

Review

Global Changes Alter the Successions of Early Colonizers of Benthic Surfaces

Emanuele Somma ^{1,2}, Antonio Terlizzi ³, Maria Costantini ⁴ , Madalena Madeira ^{1,5} and Valerio Zupo ^{1,*} 

¹ Ischia Marine Centre, Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, 80121 Naples, Italy; emanuele.somma@szn.it (E.S.)

² Department of Life Science, University of Trieste, Via L. Giorgieri, 10, 34127 Trieste, Italy

³ Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

⁴ Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Via Ammiraglio Ferdinando Acton n. 55, 80133 Napoli, Italy

⁵ Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

* Correspondence: valerio.zupo@szn.it; Tel.: +39-081-5833503

Abstract: The successions of benthic communities over time are strongly influenced by the first colonizers, because surface associations are facilitated by modifications to the adhesive properties promoted by primary colonizers, such as bacteria, protozoans, diatoms, algal propagules, spores, and invertebrate larvae. Bacteria are often the first colonizers on marine submerged surfaces, both organic (e.g., algae, seagrasses and invertebrates) and inorganic. However, they are promptly followed by diatoms and other microorganisms. Consequently, diatoms may represent key elements in the determination of the colonization patterns, although the development of epiphytic communities is a dynamic process influenced by several factors, including nutrient availability, the ability to synthesize and secrete extracellular material, the competition among species and the influence of grazers on individual colonizers. The process may be drastically impacted by global warming and ocean acidification due to the increasing atmospheric levels of CO₂. The impact of such global stressors on benthic ecosystems, especially on the primary microphytobenthic assemblages, is still poorly investigated, and may have deleterious consequences for the benthic successions. In this review, we analyze the adhesion patterns of marine microorganisms according to their surface features and the effects of global changes on critical pioneer colonizers, such as the benthic diatoms. The results are remarkable, as they highlight emergent concerns in ecosystem conservation and the prediction of benthic communities.

Keywords: biofilm; diatom; global change; global warming; ocean acidification



Citation: Somma, E.; Terlizzi, A.; Costantini, M.; Madeira, M.; Zupo, V. Global Changes Alter the Successions of Early Colonizers of Benthic Surfaces. *J. Mar. Sci. Eng.* **2023**, *11*, 1232. <https://doi.org/10.3390/jmse11061232>

Academic Editors: Aurélie Blanfuné and Daniel Rittschof

Received: 17 April 2023

Revised: 26 May 2023

Accepted: 13 June 2023

Published: 15 June 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The settlement of organisms anchoring to any submerged surface, including deposit formation, encrustation, crudding, deposition, scaling, scale formation, slagging, and sludge [1], is a well-known phenomenon which has historically been the object of basic and applied studies for its potential influence on many human activities, including the installation of structures in coastal areas and the navigation [2]. Global changes, such as ocean warming and acidification due to increasing $p\text{CO}_2$, are changing the complex relationships between organic and inorganic components, leading to new paradigms and requiring new actions to control the expansion of encrusting communities [3]. However, encrusting organisms are also important components of benthic communities, because various micro- and macro-organisms may succeed up to the reaching of mature communities. Epiphytic communities are crucial in the food webs of important marine habitats, such as those characterizing seagrasses. The complex ecosystems of seagrasses, which provide a nursery for several species of fish and invertebrates, are important for fisheries

and other human activities. They depend on the succession of organisms anchoring to the surface of leaves. Often, this succession starts with bacteria and diatoms, which grow at the base of leaves (the younger portions), followed by algae, bryozoans, polychaetes and other invertebrates (Figure 1), leading to the complete decay of the oldest portion of leaves (the upper parts), which is lost as detritus.

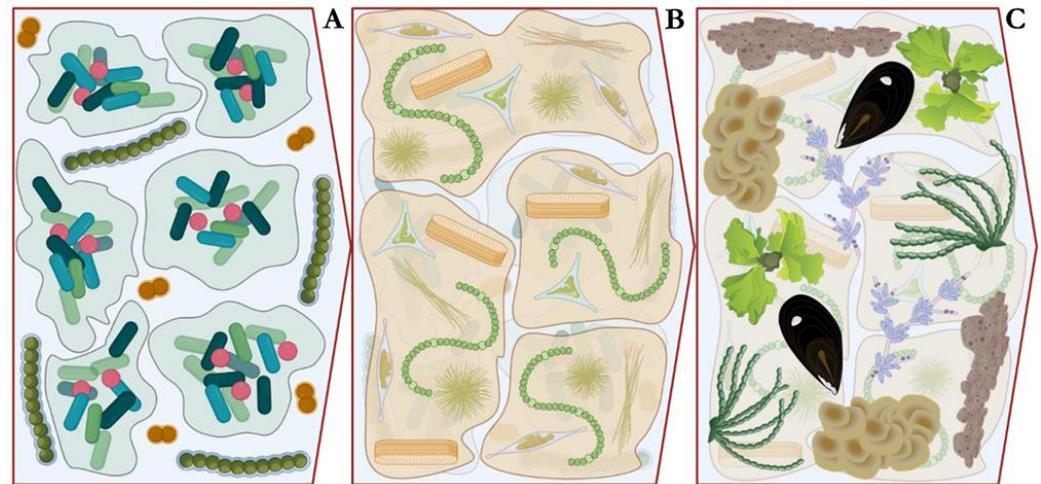


Figure 1. Bacteria are the first colonizers on almost all marine submerged surfaces (A), both live (as algae, seagrasses and invertebrates) and abiotic. They are promptly followed by diatoms and other microalgae, producing a first basal layer (B), named the “conditioning film”. This film is essential for further successions, promoting the adhesion of plant and animal macro-epibionts (C).

The detritus itself is a source of nutrition for a complex food web and a means of exporting organic matter to closer ecosystems and micro-epiphytic matter to other surfaces. Possible alterations in the epiphytic communities occur due to global changes, and they may have consequences both for the control of biofouling on submerged structures and the functioning of ecologically relevant seagrass meadows. Here, we reviewed the available literature to: i. state the role and ecology of the first colonizers; ii. analyze their actual roles in the determination of further successions; iii. analyze the effects of global stressors, such as ocean acidification and warming, on the process of colonization; iv. forecast newer management strategies to control the expansion of organisms encrusting on various submerged structures, while conserving the services provided by complex environments, such as the seagrass meadows.

2. Epiphytes and Early Colonizers

A variety of surfaces, both biotic (e.g., seagrasses leaves, algae, sessile and even motile animals) and abiotic (e.g., rocks, mineral aggregates), are available for the adhesion of benthic organisms in the marine environment [3]. Although all these surfaces are characterized by specific chemical, physical and biological properties, they all share an important feature [4]: they are, sooner or later, colonized by marine microorganisms that establish a complex biofilm [5]. Since the pioneering paper by Zobell and Allen [6], it has been acknowledged that the formation of epiphytic/epizoic communities leads to a predictable succession of events, although the specific interactions involved in the mechanisms of the initial surface adhesion are still largely unexplored [7] and may rule both the benthic communities and the epiphytic associations characterizing any natural substrate in benthic environments. However, clear evidence indicates that the succession events are largely influenced by the nature and composition of the first colonizers [8]. Benthic micro-organisms may be selected as well, according to the molecular properties of the submerged surfaces [9]. Marine biofilms are composed of heterogeneous communities and also contain metabolic by-products [8,10] such as exopolysaccharides and algal mucilage,

which will be further detailed. The development of these communities is a dynamic process influenced by several factors, such as nutrient availability, the ability to synthesize and secrete extracellular material, competition among species and the influences of grazers [11].

Biofilms facilitate the settlement of microorganisms and their shift to a lifestyle that is different from planktonic life [12]. Whether the biofilm matrix confers an ecological advantage to benthic organisms is still unknown. However, a strong evolutionary benefit for polymer-producing cells has been demonstrated [13]. The formation of a matrix starts with the shift from planktonic to benthic life [14]. This process is likely to depend on the bacterial motility and cell-to-cell communication processes, also known as *quorum sensing* (QS) [15,16]. The succession of marine organisms within a biofilm is a complex multi-step process triggered by the formation of a conditioning film composed of adsorbed molecules (mainly glycoproteins), which quickly form on each submerged surface [13].

The development of a given community within a microbial biofilm generally starts with the adhesion of a bacterial consortium to the surface [11]. Bacterial cells produce extracellular polymeric substances (EPS), mainly composed of exopolysaccharides, proteins, nucleic acids, glycoproteins, phospholipids and humic substances [17]. Bacterial colonies encased in their EPS promote the formation of a highly hydrated three-dimensional matrix on which the development and maintenance of a marine biofilm structured community depend [11]. As observed for the initial conditioning film, the EPS–bacteria complex can modify the available surfaces, forming micro-colonies that modify the physical and chemical properties of the substrate and affecting the subsequent colonization by algae, spores and larvae [18,19]. The recruitment of diatoms and cyanobacteria immediately follows, taking advantage of the changes in the surface of the substrates operated by bacteria [20]. Moreover, even organisms such as benthic dinoflagellates, fungi and protozoa can attach themselves to these surfaces by exploiting the mucilage secretions produced by previous colonizers [2]. These communities generally play important roles in determining the structure and dynamics of the overlying benthic communities by enhancing and/or inhibiting the subsequent colonization stages [21]. The settlement of further stages, including spores, macroalgal propagules and larvae of sessile invertebrates [22,23], may be influenced by marine biofilms, which, in several cases, represent a preferred substrate for their attachment [8,24–28]. However, some studies highlighted no effect or inhibitory effects on the settlement of macro-fouler larvae [18,29]. In this context, peculiar examples are provided by the polychaetes *Spirorbis* spp. [30] and the bryozoan *Bugula neritina* [31,32], for which the settlement of larvae is strongly influenced by the biofilm composition. An extreme example of the selection by the attachment surface was also reported by Wieczorek et al. [33] for cyprids of *Amphibalanus* sp. (*Balanus*) and for *Amphitrite* spp. Their larvae were induced to settle on older biofilms, which were characterized by a diverse microbial composition and abundance as compared to immature biofilms. Although some examples were provided for all kinds of effects, different experimental procedures may lead to contradictory responses regarding the relationships between macro-foulers and biofilms [34]. Although there is a noteworthy amount of literature about biofouling on benthic surfaces, the effects of primary biofilms remain partially unclear [35,36].

In addition, a wide range of biotic and abiotic factors may influence the colonization of marine surfaces [9,37]. Many surface properties influence the colonization patterns, especially at the early stages of biofilm formation [20,38]. The roughness of the surfaces represents one of the most relevant factors supporting the adhesion of marine microbes and promoting biofilm formation [39]. To this end, several marine microorganisms exploit the irregularities of the substrates, finding shelter from unfavorable environmental conditions and better adhesion properties [40]. Additional factors related to the physical and chemical properties of the surface and affecting the colonization are wettability [9], surface polarization and tension [41], hydrophobicity [42] and molecular topography [43]. As suggested by Dobretsov et al. [15], the succession patterns of marine biofilm communities are also influenced by a variety of environmental factors. Regarding the physical and chemical vari-

ables, the ones with the greatest influence on substrate colonization are temperature [26], pH [44] and dissolved CO₂ concentration [45,46].

Overall, it is likely that the marine environment will be subject to profound changes in the coming decades, calling for a change in the paradigms of the investigation of epiphytic communities. The general increase in water temperature and ultraviolet radiation, along with plastic pollution and changes in salinity and OA will affect the survival of dispersal stages and their development and recruitment on various submersed surfaces, both natural (such as seagrass leaves) and man-made (such as ship hulls, pipework, grates and ponds) [47]. Consequently, our aim is to analyze the mechanisms of adhesion and the patterns of colonization that characterize both the epiphytic colonization and the benthic communities in view of global changes. In fact, climate changes impact the features of the pioneer microbial communities and their production of metabolites, which, in turn, influence the settlement of dispersal stages and the fate and development of further communities [48]. For this reason, it is important to analyze the mechanisms of action of the first colonizers that follow the adhesion of bacterial films, which are mainly represented by diatoms and other micro-algae [49]. This topic will be analyzed in the following paragraphs, as well as the influence of global changes, to detect various important aspects for the ecosystem conservation.

In recent research, Khalaman et al. [50] investigated the communities while they were allowed to develop on the top and bottom sides of horizontal plates. After a given period of exposure, all the plates were inverted. When the substrate orientation was reversed, the community adapted to the new lighting conditions. Consequently, an increased light intensity resulted in the degradation of zoocoenosis and changed its structure. In contrast, shading did not lead to degradation or changes in the algocoenoses, but stunted algal growth. These results indicate that algocoenoses are more stable and more competitive than zoocoenoses in early communities [51]. In parallel, detailed examples of chemically mediated interactions over seagrass and seaweed surfaces were quantified in situ and a biological effect was determined. Steinberg and de Nys [52] investigated both the deterrents and inducers of colonization on various surfaces. For example, seaweed secondary metabolites, such as brown algal phlorotannins, play an ecologically important function as a chemical defense against epiphytes and they retard the colonization of epiphytic organisms. Similarly, *Fucus* sp. phlorotannins have the potential to inhibit the settlement of invertebrate larvae, but that settlement inhibition cannot explain the lower abundance of the barnacle *Balanus improvisus* on *Fucus evanescens* compared to *F. vesiculosus*, because the mechanism of *F. evanescens* acts on post-settlement stages of *B. improvisus* [53]. Consequently, Steinberg and de Nys [52] described the interactions with the associated bacterial biofilms at the surface of seaweeds and seagrasses. There is evidently a strong need to integrate ecology, cell biology, and chemistry to understand the distribution of surface-active compounds in situ and their ecological and physiological effects. This multidisciplinary approach, merging ecological knowledge to produce biotechnologies, is indispensable to research on “biofilms grown on other organisms or surfaces”. Recently, molecular tools were developed to characterize bacterial communities. These tools opened an entirely new area of research in marine chemical ecology. An integrated approach to the topic is evidently needed, because many aspects of disparate fields (e.g., larval settlement, algal pathogenesis, the molecular biology of bacterial signaling and biotechnologies linked to the control of adhesion) may be investigated within the framework of chemical mediations, facilitating the colonization of submerged surfaces.

