described in Hooper [119].

The taxonomic classification follows the updated nomenclature reported in the World Porifera Database (WPD) [120].

4.3. DNA Extraction and PCR Amplification

About 10 mg of tissue were used for DNA extraction by using *QIAamp® DNA Micro kit* (QIAGEN), according to the manufacturer's instructions. DNA quantity (ng/µL) was evaluated by NanoDrop spectrophotometer. PCR reactions were performed on the C1000 Touch Thermal Cycler (BioRad) in a 30 µL reaction mixture final volume including about 50–100 ng of genomic DNA, 6 µL of 5X Buffer GL (GeneSpin Srl, Milan, Italy), 3 µL of dNTPs (2 mM each), 2 µL of each forward and reverse primer (25 pmol/µL, Table 1), 0.2 µL of Xtra Taq Polymerase (5 U/µL, GeneSpin Srl, Milan, Italy), using different PCR programs for 18S and 28S, ITS and CO1 as follows:

(i) for 18S and 28S, a denaturation step at 95 °C for 2 min, [35 cycles denaturation step at 95 °C for 1 min, annealing step at 60 °C (A/B, [121]), 57 °C (C2/D2, [122]), 55 °C (18S-AF/18S-BR, NL4F/NL4R, [123]) for 1 min and 72 °C of primer extension for 2 min], a final extension step at 72 °C for 10 min;

(ii) ITS primers (RA2/ITS2.2, [121]), a first denaturation at 95 °C for 2 min, [35 cycles denaturation step at 95 °C for 1 min, annealing step at 67 °C for 1 min and 72 °C of primer extension for 2 min], a final extension step at 72 °C for 10 min.

(iii) CO1 primers (dgLCO1490/dgHCO2198, [124]), a first denaturation at 94 °C for 3 min, [35 cycles of denaturation at 94 °C for 30 sec, annealing at 45 °C for 30 sec and primer extension at 72 °C for 1 min].

Sequences of PCR primers are reported in Supporting Information (Table S9). PCR products were run on 1.5% agarose gel and the fragment length was evaluated by using 100 bp DNA ladder (GeneSpin Srl, Milan, Italy). PCR products were purified using *QIAquick Gel Extraction Kit* (Qiagen), according to the manufacturer's instructions. PCR amplicons were then sequenced in both strands through Applied Biosystems (Life Technologies) 3730 Analyzer (48 capillaries). Sequences produced were ~300–650 bases long in average with more than 97.5% accuracy, starting from PCR fragments. The total 18S, 28S, ITS and CO1 region were submitted to GenBank using Basic Local Alignment Search Tool (BLAST) [125] and then aligned with highly similar sequences using MultiAlin (http://multalin.toulouse.inra.fr/multalin/ accessed on 29 January 2021) [126].

4.4. Metagenomic DNA Extraction and Illumina MiSeq Sequencing

Genomic DNA for 16S rRNA sequencing was performed from about 250 mg of tissue by using *DNeasy® PowerSoil® Pro Kit* (QIAGEN), according to the manufacturer's instructions. Extractions were performed using both internal and external sponge tissue in order to obtain the whole bacterial community. DNA quantity (ng/µL) and quality (A260/280, A260/230) were evaluated by NanoDrop spectrophotometer, whereas DNA integrity was checked on 0.8% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). 20 µL of samples (30 ng/µL final concentration) were subjected to 16S V3-V4 rRNA gene library preparation and sequencing (Bio-Fab Research, Rome, Italy). Illumina adapters overhang nucleotide sequences were added to the gene specific primer sequences targeting the V3-V4 region [127]. After 16S amplification, a PCR clean-up was done to purify the V3-V4 amplicon from free primers and primer-dimer species. A subsequent limited cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters by using a Nextera XT Index Kit. Finally, libraries were normalized and pooled by denoising processes (Table S10), and sequenced on Illumina MiSeq Platform with 2x300 bp paired-end reads. Taxonomy was assigned using "home made" Naive Bayesian Classifier trained on V3-V4 16S sequences of SILVA 132 database [128]. QIIME 2 (Quantitative Insights Into Microbial Ecology) platform [129] was used for microbiome analysis from raw DNA sequencing data. QIIME analysis workflow was performed by demultiplexing, quality filtering, chimera removal

and taxonomic assignment. The full dataset of raw data has been deposited in the SRA database (submission ID: SUB8701897; BioProject ID: PRJNA687362).

4.5. Statistical Analysis

ASVs distribution and frequency in the whole dataset and for each sample are reported in the Supporting Information (Figures S5 and S6).

Heatmap was generated by using Heatmapper Software available at http://www.heatmapper.ca/ accessed on 29 January 2021 [130]. The number of features observed for each identified taxa were normalized as Log10 and scaled by column. Hierarchical clustering was applied on both rows and columns by average linkage method. For computing distance between rows and columns, Manhattan distance measurement algorithm was performed.

5. Conclusions

Our metataxonomic analysis highlights the occurrence of dominant and locally enriched microbes in the Antarctic sponges *M.* (*Oxymycale*) *acerata*, *H.* (*Rhizoniera*) *dancoi*, *H. pilosus* and *M. sarai*, characterized by morphological and molecular approaches. This can be considered a starting point in the understanding of the global Antarctic microbiome in a more complete perspective, given the scarce information in the literature for extreme environments such as the Antarctica. According to the microbial community identified, the biotechnological value should not be underestimated. In fact, our findings open new perspectives concerning the possible role of these Antarctic sponges and their symbiotic bacteria as a source of bioactive compounds. Further studies will be devoted to bioassay-guided fractionations for identifying new potential drugs useful in pharmaceutical, nutraceutical and cosmeceutical fields.

Supplementary Materials: The following are available online at https://www.mdpi.com/1660-3397/19/3/173/s1, Figure S1. Alignments of CO1 (PorCOI2fwd/PorCOI2rev) sequence from B4 sponge with (a) the first BLAST hit *Asbestopluma lycopodium* and (b) the sequence of *M. acerata* displaying low query cover, Figure S2: Alignment of CO1 (dgLCO1490/dgHCO2198) sequence from B4 sponge with the first BLAST hit (*M. acerata*), Figure S3: Alignment of CO1 (dgLCO1490/dgHCO2198) sequence from D4 sponge with the first BLAST hit (*H. pilosus*), Figure S4. Krona plot at increasing complexity levels. Regnum (a), phylum (b), class (c), order (d), family (e), genus (f) and species (g) were reported. Figure S5. Distribution of ASV's frequencies, Figure S6. Distribution of ASV's frequencies for each sample (reported as a blue bar), Table S1. BLAST results from B4 sponge (*Mycale* (*Oxymycale*) *acerata*). The primer names, sequence length in base pairs (bp), first hits (highlighted in bold), hits at low significance displaying the correct species (where present), query cover and identity percentages (%) were reported, Table S2. BLAST results from C6 sponge (*Haliclona* (*Rhizoniera*) *dancoi*). The primer names, sequence length in base pairs (bp), first hits (highlighted in bold), hits at low significance displaying the correct species (where present), query cover and identity percentages (%) were reported, Table S3. BLAST results from D4 sponge (*Hemigellius pilosus*). The primer names, sequence length in base pairs (bp), first hits (highlighted in bold), hits at low significance displaying the correct species (where present), query cover and identity percentages (%) were reported, Table S4. BLAST results from D6 sponge (*Microxina sarai*). The primer names, sequence length in base pairs (bp), first hits (highlighted in bold), query cover and identity percentages (%) were reported, Table S5. ASVs found from *M.* (*Oxymycale*) *acerata* with percentage of confidence $\geq$ 75%, Table S6. ASVs found from *H.* (*Rhizoniera*) *dancoi* with percentage of confidence $\geq$ 75%, Table S7. ASVs found from *H. pilosus* with percentage of confidence $\geq$ 75%, Table S8. ASVs found from *M. sarai* with percentage of confidence $\geq$ 75%, Table S9. Targeted region, forward and reverse names, sequences (5'$\rightarrow$ 3') and reference of primers pairs used for molecular characterization, Table S10. Denoising process.

Author Contributions: Molecular identification of sponges, data curation, formal analysis, N.R. and R.E.; Morphological analysis of sponges, M.B.; collection of sponge samples in the framework of XXXIV Italian National Antarctic Research Program (PNRA) expedition, G.Z.; metataxonomic analysis, M.S., F.A.; data curation, formal analysis, investigation, reviewing and editing, D.C., G.N., C.L., A.F.; funding acquisition, writing—reviewing and editing, D.G., A.I., C.V.; conceptualization, funding acquisition, supervision, writing—original draft, M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by PNRA16_00043, Cosmeceuticals and Nutraceuticals From Antarctic Biological REsources (CAN FARE). It was carried out in the framework of the SCAR Programme "Antarctic Thresholds–Ecosystem Resilience and Adaptation" (AnT-ERA).

Informed Consent Statement: Not applicable.

Acknowledgments: Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Peck, L.S. Antarctic marine biodiversity: Adaptations, environments and responses to change. In *Oceanography and Marine Biology: An Annual Review*; Hawkins, S.J., Evans, A.J., Dale, A.C., Firth, L.B., Smith, I.P., Eds.; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2018; Volume 56, pp. 2–133, ISBN 9781138318625.
2. Arnaud, P. Contribution à la bionomie marine benthique des régions antarctiques et subantarctiques. In *Téthys*; Station Marine d'Endoume, Centre d'Océanographie de Marseille: Marseille, France, 1974; Volume 6, pp. 465–656.
3. Konecki, J.T.; Targett, T.E. Eggs and larvae of *Nototheniops larseni* from the spongocoel of a hexactinellid sponge near Hugo island, Antarctic Peninsula. *Polar Biol.* **1989**, *10*, 197–198. [CrossRef]
4. Pearse, J.S.; Mcclintock, J.B.; Bosch, I. Reproduction of Antarctic benthic marine invertebrates: Tempos, modes, and timing. *Integr. Comp. Biol.* **1991**, *31*, 65–80. [CrossRef]
5. Dayton, P.K. Polar benthos. In *Polar oceanography-Part B-Chemistry, Biology and Geology*; Smith, W.O., Ed.; Academic Press: London, UK, 1990; pp. 631–685, ISBN 9780080925950.
6. Dayton, P.K.; Mordida, B.J.; Bacon, F. Polar marine communities. *Am. Zool.* **1994**, *34*, 90–99. [CrossRef]
7. Thompson, M.R.A.; Crame, J.A.; Thompson, J.W. *Geological Evolution of Antarctica*; Cambridge University Press: Cambridge, UK, 1991; ISBN 9780521372664.
8. Tingey, R. *The Geology of Antarctica*; Oxford University Press: Oxford, UK, 1991; ISBN 9780198544678.
9. Clarke, A. Is there a latitudinal diversity cline in the sea? *Trends Ecol. Evol.* **1992**, *7*, 286–287. [CrossRef]
10. Crame, J.; Clarke, A. The historical component of marine taxonomic diversity gradients. In *Marine Biodiversity: Patterns and Processes*; Ormond, R.F.G., Gage, J.D., Angel, M.V., Eds.; Cambridge University Press: Cambridge, UK, 1997; pp. 258–273.
11. Gray, J.S. Antarctic marine benthic biodiversity in a world-wide latitudinal context. *Polar Biol.* **2001**, *24*, 633–641. [CrossRef]
12. McClintock, J.B.; Amsler, C.D.; Baker, B.J.; Van Soest, R.W.M. Ecology of antarctic marine sponges: An overview. *Integr. Comp. Biol.* **2005**, *45*, 359–368. [CrossRef]
13. Janussen, D.; Downey, R. V Porifera. In *Biogeographic Atlas of the Southern Ocean*; De Broyer, C., Koubbi, P., Eds.; SCAR: Cambridge, UK, 2014; pp. 1–10.
14. Mangano, S.; Michaud, L.; Caruso, C.; Brilli, M.; Bruni, V.; Fani, R.; Lo Giudice, A. Antagonistic interactions between psychrotrophic cultivable bacteria isolated from Antarctic sponges: A preliminary analysis. *Res. Microbiol.* **2009**, *160*, 27–37. [CrossRef] [PubMed]
15. Hentschel, U.; Piel, J.; Degnan, S.M.; Taylor, M.W. Genomic insights into the marine sponge microbiome. *Nat. Rev. Microbiol.* **2012**, *10*, 641–654. [CrossRef]
16. Bayer, K.; Kamke, J.; Hentschel, U. Quantification of bacterial and archaeal symbionts in high and low microbial abundance sponges using real-time PCR. *FEMS Microbiol. Ecol.* **2014**, *89*, 679–690. [CrossRef]
17. Lee, Y.K.; Lee, J.H.; Lee, H.K. Microbial symbiosis in marine sponges. *J. Microbiol.* **2001**, *39*, 254–264.
18. Thoms, C.; Horn, M.; Wagner, M.; Hentschel, U.; Proksch, P. Monitoring microbial diversity and natural product profiles of the sponge *Aplysina cavernicola* following transplantation. *Mar. Biol.* **2003**, *142*, 685–692. [CrossRef]
19. Hentschel, U.; Usher, K.M.; Taylor, M.W. Marine sponges as microbial fermenters. *FEMS Microbiol. Ecol.* **2006**, *55*, 167–177. [CrossRef]
20. Calcinai, B.; Pansini, M. Four new demosponge species from Terra Nova Bay (Ross Sea, Antarctica). *Zoosystema* **2000**, *22*, 369–381.
21. Ríos, P.; Cristobo, F.J.; Urgorri, V. Poecilosclerida (Porifera, Demospongiae) collected by the Spanish Antarctic expedition BENTART-*Cah. Biol. Mar.* **2004**, *45*, 97–119.
22. Dayton, P.K.; Robilliard, G.A.; Paine, R.T.; Dayton, L.B. Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecol. Monogr.* **1974**, *44*, 105–128. [CrossRef]