3. The Role of Diatoms and the Mechanisms of Adhesion

Diatoms are a widespread and ecologically important group of microalgae (phylum Chrysophycophyta, class Bacillariophyceae). According to cell symmetry, they are divided into two groups: centric and pennate. Centric diatoms are characterized by radial symmetry and they are generally planktonic. Pennate diatoms are bilaterally symmetrical and mostly benthic [54]. Many are characterized by the *raphe*, an elongate fissure that

longitudinally crosses each valve. In terms of algal diversity, they are only surpassed by green algae, representing the most diverse group of primary producers, with approximately 200,000 known species. Diatoms are major contributors to the primary production of marine and freshwater environments [55], accounting for 40% of the global primary production of oceans [56,57].

Diatoms occupy a wide range of aquatic environments. In particular, benthic diatoms adhere to any submerged surface, contributing to primary and secondary biofilm formation [2]. Benthic diatoms are among the most frequent algal early colonizers of natural and artificial substrates, of which they are the main colonizers, strongly modifying the surface properties and the ecological functions. They can also settle on sand and rocks as epiphytes of macroalgae and seagrass leaves, or even form colonial “mats” according to the species and the local ecological conditions [58], producing conspicuous amounts of adhesive mucilage during the formation of primary biofilms [59]. Several microorganisms may form a “biofilm” (Figure 1), an assembly of adherent cells and their products on a given surface [1]. The initial event of biofilm formation on every submerged surface is represented by the adsorption of an organic layer [60]. The adsorption of the organic layer is often referred to as a conditioning film, and is quite a fast process, lasts a few seconds, and follows a predictable pattern. The so-called “molecular marine conditioning film” (MMCF) is adsorbed by a clean surface just a few seconds after its immersion in the sea. During this process, the surface chemistry of the substratum changes due to the adsorbed film and the MMCF makes the adhesion of microorganisms possible [1]. Normally, the adsorption is followed by the attachment of microorganisms (bacteria are considered to be the initial colonizers) and, further, by more complex and larger organisms (such as diatoms, other algae, and invertebrate larvae, in this order) [4,8,10]. However, there is no obligatory sequence in any succession, because various factors, both biotic and abiotic, may influence the process and, as indicated above, OA and other changes in the marine environment induce changes in the initial colonizers that are further reflected in the speed and direction of the colonization process. According to the hypothesis by Baier [61], the film is glycoproteinaceous, and the receptors that are present on the diatom surface could be similar to the sugar receptors. Such receptors should be able to bind to the terminal sugars of the carbohydrate side chains of the adsorbed layer and set in motion the cascade of events that leads to the secretion of an adhesive polymer by the cell.

It is known [62] that diatoms can attach to clean surfaces in the laboratory, even in the absence of bacteria. The cells of diatoms may contact an available substrate in many ways, primarily by chance or due to random events, such as currents, random movements of the medium or gravity [2]. Adhesive events referred to diatoms have been reviewed by Wigglesworth-Cooksey and Cooksey [63], indicating that a combination of physical and chemical signals synergistically influence the process. The first contact triggers the initial adhesion process, which is reversible, during which the cells consume energy to control the attachment, depending on the inhabitability of the surface [3]. When diatoms are suspended in the water, some polymers diffuse from the cells. To start the process of diatom adhesive synthesis, when a diatom contacts a surface, the diffusion of adhesive compounds is reduced by the proximity of the surface. Consequently, local extracellular polymer concentration will increase. If such a local increase in polymer concentration is sensed by cell surface-bound receptors, the same signal might initiate an increased polymer synthesis [63]. Chemotaxis in diatoms appears to be receptor-controlled and, as mentioned above, is mediated by Ca^{2+} -channel. At least three types of receptors for simple sugars are described but how such receptors may sense a given surface is still ignored [64]. However, in this starting phase, the cells begin to secrete extracellular polymeric substances (EPS) that are responsible for the adhesion, the motility, the sessile attachment and the habitat stabilization. Almost simultaneously, the formation of an adhesion complex (AC) is triggered. The AC formation and its important role in cell adhesion and motility has been investigated and defined by Wetherbee et al. [3] and may be visualized as a continuous process acting as a bridge between the actin filaments of the

cytoskeleton and the substrate, made by polarized molecules and associated intracellular proteins. It has been demonstrated that adhesion, as well as motility, is Ca^{2+} -dependent but not light-dependent [65]. This process requires metabolic energy to sustain the synthesis of proteins and, in particular, glycoproteins. Ca^{2+} -channel blockers act at the cell membrane and prevent the adhesion of diatoms, confirming that intracellular Ca^{2+} is required [64]. The key role played by Ca^{2+} in the adhesion process by diatoms may explain why OA so drastically influences their assemblages, taking into account its buffering influence and the mutual relationships with the $p\text{CO}_2$ and the pH. In addition, such an extensive set of biochemically and energetically expensive events is evidently under the metabolic control of the diatoms (e.g., through modulation of the transmembrane signaling processes) and it is known that pH changes may dramatically impact the physiology of cells [66].

Diatoms produce EPS, which is also used for the adhesion to any substratum and secreted through the pores and the *raphe* [67]. Consequently, EPS play a critical role in the establishment of mature epiphytic communities and the development of communities. For centric and araphid pennate diatoms, little attention has been paid regarding the primary adhesion processes, since they are mostly planktonic [2,3]. The cells are likely to adhere almost instantly to the surfaces upon first contact, facilitated by the EPS layer that envelops the entire frustule, as observed in *Toxarium undulatum* by Dugdale et al. [64]. Moreover, within a few minutes from the first contact, the AC is triggered and other adhesive mucilaginous substances are secreted, which confer stronger adhesion and stability to the cells. That mucilage, mainly composed of exopolysaccharides, glycoproteins and nucleic acids encapsulating the cells, contributes to the constitution of a kind of pad that is strongly anchored to the substratum [2].

Remarkably, the settlement on a given substrate of the raphid pennate diatoms is quite different. It is worth noting that diatoms move by gliding [68]. They cannot move when they are not adherent, and the signals that induce motility (chemotaxis) must also induce adhesive mechanisms. Consequently, after the first contact the cells may come into contact directly due to the *raphe*, leading to the immediate formation of the AC and producing EPS that provide strong attachment to the substrate [69,70]. More often, diatom cells land on their side and only the girdle band of the cell remains in contact with the surface [69]. In this case, the diatom starts to produce EPS, which protrudes through the *raphe*. Further, the cells move to a set with one *raphe* in contact with the surface, using those extensions of mucilage and the motility apparatus to pull themselves up [3]. This process was observed and described for the first time by Lind et al. [69] regarding the marine diatoms *Stauroneis decipiens*. In conclusion, it is possible to summarize that benthic diatoms represent key organisms that ride through primary and secondary biofilm formation.

It should also be noted that a natural biofilm growing on any shallow marine surface will probably contain many types of unicellular organisms, including bacteria and diatoms. In contrast, as a rule, experimental studies on the adhesion mechanisms currently consider cells that are attached to various substrates in axenic cultures. This could easily be explained by the need to model single processes because, given the difficulties concerning the study of cultures of even a single bacterial species, it is reasonable to assume that a mixed microbial population including bacteria and diatoms would be quite difficult to experimentally afford [71]. However, a few investigations (e.g., [70,72]) considered mixed populations of one species of diatoms and some bacteria. For example, diatoms of the genus *Amphora* were experimentally attached in a known ratio, along with the marine bacterium *Vibrio proteolytica*, to the surfaces of synthetic Petri dishes, and surprisingly demonstrated that bacterial growth (as measured by 3H-thymidine incorporation) was dependent on the metabolism of diatoms [70]. This result might be due to the cell damage that occurred during the harvesting process, which provided the bacteria with assimilable carbon, which is not available in the intact film. In addition, natural substrates could represent themselves as an important source of nutrients, and this element may be crucial to understand the differences in the observed colonization patterns, for example, on natural substrates (as *Posidonia oceanica* leaves) as compared to abiotic substrates such as rocks and vessel

hulls [73]. For example, marine sediments in near-shore areas are generally covered with diatoms, although the wave action and the character of the sediment may produce water turbidity and drastically reduce the light penetration [74]. Interestingly, these sediments may contain much more dissolved organic carbon in their pore water than the overlying water column, thus triggering the bloom of various microorganisms, including diatoms. Under these conditions, diatoms may be attracted by chemotactic processes. In fact, positive chemotaxis to a small number of organic compounds was demonstrated at least in two diatoms of the genus *Amphora* by Cooksey and Cooksey [63]. The chemotactic ability is common in motile, pennate diatoms. Consequently, it is likely that they use this behavior to move towards higher concentrations of nutrients, and thus may be largely involved in the biogeochemistry of sediments and of some natural substrates, such as seagrass leaves.

In contrast, phototaxis has been a well-known process since the last century, justifying the abundance of diatoms on some substrates [75]. Consequently, a microorganism capable of mixotrophy or heterotrophy in a gradient of a metabolizable compound and in reduced light is potentially offered a choice, to which it should respond by tuning the possible movements according to the signals collected through its receptors. In fact, as noted above, the operation of chemotaxis requires the synthesis of specific proteins, the differential occupancy of these receptors by chemotactically active compounds [76], the transduction of the receptor signal [64] to the motility apparatus and, at least in some diatoms, the differential interpretation of that signal at the two raphes; otherwise, directional response (turning) would not be possible [63]. The distinction between cell responses during chemotaxis and the products of transmembrane transport is significant because it is more important to have a specific uptake mechanism than a highly specific chemotactic mechanism.

Of course, one of the key reasons for much of our interest in the ecology of marine biofilms, and of diatoms in particular, derives from their nuisance value to man-made structures. It is well known [77] that the deterioration of the immersed surface of a ship, which leads to increased drag, loss of performance, increased fuel consumption and corrosion, is caused by macroinvertebrates that cause calcareous deposits (e.g., barnacles and oysters). These bear much of the responsibility for these problems, but it is also believed that the final result is due to the initial biofilms that settled on those surfaces, consisting only of microorganisms such as bacteria and diatoms. At present, we know that the consequences of the adhesion and growth of microorganisms on the hull of a ship are very significant, despite their thin and innocuous aspect. In the past, these phenomena were controlled by painting the hulls with toxic compounds such as tributyltin (TBT). At present, in most parts of the world, TBT and its derivatives can no longer be used due to their higher pollutant power and strong and documented impacts on benthic and planktonic organisms. Based on the previous considerations, it is evident that the materials used to replace them must control microbial adhesion as well as the adhesion of higher forms of life, and that the physiologic modifications due to global changes must be taken into account to forecast their effects on target foulers, as well as on the marine biota [78]. Consequently, we should improve our knowledge of the mechanisms of adhesion of diatoms, because they are essential to conserving diverse epiphytic communities in complex ecosystems and facilitating the adhesion of foulers on man-made structures.

Molecular and Genic Aspects of the Anchoring Mechanisms

Diatoms are well known for the strength of their adhesion to natural and man-made surfaces and most research on diatom adhesion aimed to develop anti-fouling coatings. Although there is little information concerning the attachment of microalgae, several extracellular proteins have been identified, which are required for the adhesion of bacterial biofilms, including amyloid-fibers for biofilm matrix cohesiveness, and flagella for bacterial attachment to abiotic surfaces [79]. The molecular mechanisms enabling the production of these substances are essential to the ecology of first colonizers in a changing climate. Diatoms adhere through a material composed of both proteins and carbohydrates, secreted by the raphe [80]. It is noteworthy that the extreme events of global changes, such

as global warming and ocean acidification, affect the molecular pathways involved in the anchoring mechanisms to modify them, with negative consequences for the control of encrusting organisms. A bioinformatic-based approach was applied to the diatom *Amphora coffeaeformis*, one of the most common pioneer colonizers, which is used in many studies as a model organism for underwater bio-adhesion [81–83], shedding light on the molecular mechanisms of underwater adhesion and biological silica formation in diatoms. A normalized transcriptome database was generated from this diatom, which permitted the identification of five proteins exhibiting unique amino acid sequences with high similarity to the amino acid composition of the tyrosine-rich adhesion proteins from mussel footpads [83]. In addition, the molecular genetic transformation of *A. coffeaeformis* enabled an investigation into the functional characterization of one of these proteins, AC3362, revealing that it is not involved in adhesion, but in the biosynthesis and/or structural stability of the cell wall. The analysis of the amino acid sequence of AC3362 revealed a high content of lysine and tyrosine residues, resembling the cingulins of the diatom *Thalassiosira pseudonana* and the silaffin-1 peptides from *Cylindrotheca fusiformis* [79], which are known to be tightly associated with the biosilica and thus cannot be extracted from the cell walls. Global stressors, such as seawater warming and acidification, could affect the expression of these genes, affecting the cellular machinery used for silica morphogenesis and mechanical performance in diatoms [84]. It is important to consider that these genes are phylogenetically well-conserved throughout the diatom realm, and essential for the biogenesis of mechanically robust diatom cell walls.

A further proteomic analysis of *A. coffeaeformis* adhesive compounds led to the identification of 21 proteins, of which 13 are diatom-specific [85]. Ten of those proteins shared a conserved C-terminal domain, termed the GDPH domain, which is not ubiquitously present but quite widespread in all diatom classes. Some of these proteins (Ac629, Ac1442 and Ac9617) are components of the adhesive trails secreted by cells on surfaces, as revealed by the immunofluorescence localization of a GDPH domain. Raphid diatoms secrete carbohydrate-rich extracellular polymeric substances (EPS) related to their motility and underwater adhesion [85]. Stress conditions may induce the up- or down-regulation of the genes that encode for these adhesive proteins, leading to adhesion problems for benthic diatoms, which may be seasonally abundant on the leaves of *P. oceanica*, a preferred food item for marine benthic invertebrates [86–89]. In parallel, similar constraints play a critical role in the adhesion of diatoms to man-made structures, influencing the development of the encrusting communities.

Several proteins related to carbohydrate metabolism were also identified, comprising one glycoside hydrolase and seven exostosin-like proteins, which are known in mammalian cells to encode for glycosyltransferases involved in the synthesis of sulphated proteoglycans present in the extracellular matrix. Interestingly, these sulphated polysaccharides/proteoglycans were also identified in the EPS and cell-wall-associated glycoproteins of other two benthic diatoms, *Stauroneis amphioxys* and *Craspedostauros australis*, suggesting their key role in EPS synthesis in diatoms [12].

In the oceans, microalgae–bacteria interactions play key roles in nutrient exchange processes and marine aggregate formation, mainly leading to the cycling, mineralization, or sedimentation of organic carbon. Aiming to study these interactions at the cellular scale, an *in vitro* interaction model system, consisting of the diatom *Thalassiosira weissflogii* and the gamma-proteobacterium *Marinobacter adhaerens* HP15, was established [90]. This bacterium is able to attach to *T. weissflogii* cells, inducing transparent exopolymeric particle formation, which can increase the formation of marine aggregates. Its investigation facilitated the detection of 30 bacterial promoters that were specifically expressed during interactions with *T. weissflogii*, whose corresponding genes could be involved in the biochemical detection of diatom cells, bacterial attachment, metabolic exchange of nitrogen compounds and resistance toward heavy metals [90]. The interaction between *M. adhaerens* HP15 and *T. weissflogii* is multifactorial, involving a complex network of physiologic processes, which could be affected by multiple environmental stressors. In fact, temperature increases can

induce shifts from the co-existence to competition in the use of inorganic and organic carbon by microalgae and bacterial communities [91].

Fu et al. [92] provided molecular evidence of the mechanisms of the morphological shifts that occur during surface colonization, identifying candidate target proteins for the screening of eco-friendly anti-biofouling compounds. G-protein-coupled receptor (GPCR) genes, activators of the surface colonization pathway, were found to be differentially expressed in the model marine diatom *Phaeodactylum tricornutum* [92]. Moreover, the transformation of *P. tricornutum* with constructs expressing individual G protein-coupled receptor (GPCR) genes were able to induce cells to adopt a morphology that is adequate for surface colonization. These transformed cells resulted in a 30% increase in resistance to UV light exposure compared to their wild-type counterparts, together with the increased silicification of cell walls, which, in turn, was usually associated with the oval morphotype. GPCRs are the largest family of membrane proteins and mediate many physiological functions through their interaction with heterotrimeric G proteins, also mediating most cellular responses to hormones and neurotransmitters, important cell recognition and communication processes.

A genome-enabled bioinformatic approach was also applied in *P. tricornutum* to identify putative molecules for cell-substratum adhesion (PDC) [93]. The analyses detected the presence of molecules that diatoms may use for cell-substratum adhesion: PDC4, an arabinogalactan fasciclin-like protein that is putatively a cell-surface molecule involved in secretion; PDC7, a c-type lectin, is a molecule involved in carbohydrate binding and cell-cell adhesion; PDC8, involved in cell-substratum adhesion by increasing the adhesive molecules that are exposed on the surface of the mucilage; PDC9, a homolog to fasciclin proteins involved in interactions within the mucilage; PDC10, a fasciclin-like molecule with a role in cross-linking. The fasciclin-like proteins in *Arabidopsis thaliana* play a role in maintaining proper cell expansion under salt-stressed conditions [94].

Benthic diatoms represent key primary producers in coastal environments, with important ecological functions in nutrient cycling and sediment stabilization. A reference genome for the marine biofilm-forming diatom *Seminavis robusta* showed that genes that were differentially expressed within diatom-bacteria interactions were strongly conserved in other benthic diatoms, while many species-specific genes were strongly upregulated during sexual reproduction [95]. The von Willebrand factor, type D domains (vWDs) was found in several extracellular proteins, and is usually involved in protein multimerization and maturation of the adhesive into multi-protein complexes. In fact, several adhesive proteins, such as zonadhesin, sea star foot protein and diatom adhesive trail proteins, contained vWDs [85,96,97], 61 of which also had the diatom-specific conserved C-terminal domain GDPH, which is important for diatom-secreted proteins involved in motility, mucilage pads and gamete fusion. The vWD family is abundant in raphid species and is differently expressed in *S. robusta*, with increases in the expression of genes related to bacterial interactions and recognition, motility and cell adhesion. This expansion of the vWD family may suggest an adaptation to highly heterogeneous benthic environments due to the presence of diverse and dense bacterial populations as compared to the water column [48]. In addition, stress conditions can produce a von Willebrand factor deficiency [97]. Three proteins containing the ancient cell adhesion domain fasciclin (FAS1) were identified, as well as eight proteins with a peptidase C2, calpain domain, and calcium-responsive intracellular proteases involved in the regulation of cell migration, integrin-mediated adhesion and actin-based membrane protrusion, enabling cell movement by modifying the adhesion sites [85]. These results indicate that the molecular aspects of the adhesion process may provide powerful tools to understand the ecology of epiphytic communities under the current environmental changes. In addition, a full comprehension of the relationships influencing the activation of specific genes will be critical under the forecasted climate changes to develop newer technologies able to reduce or retard the formation of diatom biofilms and EPS on man-made submerged structures.

4. Algal Colonization

Marine rocky bottoms and other submerged natural surfaces are rapidly colonized by micro- and macro-algae [98]. Several organisms, including macroalgae, seaweeds, seagrasses, and even sessile and motile invertebrates, may provide additional space for colonization when the competition for space increases on non-living substrates. The propagule pools of algal colonizers are diverse and are influenced by environmental factors [99]. Their probability of encountering a surface is strongly dependent on their abundance in the water mass. In epibenthic communities, including secondary surfaces produced by foundation organisms such as seagrasses, algae are usually predominant on sunlit substrates, while animals dominate shaded surfaces. Two main factors cause this pattern: (a) the more competitive nature of algae and (b) the evolved differentiation of niches that results in animals avoiding competition with algae, which is also due to chemical interactions. Organisms belonging to those pools are suspended in the water column and transported by fluid-dynamic forces. Once the propagules are in close proximity to the available surface, physical-chemical forces act upon cells, particularly electrostatic forces and van der Waals forces [100]. Furthermore, the metabolic products exuded and retained by living substrates such as seagrasses potentially promote adhesion and and/or retard colonizing propagules [101]. Bacteria and diatoms, which are present in the propagule pools, may represent the earliest colonizers of these substrates. However, they are ubiquitous components of encrusting communities because they can quickly spread on any submerged surface [101]. They start to settle on the surfaces as primary colonizers, promoting the so-called periphyton formation. Their interaction promotes the formation of a microbial consortia, resulting in biofilm formation [101], which can be observed even on the youngest seagrass leaves [102]. The colonization and the biofilm formation on seagrasses are mainly affected by light, temperature and nutrient availability [43,103]. However, their productivity is largely influenced by the intensity and quality of light [104,105]. Primary colonizers alter the initial surface properties after the formation of a biofilm complex, thus influencing the secondary colonization. Further, the surface characteristics of both the type of substrate and the cell complex play an important role in the subsequent settlers [106]. Seagrasses and seaweeds represent secondary substrates for the colonization of other encrusting organisms because marine seagrasses and macroalgae are prone to epiphytism.

The colonization of their surface may lead to severe perturbations and sometimes to the death of the host, as in the case of some tropical seagrasses, which are severely impacted by the excessive growth of epiphytes when algal grazers are scarce. This demonstrates how the adhesion process, involving the features of the primary surface (e.g., live or inorganic substrates), the first colonizers (e.g., bacteria and micro-algae) and further colonizers (as macroalgae and sessile animals), is complex and dramatically influenced by chemical relationships and the presence of grazers. Consequently, to protect themselves against settling and/or competing organisms, many algae developed chemical defenses against further colonizers by producing a wide variety of bioactive metabolites [107].

However, the surface topography may also play a critical role in the colonization of submerged surfaces (both alive and inorganic) through the colonizing stages of micro- and macro-algae. The surface topography dramatically influences the attachment of zoospores of the green macroalga *Ulva linza* and cells of the diatom *Navicula incerta* on inorganic substrates [108]. Indeed, the preferred location of cells of *N. incerta* was dominated by a positive correlation between the density of cell adherence and the amount of available attachment points, while the settlement of spores of *U. linza* was mainly regulated by both Wenzel roughness (relationship between the roughness and wettability of a surface) and the local binding geometry.

5. Seagrasses and Other Living Substrates for Epiphytes

Seagrasses are among the most relevant available natural surfaces for epiphytic organisms in the marine environment [109–114]. They provide a wide range of substrates, which are structurally different depending on the leaf morphology of individual species [115]. Sea-

grasses are colonized by a multitude of epiphytic organisms that broaden their ecological role. For this reason, they can be considered as “models” used to investigate the dynamics of colonization of micro- and macro-organisms [116]. The lifetime of their above-ground parts (leaves, stems, rhizomes) varies within species and seasonality, ensuring a temporary but continuously renewed substrate [109]. The process of colonization of a seagrass leaf is easily sketched by comparing differently aged leaves of *P. oceanica*, a seagrass endemic to the Mediterranean that is characterized by its long persistence and high sensitivity to environmental triggers. Often, the organisms that settle on the leaf surface may persist throughout its lifespan. Due to this process, the lower (i.e., younger) portions of leaves show an early colonization stage, whose age increases according to the distance from the base, reaching the maximum age at the leaf tips. Consequently, the mechanisms facilitating the anchoring to the leaf surface are more efficient at the edge of leaves and near their apex, where a thicker film of first colonizers is already present. This is due to the higher turbulence that characterizes this portion of the leaves, which favors the chances that cells and propagules encounter the available leaf surface for attachment [117]. The leaves of seagrasses, as hosts of epiphytes, differ according to various aspects, such as anatomy, morphology, surface roughness and chemistry. All these characteristics may affect the presence, distribution and abundance of epiphytes in various areas of the world [118].

As soon as they encounter the surface of a seagrass leaf, algae become the most abundant and diverse encrusting organisms on seagrasses, with a wide variety of forms ranging from unicellular diatoms and dinoflagellates, which are found on almost all seagrasses, to macro-algae, especially crustose coralline ones. Epiphyte algae play a crucial role in the whole ecosystem, enhancing the primary production [109]. Furthermore, the high generation rate and growth of epiphytic biomasses strongly contribute to food webs, providing the main food source for the associated fauna [110]. As noted above, the diversity and biomass of epiphytes is highest in the upper portions of leaves (apico-basal pattern), especially on the oldest leaf and near the leaf apex [111,112]. In most marine angiosperms, the rhizomes of seagrasses generally carry a low epiphyte load compared with the leaves, with the exception of *P. oceanica*, where a larger species richness and higher biomass on the exposed and long-lived rhizomes has been reported [113]. The low abundance of epiphytes on the rhizomes is probably due to the fact that the rhizomes of almost all seagrasses are continuously buried. Epiphytes, as part of the seagrass ecosystem, are also considered bioindicators for factors that could be causal in seagrass decline, being sensitive to environmental perturbations [101]. The process of colonization and the biofilm formation of seagrasses seems to be crucial in terms of the selection and composition of species, as well as biomass production.

Environmental perturbations may negatively affect all stages of the community and biofilm development, which represent the most vulnerable targets. In fact, global change stressors can alter beneficial interactions in structurally complex systems, leading to dramatic changes in the dynamics of the succession of seagrass epiphytes [119].

Global stressors such as OA influence both bacterial and eukaryotic epiphytes, which form distinct communities at the CO₂-impacted sites compared with control sites [120]. Both bacterial and eukaryotic epiphytes form distinct communities at low-pH sites as compared with control sites, and a site-related CO₂ effect was demonstrated in the succession pattern of microbial epiphytes on seagrasses and close stands of corals. These authors further found an increased relative sequence abundance of bacterial types associated with coral diseases at the CO₂-impacted site, whereas certain crustose coralline algae commonly related to healthy reefs exhibited lower diversity. These trends in the epiphytic community suggest that seagrasses play a role as potential vectors of pathogens for closed coral reefs and may support previous predictions of a decrease in the reef health and prevalence of diseases under future ocean acidification scenarios [121]. Consequently, global stressors might have strong effects on the succession of epiphytes on seagrasses, which may have further consequences for other environments in the same area.

In the case of seagrasses, which are considered “foundation species” due to their role in structuring complex communities, the composition of associated epiphytic communities is also influenced by the nature of the first colonizers. In fact, the epiphytic community associated with the leaf stratum of seagrasses is seasonally variable [101]. From this perspective, the overall density and distribution of seagrass meadows, along with seasonal and global environmental changes, may be more important than the seagrass species identity itself [122].

As a general rule, in addition to the concepts reported above, the structure of the epiphytic community on seagrass leaves is mainly due to: (a) the rate of leaf growth; (b) the availability of nutrients; (c) the local effect of grazer communities; (d) the effect of various stressors, such as the OA and the warming of oceans. The diversity of the associated invertebrates can be a key driver of ecosystem functioning, in addition to the still uncertain factors influencing local biodiversity [123]. In both marine and terrestrial systems, facilitation cascades where the primary foundation and/or autogenic ecosystem engineering species (as seagrasses) promote the settlement and survival of a secondary foundation/engineering species have been shown to enhance local biodiversity and ecosystem functioning. The functioning of seagrass ecosystems may be enhanced in scenarios where secondary foundation species (e.g., epiphytic organisms) specifically increase the diversity of key functional groups, such as epiphyte grazers [124].

A considerable amount of the literature demonstrates that secondary foundation species are important drivers of local biodiversity in marine ecosystems and, considering the dramatic importance of the first colonizers in determining the anchoring of secondary foundation species, the role of organisms such as bacteria, micro-algae and cyanobacteria emerges because they prime a facilitation cascade [123]. Several investigations demonstrated that epiphytic communities on seagrasses were mostly influenced by nitrogen (N) and phosphorus (P) additions [125]. The variability in the epiphytic community structure was mainly related to the uncontrolled temporal and spatial environmental heterogeneity. However, P additions increased the relative abundance of the red algae, cyanobacteria and green algae, while diatoms in these conditions showed a concomitant decrease. In particular, red algae and cyanobacteria produce a complex that can facilitate the further anchoring of other epiphytes, representing the key first colonizers of seagrass leaves. Within the diatom community, species relative abundances, species richness and diversity seem to respond weakly to nutrient additions. Phosphorus additions produce changes in the structure of the diatom community, especially in warmer conditions [125] and, consequently, global warming produces stronger changes. Other studies, conducted in warm areas, reported contrasting results and different triggers. For example, according to Campbell and Fourqurean [126], a lower pH induces declines in the abundance of coralline algae, along with increases in filamentous algae, but the nutrient enrichment induced negligible effects on epiphyte community structure or the overall epiphyte loading.