23. Cárdenas, C.A.; Font, A.; Steinert, G.; Rondon, R.; González-Aravena, M. Temporal stability of bacterial communities in Antarctic sponges. *Front. Microbiol.* **2019**, *10*, 1–14. [CrossRef] [PubMed]
24. Lo Giudice, A.; Rizzo, C. Bacteria associated with marine benthic invertebrates from polar environments: Unexplored frontiers for biodiscovery? *Diversity* **2018**, *10*, 80. [CrossRef]
25. Lo Giudice, A.; Azzaro, M.; Schiaparelli, S. Microbial symbionts of Antarctic marine benthic invertebrates. In *The Ecological Role of Micro-Organisms in the Antarctic Environment*; Castro-Sowinski, S., Ed.; Springer International Publishing: New York, NY, USA, 2019; pp. 277–296.
26. Steinert, G.; Wemheuer, B.; Janussen, D.; Erpenbeck, D.; Daniel, R.; Simon, M.; Brinkhoff, T.; Schupp, P.J. Prokaryotic diversity and community patterns in antarctic continental shelf sponges. *Front. Mar. Sci.* **2019**, *6*, 1–15. [CrossRef]
27. Savoca, S.; Lo Giudice, A.; Papale, M.; Mangano, S.; Caruso, C.; Spanò, N.; Michaud, L.; Rizzo, C. Antarctic sponges from the Terra Nova Bay (Ross Sea) host a diversified bacterial community. *Sci. Rep.* **2019**, *9*, 16135. [CrossRef] [PubMed]
28. Moreno-Pino, M.; Cristi, A.; Gillooly, J.F.; Trefault, N. Characterizing the microbiomes of Antarctic sponges: A functional metagenomic approach. *Sci. Rep.* **2020**, *10*, 645. [CrossRef]
29. Papale, M.; Rizzo, C.; Fani, R.; Bertolino, M.; Costa, G.; Paytuví-Gallart, A.; Schiaparelli, S.; Michaud, L.; Azzaro, M.; Lo Giudice, A. Exploring the diversity and metabolic profiles of bacterial communities associated with Antarctic sponges (Terra Nova Bay, Ross Sea). *Front. Ecol. Evol.* **2020**, *8*, 1–16. [CrossRef]
30. Sacristán-Soriano, O.; Pérez Criado, N.; Avila, C. Host species determines symbiotic community composition in Antarctic sponges (Porifera: Demospongiae). *Front. Mar. Sci.* **2020**, *7*, 1–36. [CrossRef]
31. Rodríguez-Marconi, S.; De La Iglesia, R.; Díez, B.; Fonseca, C.A.; Hajdu, E.; Trefault, N.; Webster, N. Characterization of bacterial, archaeal and eukaryote symbionts from antarctic sponges reveals a high diversity at a three-domain level and a particular signature for this ecosystem. *PLoS ONE* **2015**, *10*, 1–19. [CrossRef]
32. Webster, N.S.; Negri, A.P.; Munro, M.M.H.G.; Battershill, C.N. Diverse microbial communities inhabit Antarctic sponges. *Environ. Microbiol.* **2004**, *6*, 288–300. [CrossRef] [PubMed]
33. Mangano, S.; Michaud, L.; Caruso, C.; Lo Giudice, A. Metal and antibiotic resistance in psychrotrophic bacteria associated with the Antarctic sponge *Hemigellius pilosus* (Kirkpatrick, 1907). *Polar Biol.* **2014**, *37*, 227–235. [CrossRef]
34. Mangano, S.; Caruso, C.; Michaud, L.; Lo Giudice, A. First evidence of quorum sensing activity in bacteria associated with Antarctic sponges. *Polar Biol.* **2018**, *41*, 1435–1445. [CrossRef]
35. Caruso, C.; Rizzo, C.; Mangano, S.; Poli, A.; di Donato, P.; Finore, I.; Nicolaus, B.; di Marco, G.; Michaud, L.; Lo Giudice, A. Production and biotechnological potential of extracellular polymeric substances from sponge-associated Antarctic bacteria. *Appl. Environ. Microbiol.* **2018**, *84*, e01624-17. [CrossRef]
36. Brinkmann, C.M.; Marker, A.; Kurtböke, D.I. An overview on marine sponge-symbiotic bacteria as unexhausted sources for natural product discovery. *Diversity* **2017**, *9*, 40. [CrossRef]
37. Taylor, M.W.; Radax, R.; Stegar, D.; Wagner, M. Sponge-associated microorganisms: Evolution, evology, and biotechnological potential. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 295–347. [CrossRef] [PubMed]
38. Webster, N.S.; Taylor, M.W. Marine sponges and their microbial symbionts: Love and other relationships. *Environ. Microbiol.* **2012**, *14*, 335–346. [CrossRef]
39. Thacker, R.W.; Freeman, C.J. Sponge-microbe symbioses. Recent advances and new directions. In *Advances in Marine Biology*; Academic Press: Cambridge, MA, USA, 2012; Volume 62, pp. 57–111.
40. Björk, J.R.; Díez-Vives, C.; Coma, R.; Ribes, M.; Montoya, J.M. Specificity and temporal dynamics of complex bacteria–sponge symbiotic interactions. *Ecology* **2013**, *94*, 2781–2791. [CrossRef]
41. Hughes, K.A.; Pertierra, L.R.; Walton, D.W.H. Area protection in Antarctica: How can conservation and scientific research goals be managed compatibly? *Environ. Sci. Policy* **2013**, *31*, 120–132. [CrossRef]
42. Kennedy, J.; Codling, C.E.; Jones, B.V.; Dobson, A.D.W.; Marchesi, J.R. Diversity of microbes associated with the marine sponge, *Haliclona simulans*, isolated from Irish waters and identification of polyketide synthase genes from the sponge metagenome. *Environ. Microbiol.* **2008**, *10*, 1888–1902. [CrossRef] [PubMed]
43. Dupont, S.; Carré-Mlouka, A.; Descarrega, F.; Ereskovsky, A.; Longeon, A.; Mouray, E.; Florent, I.; Bourguet-Kondracki, M.L. Diversity and biological activities of the bacterial community associated with the marine sponge *Phorbas tenacior* (Porifera, Demospongiae). *Lett. Appl. Microbiol.* **2014**, *58*, 42–52. [CrossRef] [PubMed]
44. Karimi, E.; Ramos, M.; Gonçalves, J.M.S.; Xavier, J.R.; Reis, M.P.; Costa, R. Comparative metagenomics reveals the distinctive adaptive features of the *Spongia officinalis* endosymbiotic consortium. *Front. Microbiol.* **2017**, *8*, 1–16. [CrossRef] [PubMed]
45. Slaby, B.M.; Hackl, T.; Horn, H.; Bayer, K.; Hentschel, U. Metagenomic binning of a marine sponge microbiome reveals unity in defense but metabolic specialization. *ISME J.* **2017**, *11*, 2465–2478. [CrossRef]
46. Najafi, A.; Moradinasab, M.; Nabipour, I. First record of microbiomes of sponges collected from the Persian Gulf, using tag pyrosequencing. *Front. Microbiol.* **2018**, *9*, 1–18. [CrossRef] [PubMed]
47. Storey, M.A.; Andreassend, S.K.; Bracegirdle, J.; Brown, A.; Keyzers, R.A.; Ackerley, D.F.; Northcote, P.T.; Owen, J.G. Metagenomic exploration of the marine sponge *Mycale hentscheli* uncovers multiple polyketide-producing bacterial symbionts. *MBio* **2020**, *11*, 1–16. [CrossRef] [PubMed]
48. Cao, S.; Zhang, W.; Ding, W.; Wang, M.; Fan, S.; Yang, B.; McMinn, A.; Wang, M.; Xie, B.B.; Qin, Q.L.; et al. Structure and function of the Arctic and Antarctic marine microbiota as revealed by metagenomics. *Microbiome* **2020**, *8*, 47. [CrossRef] [PubMed]

49. Stevens, H.; Stübner, M.; Simon, M.; Brinkhoff, T. Phylogeny of Proteobacteria and Bacteroidetes from oxic habitats of a tidal flat ecosystem. *FEMS Microbiol. Ecol.* **2005**, *54*, 351–365. [CrossRef] [PubMed]
50. Bibi, F.; Alvi, S.A.; Al-Sofyani, A.; Naseer, M.I.; Yasir, M.; Azhar, E.I. Pyrosequencing reveals sponge specific bacterial communities in marine sponges of Red Sea, Saudi Arabia. *Saudi J. Biol. Sci.* **2020**, *27*, 67–73. [CrossRef] [PubMed]
51. Joye, S.B.; Kleindienst, S.; Gilbert, J.A.; Handley, K.M.; Weisenhorn, P.; Overholt, W.A.; Kostka, J.E. Responses of microbial communities to hydrocarbon exposures. *Oceanography* **2016**, *29*, 136–149. [CrossRef]
52. McGenity, T.J. *Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes*; Springer International Publishing: New York, NY, USA, 2019; ISBN 9783319600536.
53. Hamdan, L.J.; Coffin, R.B.; Sikaroodi, M.; Greinert, J.; Treude, T.; Gillevet, P.M. Ocean currents shape the microbiome of Arctic marine sediments. *ISME J.* **2013**, *7*, 685–696. [CrossRef]
54. Williams, T.J.; Cavicchioli, R. Marine metaproteomics: Deciphering the microbial metabolic food web. *Trends Microbiol.* **2014**, *22*, 248–260. [CrossRef]
55. Shivaji, S.; Reddy, G.S.N.; Chattopadhyay, M.K. Bacterial biodiversity, cold adaptation and biotechnological importance of bacteria occurring in Antarctica. *Proc. Ind. Natl. Sci. Acad. USA* **2017**, *83*, 327–352. [CrossRef]
56. Picazo, A.; Rochera, C.; Villaescusa, J.A.; Miralles-Lorenzo, J.; Velázquez, D.; Quesada, A.; Camacho, A. Bacterioplankton community composition along environmental gradients in lakes from Byers peninsula (Maritime Antarctica) as determined by next-generation sequencing. *Front. Microbiol.* **2019**, *10*, 1–12. [CrossRef] [PubMed]
57. Buijs, Y.; Bech, P.K.; Vazquez-Albacete, D.; Bentzon-Tilia, M.; Sonnenschein, E.C.; Gram, L.; Zhang, S. Da Marine Proteobacteria as a source of natural products: Advances in molecular tools and strategies. *Nat. Prod. Rep.* **2019**, *36*, 1333–1350. [CrossRef]
58. Reen, F.; Gutiérrez-Barranquero, J.; Dobson, A.; Adams, C.; O'Gara, F. Emerging Concepts Promising New Horizons for Marine Biodiscovery and Synthetic Biology. *Mar. Drugs* **2015**, *13*, 2924–2954. [CrossRef] [PubMed]
59. Bode, H.B.; Bethe, B.; Höfs, R.; Zeeck, A. Big effects from small changes: Possible ways to explore nature's chemical diversity. *ChemBioChem* **2002**, *3*, 619–627. [CrossRef]
60. Santos, O.C.S.; Pontes, P.V.M.L.; Santos, J.F.M.; Muricy, G.; Giambiagi-deMarval, M.; Laport, M.S. Isolation, characterization and phylogeny of sponge-associated bacteria with antimicrobial activities from Brazil. *Res. Microbiol.* **2010**, *161*, 604–612. [CrossRef]
61. Lee, L.H.; Cheah, Y.K.; Nurul Syakima, A.M.; Shiran, M.S.; Tang, Y.L.; Lin, H.P.; Hong, K. Analysis of Antarctic protobacteria by PCR fingerprinting and screening for antimicrobial secondary metabolites. *Genet. Mol. Res.* **2012**, *11*, 1627–1641. [CrossRef] [PubMed]
62. Desriac, F.; Jégou, C.; Balnois, E.; Brillet, B.; Le Chevalier, P.; Fleury, Y. Antimicrobial peptides from marine proteobacteria. *Mar. Drugs* **2013**, *11*, 3632–3660. [CrossRef] [PubMed]
63. Bibi, F.; Yasir, M.; Al-Sofyani, A.; Naseer, M.I.; Azhar, E.I. Antimicrobial activity of bacteria from marine sponge *Suberea mollis* and bioactive metabolites of *Vibrio* sp. *EASaudi J. Biol. Sci.* **2020**, *27*, 1139–1147. [CrossRef] [PubMed]
64. Haber, M.; Ilan, M. Diversity and antibacterial activity of bacteria cultured from Mediterranean *Axinella* spp. sponges. *J. Appl. Microbiol.* **2014**, *116*, 519–532. [CrossRef]
65. Rua, C.P.J.; Trindade-Silva, A.E.; Appolinario, L.R.; Venas, T.M.; Garcia, G.D.; Carvalho, L.S.; Lima, A.; Kruger, R.; Pereira, R.C.; Berlinck, R.G.S.; et al. Diversity and antimicrobial potential of culturable heterotrophic bacteria associated with the endemic marine sponge *Arenosclera Brasiliensis*. *PeerJ* **2014**, *2014*, 1–14. [CrossRef]
66. Lin, J.; Meng, Y.; Shi, Y.; Lin, X. Complete genome sequences of *Colwellia* sp. Arc7-635, a denitrifying bacterium isolated from Arctic seawater. *Curr. Microbiol.* **2019**, *76*, 1061–1065. [CrossRef]
67. Casillo, A.; Parrilli, E.; Sannino, F.; Mitchell, D.E.; Gibson, M.I.; Marino, G.; Lanzetta, R.; Parrilli, M.; Cosconati, S.; Novellino, E.; et al. Structure-activity relationship of the exopolysaccharide from a psychrophilic bacterium: A strategy for cryoprotection. *Carbohydr. Polym.* **2017**, *156*, 364–371. [CrossRef]
68. Wang, Z.J.; Zhou, H.; Zhong, G.; Huo, L.; Tang, Y.J.; Zhang, Y.; Bian, X. Genome mining and biosynthesis of primary amine-acylated desferrioxamines in a marine gliding bacterium. *Org. Lett.* **2020**, *22*, 939–943. [CrossRef]
69. Choi, T.H.; Lee, H.K.; Lee, K.; Cho, J.C. *Ulvibacter antarcticus* sp. nov., isolated from Antarctic coastal seawater. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2922–2925. [CrossRef] [PubMed]
70. McBride, M.J. The Family Flavobacteriaceae. In *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*; Rosenberg, E., De Long, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer-Verlag: Berlin/Heidelberg, Germany, 2014; pp. 1–1028.
71. Enisoglu-Atalay, V.; Atasever-Arslan, B.; Yaman, B.; Cebecioglu, R.; Kul, A.; Ozilhan, S.; Ozen, F.; Cata, T. Chemical and molecular characterization of metabolites from *Flavobacterium* sp. *PLoS ONE* **2018**, *13*, e0205817. [CrossRef]
72. Scheuermayer, M.; Gulder, T.A.M.; Bringmann, G.; Hentschel, U. *Rubritalea marina* gen. nov., sp. nov., a marine representative of the phylum "*Verrucomicrobia*", isolated from a sponge (*Porifera*). *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2119–2124. [CrossRef]
73. Kasai, H.; Katsuta, A.; Sekiguchi, H.; Matsuda, S.; Adachi, K.; Shindo, K.; Yoon, J.; Yokota, A.; Shizuri, Y. *Rubritalea squalenifaciens* sp. nov., a squalene-producing marine bacterium belonging to subdivision 1 of the phylum "*Verrucomicrobia*". *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1630–1634. [CrossRef]
74. Yoon, J.; Matsuo, Y.; Matsuda, S.; Adachi, K.; Kasai, H.; Yokota, A. *Rubritalea spongiae* sp. nov. and *Rubritalea tangerina* sp. nov., two carotenoid- and squalene-producing marine bacteria of the family *Verrucomicrobiaceae* within the phylum "*Verrucomicrobia*", isolated from marine animals. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2337–2343. [CrossRef]

75. Cuc, N.T.K.; Dat, T.T.H.; Hong, T.T.; Cuong, P.V. Phylogenetic diversity of microorganisms associated with three marine sponges from Mien Trung Sea of Vietnam. *J. Sci. Technol.* **2017**, *55*, 168–177. [CrossRef]
76. Kaluzhnaya, O.; Itskovich, V. Diversity of potential producers of bioactive metabolites having polyketide nature in the Baikal sponge community of *Rezinkovia echinata*. *Limnol. Freshw. Biol.* **2020**, *2020*, 423–428. [CrossRef]
77. Sizikov, S.; Burgsdorf, I.; Handley, K.M.; Lahyani, M.; Haber, M.; Steindler, L. Characterization of sponge-associated *Verrucomicrobia*: Microcompartment-based sugar utilization and enhanced toxin–antitoxin modules as features of host-associated *Opitutales*. *Environ. Microbiol.* **2020**, *22*, 4669–4688. [CrossRef] [PubMed]
78. Wagner, M.; Horn, M. The *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. *Curr. Opin. Biotechnol.* **2006**, *17*, 241–249. [CrossRef] [PubMed]
79. DeVos, D.P.; Ward, N.L. Mind the PVCs. *Environ. Microbiol.* **2014**, *16*, 1217–1221. [CrossRef]
80. Spring, S.; Bunk, B.; Spröer, C.; Schumann, P.; Rohde, M.; Tindall, B.J.; Klenk, H.P. Characterization of the first cultured representative of *Verrucomicrobia* subdivision 5 indicates the proposal of a novel phylum. *ISME J.* **2016**, *10*, 2801–2816. [CrossRef] [PubMed]
81. Yoon, J.; Matsuo, Y.; Matsuda, S.; Adachi, K.; Kasai, H.; Yokota, A. *Rubritalea sabuli* sp. nov., a carotenoid- and squalene-producing member of the family *Verrucomicrobiaceae*, isolated from marine sediment. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 992–997. [CrossRef]
82. Yoon, J.; Matsuda, S.; Adachi, K.; Kasai, H.; Yokota, A. *Rubritalea halochordaticola* sp. nov., a carotenoid producing verrucomicrobial species isolated from a marine chordate. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 1515–1520. [CrossRef] [PubMed]
83. Shindo, K.; Asagi, E.; Sano, A.; Hotta, E.; Minemura, N.; Mikami, K.; Tamesada, E.; Misawa, N.; Maoka, T. Diapolycopenedioic acid xylosyl esters A, B, and C, novel antioxidative glyco-C30-carotenoic acids produced by a new marine bacterium *Rubritalea Squalenifaciens*. *J. Antibiot.* **2008**, *61*, 185–191. [CrossRef] [PubMed]
84. Shindo, K.; Misawa, N. New and rare carotenoids isolated from marine bacteria and their antioxidant activities. *Mar. Drugs* **2014**, *12*, 1690–1698. [CrossRef] [PubMed]
85. Choudhary, A.; Naughton, L.M.; Dobson, A.D.W.; Rai, D.K. High-performance liquid chromatography/electrospray ionisation mass spectrometric characterisation of metabolites produced by *Pseudovibrio* sp. W64, a marine sponge derived bacterium isolated from Irish waters. *Rapid Commun. Mass Spectrom.* **2018**, *32*, 1737–1745. [CrossRef]
86. Romano, S. Ecology and biotechnological potential of bacteria belonging to the genus *Pseudovibrio*. *Appl. Environ. Microbiol.* **2018**, *84*, e02516-17. [CrossRef]
87. Brinkhoff, T.; Bach, G.; Heidorn, T.; Liang, L.; Schlingloff, A.; Simon, M. Antibiotic production by a *Roseobacter* clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. *Appl. Environ. Microbiol.* **2004**, *70*, 2560–2565. [CrossRef] [PubMed]
88. Bruhn, J.B.; Gram, L.; Belas, R. Production of antibacterial compounds and biofilm formation by *Roseobacter* species are influenced by culture conditions. *Appl. Environ. Microbiol.* **2007**, *73*, 442–450. [CrossRef] [PubMed]
89. Cerqueda-García, D.; García-Maldonado, J.Q.; Aguirre-Macedo, L.; García-Cruz, U. A succession of marine bacterial communities in batch reactor experiments during the degradation of five different petroleum types. *Mar. Pollut. Bull.* **2020**, *150*, 110775. [CrossRef]
90. Peng, C.; Tang, Y.; Yang, H.; He, Y.; Liu, Y.; Liu, D.; Qian, Y.; Lu, L. Time- and compound-dependent microbial community compositions and oil hydrocarbon degrading activities in seawater near the Chinese Zhoushan Archipelago. *Mar. Pollut. Bull.* **2020**, *152*, 110907. [CrossRef] [PubMed]
91. Andreo-Vidal, A.; Sanchez-Amat, A.; Campillo-Brocal, J.C. The *Pseudoalteromonas luteoviolacea* L-amino acid oxidase with antimicrobial activity is a flavoenzyme. *Mar. Drugs* **2018**, *16*, 499. [CrossRef]
92. Montalvo, N.F.; Mohamed, N.M.; Enticknap, J.J.; Hill, R.T. Novel actinobacteria from marine sponges. *Antonie Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2005**, *87*, 29–36. [CrossRef]
93. Zhang, H.; Zhang, W.; Jin, Y.; Jin, M.; Yu, X. A comparative study on the phylogenetic diversity of culturable actinobacteria isolated from five marine sponge species. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2008**, *93*, 241–248. [CrossRef]
94. Xin, Y.; Huang, J.; Deng, M.; Zhang, W. Culture-independent nested PCR method reveals high diversity of actinobacteria associated with the marine sponges *Hymeniacidon perleve* and *Sponge* sp. *Antonie Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2008**, *94*, 533–542. [CrossRef]
95. Selvin, J.; Gandhimathi, R.; Kiran, G.S.; Priya, S.S.; Ravji, T.R.; Hema, T.A. Culturable heterotrophic bacteria from the marine sponge *Dendrilla nigra*: Isolation and phylogenetic diversity of actinobacteria. *Helgol. Mar. Res.* **2009**, *63*, 239–247. [CrossRef]
96. Sun, W.; Dai, S.; Jiang, S.; Wang, G.; Liu, G.; Wu, H.; Li, X. Culture-dependent and culture-independent diversity of *Actinobacteria* associated with the marine sponge *Hymeniacidon perleve* from the South China Sea. *Antonie Leeuwenhoek, Int. J. Gen. Mol. Microbiol.* **2010**, *98*, 65–75. [CrossRef]
97. Li, L.; Zhang, D.; Tang, W.Z.; Lin, H.W.; Lu, Y.H. *Geodermatophilus marinus* sp. nov., isolated from the marine sponge *Leucetta chagosensis*. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 2966–2971. [CrossRef]
98. Dita, S.F.; Budiarti, S.R.I.; Lestari, Y. Sponge-associated Actinobacteria: Morphological character and antibacterial activity against pathogenic bacteria. *J. Sumberd. Hayati* **2017**, *3*, 21–26.
99. Xu, D.; Han, L.; Li, C.; Cao, Q.; Zhu, D.; Barrett, N.H.; Harmody, D.; Chen, J.; Zhu, H.; McCarthy, P.J.; et al. Bioprospecting deep-sea actinobacteria for novel anti-infective natural products. *Front. Microbiol.* **2018**, *9*, 1–9. [CrossRef] [PubMed]

100. Kasanah, N.; Triyanto, T. Bioactivities of halometabolites from marine Actinobacteria. *Biomolecules* **2019**, *9*, 225. [CrossRef] [PubMed]
101. Liu, T.; Wu, S.; Zhang, R.; Wang, D.; Chen, J.; Zhao, J. Diversity and antimicrobial potential of *Actinobacteria* isolated from diverse marine sponges along the Beibu Gulf of the South China Sea. *FEMS Microbiol. Ecol.* **2019**, *95*, 1–10. [CrossRef] [PubMed]
102. Phuong, T.V.; Thi, N.; Dung, P.; Diep, C.N. Diversified collection of novel sponge-associated actinobacteria of Ha Tien sea, Kien Giang province, Vietnam. *Int. J. Innov. Eng. Technol.* **2019**, *14*, 33–41.
103. Puttaswamygowda, G.H.; Olakkaran, S.; Antony, A.; Purayil, A.K. Present status and future perspectives of marine actinobacterial metabolites. In *Recent Developments in Applied Microbiology and Biochemistry*; Buddolla, V., Ed.; Academic Press: Cambridge, CA, USA, 2019; pp. 307–319.
104. Santos, J.D.; Vitorino, I.; De La Cruz, M.; Díaz, C.; Cautain, B.; Annang, F.; Pérez-Moreno, G.; Martinez, I.G.; Tormo, J.R.; Martín, J.M.; et al. Bioactivities and extract dereplication of *Actinomycetales* isolated from marine sponges. *Front. Microbiol.* **2019**, *10*, 1–11. [CrossRef]
105. Baig, U.; Dahanukar, N.; Shintre, N.; Holkar, K.; Pund, A.; Lele, U.; Gujarathi, T.; Patel, K.; Jakati, A.; Singh, R.; et al. Phylogenetic diversity and activity screening of cultivable actinobacteria isolated from marine sponges and associated environments from the western coast of India. *bioRxiv Prepr. Serv.* **2020**, 1–36. [CrossRef]
106. Gohain, A.; Manpoong, C.; Saikia, R.; De Mandal, S. Actinobacteria: Diversity and biotechnological applications. In *Recent Advancements in Microbial Diversity*; De Mandal, S., Bhatt, P., Eds.; Elsevier Inc.: Berlin/Heidelberg, Germany, 2020; pp. 217–231.
107. Kamala, K.; Sivaperumal, P.; Kamath, S.M.; Thilagaraj, W.R.; Rajaram, R. Marine actinobacteria as a source for emerging biopharmaceuticals. In *Encyclopedia of Marine Biotechnology*; Se-Kwon, K., Ed.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2020; pp. 2095–2105.
108. Jiang, S.; Sun, W.; Chen, M.; Dai, S.; Zhang, L.; Liu, Y.; Lee, K.J.; Li, X. Diversity of culturable actinobacteria isolated from marine sponge *Haliclona* sp. *Antonie Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2007**, *92*, 405–416. [CrossRef]
109. Jiang, S.; Li, X.; Zhang, L.; Sun, W.; Dai, S.; Xie, L.; Liu, Y.; Lee, K.J. Culturable actinobacteria isolated from marine sponge *Iotrochota* sp. *Mar. Biol.* **2008**, *153*, 945–952. [CrossRef]
110. Schneemann, I.; Nagel, K.; Kajahn, I.; Labes, A.; Wiese, J.; Imhoff, J.F. Comprehensive investigation of marine actinobacteria associated with the sponge *Halichondria panicea*. *Appl. Environ. Microbiol.* **2010**, *76*, 3702–3714. [CrossRef] [PubMed]
111. Selvin, J.; Sathiyanarayanan, G.; Lipton, A.N.; Al-Dhabi, N.A.; Arasu, M.V.; Kiran, G.S. Ketide Synthase (KS) domain prediction and analysis of iterative type II PKS gene in marine sponge-associated actinobacteria producing biosurfactants and antimicrobial agents. *Front. Microbiol.* **2016**, *7*, 1–12. [CrossRef]
112. Huang, X.; Kong, F.; Zhou, S.; Huang, D.; Zheng, J.; Zhu, W. *Streptomyces tirandamycinicus* sp. nov., a novel marine sponge-derived actinobacterium with antibacterial potential against *Streptococcus Agalactiae*. *Front. Microbiol.* **2019**, *10*, 1–11. [CrossRef]
113. Madhusoodanan, J. Probing predatory bacteria as an antibacterial remedy. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 22887–22890. [CrossRef]
114. Williams, L.E.; Cullen, N.; Degiorgis, J.A.; Martinez, K.J.; Mellone, J.; Oser, M.; Wang, J.; Zhang, Y. Variation in genome content and predatory phenotypes between *Bdellovibrio* sp. NC01 isolated from soil and *B. bacteriovorus* type strain HD100. *Microbiology* **2019**, *165*, 1315–1330. [CrossRef]
115. Bratanis, E.; Andersson, T.; Lood, R.; Bukowska-Faniband, E. Biotechnological potential of *Bdellovibrio* and like organisms and their secreted enzymes. *Front. Microbiol.* **2020**, *11*, 1–14. [CrossRef] [PubMed]
116. Sun, M.L.; Zhao, F.; Shi, M.; Zhang, X.Y.; Zhou, B.C.; Zhang, Y.Z.; Chen, X.L. Characterization and biotechnological potential analysis of a new exopolysaccharide from the Arctic marine bacterium *Polaribacter* sp. *SMSci. Rep.* **2015**, *5*, 1–12. [CrossRef] [PubMed]
117. Sun, M.L.; Zhao, F.; Zhang, X.K.; Zhang, X.Y.; Zhang, Y.Z.; Song, X.Y.; Chen, X.L. Improvement of the production of an Arctic bacterial exopolysaccharide with protective effect on human skin cells against UV-induced oxidative stress. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 4863–4875. [CrossRef] [PubMed]
118. Rützler, K. Sponges in coral reefs. In *Coral reefs: Research Methods, Monographs on Oceanographic Methodology*; Stoddart, D.R., Johannes, R.E., Eds.; UNESCO: Paris, France, 1978; pp. 299–313.
119. Hooper, J.N.A. 'Sponguide'. Guide to sponge collection and identification. Available online: https://www.academia.edu/34258606/SPONGE_GUIDE_GUIDE_TO_SPONGE_COLLECTION_AND_IDENTIFICATION_Version_August_2000 (accessed on 17 March 2021).
120. Van Soest, R.W.M.; Boury-Esnault, N.; Hooper, J.N.A.; Rützler, K.; de Voogd, N.J.; Alvarez, B.; Hajdu, E.; Pisera, A.B.; Manconi, R.; Schönberg, C.; et al. World Porifera Database. Available online: http://www.marinespecies.org/porifera/ (accessed on 17 March 2021).
121. Schmitt, S.; Hentschel, U.; Zea, S.; Dandekar, T.; Wolf, M. ITS-2 and 18S rRNA gene phylogeny of Aplysinidae (Verongida, Demospongiae). *J. Mol. Evol.* **2005**, *60*, 327–336. [CrossRef]
122. Chombard, C.; Boury-Esnault, N.; Tillier, S. Reassessment of homology of morphological characters in Tetractinellid sponges based on molecular data. *Syst. Biol.* **1998**, *47*, 351–366. [CrossRef]
123. Dohrmann, M.; Janussen, D.; Reitner, J.; Collins, A.G.; Wörheide, G. Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). *Syst. Biol.* **2008**, *57*, 388–405. [CrossRef]