In addition, OA alters the relationships between epiphytes, seagrasses and animal grazers. It has been demonstrated that the deterrent compound contents may change in both the seagrass leaves and in the epiphytic layer, and that the wound-activated infochemicals produced by epiphytes are modified by changes in the pH of the medium [127]. Leaves at normal pH (8.1) sites showed, according to Berlinghof et al. [128], a 25% higher total epiphytic cover compared to acidified sites. In normal conditions, encrusting red algae dominated the epiphytic community (32% cover) of leaves. In contrast, leaves of *P. oceanica* living in acidified conditions were dominated by hydrozoans (21%), probably due to the negative effects of OA on calcifying epiphytes. Leaves with and without epiphytes, collected from a low-pH meadow, produced and respired significantly more oxygen than leaves from the normal pH, showing an average increase of $47 \pm 21\%$ (mean \pm SE) in epiphytes to net primary production (NPP) and $50 \pm 4\%$ in the respiration, respectively. Epiphytes, however, made little contribution to the increase in respiration. In contrast, their contribution to NPP was quite relevant ($56 \pm 6\%$ of the total flux). In addition, the volatile organic compounds (VOCs) produced by individual diatoms are likely to

trigger contrasting reactions in invertebrates according to pH [129]. The perception of epiphyte VOCs varies due to alterations in species' ability to perceive and/or interpret chemical cues as infochemicals or due to changes in the structure of the VOCs themselves. Consequently, OA alters the fine-tuned chemical cross-talks between seagrass epiphytes and associated invertebrates, with consequences for the structure of communities and food webs of seagrass ecosystems [127,130]. These variations induce dramatic differences in the colonization patterns because they represent an indirect influence of the biodiversity in the epiphytic layer, over-imposed on the direct effects of OA on the survival of individual species of micro- and macro-algae [125].

However, we should consider that seagrasses may defend against epiphytic colonizers by adopting mechanisms of chemical mimicry [131]. The seasonal dynamics of chemical defenses against micro-organisms in seagrasses and the drivers of these dynamics have been investigated, demonstrating that the seagrass *Zostera marina* is prone to micro-colonizers, although this seagrass, as all marine spermatophytes, can chemically defend against settlers and grazers [130]. Besides these biotic factors, the seasonal changes in environmental factors such as nitrogen availability and temperature regimes also affect the defense levels in this seagrass, either directly or through indirect effects on its microbial settlers. The relationships between seagrass leaf surface and micro-epiphytes may be useful, therefore, in identifying newer compounds that retard the accumulation of encrusting organisms, because the release of several toxic antifouling chemicals into seawater has led to marine environmental degradation. From this perspective, the development of new methods based on biomimicry, as sketched by seagrass leaf ecology, could provide nontoxic or less toxic solutions for the deterrence of unwanted settlers [132]. In addition, natural mechanisms developed by various seagrasses and algae are often highly dynamic, and biotechnological applications may need to mimic not only defensive compounds but also their dynamics in order to exploit the full potential of biomimicry for marine applications. For example, the effectiveness of zosteric acid, a natural antifoulant extracted from the marine seagrass *Z. marina*, in preventing the attachment of several encrusting organisms was demonstrated by previous research [133]. Accordingly, understanding the primary and interactive effects of the first colonizers and the abundance of algal epifaunal assemblages in relation to global stressors such as ocean acidification and warming is important for seagrass ecology and management, especially in fragmented habitats [73], as well as for the development of new technologies.

6. Ocean Acidification Effects on Epiphytes

The uptake of carbon dioxide by the oceans, enhanced by its rising concentrations in the atmosphere, mostly due to anthropogenic emissions, is a global environmental concern known as ocean acidification (OA). This phenomenon is drastically changing the carbonate chemistry and reducing the pH of the oceans [134–140], with far-reaching consequences for marine communities and ecosystem dynamics. Ocean acidification, at a global scale, has deleterious effects on various taxa of marine organisms (Table 1), negatively affecting the correct function and services of the ecosystems [141].

Several species respond to the acidification of the seawater (Table 1), but the direction and magnitude of their responses will ultimately depend on the interspecific and ontogenetic variations in their physiology and the importance of calcification (e.g., for the bioconstruction of skeletons). Factors influencing the ecology of the first colonizers will likely modify both the structure and the diversity of benthic communities [142]. Seawater acidification significantly alters the community structure by influencing the relative abundance of several species and triggering a reduced community variability (Figure 2). This results in a “flattening” of benthic communities in acidified environments [143].

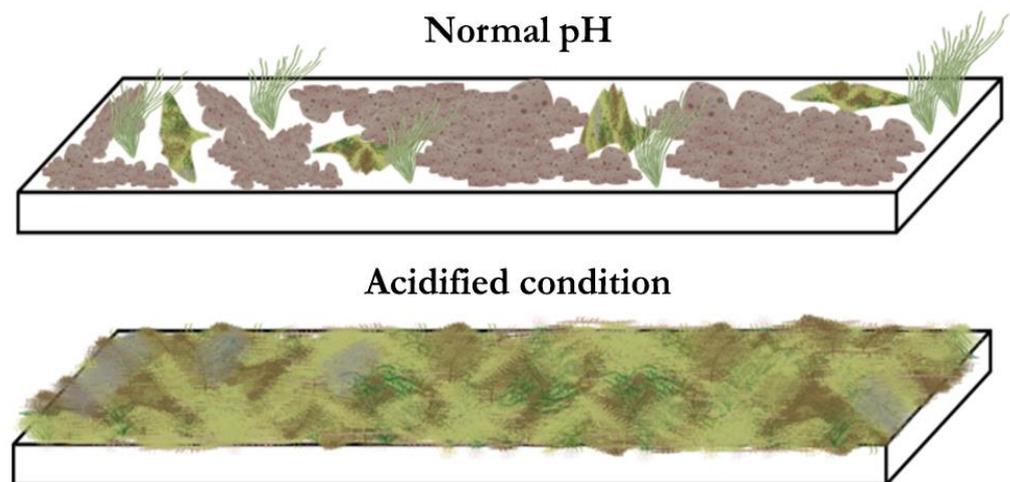


Figure 2. The alterations in epiphytic communities triggered by OA induce a reduction in the first basal colonizers and a modified conditioning film, leading to an impoverished mature community. Such variations result in a “flattening” of communities in acidified environments and an altered ecological stability, with consequences for biodiversity (lower levels) and the abundance of some invasive and stronger organisms (larger biomass).

However, the responses of individual organisms may be contrasting. OA does not affect the growth of calcified organisms such as adult mussels and barnacles, while the growth of non-calcifying organisms, such as some hydroids, is significantly reduced in elevated CO_2 treatments [128]. Conversely, the percentage cover of some adult bryozoan colonies may be higher under acidified conditions. On the overall, these changes lead to a decrease in diversity (41% lower) in the acidified treatment relative to ambient conditions, according to Brown et al. [142]. This topic reaches paramount importance when seagrass meadows are considered, because they represent stable but sensitive environments and produce habitats for ecologically and economically important coastal communities [126]. Seagrasses are among the most diverse ecosystems in the world. Severe natural and anthropogenic changes such as ocean warming [144], OA [145], the physical impacts of the fishery, the higher presence of particles (due to industrial or urban pollution), and microplastics are extremely detrimental to these ecosystems [146]. In particular, the effects that OA has on the physiology of the first colonizers will have a remarkable effect on the composition of seagrass-associated communities.

The obtained results will also be useful for a comprehensive approach to various biotechnologies, because the shifts in the colonization *consortia* induced by global changes require newer approaches to the field of anti-fouling coatings for several marine applications [143]. It is forecasted that OA will reduce the biodiversity of marine communities by acting on important ecological processes that ultimately determine the community structure [146,147]. Consequently, OA will increasingly interact, in the future, with the ecology of encrusting organisms, posing large threats to coastal ecosystems. Overall, acidification-driven changes in the benthic community might produce important implications for ecosystem functions and food-web dynamics in various environments. Seagrass meadows are likely to be significantly impacted as well, due to the importance of relationships [148] between the leaf growth and the succession of associated organisms building a complex epiphytic layer. In addition, the indirect effects of OA will alter the chemical relationships between epiphytes and various animal consumers. For example, OA remarkably influences the production patterns of various algal infochemicals, and this produces dramatic changes in the chemical relationships among species within the same community [126,127,149].

However, the fate of highly invasive encrusting species in an acidified ocean remains poorly understood, due to lack of information on the changes in successional stages and at various levels of biological organization. Previous investigations [142] demonstrated that the ecological succession in an acidified site was initially delayed and the community remained in an early stage for at least 8 weeks. The changes observed in the succession led to the homogenization of communities in acidified conditions and altered the community structure, reflecting both short- and longer-term acidification histories. The observed shifts in the biodiversity and structure of the communities are a result of interspecific variability in response to increased $p\text{CO}_2$ and shifts in key species interactions [135]. It is worth considering that OA altered the development of initial biofilms, allowing for some polychaetes to perform best under acidified conditions, although the early negative effects of acidification were demonstrated in the recruitment stages of various worms [23,150]. In contrast, the effect of OA on initial biofilms facilitates the adhesion of the ascidians *Diplosoma* sp. and *Botryllus* sp. They settled later, but were more tolerant to acidification, probably due to the absence of calcareous structures [26,151].

Our knowledge about the effects of OA relies on studies about the biology, physiology and behavior of various marine species [149–154]. Unfortunately, assessment of the effects of OA on marine communities is difficult; for this reason, it is necessary to disentangle and clarify the effects of acidification on the succession and development of marine communities, as highlighted by Brown et al. [46]. Moreover, since the saturation state of calcium carbonate falls due to the decrease in pH, and the solution equilibrium shifts towards higher dissolved concentrations of calcium ions, research has predominantly focused on primary producer calcifying organisms [145,155,156] because their skeletons are naturally corroded by low pH levels [144,157].

As shown above, most studies involved manipulative laboratory experiments [152] and long-term mesocosm observations [146]. In order to facilitate the comprehension of the effect of OA on marine communities, scientific research is now moving towards in-situ $p\text{CO}_2$ manipulations [142,158] and studies exploiting natural CO_2 vents [159–161]. The latter provides insights into the effects of seawater acidification at community and ecosystem levels [46]. Many studies carried out in those natural laboratories show an alteration in the community composition, which led to a reduction in the ecological complexity and a decrease in biodiversity [147,162].

In order to increase our understanding of the direct and indirect effect of OA on coastal benthic ecosystems, it is essential to determine what kinds of changes might occur at the primary producers' level (Table 1). Despite this, the impact of high CO_2 concentration on benthic ecosystems, especially on the microphytobenthic assemblage dynamics, remains poorly studied and explored. Changes in the community composition are represented by a clear pattern [163–166], triggering a shift from calcifying organism (e.g., coralline algae) towards non-calcified species (especially brown algae and diatoms). Similar changes in the patterns of colonization were also observed by the study conducted by Porzio et al. [160] of the early colonizers in the CO_2 vents off the Island of Ischia (Bay of Naples, Italy), which highlighted a progressive decrease in the number of species along the pH gradient. Harvey et al. [164] also supported this evidence, asserting that OA can set the course of successional development in the phytobenthos. This facilitates turf algae formation at the expense of the biomass and diversity of the whole benthic community, supporting the concept that the OA may lock the algal community at an earlier stage, dominated by r-selected species [167].

Due to the high solubility of their skeleton, which has a high magnesium content [168], encrusting red algae (Corallinales) show strong vulnerability to the phenomenon of acidification [169]. Under normal conditions, they act as pioneer species in the colonization of the leaf surface of *P. oceanica* that, once established, help to condition the adhesion and colonization of other groups of organisms [170]. While corallinales often represent the dominant group of epiphytic colonizers along the leaves of *P. oceanica*, in acidified sites the algal epiphytic assemblage is mainly composed of flashy algae, both encrusting and erect brown and green algae. In those environments, the community structure of epiphytic

assemblages undergoes a strong reduction in terms of taxonomic diversity due to the decrease/absence of corallinales, leading a community dominated by flashy and turf algae. The resulting epiphytic community faces a trivialization of its biodiversity, appearing more homogeneous and strongly impoverished. After the initial colonization, the assemblages of epiphytes present in natural acidified areas seem not to evolve, remaining in their initial state [171]. The disappearance of the coralline red algae could affect the subsequent stages of colonization, causing cascade effects on the trophic web associated with the canopy and the functioning of the entire ecosystem [172]. Furthermore, the scarce or totally absent corallinales cover may induce a great palatability of the leaves, enhancing the grazing activity, especially by some fish, such as *Sarpa salpa*, whose abundance tripled close to the vents compared to ambient pH sites [173]. The increased grazing led to evident modifications in the leaf canopy of *P. oceanica* in acidified areas, which could suggest a possible correlation between the phenology of the seagrass and the structure of the epiphytic community [171]. Although the canopy presents a high shoot density in acidified zones, the *Posidonia* leaves appear narrower and shorter than under normal pH conditions, resulting in a decrease in available surface area for epiphyte colonizers.

Table 1. Summary of the effects of OA on the early stages of plant and animal colonizers.

Colonizer Organism	Effect on Colonization	Environment/Substrate	References
Calcified epiphytes	Strong reduction in the cover	<i>P. oceanica</i> leaves in shallow volcanic CO ₂ vents in Ischia island	[159]
Calcareous algae Non-calcified algae Turf algae	Disappearance within the acidified site Become dominant in acidified site Declined the most in diversity and abundance	Rocky shore in Ischia island shallow volcanic CO ₂ vents	[161]
Algal species with calcareous structures Turf algae	Decline and disappear along the pH gradient Increase cover along the pH gradient	Rocky reef in CO ₂ shallow volcanic vents in Ischia Island	[147]
Cover of coralline algae Large canopy forming macroalgae Low-profile algae Turf algae	Significantly reduced Significantly reduced abundance Increased in cover Increased in cover	Shikine island in Japan CO ₂ seeps in shallow waters	[163]
Coralline algae Non-calcified macroalgae	Sharp decline in coralline species diversity in both sites More abundant	Seeps off the volcanic coasts of Italy (Vulcano Island, Mediterranean Sea) and Japan (Shikine Island, North-Western Pacific)	[165]
Coralline algae Filamentous turfs cover	Significant negative effect of high pCO ₂ on calcification with partial tissue mortality at very high pCO ₂ Increase	Laboratory experiment—mesocosm	[166]
Coralline algae cover	Declines consistently with decrease in pH	Great Barrier Reef World Heritage Area (Australia)	[167]
Taxonomic diversity Encrusting red Algae (Corallinales) Encrusting and erect brown and green algae (Fleshy and Turf algae)	Strong reduction Disappearance with the increasing acidification of seawater Become dominant with acidification	<i>P. oceanica</i> orthotropic shoots collected by CO ₂ shallow volcanic vents in Ischia Island	[171]
Epiphytic coralline algal cover	Significant reduction with increased acidification of seawater	<i>P. oceanica</i> blades collected by CO ₂ shallow volcanic vents in Ischia Island	[172]

It is also appropriate to note that all these variations in terms of the abundance and biodiversity of epiphytes are seasonally dependent and the pathways of colonization vary according to the physical and chemical features of the substrates. In addition, multi-stressor approaches indicate multiple effects of OA when coupled with temperature increases, which could facilitate the introduction of invasive species. Many studies showed negative [174] or irrelevant effects [175] on diatoms after high CO₂ exposure in laboratory experiments, even under multi-stressor scenarios. In contrast, some other research works demonstrated that diatoms benefit from the increase in pCO₂, especially through the reduction in the energetic costs of their “CO₂ Concentrating Mechanisms” (CCMs) [45,176]. In their study, Johnson et al. [45] also demonstrated that high CO₂ levels stimulate primary productivity in the periphyton, as proven by the increase in the photosynthetic standing

crop of the above-mentioned benthic assemblages, mainly due to the increased presence of diatoms. The same study also showed a non-uniform response of benthic diatoms to OA.