124. Meyer, C.P.; Geller, J.B.; Paulay, G. Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. *Evolution (N. Y).* **2005**, *59*, 113–125. [CrossRef]
125. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
126. Corpet, F. Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res.* **1988**, *16*, 10881–10890. [CrossRef]
127. Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F.O. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **2013**, *41*, e1. [CrossRef]
128. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, 590–596. [CrossRef]
129. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
130. Babicki, S.; Arndt, D.; Marcu, A.; Liang, Y.; Grant, J.R.; Maciejewski, A.; Wishart, D.S. Heatmapper: Web-enabled heat mapping for all. *Nucleic Acids Res.* **2016**, *44*, W147–W153. [CrossRef] [PubMed]

Organic extract of *Geodia cydonium* induces cell cycle block in human mesothelioma cells

FRANCESCO DI MEO[1*], ROBERTA ESPOSITO[2*], ROSSANA CUCINIELLO[1,3], GREGORIO FAVALE[1], MARIO ARENGA[1], NADIA RUOCCO[2], GENOVEFFA NUZZO[4], ANGELO FONTANA[4], STEFANIA FILOSA[1,3], STEFANIA CRISPI[1,2] and MARIA COSTANTINI[2]

[1]Department of Biology, Agriculture and Food Science, Institute of Biosciences and BioResources-UOS Naples, National Research Council, I-80131 Naples; [2]Department of Ecosustainable Marine Biotechnology, Zoological Station Anton Dohrn, I-80121 Naples; [3]Scientific Institute for Research, Hospitalization and Healthcare-IRCCS Neuromed, I-86077 Pozzilli; [4]Department of Chemical Sciences and Materials Technologies, Institute of Biomolecular Chemistry, National Research Council, I-80078 Naples, Italy

Received April 5, 2022; Accepted May 23, 2022

DOI: 10.3892/ol.2022.13406

Abstract. The serious side effects caused by chemotherapeutics and the development of cancer chemoresistance represent the most significant limitations in the treatment of cancer. Some alternative approaches have been developed in recent years, which are based on natural compounds, and have allowed important advances in cancer therapeutics. During the last 50 years, sponges have been considered a promising source of natural products from the marine environment, representing ~30% of all marine natural products. Among sponges, the Mediterranean species *Geodia cydonium* represents a potential source of these type of products with considerable biotechnological interest as pharmaceutical agents. The present study demonstrated the antiproliferative effect of an organic *G. cydonium* extract (GEOCYDO) against three human mesothelioma cell lines, MSTO-211H (MSTO), NCI-H2452 (NCI) and Ist-Mes2 (Mes2), which differ in their sensitivity (MSTO and NCI) and resistance (Mes2) to standard combined treatment with cisplatin and piroxicam. To this aim, the activity of the extract was evaluated by analyzing its effects on cell viability, cancer properties and cell cycle progression by means of colony formation assay, cell cycle analysis and protein expression analysis. The results revealed, in mesothelioma, this extract was able to reduce self-renewal, cell migration and it could induce cell cycle arrest in G_0/G_1 stage, thus blocking cell proliferation. In conclusion, to the best of our knowledge, the present results indicated for the first time that GEOCYDO can contain active compounds able to affect cell proliferation in mesothelioma, suggesting that it could be considered as a potential novel drug source for cancer treatment.

Correspondence to: Dr Stefania Crispi, Department of Biology, Agriculture and Food Science, Institute of Biosciences and BioResources-UOS Naples, National Research Council, Via Pietro Castellino 111, I-80131 Naples, Italy
E-mail: stefania.crispi@ibbr.cnr.it

Dr Maria Costantini, Department of Ecosustainable Marine Biotechnology, Zoological Station Anton Dohrn, Via Acton 55, Villa Comunale, I-80121 Naples, Italy
E-mail: maria.costantini@szn.it

[*]Contributed equally

Key words: *Geodia cydonium* extract, mesothelioma, antiproliferative effect, cell cycle block, solid-phase extraction

Introduction

In recent decades, the search for active compounds from natural sources, mainly for pharmacological applications, has represented an important challenge (1). The increasing incidence of severe diseases, such as cancer, demand an urgent need to discover new drugs. Standard drugs have notable toxicity and their use is often associated with tumor resistance, thus the development of more effective therapies is required. In addition, natural anticancer compounds, unlike synthetic drugs, are able to inhibit tumor growth with minimal side effects (2). Particularly intriguing is the identification of molecules from the marine environment to be used in pharmaceuticals. Notably, oceans cover >70% of the earth surface and display higher biodiversity than the terrestrial environment; therefore, they have become an interesting source for the discovery of novel drugs. To date, oceans are still a largely unexplored environment, suggesting them as promising candidates for sources of biologically active natural compounds (3,4). Notably, soft-bodied sessile marine invertebrates, such as sponges, are able to produce several secondary metabolites for their survival in different habitats, which are used to counterattack predators, and in competition for space and nutrients. These bioactive compounds could be useful in pharmacological, nutraceutical and cosmeceutical applications (5,6).

Sponges represent the most studied marine organisms as sources of bioactive compounds (1,3,4). Previous studies have reported the bioactivity of different marine sponge extracts in several diseases and some extracts have been used to produce commercial anticancer drugs (7). Among sponges, the bioactivity of the Mediterranean sponge *Geodia cydonium* has been poorly characterized. Our previous study reported the anti-inflammatory and anticancer effects of *G. cydonium* organic extracts on breast cancer cells (8,9).

To the best of our knowledge, the present study was the first to evaluate the antiproliferative potential of *G. cydonium* extract (GEOCYDO) in mesothelioma, which is a rare and aggressive type of cancer associated with exposure to asbestos fibers that exhibits high chemoresistance (10). The present study used three human mesothelioma cell lines, MSTO-211H (MSTO), NCI-H2452 (NCI) and Ist-Mes2 (Mes2), which differ in their sensitivity (MSTO and NCI) and resistance (Mes2) to standard combined treatment with cisplatin and piroxicam (11). The aim of the present study was to analyze the effect of GEOCYDO on mesothelioma, a type of cancer characterized by high chemoresistance. The present study indicated that the extract affects mesothelioma cell viability and that the fraction C could be the one responsible for its antiproliferative effects, being the most active against the three mesothelioma cell lines. Furthermore, preliminary chemical analysis of fraction C (12) revealed a complex metabolic profile, which requires further fractionation for identification of the active metabolite. To the best of our knowledge, the present study provided novel findings, as despite the large number of marine compounds assessed as drug candidates in various types of cancer (13), no previous study has referred to their use in mesothelioma.

Materials and methods

Collection of biological material. The present study did not involve protected species. *G. cydonium* (order, Tetractinellida; family, Geodiidae) samples were collected at 20 m in depth by scuba diving at the 'Parco Sommerso di Baia' (Naples, Italy). As soon as the samples were collected, they were stored at -20˚C until further analysis.

G. cydonium extraction. Lyophilized sponge tissue (200 g wet weight) was extracted with methanol at room temperature. After sonication (5 min, 59 KHz, 26˚C), the organic extract was dried under nitrogen flow and maintained at -20˚C until further use. The extraction step was repeated three times. The extract was filtered through Whatman filter paper to recover solvent residues, and was then evaporated at low pressure in a rotavapor at 28˚C and dissolved in methanol. The final extract was dried and stored at -20˚C until use. For the NMR analysis (Pulprog: zg), the dry extract was dissolved in deuterated methanol (CD_3OD) and transferred to a NMR tube. NMR spectra were recorded on Bruker DRX 600 spectrometer equipped with an inverse TCI CryoProbe (Bruker Corporation). Chemical shift values are reported in ppm (δ) and referenced to internal signals of residual protons (CD_3OD, ^1H 3.34).

Cell culture and chemicals. Human mesothelioma cell lines MSTO and NCI, and the human mesothelial cell line MeT-5A were grown in RPMI supplemented with 10% FBS (both from Euroclone SpA), glutamine (2 mM), sodium pyruvate and antibiotics (0.02 IU/ml penicillin and 0.02 mg/ml streptomycin). Human mesothelioma Mes2 cells were cultured in DMEM (Euroclone SpA), supplemented with 10% FBS, glutamine (2 mM), 1% nonessential amino acids and antibiotics (0.02 IU/ml penicillin and 0.02 mg/ml streptomycin). MSTO, NCI and MeT-5A cells were obtained from the American Type Culture Collection, and Mes2 cells were obtained from the Istituto Nazionale per la Ricercasul Cancro-Genova.

Fractionation of the methanolic extract of G. cydonium. A small amount of the active methanol extract (~40 mg) was subject to SPE using CHROMABOND® HRX cartridges (6 ml/500 mg; Macherey-Nagel) on a GX-271 ASPEC (Gilson, Inc.) (12). The extract was suspended in 1 ml distilled water and sonicated (59 KHz, 26˚C), for a few seconds in an ultrasonic bath before loading onto the column, which was previously conditioned with 3 ml methanol and equilibrated with 6 ml distilled water. This fractionation yielded five fractions (A, B, C, D and E) obtained by stepwise elution with H_2O (6 ml), CH_3OH/H_2O 7:3 (9 ml), CH_3CN/H_2O 7:3 (9 ml), CH_3CN (9 ml) and CH_2Cl_2/CH_3OH 9:1 (9 ml), respectively. The total extract (TE) and SPE fractions B-E were tested for cytotoxicity. The organic extract and fractions were then analyzed by thin layer chromatography (TLC) stained with $Ce(SO_4)_2$ and a preliminary chemical characterization was carried out by ^1H-NMR spectrum in CD_3OD. Each SPE fraction was dissolved in deuterated solvent (CD_3OD for GCYD-2B and 2C; $CDCl_3$ for GCYD-2D and 2E) and transferred to an NMR tube to acquire ^1H-NMR spectra (Pulprog: zg), as already reported for *G. cydonium* extract. Bidimensional NMR experiments heteronuclear single quantum coherence edited (HSQCed) (Fig. S1) and heteronuclear multiple bond correlation (HMBC) (Fig. S2) in CD_3OD were also acquired on the active SPE fraction C. NMR spectra were recorded on a Bruker DRX 600 spectrometer equipped with an inverse TCI CryoProbe. Chemical shift values are reported in ppm (δ) and referenced to internal signals of residual protons (CD_3OD, ^1H 3.34; $CDCl_3$, ^1H 7.26).

The extract and fractions were dissolved in 100 mM DMSO and dilutions were made to obtain the different concentrations to be tested, with a final concentration of 0.05% DMSO. Details on fractionation and analysis are reported in Figs. S1 and S2.

To evaluate the bioactivity of SPE fractions, MSTO, NCI and Mes2 cells were treated with 200 μg/ml of the four enriched samples (B-E) for 24 h at 37˚C; this concentration was selected as this concentration of the total extract did not affect cell viability.

Cell viability assay. For each cell line, ~$1x10^4$ cells/well were plated in 48-well plates and were treated with GEOCYDO at different concentrations (50, 150, 300 and 500 μg/ml) for 16, 24 or 48 h at 37˚C in humified atmosphere containing 5% CO_2. For fraction bioactivity analysis, cells were treated with 200 μg/ml SPE fractions B, C, D and E for 24 h at 37˚C. Subsequent analysis with 50, 100, 150 and 200 μg/ml fraction C for 24 h at 37˚C was performed done to establish the half maximal inhibitory concentration (IC_{50}) concentration. For all experiments, cells treated with the same amount of

vehicle (0.1% DMSO; MilliporeSigma) present in the extract were used as a control.

Cell viability was evaluated counting live cells using MTS assay (CellTiter 96; Promega Corporation) according to the manufacturers' instructions. For the MTS assay, treated cells were incubated with 20 μl MTS reagent for 2 h at 37˚C. The absorbance was recorded on a microplate reader at a wavelength of 490 nm (VICTOR Multilabel Plate Reader; PerkinElmer, Inc.). All experiments were performed in triplicate and data are expressed as the mean ± SD.