Variations in the chemistry of the seawater due to the higher $p\text{CO}_2$ have different effects on the periphyton communities, causing increases in some populations and decreases in some others. A reduction in diversity in the assemblages occurs when moving along the $p\text{CO}_2$ gradient: as pH decreases, diatoms characterized by larger size become dominant in the biofilm community. Under the present $p\text{CO}_2$ conditions, smaller cell-size genera maximize the uptake of carbon thanks to the higher surface:volume ratio (SVr). With the increase in cell size and the consequent reduction in SVr, a limitation starts to occur in the diffusion path [177]. The actual incremental trend of CO_2 levels might provide an advantage to larger species by inverting the competitive skills of different size diatom classes.

These community shifts are likely a result of interspecific variability in response to OA and changes in species interactions. High $p\text{CO}_2$ altered biofilm development, with detrimental consequences on whole ecosystems, triggering a dramatic cascade of effects. In benthic communities, where the time stability of plant and animal associations is a key factor to assure the resilience and conservation of species, pH changes in the seawater may have dramatic consequences for the assemblages of species. In these environments, well-known time successions lead to stable associations that assure the functioning of ecosystems [178]. Slight changes in the assemblages of first colonizers may totally change the time evolution of benthic communities, leading to still-unknown variations in their composition and functioning. The synergistic actions of several factors of global change (OA and human disturbances, such as nutrient increases) could lead to a rapid acceleration of the whole process, causing winners and losers among species in marine benthic communities. Those variations in phenology and epiphyte communities can be considered relevant aspects of the effects of OA and could represent drastic modifications that seagrass systems are going to face.

7. Global Warming Modifies Epiphytic Communities

Another key stressor is the well-known increase in temperature affecting all oceans in the last five decades [179]. In terrestrial systems, the influence of warming on community dynamics has been largely investigated through field manipulations. However, only a few warming experiments were conducted in the marine environment to determine models for the adhesion and progression of communities (Table 2). In many ecosystems, it is still unclear which interactions among surface colonizers are most likely to be altered under increasing thermal stress and what impacts, if any, such altered interactions will have on the structure of mature communities. An increased temperature is arguably one of the most important facets of global climate changes, because temperature influences various processes across all the biological scales, including the survival and recruitment of plants and invertebrates [180]. Global warming can alter the multifunctionality of plant and animal communities associated with structurally complex ecosystems by modifying the co-existence of engineers and the diversity of the functional traits present in a landscape [122].

Table 2. Summary of the effects of rising temperature of seawater at the early stages of plants and animals.

Colonizer Organism	Effect on Colonization	Environment/Substrate	Reference
Macroalgae cover (<i>Gracilaria gracilis</i>) Seagrass cover Macrofaunal abundance	Increase Reduction Reduction	Seaweed beds and unvegetated sandflats	[181]
<i>P. oceanica</i> abundance	Reduction, becoming steep when the temperature reaches 28 °C	<i>P. oceanica</i> meadows of Cabrera Island (Algerian subbasin of the Western Mediterranean)	[182]
Phytoplankton	Indirect reduction by increased grazing	Coastal regions	[183]

Table 2. Cont.

Colonizer Organism	Effect on Colonization	Environment/Substrate	Reference
Phytoplankton	Reduction and shift towards assemblage dominated by smaller species	Nutrient-limited water (oligotrophic oceans)	[184,185]
<i>Hydroides elegans</i>	Production of stronger tubes more resistant to simulated predator attacks	Laboratory experiment—mesocosm	[186]
Bacterial biofilm	Most diversified bacterial communities at high temperature conditions	Laboratory experiment—mesocosm	[187]
Bacterial biofilm	Higher biomass production and higher diversity in the biofilm at higher temperature	Laboratory experiment—mesocosm	[26]
Crustose coralline algae	Lack of seasonality growth and high mortality	Marmion Lagoon, South-Western coast of Western Australia	[188]
Kelp forests (<i>Nereocystis luetkeana</i>)	Clear decrease without full recovery	Coastline in northern California	[189]
Diatom <i>Chaetoceros socialis</i>	Reduction of production and cell size; poleward shift in biogeographic distribution	Laboratory experiment—mesocosm	[190]
Coralline algae	Clear reduction in cover and negative effect on photosynthetic efficiency	Laboratory experiment—mesocosm	[191]

This effect is mediated by warming, favoring the retention of tolerant engineer traits over sensitive ones and the functional characteristics of residents nested within engineered patches. In situ comparisons of functional traits and community metrics of macrofauna inhabiting beds of seagrasses and macroalgae, as well as unvegetated sandflats, indicated unique assemblages in seagrass habitats and a dominance of surface-dwelling fauna due to global warming [192]. Macrofaunal abundance is reduced in seaweed beds and unvegetated sandflats, which are dominated by burrowing infauna [181]. Experimental findings indicate that increases in temperature in temperate coastal systems may lead to declines in seagrass, but increased seaweed cover. This shift is likely to lower ecosystem heterogeneity due to the faunal similarity between unvegetated sandflats and seaweed beds and, in parallel, reduce the habitat for critically endangered specialist seagrass inhabitants [181]. However, community responses to short-term increases in temperature are variable, and they are influenced by the individual responses of key species [193]. The importance of community-level, field-based manipulations of environmental change factors, which examine interactions between all specific members of the local species pools, should be recognized, because the strength and direction of interspecific interactions governing complex benthic communities are expected to change according to the increasing global temperatures.

The increased water temperatures associated with global changes led to the formation of diverse biofilm communities, affecting the subsequent settlement of other organisms. In their study, Whalan and Webster [194] demonstrated that the most successful larval settlement of the abundant Great Barrier Reef sponge *Rhopaloeides odorabile* occurred on biofilms that developed at higher temperatures. This is likely to be related to the more diversified communities that developed compared with the biofilms that developed at lower temperatures. Similar pathways are detectable in the study conducted by Lau et al. [26], in which they tested the role of biofilm in the larval settlement of two species of barnacles (*Balanus amphitrite* and *B. trigonus*) and the polychaete *Hydroides elegans*. Biofilms were qualitatively different in terms of their bacterial community composition, and also quantitatively differed in the density and total biomass produced. The settlement of the larvae of the two species of barnacles was strongly induced by the biofilm that developed under higher-temperature conditions, while the surfaces colonized by microbial communities exposed to lower temperatures inhibited (*B. trigonus*) or did not affect (*B. amphitrite*) the larval settlement [26]. This response to the colonization process of the larvae coincides

with the differences expressed in terms of bacterial community composition of the biofilm, rather than the biomass or the density produced.

Several studies have also investigated the effect of global warming on the survival and distribution of seagrasses, especially in *P. oceanica* [194,195]. *P. oceanica* is endemic to the Mediterranean Sea, which is warming up three times faster than the global ocean [196]. Therefore, it has more significant effects on the marine organisms inhabiting this local area. According to Marbà et al. [182], warming of the Mediterranean triggers *P. oceanica* mortality, and a further increase in mortality was observed when seawater surpassed 28 °C. Under these warming conditions, other seagrass species can occur. However, *P. oceanica* exhibits distinct structures and functions, including the contribution of such species as the mussel *Pinna nobilis*, which only occurs in its meadows [197]. Pillay and Waspe [119] in another study, investigated a mesocosm to quantify the effects of ocean warming on the epiphytic organisms of the temperate seagrass *Zostera capensis*, using a sympatric specialist (*Siphonaria compressa*) and a generalist organism (*Fissurella mutabilis*) to exemplify two limpet models of grazers [98]. Their results indicated changes in epiphytes loads due to warming, which increased up to 15 times in the absence of grazers. Grazers differentially impacted the community, with the specialist limpet providing a stronger cleaning function at high temperatures. The specialist species exhibited higher survival rates at higher temperatures, while the survival rates of the generalist limpet reduced. These findings highlight the need for specialist, warm-tolerant grazers to be prioritized in conservation/restoration programs for seagrasses. Such consideration will produce positive feedback loops, benefitting both seagrasses and grazers under global change conditions, with positive community- and ecosystem-wide ramifications.

The warming of the oceans has also been related to a decline in phytoplankton biomass [198–200]. However, the effect of temperature may vary in different ecosystems. In coastal regions (nutrient-replete waters), phytoplankton is dominated by large diatoms (20–200 µm), which are the preferred food source for overwintering copepods [201]. In these regions, temperature causes metabolic changes in plankton populations. Therefore, due to the warming of oceans, there is an increase in the grazing pressure caused by copepods, reducing phytoplankton abundance [183]. On the other hand, in nutrient-limited waters, temperature mainly influences plankton biomass through physical mechanisms [183]. In the water column, the growth of larger diatoms is limited under low-nutrient conditions, favoring small phytoplankton (<20 µm) and flagellates, while an increase in ocean temperature increases nutrient limitations and reduces phytoplankton biomass. This causes a shift towards an assemblage of phytoplankton, dominated by smaller species [184,185], resulting in a shift in copepod food sources from large diatoms to ciliates [202]. These changes in the marine plankton community vary according to the surrounding nutrient regime; nonetheless, they can affect biogeochemical cycles, climate patterns, and, ultimately, the function and structure of benthic ecosystems [183]. Similar relationships might be hypothesized for benthic diatoms colonizing seagrass leaves, although no information is available from previous investigations as far as we know.

As mentioned above, multi-stressor scenarios are most likely to be important when forecasting future variations in the ecology of key early colonizers. Among others, Li et al. [186] investigated the polychaete *Hydroides elegans*, because the calcareous tube it builds can be used as an example to evaluate the individual and interactive effects of OA, temperature increases and reduced salinity, on the mechanical properties of its tube. Under OA (pH 7.8), polychaetes produced a mechanically weaker tube, exhibiting less resistance to simulated predator attacks. Seawater warming of up to 29 °C induced an increase in the tube volume, the tube mineral density and the tube resistance to a simulated predatory attack. The observed weakening effect by OA did not make the removal of tubeworms from the substrate easier, except for the earliest stage, when warming had a weak effect. Reduced salinity (down to 27 PSU) did not affect the structure and the resistance of the tubes. These investigations demonstrate that multi-stressor scenarios are needed to obtain realistic previsions, and that both mechanical analysis and computational modeling should

be integrated to provide insights into how communities might develop in future conditions according to the effect of various stressors on the ecology of marine early colonizers.

8. Biotechnological Issues

The prevention of biogenic encrustations is one of the largest challenges faced by the maritime industry, but antifouling agents commonly impact marine ecosystems [77]. The development of antifouling strategies for the protection of submerged structures requires a clear understanding of how various organisms may respond to environmental stressors associated with climate changes [46]. Climate changes facilitate invasions and induce shifts in community composition, especially in complex and stable ecosystems. Invasive alien species, whose introduction is facilitated by global warming, can tolerate significantly higher temperatures than native species and, consequently, climate changes are likely to have disproportionately negative impacts on native species [186]. Global change stressors can alter mutually beneficial interactions in structurally complex systems, leading to impaired functionality and service provision. Based on the temperature tolerance, survival, and growth results, Sorte et al. [47] predicted that native species in a California Bay will decrease in abundance according to the increase in ocean temperatures, being substituted by introduced species. The facilitation of invasions by climate change is underway. Over the last 40 years, invasive dominance has increased concurrently with ocean warming [142] and this has had substantial ecological and economic impacts. From this perspective, the effects of the first colonizers on further anchoring processes may vary due to changes in the temperature and pH of the medium. As a general rule, higher recruitment rates are observed at warmer seawater temperatures, but the highest recruitment values may shift from resident species to alien species, dramatically modifying the trends of ecological successions observed on submerged surfaces according to a complex series of interactions among invasive and resident species as they relate to seawater temperature [44].

In addition, modern approaches to the control of encrusting organisms should avoid the use of toxic compounds, which are largely demonstrated to impact key coastal and planktonic environments [21]. At present, several environmentally friendly approaches are being tested worldwide to reduce the toxic effects of biocides on non-target species. The use of natural products has had significant effects. For example, it has been demonstrated that the international ban on the toxic antifoulant tributyltin (TBT) had positive effects on “green tide” phenomena, in addition to the reduction in its toxicant power on natural stocks of mollusks and crustaceans in several water bodies. Advances also include the use of nanomaterials, but some physiological constraints are elicited by the increase in seawater temperature, reducing the effectiveness of anti-fouling coatings [64]. Other approaches include the encapsulation/immobilization of commercially available biocides to achieve control over the leaching rate and the overall realization of low-adhesion surfaces that can retard the production of first colonization films, priming the further colonization of larger foulers [21,203]. However, several compounds used to obtain a non-polluting control of foulers may change their chemical properties under acidified or warm conditions. In this case, the mechanisms of oxidative stress, detoxification and neurotransmission in aquatic organisms might be impacted by the newer conditions imposed by global changes. Consequently, the antioxidant patterns that characterize the exposure of marine organisms to anti-fouling agents could vary. These effects indicate the need to identify newer candidate compounds and new strategies to allow for the activity of plant and animal foulers with an efficient and eco-friendly strategy.

Unfortunately, the impacts of OA on encrusting communities, which may be some of the initial colonizers of hard substrates, with a high economic importance, require further investigation. There are well-known effects of OA on first colonizers as diatom and algal communities, indicating that they are often poorly developed in low-pH conditions, which allows for the easy prediction that encrusting communities will face dramatic changes, with reductions in groups bearing exposed exoskeletons. However, our actual knowledge

of the long-term changes in benthic communities according to the forecasted decreases of pH is almost null [46].