Colony formation assay. Colony formation was assessed as previously reported by our group (14). For each cell line, 500 cells/well were plated in six-well plates, incubated for 7 days and then treated with 500 μg/ml GEOCYDO for 24 or 48 h under culture conditions before replacing the media. The growth was assessed for a further 7 days and then crystal violet was used to stain the colonies, which were successively counted. Briefly, cells were fixed with formaldehyde (3.7%) for 10 min at room temperature, then washed with PBS and stained for 10 min with crystal violet (0.5%) at room temperature. The absorbance was measured at 595 nm using a microplate reader (Cytation3 ASHI; BioTek Corporation). A scanner (Epson Stylus Photo, PX 650; Epson) was used to capture images of representative plates. All experiments were performed in triplicate and data are expressed as the mean ± SD.

Wound-healing assay. The wound-healing assay was performed as previously reported (14,15). For each cell line, ~$3x10^5$ cells/well were seeded in six-well plates. After overnight incubation at 37˚C, cells were at 90% confluence and wounds were created using a 200-μl pipette tip. After wound generation, cells were treated using the aforementioned culture medium, with 500 μg/ml GEOCYDO or 0.1% DMSO for 24 or 48 h. To analyze cell migration, at least six representative images for each scratch were taken in different areas at different time points. A phase contrast light microscope (DMI8; Leica Microsystems GmbH) was used to capture images of the representative plates. ImageJ software (version 1.52; National Institutes of Health) and its wound healing assay macro was used to measure wound healing. All experiments were performed in triplicate and data are expressed as the mean ± SD.

Cell cycle analysis. After overnight incubation, ~$7.5x10^5$ cells/well were plated in 100-mm plates, serum starved for 24 h and then treated with 500 μg/ml GEOCYDO for 16, 24 or 48 h at 37˚C. PBS was used to wash the cells, which were then fixed in cold 70% ethanol for 30 min at 4˚C. After centrifugation at 850 x g for 8 min at 4˚C, cells were washed twice with cold PBS and cell pellets were dissolved in 500 μl cold PBS. To ensure only DNA was stained, cells were digested for 30 min at 37˚C with 100 μg/ml RNase A. Propidium iodide (50 μg/ml) was then used to stain cells for 30 min at room temperature and the cells were then analyzed by flow cytometry (FACSCanto; BD Biosciences) using FACSDiva software (version 6.1.3; BD Biosciences). A total of $20x10^4$ events were recorded for each sample and the percentage of cell fractions in all cell cycle phases was calculating. All experiments were performed in triplicate and data are expressed as the mean ± SD.

Western blot analysis. Protein extracts were obtained from cells treated with GEOCYDO (500 μg/ml) for 16, 24 or 48 h at 37˚C as previously described (11). Total cell lysates (20 μg) were separated on 4-15% Tris-glycine gels by SDS-PAGE (Bio-Rad Laboratories, Inc.) at 100 V and proteins were then transferred to PVDF membranes (Bio-Rad Laboratories, Inc.). The membranes were blocked in 5% milk in TBS/5% Tween at room temperature for 1 h and were then probed overnight at 4˚C with the specific primary antibodies, and then with horseradish peroxidase-conjugated secondary antibodies (1:10,000; cat. no. A6154; MilliporeSigma) for 1 h at room temperature according to the manufacturer's indications. The primary antibodies used for western blotting include: Anti-cyclin E (cat. no. sc-481; Santa Cruz Biotechnology, Inc), anti-cyclin A (cat. no. sc-596; Santa Cruz Biotechnology, Inc.), anti-cyclin B1 (cat. no. sc-245; Santa Cruz Biotechnology, Inc.), anti-p21 (cat. no. 2947; Cell Signaling Technology, Inc.), anti-p27 (cat. no. sc-528; Santa Cruz Biotechnology, Inc.) and anti-β-actin (cat. no. 3700; Cell Signaling Technology, Inc.), which was used as a loading control, at the concentrations suggested by manufacturers (1:1,000). Clarity western ECL (Bio-Rad Laboratories, Inc.) was used to detect protein bands and the blots were semi-quantified with ImageJ software. All experiments were performed in triplicate and data are expressed as the mean ± SD.

Statistical analysis. Graph Pad Prism 6.0 (GraphPad Software, Inc.) analysis was used to evaluate the difference between control and treatment groups. One-way ANOVA was used to evaluate the significance of the differences among means. Dunnett's multiple comparison test with Bonferroni post hoc correction was used to assess the significance between each treatment group and the control group. $P \leq 0.05$ was considered to indicate a statistically significant difference.

Results

GEOCYDO affects cell viability. To analyze the bioactivity of GEOCYDO on mesothelioma cells, the present study first determined, in a dose-response curve at 24 h, the amount of extract that had a lethal effect on MSTO cells. Results revealed that GEOCYDO had a IC_{50} of ~500 μg/ml in MSTO cells (Fig. 1A). Therefore, a concentration of 500 μg/ml was used for subsequent experiments. By contrast, the same concentration of GEOCYDO had no effect on wild-type Met-5A mesothelial cells (Fig. 1B). The present study also analyzed if the effects of GEOCYDO were increased over the time. As shown in Fig. 1C, cell viability decreased from 16 to 48 h; it was reduced by 75% in MSTO, 70% in NCI and 80% in Mes2 cells at 48 h compared with in the vehicle-treated cells.

Subsequently, cell proliferation and migration were analyzed to evaluate the anticancer potential of GEOCYDO. The results clearly showed that GEOCYDO was able to impair cell proliferation, reducing cellular self-renewal ability and long-term proliferative potential in all of the cell lines tested. Notably, treatment with 500 μg/ml GEOCYDO inhibited colony formation after 24 and 48 h, and the ability to produce colonies was reduced by ~50% after 24 h, and by 65, 60 and 70% after 48 h in MSTO, NCI and Mes2 cells, respectively (Fig. 2A and B). In addition, to measure the migratory

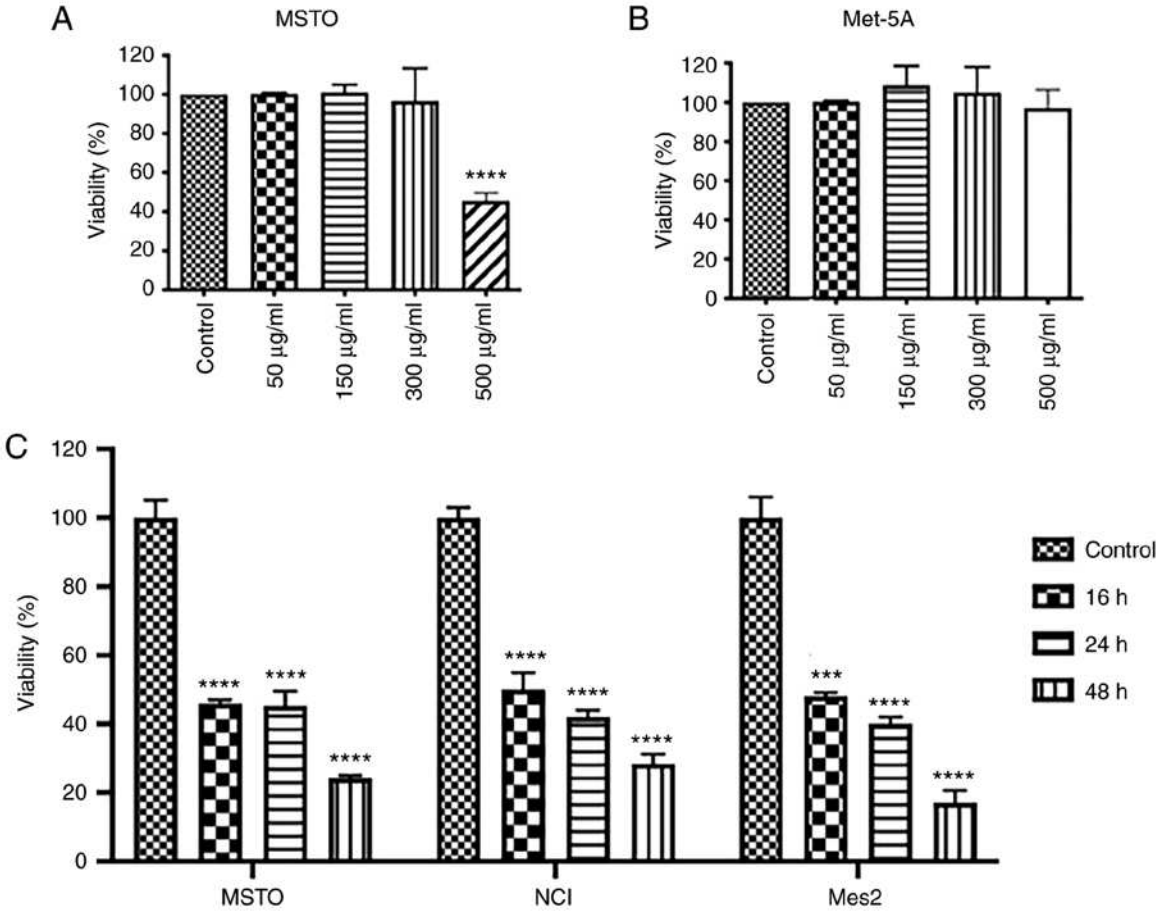

Figure 1. GEOCYDO treatment affects cell viability. Cell viability after treatment with various concentrations of GEOCYDO for 24 h in (A) MSTO or (B) Met-5A cells. (C) Cell viability after treatment of MSTO, NCI and Mes2 cells with 500 μg/ml GEOCYDO for different durations. Cells treated with vehicle only (0.1% DMSO) were used as a control. Data are presented as the mean ± standard deviation (n=3). ***P<0.005, ****P<0.001 vs. control. MSTO, MSTO-211H; NCI, NCI-H2452; Mes2, Ist-Mes2; GEOCYDO, *Geodia cydonium* extract.

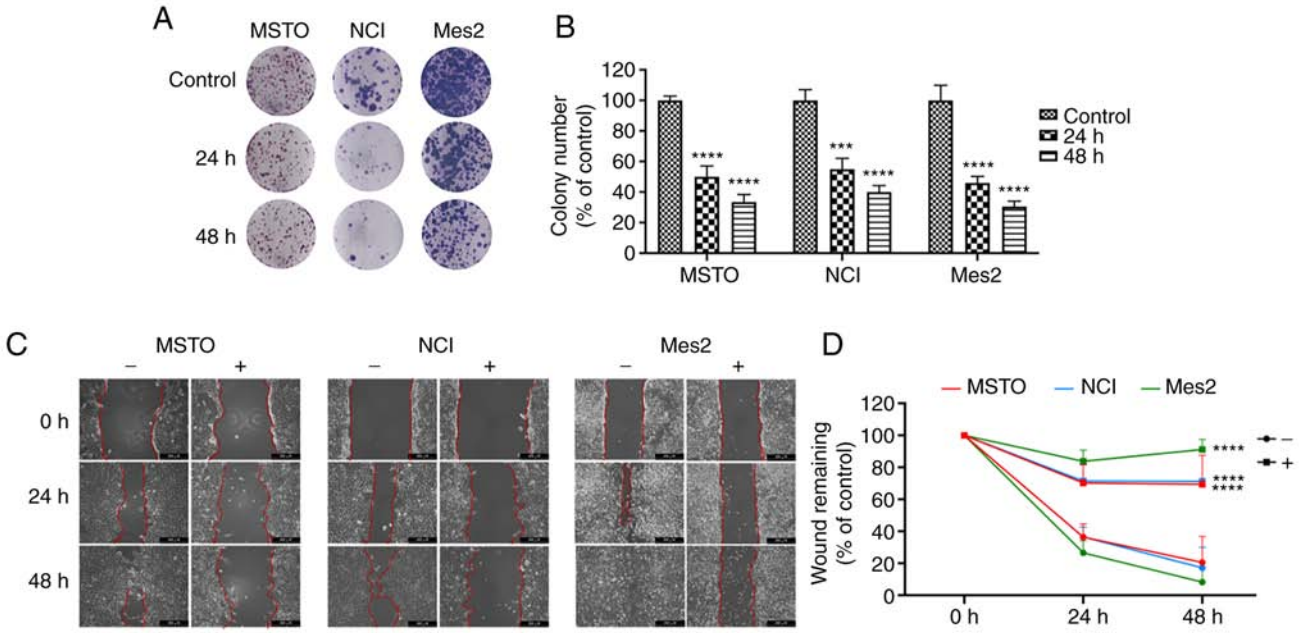

Figure 2. GEOCYDO treatment affects tumorigenic properties. (A) Colony formation assay on mesothelioma cell lines following GEOCYDO treatment. Representative plate images after crystal violet staining are shown. (B) Histograms of the average colony numbers. (C) Wound-healing assay. The wound closure rate was measured by detecting the closure distance at the time indicated in MSTO, NCI and Mes2 cells treated with 500 μg/ml GEOCYDO (+). Cells treated with vehicle only (0.1% DMSO) were used as a control (-). Representative micrographs under a phase contrast microscope are shown. Scale bar, 200 μm. (D) Quantification of wound gap distance. Data are presented as the mean ± standard deviation (n=3). ***P<0.005, ****P<0.001 vs. control. MSTO, MSTO-211H; NCI, NCI-H2452; Mes2, Ist-Mes2; GEOCYDO, *Geodia cydonium* extract.

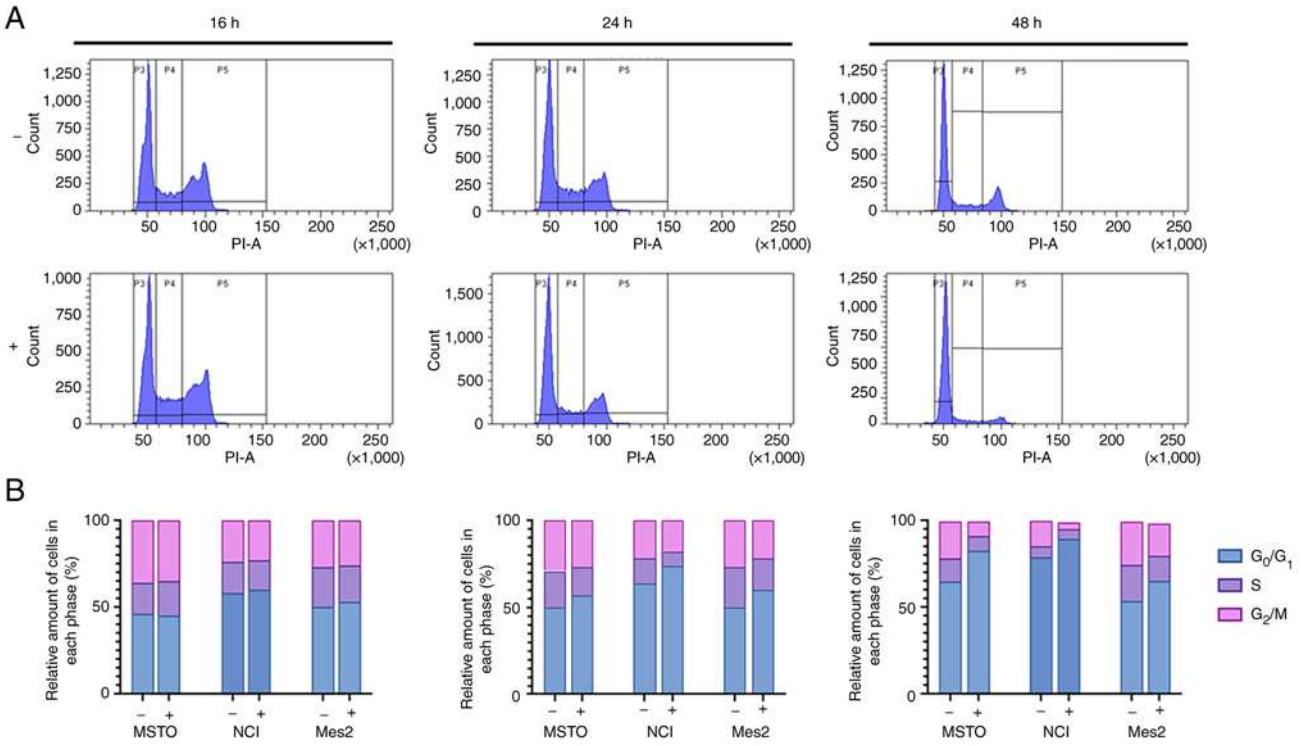

Figure 3. Effect of GEOCYDO on cell cycle progression. (A) Distribution of MSTO cell population during the cell cycle after 16, 24 and 48 h of treatment with 500 μg/ml GEOCYDO (+) analyzed by flow cytometry. Cells treated with vehicle only (0.1% DMSO) were used as a control (-). (B) Histograms of the cell cycle distribution in MSTO, NCI and Mes2 cells. Data are presented as the mean ± standard deviation (n=3). MSTO, MSTO-211H; NCI, NCI-H2452; Mes2, Ist-Mes2; GEOCYDO, *Geodia cydonium* extract; P3, G_0/G_1 cell population; P4, S cell population; P5, G_2/M cell population.

capability of cells, a wound-healing assay was performed, where scratched cells were treated with GEOCYDO. The results showed that GEOCYDO significantly inhibited the ability of cells to close the gap in all of the cell lines (Fig. 2C and D). By contrast, in the untreated cells, the wound gap was closed at the end of the treatment. Notably, this experiment does not completely distinguish if GEOCYDO treatment affects cell migration or proliferation, since it was not performed in low serum conditions; thus, proliferation inhibition was analyzed in more detail.

GEOCYDO extract induces G_0/G_1 cell cycle arrest. To explore the mechanisms underlying the inhibition of mesothelioma cells induced by GEOCYDO, cell cycle distribution was analyzed by flow cytometry. Cells were analyzed following treatment with GEOCYDO for 16, 24 or 48 h, in order to analyze cell proliferation modifications. As shown in Figs. 3 and S1, untreated MSTO cells exhibited a typical cell cycle distribution over time, whereas GEOCYDO treatment induced a cell cycle arrest at 24 and at 48 h, as shown by an increased percentage of the cell population in the G_0/G_1 phase. Notably, alongside the increase in the G_0/G_1 cell population, there was a corresponding reduction in the population of cells in S and G_2/M phases. Similar results were obtained with NCI and Mes2 cells (Fig. 3 and Fig. S3). Specifically, the percentage of cells arrested in G_0/G_1 phase was increased after 24 and 48 h in all of the cell lines analyzed; conversely, a slight decrease in the percentage of cells in S and G_2/M phases was found.

To assess the effects of GEOCYDO, the expression levels of different cyclins and of two CDK inhibitors (CDKIs), p21 and p27, which are crucial for cell cycle progression, were detected. In particular, the expression levels of cyclin E, which is required for cell cycle G_1/S transition, of cyclin A, which is needed for the G_2/M transition, and of cyclin B1, which is the mitotic cyclin, were detected. The analysis indicated a decrease in the expression levels of cyclin E in MSTO, NCI and Mes2 cells, which was in agreement with the observed cell cycle arrest (Fig. 4). Considering that cyclin E can regulate the passage between phase G_1 and S, these results confirmed that GEOCYDO blocked the transition between those two cell cycle phases. Cyclin A exhibited a similar decreased expression in MSTO and NCI cells, whereas it was not modulated in Mes2 treated cells compared with control cells. By contrast, cyclin B1 expression was decreased at 16 and 24 h in MSTO and NCI treated cells compared with in the control cells, and at all timepoints in Mes2 treated cells compared with in the control cells. The differences in the expression of cyclin B1 between the different cell lines could be related to the tumorigenicity of the cell lines. Finally, the decreased expression level of cyclins was accompanied by an increase in the expression levels of p21 and p27 in MSTO and NCI treated cells compared with in the control cells, and of p27 in Mes2 treated cells compared with in the control cells.

Analysis of GEOCYDO and SPE fractions. Spectroscopic analysis of GEOCYDO indicated that the active methanolic extract was very rich in metabolites. Proton signals in the region at low field showed the presence of aromatic compounds (blue arrows in Fig. 5), whereas the abundance of methyl singlets in the region between 2.7 and 3.7 ppm suggested the

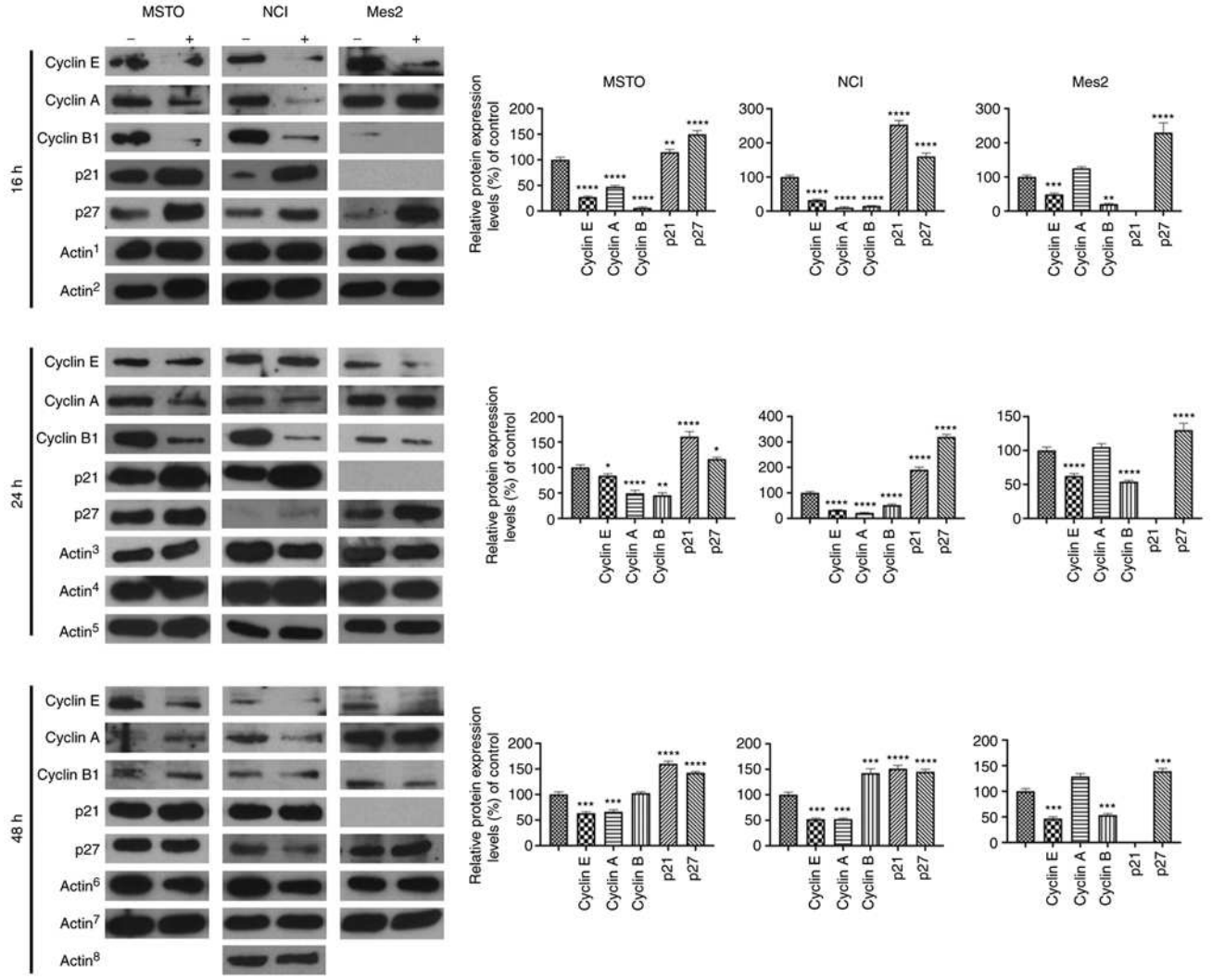

Figure 4. GEOCYDO induces cell cycle arrest at G_0/G_1 phase through modulation of cyclins and CDK inhibitors. Western blot analysis of the expression levels of cyclin E, cyclin A, cyclin B1, p21 and p27 16 h, 24 or 48 h after treatment with GEOCYDO. β-actin expression was used as a loading control. Actin[1] refers to the control for cyclin E, cyclin B1 and p21; Actin[2] refers to the control for cyclin A and p27; Actin[3] refers to the control for cyclin E, cyclin B1 and cyclin A (for NCI cells); Actin[4] refers to the control for p21; Actin[5] refers to the control for p27 and cyclin A (for MSTO and Mes2 cells); Actin[6] refers to the control for cyclin E, cyclin B1 and p21; Actin[7] refers to the control for p27 and cyclin A (for MSTO and Mes2 cells); Actin[8] refers to the control for cyclin A (for NCI cells). Histograms represent the relative expression levels relative to the control. All the controls were set at 100%. Data are presented as the mean ± standard deviation (n=3). *P<0.05, **P<0.01, ***P<0.005, ****P<0.001 vs. control. MSTO, MSTO-211H; NCI, NCI-H2452; Mes2, Ist-Mes2; GEOCYDO, *Geodia cydonium* extract.

occurrence of heteroatom-containing compounds (purple arrows in Fig. 5). Fractionation of GEOCYDO by SPE-HRX column led to five new samples: Salts were eluted in fraction A, whereas the enriched fractions B-E, contained different classes of metabolites. As shown in Fig. 6, according to the expected resolution of the method (12), chemical analysis of the SPE fractions clearly indicated the presence of water-soluble metabolites in fraction A (mainly sugars); nucleosides and nitrogen-containing compounds in fraction B; complex lipids, including sphingolipids, lipopeptides, glycolipids and phospholipids in fraction C; sterols, terpenes, alcohols and fatty acids in fraction D; and triglycerides and neutral lipids in fraction E.

To evaluate the bioactivity of SPE fractions, MSTO, NCI and Mes2 cells were treated with 200 μg/ml of the four enriched samples (B-E) for 24 h; this concentration was selected as this concentration of the total extract did not affect cell viability. Cell viability analysis indicated that only fraction C was able to decrease viability in all cell lines (Fig. 7A). Subsequent analysis using various concentrations of fraction C (50-200 μg/ml) for 24 h confirmed its strong cytotoxicity on all mesothelioma cell lines analyzed, lowering the IC_{50} of GEOCYDO to ~150 μg/ml (Fig. 7B).

Preliminary ^{1}H-NMR and TLC analysis of the enriched fraction C indicated a prevalence in this fraction of polar minor metabolites (Fig. 6). However, although the composition of fraction C is very complex and produced a crowded NMR spectra with several overlapping signals, these data are consistent with the presence of molecules with cyclodepsipeptide or a macrolide skeleton (Figs. S2 and S3). In detail, spectroscopic data of this fraction revealed a chemical signature with several methine groups between 4 and 5 ppm, coupled with carbon between 50 and 60 ppm that could be diagnostic of amino acid skeletons, and various methine and methylene signals between 3.5 and 4.10 ppm coupled with oxygen-bearing carbon between 62 and 75 ppm (Fig. S1). In the HMBC spectrum (Fig. S2),

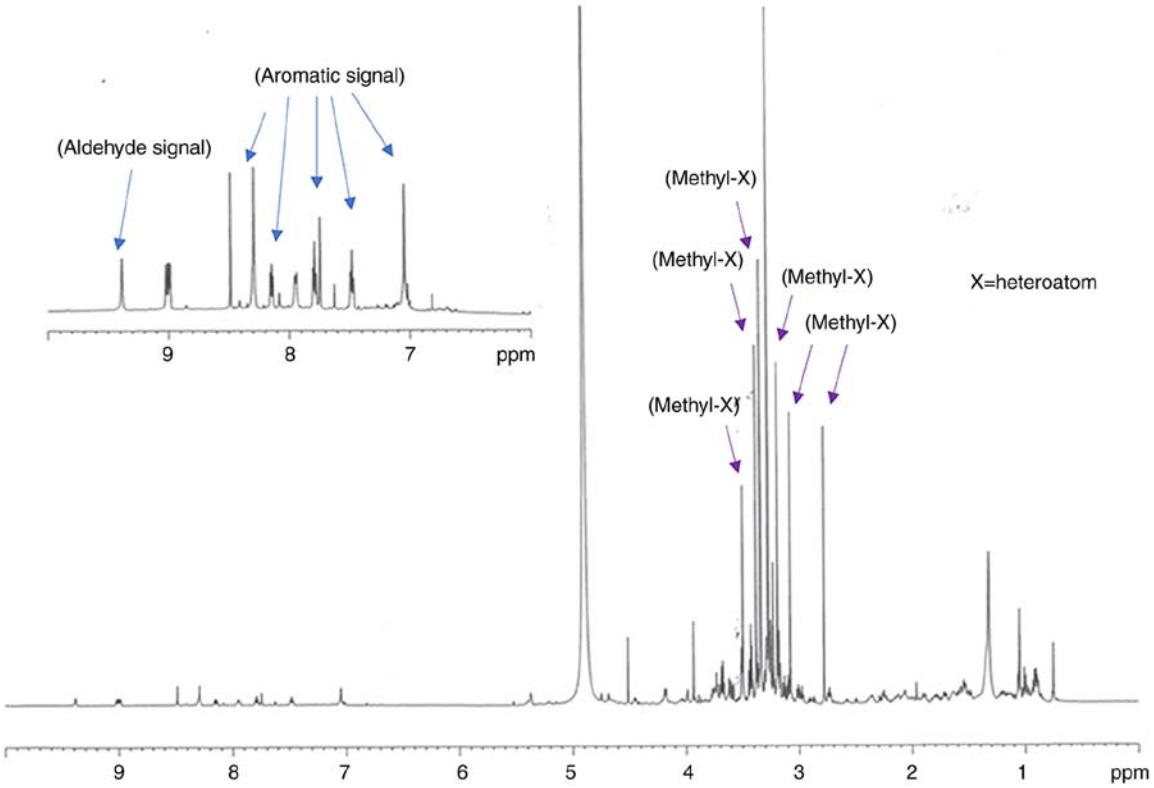

Figure 5. Chemical analysis of GEOCYDO. Proton nuclear magnetic resonance profile of methanolic GEOCYDO (total extract) in CD_3OD at Bruker 600 MHz. The spectrum mainly showed the presence of aromatic compounds (blue arrows), whereas the occurrence of heteroatom-containing compounds was suggested by methyl singlets (purple arrows) in the region between 2.7 and 3.7 ppm. X, heteroatom; GEOCYDO, *Geodia cydonium* extract.