While the impact of ocean warming on single species has been revealed, its global impact on benthic communities is almost totally unknown [204]. According to the above-mentioned studies, the future warming of oceans will likely increase the pressure on encrusting organisms, while decreasing the diversity of communities due to the introduction of alien species, particularly in habitats where organisms exist at their upper tolerance limits in terms of temperature [118]. Overall, the combined effect of various stressors and their influence on the efficacy of protective coatings is little investigated. Consequently, investigations taking into account both the chemical ecology relationships and the features of foulers influenced by global changes are urgently needed.

9. Conclusions

Climate change at a global scale affects marine environments, with dramatic consequences at various levels of complexity [199]. Both OA and rising temperatures are changing the benthic communities, primarily (but not only) acting on the settlement of early colonization stages. Several recent research papers have explained the effects of those stressors on individual species [151]. Although the direct effects that OA has on calcified organisms are evident and very well detailed in various studies [148], the indirect effects are less evident but might be deleterious as well. This review sheds light on the effects of two key stressors (higher $p\text{CO}_2$ /lower pH and higher temperature) on the primary colonizers, the pioneer organisms, paying special attention to seagrass meadows due to their critical role in the ecology of coastal areas. Environmental changes may affect whole ecosystems, interrupting or disturbing vital relationships among organisms, such as chemical communications [129]. In benthic communities, where the time stability of plant and animal associations are key factors to assure the resilience and conservation of species, variations in the temperature or pH of the seawater may have dramatic consequences for the final species assemblages [14]. In these environments, well-known time successions bring stable associations. Slight changes in the assemblages of first colonizers, and thus in the biofilm development and composition, may dramatically change the time evolution of benthic communities, producing still-unknown variations in the composition and functioning of the entire ecosystem [21]. The biodiversity of ecosystems is largely controlled by these successions [101]. Furthermore, biodiversity helps determining the services provided by those environments, and their economical exploitation. Thus, the correct forecast of the indirect effects of global changes on local biodiversity may be crucial to allow for the sustainable management of marine biodiversity and its related resources [122,146].

Several modern strategies to reduce the attachment of encrusting organisms partially lose their efficacy in the light of changes produced by global stressors, because both the chemistry of anti-fouling coatings and the physiology of biofoulers are impacted by key stressors, such as the OA and the warming of oceans [46]. For this reason, further research will be needed to identify the factors priming the anchoring of biofoulers, also considering the physiology and molecular ecology of microorganisms such as bacteria and micro-algae, facilitating the further colonization of other organisms. The data reported here represent a first step towards the development of new strategies, taking into account the properties of diatoms and bacteria as pioneering adhesive organisms [2]. We indicated how they determine the final destiny of benthic communities on both man-made structures and in stable and structurally complex ecosystems such as seagrass meadows. The changes still occurring in the chemical and physical environment of oceans might impact and dramatically change the ecology of pioneer adhesive organisms [149,152], priming the colonization of economically important ecosystems worldwide.

Author Contributions: Conceptualization, E.S. and V.Z.; data curation, E.S. and M.M.; writing—original draft preparation, E.S., M.C. and V.Z.; writing—review and editing, A.T., M.C. and V.Z.; supervision, A.T. 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: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Emanuele Somma was supported by a PhD fellowship funded by Stazione Zoologica Anton Dohrn and University of Trieste. Madalena Madeira contributed to this review within her “Estágio da Licenciatura em Biologia” from the University of Aveiro. We thank Alice Mirasole for her support in the revision of the citations and references.

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

References

- Characklis, W.G.; Cooksey, K.E. Biofilms and Microbial Fouling. In *Advances in Applied Microbiology*; Laskin, A.I., Ed.; Academic Press: Cambridge, MA, USA, 1983; Volume 29, pp. 93–138.
- Molino, P.J.; Wetherbee, R. The Biology of Biofouling Diatoms and Their Role in the Development of Microbial Slimes. *Biofouling J. Bioadhesion Biofilm Res.* **2008**, *24*, 365–379. [[CrossRef](#)] [[PubMed](#)]
- Wetherbee, R.; Lind, J.L.; Burke, J.; Quatrano, R.S. The First Kiss: Establishment and Control of Initial Adhesion by Raphid Diatoms. *J. Phycol.* **1998**, *34*, 9–15. [[CrossRef](#)]
- Huggett, M.J.; Nedved, B.T.; Hadfield, M.G. Effects of Initial Surface Wettability on Biofilm Formation and Subsequent Settlement of *Hydroides elegans*. *Biofouling J. Bioadhesion Biofilm Res.* **2009**, *25*, 387–399. [[CrossRef](#)] [[PubMed](#)]
- Davey, M.E.; O’toole, G.A. Microbial Biofilms: From Ecology to Molecular Genetics. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 847. [[CrossRef](#)]
- Zobell, C.E.; Allen, E.C. The Significance of Marine Bacteria in the Fouling of Submerged Surfaces. *J. Bacteriol.* **1935**, *29*, 239. [[CrossRef](#)]
- Wahl, M.; Goecke, F.; Labes, A.; Dobretsov, S.; Weinberger, F. The Second Skin: Ecological Role of Epibiotic Biofilms on Marine Organisms. *Front. Microbiol.* **2012**, *3*, 292. [[CrossRef](#)]
- Salta, M.; Wharton, J.A.; Blache, Y.; Stokes, K.R.; Briand, J.-F. Marine Biofilms on Artificial Surfaces: Structure and Dynamics. *Environ. Microbiol.* **2013**, *15*, 2879–2893. [[CrossRef](#)]
- Dang, H.; Lovell, C.R. Bacterial Primary Colonization and Early Succession on Surfaces in Marine Waters as Determined by Amplified rRNA Gene Restriction Analysis and Sequence Analysis of 16S rRNA Genes. *Appl. Environ. Microbiol.* **2000**, *66*, 467. [[CrossRef](#)]
- Allison, D.G. The Biofilm Matrix. *Biofouling J. Bioadhesion Biofilm Res.* **2010**, *19*, 139–149. [[CrossRef](#)]
- Flemming, H.; Wingender, F.; Szewzyk, U.; Steinberg, P.; Rice, S.; Kjelleberg, S. Biofilms: An Emergent Form of Bacterial Life. *Nat. Rev. Microbiol.* **2016**, *14*, 563–575. [[CrossRef](#)]
- Annuk, H.; Moran, A. Microbial Biofilm-Related Polysaccharides in Biofouling and Corrosion. In *Microbial Glycobiology*; Academic Press: Cambridge, MA, USA, 2010. [[CrossRef](#)]
- Flemming, H.-C.; Wingender, J. The Biofilm Matrix. *Nat. Rev. Microbiol.* **2010**, *8*, 623–633. [[CrossRef](#)]
- Pawlik, J.R. Chemical ecology of the settlement of benthic marine invertebrates. In *Oceanography and Marine Biology: An Annual Review*; UCL Press: London, UK, 1992.
- Dobretsov, S.; Teplitski, M.; Paul, V. Mini-Review: Quorum Sensing in the Marine Environment and Its Relationship to Biofouling. *Biofouling J. Bioadhesion Biofilm Res.* **2009**, *25*, 413–427. [[CrossRef](#)]
- Landini, P.; Antoniani, D.; Burgess, J.G.; Nijland, R. Molecular Mechanisms of Compounds Affecting Bacterial Biofilm Formation and Dispersal. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 813–823. [[CrossRef](#)]
- Vu, B.; Chen, M.; Crawford, R.J.; Ivanova, E.P. Bacterial Extracellular Polysaccharides Involved in Biofilm Formation. *Molecules* **2009**, *14*, 2535–2554. [[CrossRef](#)]
- Hadfield, M.G. Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. *Annu. Rev. Mar. Sci.* **2011**, *3*, 453–470. [[CrossRef](#)]
- Mieszkin, S.; Martin-Tanchereau, P.; Callow, M.E.; Callow, J.A. Effect of Bacterial Biofilms Formed on Fouling-Release Coatings from Natural Seawater and *Cobetia marina*, on the Adhesion of Two Marine Algae. *Biofouling J. Bioadhesion Biofilm Res.* **2012**, *28*, 953–968. [[CrossRef](#)]
- Dang, H.; Lovell, C.R. Microbial Surface Colonization and Biofilm Development in Marine Environments. *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 91–138. [[CrossRef](#)]
- Terlizzi, A.; Conte, E.; Zupo, V.; Mazzella, L. Biological Succession on Silicone Fouling-Release Surfaces: Long-Term Exposure Tests in the Harbour of Ischia, Italy. *Biofouling* **2000**, *15*, 327–342. [[CrossRef](#)]
- Freckelton, M.L.; Nedved, B.T.; Hadfield, M.G. Induction of Invertebrate Larval Settlement; Different Bacteria, Different Mechanisms? *Sci. Rep.* **2017**, *7*, 42557. [[CrossRef](#)]
- Hadfield, M.G.; Nedved, B.T.; Wilbur, S.; Koehl, M.A.R. Biofilm Cue for Larval Settlement in *Hydroides elegans* (Polychaeta): Is Contact Necessary? *Mar. Biol.* **2014**, *161*, 2577–2587. [[CrossRef](#)]

24. Jenkins, S.R.; Arenas, F.; Arrontes, J.; Bussell, J.; Castro, J.; Coleman, R.A.; Hawkins, S.J.; Kay, S.; Martínez, B.; Oliveros, J.; et al. European-Scale Analysis of Seasonal Variability in Limpet Grazing Activity and Microalgal Abundance. *Mar. Ecol. Prog. Ser.* **2001**, *211*, 193–203. [[CrossRef](#)]
25. Patel, P.; Callow, M.E.; Joint, I.; Callow, J.A. Specificity in the Settlement—Modifying Response of Bacterial Biofilms towards Zoospores of the Marine Alga *Enteromorpha*. *Environ. Microbiol.* **2003**, *5*, 338–349. [[CrossRef](#)] [[PubMed](#)]
26. Lau, S.C.K.; Thiyagarajan, V.; Cheung, S.C.K.; Qian, P.-Y. Roles of Bacterial Community Composition in Biofilms as a Mediator for Larval Settlement of Three Marine Invertebrates. *Aquat. Microb. Ecol.* **2005**, *38*, 41–51. [[CrossRef](#)]
27. Joint, I.; Callow, M.E.; Callow, J.A.; Clarke, K.R. The Attachment of *Enteromorpha* Zoospores to a Bacterial Biofilm Assemblage. *Biofouling J. Bioadhesion Biofilm Res.* **2009**, *16*, 151–158. [[CrossRef](#)]
28. Wang, C.; Bao, W.-Y.; Gu, Z.-Q.; Li, Y.-F.; Liang, X.; Ling, Y.; Cai, S.-L.; Shen, H.-D.; Yang, J.-L. Larval Settlement and Metamorphosis of the Mussel *Mytilus coruscus* in Response to Natural Biofilms. *Biofouling J. Bioadhesion Biofilm Res.* **2012**, *28*, 249–256. [[CrossRef](#)]
29. Hadfield, M.; Paul, V. Natural Chemical Cues for Settlement and Metamorphosis of Marine-Invertebrate Larvae. In *Marine Chemical Ecology*; CRC Press: Boca Raton, FL, USA, 2001. [[CrossRef](#)]
30. Krug, P.J. Defense of Benthic Invertebrates against Surface Colonization by Larvae: A Chemical Arms Race. In *Antifouling Compounds*; Marine Molecular Biotechnology; Springer: Berlin/Heidelberg, Germany, 2006; Volume 42, pp. 1–53. [[CrossRef](#)]
31. Dahms, H.-U.; Dobretsov, S.; Qian, P.-Y. The Effect of Bacterial and Diatom Biofilms on the Settlement of the Bryozoan Bugula Neritina. *J. Exp. Mar. Biol. Ecol.* **2004**, *313*, 191–209. [[CrossRef](#)]
32. Dobretsov, S.; Qian, P.-Y. Facilitation and Inhibition of Larval Attachment of the Bryozoan Bugula Neritina in Association with Mono-Species and Multi-Species Biofilms. *J. Exp. Mar. Biol. Ecol.* **2006**, *333*, 263–274. [[CrossRef](#)]
33. Wieczorek, S.K.; Clare, A.S.; Todd, C.D. Inhibitory and Facilitatory Effects of Microbial Films on Settlement of Balanus Amphitrite Amphitrite Larvae. *Mar. Ecol. Prog. Ser.* **1995**, *119*, 221–228. [[CrossRef](#)]
34. De Carvalho, C.C.C.R. Marine Biofilms: A Successful Microbial Strategy With Economic Implications. *Front. Mar. Sci.* **2018**, *5*, 126. [[CrossRef](#)]
35. Sweat, L.H.; Swain, G.W.; Hunsucker, K.Z.; Johnson, K.B. Transported Biofilms and Their Influence on Subsequent Macrofouling Colonization. *Biofouling* **2017**, *33*, 433–449. [[CrossRef](#)]
36. Cacabelos, E.; Ramalhosa, P.; Canning-Clode, J.; Troncoso, J.S.; Olabarria, C.; Delgado, C.; Dobretsov, S.; Gestoso, I. The Role of Biofilms Developed under Different Anthropogenic Pressure on Recruitment of Macro-Invertebrates. *IJMS* **2020**, *21*, 2030. [[CrossRef](#)]
37. Stegen, J.C.; Lin, X.; Konopka, A.E.; Fredrickson, J.K. Stochastic and Deterministic Assembly Processes in Subsurface Microbial Communities. *ISME J.* **2012**, *6*, 1653–1664. [[CrossRef](#)]
38. Scheuerman, T.R.; Camper, A.K.; Hamilton, M.A. Effects of Substratum Topography on Bacterial Adhesion. *J. Colloid Interface Sci.* **1998**, *208*, 23–33. [[CrossRef](#)]
39. Crawford, R.J.; Webb, H.K.; Truong, V.K.; Hasan, J.; Ivanova, E.P. Surface Topographical Factors Influencing Bacterial Attachment. *Adv. Colloid Interface Sci.* **2012**, *179–182*, 142–149. [[CrossRef](#)]
40. Kardar, P.; Amini, R. A Study on the Effect of Surface Topography of Antifouling Coatings on the Settlement of Fouling Organisms. *Pigment. Resin Technol.* **2022**. *ahead-of-print*. [[CrossRef](#)]
41. Jones, P.R.; Cottrell, M.T.; Kirchman, D.L.; Dexter, S.C. Bacterial Community Structure of Biofilms on Artificial Surfaces in an Estuary. *Microb. Ecol.* **2006**, *53*, 153–162. [[CrossRef](#)]
42. Fletcher, M.; Loeb, G.I. Influence of Substratum Characteristics on the Attachment of a Marine Pseudomonad to Solid Surfaces. *Appl. Environ. Microbiol.* **1979**, *37*, 67. [[CrossRef](#)]
43. Lee, J.-W.; Nam, J.-H.; Kim, Y.-H.; Lee, K.-H.; Lee, D.-H. Bacterial Communities in the Initial Stage of Marine Biofilm Formation on Artificial Surfaces. *J. Microbiol.* **2008**, *46*, 174–182. [[CrossRef](#)]
44. Baragi, L.V.; Anil, A.C. Synergistic Effect of Elevated Temperature, $p\text{CO}_2$ and Nutrients on Marine Biofilm. *Mar. Pollut. Bull.* **2016**, *105*, 102–109. [[CrossRef](#)]
45. Johnson, V.R.; Brownlee, C.; Rickaby, R.E.M.; Graziano, M.; Milazzo, M.; Hall-Spencer, J.M. Responses of Marine Benthic Microalgae to Elevated CO_2 . *Mar. Biol.* **2013**, *160*, 1813–1824. [[CrossRef](#)]
46. Brown, N.E.M.; Milazzo, M.; Rastrick, S.P.S.; Hall-Spencer, J.M.; Therriault, T.W.; Harley, C.D.G. Natural Acidification Changes the Timing and Rate of Succession, Alters Community Structure, and Increases Homogeneity in Marine Biofouling Communities. *Glob. Chang. Biol.* **2018**, *24*, e112–e127. [[CrossRef](#)] [[PubMed](#)]
47. Sorte, C.J.B.; Williams, S.L.; Zerebecki, R.A. Ocean Warming Increases Threat of Invasive Species in a Marine Fouling Community. *Ecology* **2010**, *91*, 2198–2204. [[CrossRef](#)] [[PubMed](#)]
48. Nasrolahi, A.; Stratil, S.B.; Jacob, K.J.; Wahl, M. A Protective Coat of Microorganisms on Macroalgae: Inhibitory Effects of Bacterial Biofilms and Epibiotic Microbial Assemblages on Barnacle Attachment. *FEMS Microbiol. Ecol.* **2012**, *81*, 583–595. [[CrossRef](#)] [[PubMed](#)]
49. Khandeparker, L.; D’Costa, P.M.; Anil, A.C.; Sawant, S.S. Interactions of Bacteria with Diatoms: Influence on Natural Marine Biofilms. *Mar. Ecol.* **2014**, *35*, 233–248. [[CrossRef](#)]
50. Khalaman, V.V.; Komendantov, A.Y.; Malavenda, S.S.; Mikhaylova, T.A. Algae versus Animals in Early Fouling Communities of the White Sea. *Mar. Ecol. Prog. Ser.* **2016**, *553*, 13–32. [[CrossRef](#)]
51. Levich, A.P. Variational Modelling Theorems and Algoecoenoses Functioning Principles. *Ecol. Model.* **2000**, *131*, 207–227. [[CrossRef](#)]

52. Steinberg, P.D.; De Nys, R. Chemical Mediation of Colonization of Seaweed Surfaces. *J. Phycol.* **2002**, *38*, 621–629. [[CrossRef](#)]
53. Wikström, S.A.; Pavia, H. Chemical Settlement Inhibition versus Post-Settlement Mortality as an Explanation for Differential Fouling of Two Congeneric Seaweeds. *Oecologia* **2004**, *138*, 223–230. [[CrossRef](#)]
54. Round, F.E.; Crawford, R.M.; Mann, D.G. *The Diatoms: Biology & Morphology of the Genera*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 1990; ISBN 978-0-521-36318-1.
55. Geider, R.J.; Delucia, E.H.; Falkowski, P.G.; Finzi, A.C.; Philip Grime, J.; Grace, J.; Kana, T.M.; La Roche, J.; Long, S.P.; Osborne, B.A.; et al. Primary Productivity of Planet Earth: Biological Determinants and Physical Constraints in Terrestrial and Aquatic Habitats. *Glob. Chang. Biol.* **2001**, *7*, 849–882. [[CrossRef](#)]
56. Falciatore, A.; Bowler, C. Revealing the Molecular Secrets of Marine Diatoms. *Annu. Rev. Plant Biol.* **2002**, *53*, 109–130. [[CrossRef](#)]
57. Medlin, L.K. Why Silica or Better yet Why Not Silica? Speculations as to Why the Diatoms Utilise Silica as Their Cell Wall Material. *Diatom Res.* **2002**, *17*, 453–459. [[CrossRef](#)]
58. Underwood, G.J.C.; Paterson, D.M. The Importance of Extracellular Carbohydrate Production by Marine Epipellic Diatoms. *Adv. Bot. Res.* **2003**, *40*, 183–240. [[CrossRef](#)]
59. Hoagland, K.D.; Rosowski, J.R.; Gretz, M.R.; Roemer, S.C. Diatom Extracellular Polymeric Substances: Function, Fine Structure, Chemistry and Physiology. *J. Phycol.* **1993**, *29*, 537–566. [[CrossRef](#)]
60. Cooksey, K.E.; Wigglesworth-Cooksey, B. Adhesion of Bacteria and Diatoms to Surfaces in the Sea: A Review. *Aquat. Microb. Ecol.* **1995**, *9*, 87–96. [[CrossRef](#)]
61. Baier, R.E. Substrata Influences on Adhesion of Microorganisms and Their Resultant New Surface Properties. In *Adsorption of Microorganisms to Surfaces*; Wiley-Interscience: New York, NY, USA, 1980; pp. 59–104.
62. Cooksey, K.E. Requirement for Calcium in Adhesion of a Fouling Diatom to Glass. *Appl. Environ. Microbiol.* **1981**, *41*, 1378–1382. [[CrossRef](#)]
63. Cooksey, B.; Cooksey, K. Chemical Signal-Response in Diatoms of the Genus *Amphora*. *J. Cell Sci.* **1988**, *91*, 523–529. [[CrossRef](#)]
64. Dugdale, T.M.; Dagastine, R.; Chiovitti, A.; Mulvaney, P.; Wetherbee, R. Single Adhesive Nanofibers from a Live Diatom Have the Signature Fingerprint of Modular Proteins. *Biophys. J.* **2005**, *89*, 4252–4260. [[CrossRef](#)]
65. McLachlan, D.H.; Underwood, G.J.C.; Taylor, A.R.; Brownlee, C. Calcium Release From Intracellular Stores Is Necessary For The Photophobic Response In The Benthic Diatom *Navicula perminuta* (Bacillariophyceae). *J. Phycol.* **2012**, *48*, 675–681. [[CrossRef](#)]
66. Heath, C.R.; Leadbeater, B.C.S.; Callow, M.E. Effect of Inhibitors on Calcium Carbonate Deposition Mediated by Freshwater Algae. *J. Appl. Phycol.* **1995**, *7*, 367–380. [[CrossRef](#)]
67. Gutiérrez-Medina, B.; Peña Maldonado, A.I.; García-Meza, J.V. Mechanical Testing of Particle Streaming and Intact Extracellular Mucilage Nanofibers Reveal a Role of Elastic Force in Diatom Motility. *Phys. Biol.* **2022**, *19*, 056002. [[CrossRef](#)]
68. Bedoshvili, Y.D.; Gneusheva, K.V.; Popova, M.S.; Avezova, T.N.; Arsenyev, K.Y.; Likhoshway, Y.V. Frustule Morphogenesis of Raphid Pennate Diatom *Encyonema ventricosum* (Agardh) Grunow. *Protoplasma* **2018**, *255*, 911–921. [[CrossRef](#)] [[PubMed](#)]
69. Lind, J.L.; Heimann, K.; Miller, E.A.; Van Vliet, C.; Hoogenraad, N.J.; Wetherbee, R. Substratum Adhesion and Gliding in a Diatom Are Mediated by Extracellular Proteoglycans. *Planta* **1997**, *203*, 213–221. [[CrossRef](#)] [[PubMed](#)]
70. Murray, R.E.; Cooksey, K.E.; Priscu, J.C. Stimulation of Bacterial DNA Synthesis by Algal Exudates in Attached Algal-Bacterial Consortia. *Appl. Environ. Microbiol.* **1986**, *52*, 1177–1182. [[CrossRef](#)] [[PubMed](#)]
71. Okabe, S.; Hirata, K.; Watanabe, Y. Significance of the Spatial Distribution of Microbial Species in Mixed-population Biofilms. *Biofouling* **1997**, *11*, 119–136. [[CrossRef](#)]
72. Murray, R.E.; Cooksey, K.E.; Priscu, J.C. Influence of Physical Disruption on Growth of Attached Bacteria. *Appl. Environ. Microbiol.* **1987**, *53*, 2997–2999. [[CrossRef](#)]
73. Källén, J.; Muller, H.; Franken, M.L.; Crisp, A.; Stroh, C.; Pillay, D.; Lawrence, C. Seagrass-Epifauna Relationships in a Temperate South African Estuary: Interplay between Patch-Size, within-Patch Location and Algal Fouling. *Estuar. Coast. Shelf Sci.* **2012**, *113*, 213–220. [[CrossRef](#)]
74. Chen, M.; Qi, H.; Intasen, W.; Kanchanapant, A.; Wang, C.; Zhang, A. Distributions of Diatoms in Surface Sediments from the Chanthaburi Coast, Gulf of Thailand, and Correlations with Environmental Factors. *Reg. Stud. Mar. Sci.* **2020**, *34*, 100991. [[CrossRef](#)]
75. Lewin, R.A. (Ed.) *Physiology and Biochemistry of Algae*; Academic Press: New York, NY, USA, 1962; ISBN 978-0-12-446150-5.
76. Cooksey, K.E.; Chansang, H. Isolation and Physiological Studies on Three Isolates of *Amphora* (Bacillariophyceae). *J. Phycol.* **1976**, *12*, 455–460. [[CrossRef](#)]
77. Loeb, G.; Laster, D.; Gracik, T.; Taylor, D. The Influence of Microbial Fouling Films on Hydrodynamic Drag of Rotating Discs. In *Marine Biodeterioration: An Interdisciplinary Study*; Springer: New York, NY, USA, 1984; pp. 88–94. ISBN 978-1-4615-9722-3.
78. Terlizzi, A.; Frascchetti, S.; Gianguzza, P.; Faimali, M.; Boero, F. Environmental Impact of Antifouling Technologies: State of the Art and Perspectives. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2001**, *11*, 311–317. [[CrossRef](#)]
79. Buhmann, M.T.; Poulsen, N.; Klemm, J.; Kennedy, M.R.; Sherrill, C.D.; Kröger, N. A Tyrosine-Rich Cell Surface Protein in the Diatom *Amphora coffeaeformis* Identified through Transcriptome Analysis and Genetic Transformation. *PLoS ONE* **2014**, *9*, e110369. [[CrossRef](#)]
80. Poulsen, N.; Kröger, N.; Harrington, M.J.; Brunner, E.; Paasch, S.; Buhmann, M.T. Isolation and Biochemical Characterization of Underwater Adhesives from Diatoms. *Biofouling* **2014**, *30*, 513–523. [[CrossRef](#)]