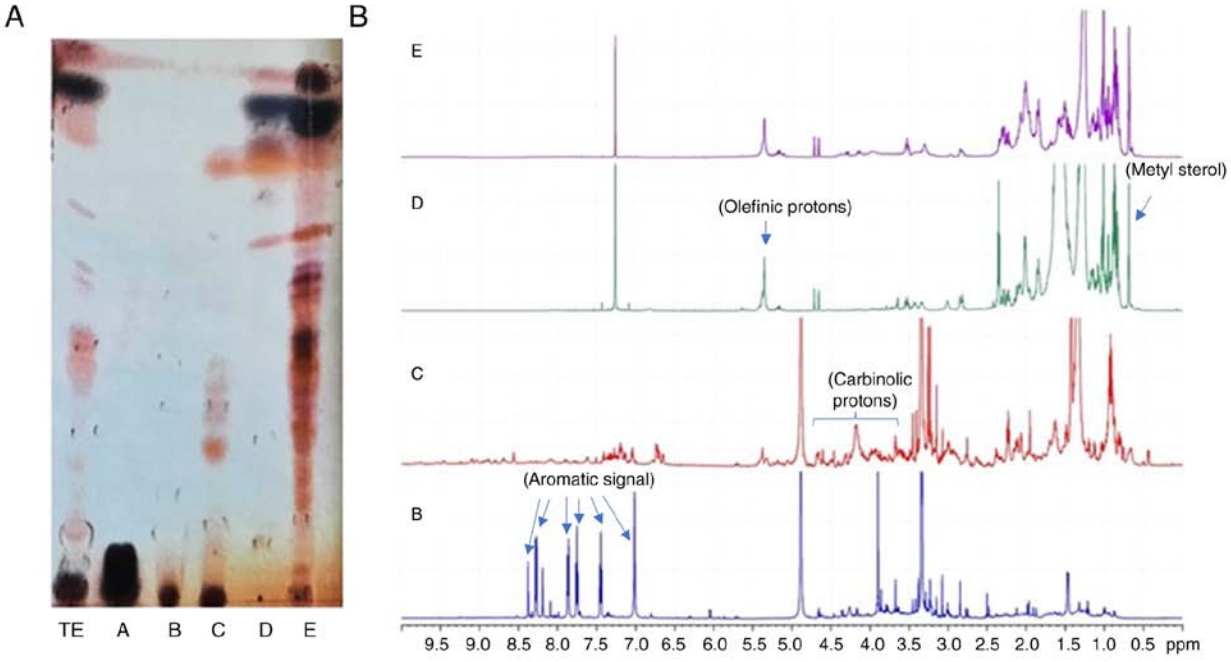

Figure 6. Chemical analysis of GEOCYDO fractions. (A) Thin layer chromatography in $CHCl_3$/MeOH/H_2O 65:25:4 of GEOCYDO TE and enriched SPE-HRX fractions (fractions A-E). (B) The metabolic profile acquired by proton nuclear magnetic resonance of the corresponding SPE-HRX fractions B-E (600 MHz, GCYD-2B and 2C were acquired in CD_3OD; GCYD-2D and 2E were acquired in $CDCl_3$). Blue arrows indicate the highest peaks. The blue bracket indicates carbinolic protons. GEOCYDO, *Geodia cydonium* extract; TE, total extract; SPE, solid-phase extraction.

these signals also showed correlations with ester functions at 170-175 ppm. In addition to methoxy moieties, the NMR spectra supported the presence of a carbonyl group below 210 ppm (Fig. S2), several methyl groups (^{1}H NMR signals at 0.57, 0.76, 0.82, 1.03 ppm; ^{13}C NMR signals at 25.3, 21.1, 15.9, 19.8 ppm), and various down-shifted protons between 6.5 and 7.3 ppm of

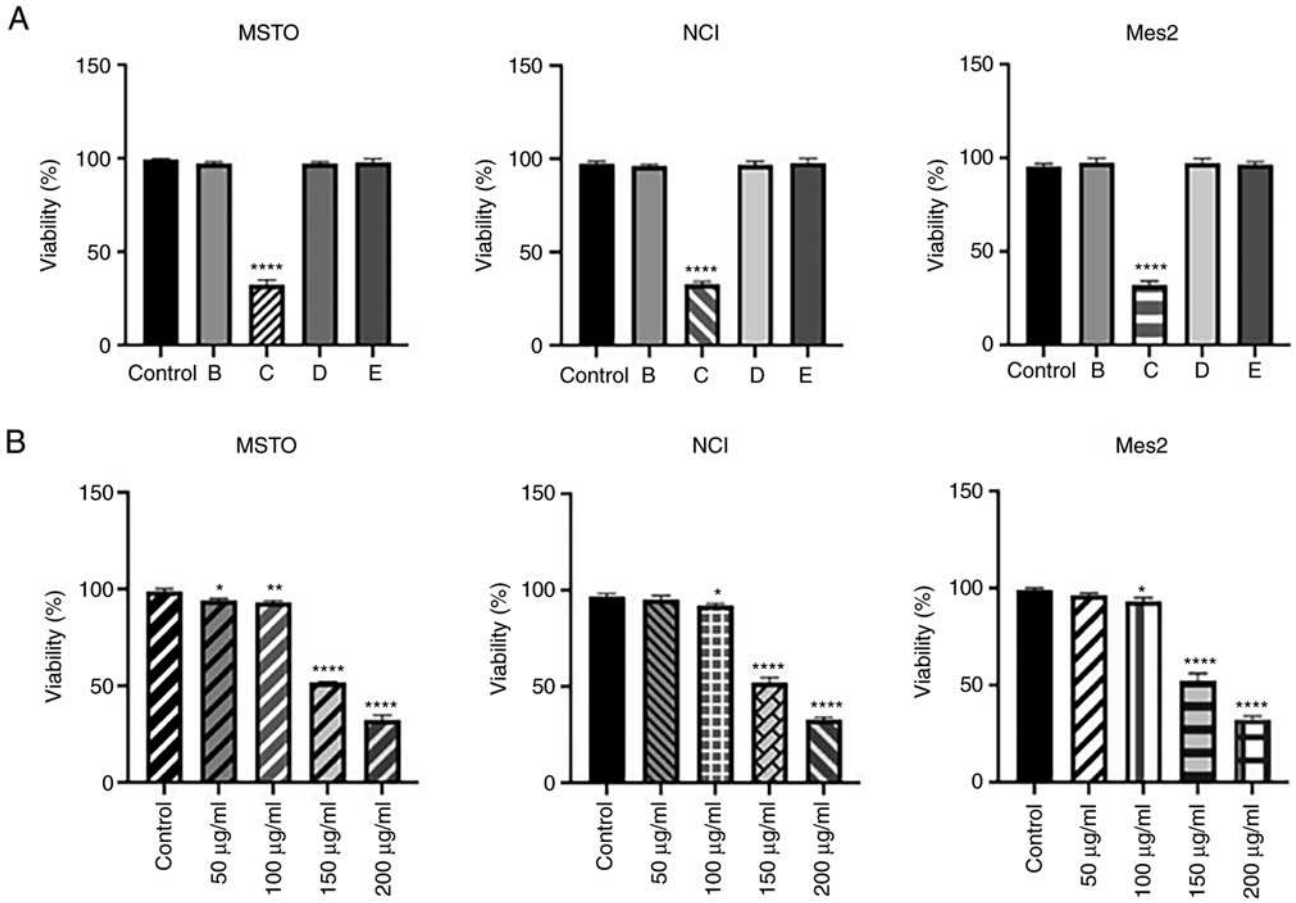

Figure 7. Fraction C affects cell viability. (A) MSTO, NCI and Mes2 cells were treated with 200 mg/ml solid-phase extraction fractions B-E for 24 h. (B) MSTO, NCI and Mes2 cells were treated with 50-200 μg/ml fraction C for 24 h. Cells treated with vehicle only (0.1% DMSO) were used as a control. Data are presented as the mean ± SD of independent experiments (n=3). *P<0.05, **P<0.01, ****P<0.0001 vs. control. MSTO, MSTO-211H; NCI, NCI-H2452; Mes2, Ist-Mes2.

conjugated unsaturated systems (Fig. S1). Unfortunately, the amount of fraction C available was not enough to complete the characterization of the active metabolite.

Discussion

To the best of our knowledge, the present study was the first to describe the *in vitro* antiproliferative effect of GEOCYDO extract on mesothelioma, a rare but very aggressive cancer characterized by high chemoresistance (10,14). Mesothelioma displays a long latency period (30-40 years) and a generally unfavorable outcome (10). Despite several reports in the literature on marine sponges (2,16-18), few studies have assessed the biotechnological applications of one of the major sources of bioactive natural products, *G. cydonium*. Our group previously reported that a methanolic extract from this sponge had an anti-inflammatory and pro-apoptotic effect on breast cancer cell lines (8,9).

The results of the present study clearly indicated that GEOCYDO was able to affect mesothelioma cancer properties acting on cell proliferation in a time-dependent manner. Cytofluorimetric analysis revealed that GEOCYDO induced cell cycle arrest at G_0/G_1 phase, which may induce inhibition of proliferation in mesothelioma. The present findings were confirmed by expression analysis of proteins involved in cell cycle arrest. Notably, specific alterations in the expression levels of cyclins A, E and B1, and of CDKIs p21 and p27, were detected (19).

Cell cycle progression is a finely tuned event regulated by protein kinase complexes containing cyclins and CDKs (20). In response to DNA damage, cell proliferation undergoes arrest until DNA is repaired and correctly replicated. Cell cycle arrest can occur at two specific checkpoints of the cell cycle: G_1/S and G_2/M (21). It is known that cyclin E represents the key molecule of the G1/S checkpoint. The binding of endogenous CDKis, such as p21 and p27, regulates the activity of the complex cyclin E-CDK2 (22). Furthermore, the induction of p21 and p27 arrests cell cycle in the G_1 phase, thus inhibiting the cells from entering the S phase for replication (23). It is known that cyclin E overexpression can promote cancer progression, whereas its downregulation, by limiting cell cycle progression to the G_0/G_1 phase, can decrease and inhibit tumor cell proliferation (24,25). Similarly, p21 and p27 act as tumor suppressors by controlling cell cycle progression and cell proliferation (26-28); decreased expression levels of p21 and p27 have been detected in various types of human cancer (29-31). Finally, the efficacy of GEOCYDO in Mes2 cells that do not express p21, can be ascribed to the activity of p27 that regulates cell cycle progression through decreasing p21 expression (32).

The ability of GEOCYDO to induce G_1/S cell cycle arrest was confirmed by protein analysis, showing a downregulation in cyclin E, cyclin A and cyclin B1 expression, and a concomitant activation of p21 and p27. A hallmark of cancer is represented by uncontrolled and rapid cell division (2); therefore, the inhibition of cell cycle progression may be a powerful anticancer approach.

Natural marine compounds are known to exert anticancer activity *in vitro* and *in vivo* (33,34), as well as in clinical settings (35), by acting on the cell cycle (36). Several extracts have been described to induce cell cycle perturbations in various tumor cell lines. Extracts from the *Lissodendorix* sponge have been described to act by preventing microtubule assembly (37-39). The antiproliferative activity of an extract from the *Negombata magnifica* sponge has been related to specific G_0/G_1 and G_2/M cell cycle block (40). Furthermore, marine sponge compounds derived from *Jaspis stellifera* and *Monanchora viridis* have been reported to induce cell cycle arrest through the reduction of cyclin D1 expression (2,41). These findings are in agreement with the present findings, which demonstrated that GEOCYDO blocked the transition between phase G_1 and S, as indicated by the decrease in the expression levels of cyclin E, the key protein that regulates this passage.

Finally, to the best of our knowledge, the preliminary results from spectroscopic analysis indicated for the first time that GEOCYDO contained different active metabolites. The results are particularly promising since, to date, the knowledge of secondary metabolites derived from members of the genus *Geodia* (class, Demospongiae; family, Geodiidae) remains limited. The bioactivity of GEOCYDO was initially tested using the TE, after which the TE was fractionated and the different fractions were analyzed to identify the active fraction; the results revealed that fraction C was responsible for the bioactivity of GEOCYDO.

Molecular networking analysis of the bioactive GEOCYDO extract revealed that it may contain different molecules, such as nucleosides and amino acids, which are currently providing lead compounds for new drugs as main constituents (9). In particular, previous studies have reported that different nucleosides exert antiviral, anticancer and hypertensive effects (42,43); and that some amino acids have a high specificity against cancer cells (44). Moreover, GEOCYDO was shown to contain 3-hydroxyquinaldic acid, a chromophore present in natural antitumor agents that is required for DNA intercalation being able to binds duplex (45). In addition, the SPE fraction C exerted the strongest activity on mesothelioma cancer cell lines, as revealed by the IC_{50} value, suggesting this fraction may be enriched in a specific molecule that is responsible of the observed effect. Although fractionation of the extract highlighted the general characteristics of the compounds that are likely responsible for the activity of the extract, fraction C is still a complex mixture of metabolites; therefore, further chemical purifications are required to isolate and characterize the active compound.

The present results are novel since, despite the large number of marine compounds used as drug candidates in various types of cancer, to the best of our knowledge, none have assessed their effects on mesothelioma. The results of the present study also suggested that new molecules from marine organisms could be further investigated for novel treatment of this type of cancer, which is characterized by high chemoresistance.

In conclusion, GEOCYDO extract from the marine sponge *G. cydonium* could be considered a novel candidate as a potential antitumor drug for human malignant mesothelioma. This is a very important step in development of alternative and more effective therapies to cure mesothelioma, considering that to date the standard therapies, including chemotherapy, surgery and radiotherapy, have produced unsatisfactory outcomes (46).

Acknowledgements

The authors would like to thank the Dr Laura Pisapia (IGB-CNR FACS Core Facility); Dr Maria Rosaria Aletta (IGB-CNR) and Dr Chiara Nobile (IBBR-CNR) for bibliographic assistance; and Dr Valentina Brasiello (IBBR-CNR) for editing assistance. The authors would also like thank Dr. Enrico Gallocchio ('Parco Sommerso di Baia', Naples), Dr Francesco Terlizzi and Dr Marco Cannavacciuolo (Fishing Service of Stazione Zoologica Anton Dohrn) for providing *Geodia cydonium*; and Dr Davide Caramiello (Marine Organisms Core Facility, Stazione Zoologica) for his technical support in sponge maintenance.

Funding

This research was partially funded by CNR project NUTR-AGE (grant nos. FOE-2019 and DSB.AD004.271). Francesco Di Meo's PhD fellowship in Biology is supported by MIUR project PON 'Dottorati Innovativi con caratterizzazione industriale' 2017-2018. Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. Rossana Cuciniello was supported by a PhD (PhD in Biology XXXVI cycle, University of Naples Federico II) fellowship funded by CNR/IRCCS Neuromed. Nadia Ruocco was supported by a research grant 'Antitumor Drugs and Vaccines from the Sea (ADViSE)' project (PG/2018/0494374).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SC, MC, SF and AF conceptualized the study. FDM, RC, MA and GF performed cellular and molecular biology experiments. GN, AF, NR and RE performed chemical extraction. FDM, RC, MA, GF, NR and RE performed data analysis. SC, MC, NR, RE, FDM and RC were responsible for original draft preparation. FDM, RE, RC, GF, MA, NR, GN, AF, SF, SC and MC confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Skropeta D, Pastro N and Zivanovic A: Kinase inhibitors from marine sponges. Mar Drugs 9: 2131-2154, 2011.
2. Bailon-Moscoso N, Cevallos-Solorzano G, Romero-Benavides JC and Orellana MI: Natural compounds as modulators of cell cycle arrest: Application for anticancer chemotherapies. Curr Genomics 18: 106-131, 2017.
3. Blunt JW, Copp BR, Keyzers RA, Munro MH and Prinsep MR: Marine natural products. Nat Prod Rep 31: 160-258, 2014.
4. Mehbub MF, Lei J, Franco C and Zhang W: Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. Mar Drugs 12: 4539-4577, 2014.
5. Romano G, Costantini M, Sansone C, Lauritano C, Ruocco N and Ianora A: Marine microorganisms as a promising and sustainable source of bioactive molecules. Mar Environ Res 128: 58-69, 2017.
6. Giordano D, Costantini M, Coppola D, Lauritano C, Pons LN, Ruocco N, di Prisco G, Ianora A and Verde C: Biotechnological applications of bioactive peptides from marine sources. Adv Microb Physiol 73: 171-220, 2018. Malve H: Exploring the ocean for new drug developments: Marine pharmacology. J Pharm Bioallied Sci 8: 83-91, 2016.
7. Costantini S, Romano G, Rusolo F, Capone F, Guerriero E, Colonna G, Ianora A, Ciliberto G and Costantini M: Anti-inflammatory effects of a methanol extract from the marine sponge geodia cydonium on the human breast cancer MCF-7 cell line. Mediators Inflamm 2015: 204975, 2015.
8. Costantini S, Guerriero E, Teta R, Capone F, Caso A, Sorice A, Romano G, Ianora A, Ruocco N, Budillon A, *et al*: Evaluating the effects of an organic extract from the mediterranean sponge geodia cydonium on human breast cancer cell lines. Int J Mol Sci 18: 2112, 2017.
9. Crispi S, Cardillo I, Spugnini EP, Citro G, Menegozzo S and Baldi A: Biological agents involved in malignant mesothelioma: Relevance as biomarkers or therapeutic targets. Curr Cancer Drug Targets 10: 19-26, 2010.
10. Baldi A, Piccolo MT, Boccellino MR, Donizetti A, Cardillo I, La Porta R, Quagliuolo L, Spugnini EP, Cordero F, Citro G, *et al*: Apoptosis induced by piroxicam plus cisplatin combined treatment is triggered by p21 in mesothelioma. PLoS One 6: e23569, 2011.
11. Cutignano A, Nuzzo G, Ianora A, Luongo E, Romano G, Gallo C, Sansone C, Aprea S, Mancini F, D'Oro U and Fontana A: Development and application of a novel SPE-method for bioassay-guided fractionation of marine extracts. Mar Drugs 13: 5736-5749, 2015.
12. Calcabrini C, Catanzaro E, Bishayee A, Turrini E and Fimognari C: Marine sponge natural products with anticancer potential: An updated review. Mar Drugs 15: 310, 2017.
13. Di Meo F, Filosa S, Madonna M, Giello G, Di Pardo A, Maglione V, Baldi A and Crispi S: Curcumin C3 complex®/Bioperine® has antineoplastic activity in mesothelioma: An in vitro and in vivo analysis. J Exp Clin Cancer Res 38: 360, 2019.
14. Kauanova S, Urazbayev A and Vorobjev I: The frequent sampling of wound scratch assay reveals the 'Opportunity' window for quantitative evaluation of cell motility-impeding drugs. Front Cell Dev Biol 9: 640972, 2021.
15. Wellington KD, Cambie RC, Rutledge PS and Bergquist PR: Chemistry of sponges. 19. Novel bioactive metabolites from Hamigera tarangaensis. J Nat Prod 63: 79-85, 2000.
16. Sipkema D, Franssen MC, Osinga R, Tramper J and Wijffels RH: Marine sponges as pharmacy. Mar Biotechnol (NY) 7: 142-162, 2005.
17. Varijakzhan D, Loh JY, Yap WS, Yusoff K, Seboussi R, Lim SHE, Lai KS and Chong CM: Bioactive compounds from marine sponges: Fundamentals and applications. Mar Drugs 19: 246, 2021.
18. Malumbres M and Barbacid M: To cycle or not to cycle: A critical decision in cancer. Nat Rev Cancer 1: 222-231, 2001.
19. Lim S and Kaldis P: Cdks cyclins and CKIs: Roles beyond cell cycle regulation. Development 140: 3079-3093, 2013.
20. Kastan MB and Bartek J: Cell-cycle checkpoints and cancer. Nature 432: 316-323, 2004.
21. Bresnahan WA, Boldogh I, Ma T, Albrecht T and Thompson EA: Cyclin E/Cdk2 activity is controlled by different mechanisms in the G0 and G1 phases of the cell cycle. Cell Growth Differ 7: 1283-1290, 1996.
22. Harper JW, Elledge SJ, Keyomarsi K, Dynlacht B, Tsai LH, Zhang P, Dobrowolski S, Bai C, Connell-Crowley L and Swindell E: Inhibition of cyclin-dependent kinases by p21. Mol Biol Cell 6: 387-400, 1995.
23. Lodén M, Nielsen NH, Roos G, Emdin SO and Landberg G: Cyclin E dependent kinase activity in human breast cancer in relation to cyclin E, p27 and p21 expression and retinoblastoma protein phosphorylation. Oncogene 18: 2557-2566, 1999.
24. Lodén M, Stighall M, Nielsen NH, Roos G, Emdin SO, Ostlund H and Landberg G: The cyclin D1 high and cyclin E high subgroups of breast cancer: Separate pathways in tumorogenesis based on pattern of genetic aberrations and inactivation of the pRb node. Oncogene 21: 4680-4690, 2002.
25. Besson A, Dowdy SF and Roberts JM: CDK inhibitors: Cell cycle regulators and beyond. Dev Cell 14: 159-169, 2008.
26. Chu IM, Hengst L and Slingerland JM: The Cdk inhibitor p27 in human cancer: Prognostic potential and relevance to anticancer therapy. Nat Rev Cancer 8: 253-267, 2008.
27. Kreis NN, Louwen F and Yuan J: The multifaceted p21 (Cip1/Waf1/*CDKN1A*) in cell differentiation, migration and cancer therapy. Cancers (Basel) 11: 1220, 2019.
28. Baldi A, De Luca A, Esposito V, Campioni M, Spugnini EP and Citro G: Tumor suppressors and cell-cycle proteins in lung cancer. Patholog Res Int 2011: 605042, 2011.
29. Bachs O, Gallastegui E, Orlando S, Bigas A, Morante-Redolat JM, Serratosa J, Fariñas I, Aligué R and Pujol MJ: Role of p27 ^{Kip1} as a transcriptional regulator. Oncotarget 9: 26259-26278, 2018.
30. Razavipour SF, Harikumar KB and Slingerland JM: p27 as a transcriptional regulator: New roles in development and cancer. Cancer Res 80: 3451-3458, 2020.
31. Gallastegui E, Biçer A, Orlando S, Besson A, Pujol MJ and Bachs O: p27 ^{Kip1} represses the Pitx2-mediated expression of p21 ^{Cip1} and regulates DNA replication during cell cycle progression. Oncogene 36: 350-361, 2017.
32. Ahn JH, Woo JH, Rho JR and Choi JH: Anticancer activity of gukulenin A isolated from the marine sponge. Mar Drugs 17: 126, 2019.
33. Chikamatsu S, Saijo K, Imai H, Narita K, Kawamura Y, Katoh T and Ishioka C: In vitro and in vivo antitumor activity and the mechanism of siphonodictyal B in human colon cancer cells. Cancer Med 8: 5662-5672, 2019.
34. Nastrucci C, Cesario A and Russo P: Anticancer drug discovery from the marine environment. Recent Pat Anticancer Drug Discov 7: 218-232, 2012.
35. Khalifa SAM, Elias N, Farag MA, Chen L, Saeed A, Hegazy MEF, Moustafa MS, El-Wahed AB, Al-Mousawi SM, Musharraf SG, *et al*: Marine natural products: A source of novel anticancer drugs. Mar Drugs 17: 491, 2019.
36. Bergamaschi D, Ronzoni S, Taverna S, Faretta M, De Feudis P, Faircloth G, Jimeno J, Erba E and D'Incalci M: Cell cycle perturbations and apoptosis induced by isohomohalichondrin B (IHB), a natural marine compound. Br J Cancer 79: 267-277, 1999.
37. Bitzer J, Grosse T, Wang L, Lang S, Beil W and Zeeck A: New aminophenoxazinones from a marine Halomonas sp: Fermentation structure elucidation and biological activity. J Antibiot (Tokyo) 59: 86-92, 2006.
38. Sagar S, Esau L, Holtermann K, Hikmawan T, Zhang G, Stingl U, Bajic VB and Kaur M: Induction of apoptosis in cancer cell lines by the red sea brine pool bacterial extracts. BMC Complement Altern Med 13: 344, 2013.
39. Rady HM, Hassan AZ, Salem SM, Mohamed TK, Esmaiel NN, Ez-El-Arab MA, Ibrahim MA and Fouda FK: Induction of apoptosis and cell cycle arrest by Negombata magnifica sponge in hepatocellular carcinoma. Medicinal Chemistry Research 25: 456-465, 2016.
40. Wang R, Zhang Q, Peng X, Zhou C, Zhong Y, Chen X, Qiu Y, Jin M, Gong M and Kong D: Stellettin B induces G1 arrest apoptosis and autophagy in human non-small cell lung cancer A549 cells via blocking PI3K/Akt/mTOR pathway. Sci Rep 6: 27071, 2016.
41. Huang RM, Chen YN, Zeng Z, Gao CH, Su X and Peng Y: Marine nucleosides: Structure, bioactivity synthesis and biosynthesis. Mar Drugs 12: 5817-5838, 2014.

42. Rajan R, Sabnani MK, Mavinkurve V, Shmeeda H, Mansouri H, Bonkoungou S, Le AD, Wood LM, Gabizon AA and La-Beck NM: Liposome-induced immunosuppression and tumor growth is mediated by macrophages and mitigated by liposome-encapsulated alendronate. J Control Release 271: 139-148, 2018.
43. Negi B, Kumar D and Rawat DS: Marine peptides as anticancer agents: A remedy to mankind by nature. Curr Protein Pept Sci 18: 885-904, 2017.
44. Sheoran A, King A, Velasco A, Pero JM and Garneau-Tsodikova S: Characterization of tioF, a tryptophan 2,3-dioxygenase involved in 3-hydroxyquinaldic acid formation during thiocoraline biosynthesis. Mol Biosyst 4: 622-628, 2008.
45. Baldi A, De Luca A, Maiorano P, D'Angelo C and Giordano A: Curcumin as an anticancer agent in malignant mesothelioma: A Review. Int J Mol Sci 21: 1839, 2020.