81. Hudon, C.; Bourget, E. Initial Colonization of Artificial Substrate: Community Development and Structure Studied by Scanning Electron Microscopy. *Can. J. Fish. Aquat. Sci.* **1981**, *38*, 1371–1384. [[CrossRef](#)]
82. Hodson, O.M.; Monty, J.P.; Molino, P.J.; Wetherbee, R. Novel Whole Cell Adhesion Assays of Three Isolates of the Fouling Diatom *Amphora coffeaeformis* Reveal Diverse Responses to Surfaces of Different Wettability. *Biofouling* **2012**, *28*, 381–393. [[CrossRef](#)]
83. Finlay, J.A.; Callow, M.E.; Ista, L.K.; Lopez, G.P.; Callow, J.A. The Influence of Surface Wettability on the Adhesion Strength of Settled Spores of the Green Alga *Enteromorpha* and the Diatom *Amphora*. *Integr. Comp. Biol.* **2002**, *42*, 1116–1122. [[CrossRef](#)]
84. Görlich, S.; Pawolski, D.; Zlotnikov, I.; Kröger, N. Control of Biosilica Morphology and Mechanical Performance by the Conserved Diatom Gene Silicanin-1. *Commun. Biol.* **2019**, *2*, 245. [[CrossRef](#)]
85. Lachnit, M.; Buhmann, M.T.; Klemm, J.; Kröger, N.; Poulsen, N. Identification of Proteins in the Adhesive Trails of the Diatom *Amphora coffeaeformis*. *Philos. Trans. R. Soc. B Biol. Sci.* **2019**, *374*, 20190196. [[CrossRef](#)]
86. Mazzella, L.; Spinoccia, L. Epiphytic Diatoms of Leaf Blades of the Mediterranean Seagrass *Posidonia oceanica* (L.) Delile. *G. Bot. Ital.* **1992**, *126*, 752–754. [[CrossRef](#)]
87. De Stefano, M.; Marino, D.; Mazzella, L. Marine taxa of *Cocconeis* on leaves of *Posidonia oceanica*, including a new species and two new varieties. *Eur. J. Phycol.* **2000**, *35*, 225–242. [[CrossRef](#)]
88. Mabrouk, L.; Ben Brahim, M.; Hamza, A.; Mahfoudhi, M.; Bradai, M.N. A Comparison of Abundance and Diversity of Epiphytic Microalgal Assemblages on the Leaves of the Seagrasses *Posidonia oceanica* (L.) and *Cymodocea nodosa* (Ucria) Asch in Eastern Tunisia. *J. Mar. Sci.* **2014**, *2014*, 275305. [[CrossRef](#)]
89. Kanjer, L.; Mucko, M.; Car, A.; Bosak, S. Epiphytic Diatoms on *Posidonia oceanica* (L.) Delile Leaves from Eastern Adriatic Sea. *Nat. Croat.* **2019**, *28*, 1–20.
90. Torres-Monroy, I.; Ullrich, M.S. Identification of Bacterial Genes Expressed During Diatom-Bacteria Interactions Using an in Vivo Expression Technology Approach. *Front. Mar. Sci.* **2018**, *5*, 200. [[CrossRef](#)]
91. Sörenson, E.; Capo, E.; Farnelid, H.; Lindehoff, E.; Legrand, C. Temperature Stress Induces Shift From Co-Existence to Competition for Organic Carbon in Microalgae-Bacterial Photobioreactor Community—Enabling Continuous Production of Microalgal Biomass. *Front. Microbiol.* **2021**, *12*, 607601. [[CrossRef](#)] [[PubMed](#)]
92. Fu, W.; Chaiboonchoe, A.; Dohai, B.; Sultana, M.; Baffour, K.; Alzahmi, A.; Weston, J.; Al Khairy, D.; Daakour, S.; Jaiswal, A.; et al. GPCR Genes as Activators of Surface Colonization Pathways in a Model Marine Diatom. *iScience* **2020**, *23*, 101424. [[CrossRef](#)] [[PubMed](#)]
93. Willis, A.; Eason Hubbard, M.; Hodson, O.; Bowler, C.; Wetherbee, R. Adhesion Molecules from the Diatom *Phaeodactylum tricorutum* (Bacillariophyceae): Genomic Identification by Amino-Acid Profiling and in Vivo Analysis. *J. Phycol.* **2014**, *50*, 837–849. [[CrossRef](#)]
94. Johnson, K.L.; Kibble, N.A.J.; Bacic, A.; Schultz, C.J. A Fasciclin-Like Arabinogalactan-Protein (FLA) Mutant of *Arabidopsis thaliana*, *fla1*, Shows Defects in Shoot Regeneration. *PLoS ONE* **2011**, *6*, e25154. [[CrossRef](#)]
95. Osuna-Cruz, C.M.; Bilcke, G.; Vancaester, E.; De Decker, S.; Bones, A.M.; Winge, P.; Poulsen, N.; Bulankova, P.; Verhelst, B.; Audoor, S.; et al. The *Seminavis robusta* Genome Provides Insights into the Evolutionary Adaptations of Benthic Diatoms. *Nat. Commun.* **2020**, *11*, 3320. [[CrossRef](#)]
96. Hennebert, E.; Wattiez, R.; Waite, J.H.; Flammang, P. Characterization of the Protein Fraction of the Temporary Adhesive Secreted by the Tube Feet of the Sea Star *Asterias rubens*. *Biofouling* **2012**, *28*, 289–303. [[CrossRef](#)]
97. Subramanian, K.; Pravalika, M.; Menon, V. Evidence for stress-induced bleeding in a patient with von Willebrand factor deficiency. *Indian J. Psychol. Med.* **2018**, *40*, 292–295. [[CrossRef](#)]
98. Lebret, K.; Thabard, M.; Helliou, C. Algae as Marine Fouling Organisms: Adhesion Damage and Prevention. In *Advances in Marine Antifouling Coatings and Technologies*; CRC Press: Boca Raton, FL, USA, 2009; pp. 80–112.
99. Clare, A.; Aldred, N. Surface Colonisation by Marine Organisms and Its Impact on Antifouling Research. In *Advances in Marine Antifouling Coatings and Technologies*; CRC Press: Boca Raton, FL, USA, 2009; pp. 46–79. ISBN 978-1-84569-386-2.
100. Van Loosdrecht, M.C.M.; Lyklema, J.; Norde, W.; Zehnder, A.J.B. Bacterial Adhesion: A Physicochemical Approach. *Microb. Ecol.* **1989**, *17*, 1–15. [[CrossRef](#)]
101. Michael, T.S.; Shin, H.W.; Hanna, R.; Spafford, D.C. A Review of Epiphyte Community Development: Surface Interactions and Settlement on Seagrass. *J. Environ. Biol.* **2008**, *29*, 629–638.
102. Sterrenburg, F.A.S.; Erfemeijer, P.L.A.; Nienhuis, P.H. Diatoms as Epiphytes on Seagrasses in South Sulawesi (Indonesia) Comparison with Growth on Inert Substrata. *Bot. Mar.* **1995**, *38*, 1–8. [[CrossRef](#)]
103. Prado, P.; Alcoverro, T.; Martínez-Crego, B.; Vergés, A.; Pérez, M.; Romero, J. Macrograzers Strongly Influence Patterns of Epiphytic Assemblages in Seagrass Meadows. *J. Exp. Mar. Biol. Ecol.* **2007**, *350*, 130–143. [[CrossRef](#)]
104. Drake, L.A.; Dobbs, F.C.; Zimmerman, R.C. Effects of Epiphyte Load on Optical Properties and Photosynthetic Potential of the Seagrasses *Thalassia testudinum* Banks Ex König and *Zostera marina* L. *Limnol. Oceanogr.* **2003**, *48*, 456–463. [[CrossRef](#)]
105. Mazzella, L.; Alberte, R.S. Light Adaptation and the Role of Autotrophic Epiphytes in Primary Production of the Temperate Seagrass, *Zostera marina* L. *J. Exp. Mar. Biol. Ecol.* **1986**, *100*, 165–180. [[CrossRef](#)]
106. Michael, T.; Smith, C. Lectins Probe Molecular Films in Biofouling: Characterization of Early Films on Non-Living and Living Surfaces. *Mar. Ecol. Prog. Ser.* **1995**, *119*, 229–236. [[CrossRef](#)]
107. Callow, M.E.; Callow, J.A. Enhanced Adhesion and Chemoattraction of Zoospores of the Fouling Alga *Enteromorpha* to Some Foul-release Silicone Elastomers. *Biofouling* **1998**, *13*, 157–172. [[CrossRef](#)]

108. Xiao, L.; Finlay, J.A.; Röhrig, M.; Mieszkin, S.; Worgull, M.; Hölscher, H.; Callow, J.A.; Callow, M.E.; Grunze, M.; Rosenhahn, A. Topographic Cues Guide the Attachment of Diatom Cells and Algal Zoospores. *Biofouling* **2018**, *34*, 86–97. [[CrossRef](#)]
109. Borowitzka, M.A.; Lavery, P.S.; van Keulen, M. Epiphytes of Seagrasses. In *Seagrasses: Biology, Ecology and Conservation*; Springer: Dordrecht, The Netherlands, 2006; pp. 441–461. ISBN 978-1-4020-2942-4.
110. Buia, M.C.; Zupo, V.; Mazzella, L. Primary Production and Growth Dynamics in *Posidonia oceanica*. *Mar. Ecol.* **1992**, *13*, 2–16. [[CrossRef](#)]
111. Reyes, J.; Sansón, M.; Afonso-Carrillo, J. Distribution of the Epiphytes along the Leaves of *Cymodocea nodosa* in the Canary Islands. *Bot. Mar.* **1998**, *41*, 543–551. [[CrossRef](#)]
112. Uku, J.; Björk, M. The Distribution of Epiphytic Algae on Three Kenyan Seagrass Species. *South Afr. J. Bot.* **2001**, *67*, 475–482. [[CrossRef](#)]
113. Mateo, M.; Cebrian, J.; Dunton, K.; Mutchler, T.; Larkum, A.; Orth, R.; Duarte, C. Carbon Flux in Seagrass Ecosystems. In *Seagrasses: Biology, Ecology and Conservation*; Springer: Dordrecht, The Netherlands, 2006; pp. 159–192. ISBN 978-1-4020-2942-4.
114. Mazzella, L.; Scipione, M.; Gambi, M.C.; Buia, M.; Lorenti, M.; Zupo, V.; Cancemi, G. The Mediterranean Seagrass *Posidonia oceanica* and *Cymodocea nodosa*. In Proceedings of the First International Conference on the Mediterranean Coastal Environment, MEDCOAST 93, Antalya, Turkey, 2–5 November 1993; pp. 103–116.
115. Kuo, J.; Hartog, C. den Seagrass Morphology, Anatomy, and Ultrastructure. In *Seagrasses: Biology, Ecology and Conservation*; Larkum, A.W.D., Orth, R.J., Duarte, C.M., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 51–87. ISBN 978-1-4020-2983-7.
116. Onuf, C.P. Biofouling and the Continuous Monitoring of Underwater Light from a Seagrass Perspective. *Estuaries Coasts* **2006**, *29*, 8. [[CrossRef](#)]
117. Trautman, D.A.; Borowitzka, M.A. Distribution of the Epiphytic Organisms on *Posidonia australis* and *P. sinuosa*, Two Seagrasses with Differing Leaf Morphology. *Mar. Ecol. Prog. Ser.* **1999**, *179*, 215–229. [[CrossRef](#)]
118. Sekar, R.; Venugopalan, V.P.; Satpathy, K.K.; Nair, K.V.K.; Rao, V.N.R. Laboratory Studies on Adhesion of Microalgae to Hard Substrates. *Hydrobiologia* **2004**, *512*, 109–116. [[CrossRef](#)]
119. Pillay, D.; Waspe, C. Grazer Specialisation and Temperature Effects on Epiphytic Fouling: Conservation Implications for a Temperate African Seagrass (*Zostera capensis*). *Mar. Ecol. Prog. Ser.* **2019**, *629*, 235–241. [[CrossRef](#)]
120. Hassenrück, C.; Hofmann, L.C.; Bischof, K.; Ramette, A. Seagrass Biofilm Communities at a Naturally CO₂-Rich Vent. *Environ. Microbiol. Rep.* **2015**, *7*, 516–525. [[CrossRef](#)]
121. Heitzman, J.M.; Caputo, N.; Yang, S.-Y.; Harvey, B.P.; Agostini, S. Recurrent Disease Outbreak in a Warm Temperate Marginal Coral Community. *Mar. Pollut. Bull.* **2022**, *182*, 113954. [[CrossRef](#)]
122. Moore, A.; Duffy, J. Foundation Species Identity and Trophic Complexity Affect Experimental Seagrass Communities. *Mar. Ecol. Prog. Ser.* **2016**, *556*, 105–121. [[CrossRef](#)]
123. Zhang, Y.S.; Silliman, B.R. A Facilitation Cascade Enhances Local Biodiversity in Seagrass Beds. *Diversity* **2019**, *11*, 30. [[CrossRef](#)]
124. Elven, B.; Lavery, P.; Kendrick, G. Reefs as Contributors to Diversity of Epiphytic Macroalgae Assemblages in Seagrass Meadows. *Mar. Ecol. Prog. Ser.* **2004**, *276*, 71–83. [[CrossRef](#)]
125. Frankovich, T.A.; Armitage, A.R.; Wachnicka, A.H.; Gaiser, E.E.; Fourqurean, J.W. Nutrient Effects on Seagrass Epiphyte Community Structure in Florida Bay. *J. Phycol.* **2009**, *45*, 1010–1020. [[CrossRef](#)]
126. Campbell, J.E.; Fourqurean, J.W. Ocean Acidification Outweighs Nutrient Effects in Structuring Seagrass Epiphyte Communities. *J. Ecol.* **2014**, *102*, 730–737. [[CrossRef](#)]
127. Mutalipassi, M.; Fink, P.; Maibam, C.; Porzio, L.; Buia, M.C.; Gambi, M.C.; Patti, F.P.; Scipione, M.B.; Lorenti, M.; Zupo, V. Ocean Acidification Alters the Responses of Invertebrates to Wound-Activated Infochemicals Produced by Epiphytes of the Seagrass *Posidonia oceanica*. *J. Exp. Mar. Biol. Ecol.* **2020**, *530–531*, 151435. [[CrossRef](#)]
128. Berlinghof, J.; Peiffer, F.; Marzocchi, U.; Munari, M.; Quero, G.M.; Dennis, L.; Wild, C.; Cardini, U. The Role of Epiphytes in Seagrass Productivity under Ocean Acidification. *Sci. Rep.* **2022**, *12*, 6249. [[CrossRef](#)]
129. Mutalipassi, M.; Mazzella, V.; Schott, M.; Fink, P.; Glaviano, F.; Porzio, L.; Lorenti, M.; Buia, M.C.; von Elert, E.; Zupo, V. Ocean Acidification Affects Volatile Infochemicals Production and Perception in Fauna and Flora Associated With *Posidonia oceanica* (L.) Delile. *Front. Mar. Sci.* **2022**, *9*, 101. [[CrossRef](#)]
130. Guan, C.; Saha, M.; Weinberger, F. Chemical Defence of a Seagrass against Microfoulers and Its Seasonal Dynamics. *Appl. Sci.* **2019**, *9*, 1258. [[CrossRef](#)]
131. 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](#)]
132. Sammarco, P.W.; Coll, J.C. Chemical Adaptations in the Octocorallia: Evolutionary Considerations. *Mar. Ecol. Prog. Ser.* **1992**, *88*, 93–104. [[CrossRef](#)]
133. Haslbeck, E.G.; Kavanagh, C.J.; Shin, H.W.; Banta, W.C.; Song, P.; Loeb, G.I. Minimum Effective Release Rate of Antifoulants (2): Measurement of the Effect of TBT and Zosteric Acid on Hard Fouling. *Biofouling* **1996**, *10*, 175–186. [[CrossRef](#)]
134. Caldeira, K.; Wickett, M.E. Anthropogenic Carbon and Ocean PH. *Nature* **2003**, *425*, 365. [[CrossRef](#)]
135. Doney, S.C.; Fabry, V.J.; Feely, R.A.; Kleypas, J.A. Ocean Acidification: The Other CO₂ Problem. *Annu. Rev. Mar. Sci.* **2009**, *1*, 169–192. [[CrossRef](#)]
136. Feely, R.A.; Sabine, C.L.; Lee, K.; Berelson, W.; Kleypas, J.; Fabry, V.J.; Millero, F.J. Impact of Anthropogenic CO₂ on the CaCO₃ System in the Oceans. *Science* **2004**, *305*, 362–366. [[CrossRef](#)] [[PubMed](#)]

137. Gattuso, J.-P.; Hansson, L. *Ocean Acidification*; OUP: Oxford, UK, 2011; ISBN 978-0-19-959109-1.
138. Hönisch, B.; Ridgwell, A.; Schmidt, D.N.; Thomas, E.; Gibbs, S.J.; Sluijs, A.; Zeebe, R.; Kump, L.; Martindale, R.C.; Greene, S.E.; et al. The Geological Record of Ocean Acidification. *Science* **2012**, *335*, 1058–1063. [[CrossRef](#)] [[PubMed](#)]
139. Orr, J.C.; Fabry, V.J.; Aumont, O.; Bopp, L.; Doney, S.C.; Feely, R.A.; Gnanadesikan, A.; Gruber, N.; Ishida, A.; Joos, F.; et al. Anthropogenic Ocean Acidification over the Twenty-First Century and Its Impact on Calcifying Organisms. *Nature* **2005**, *437*, 681–686. [[CrossRef](#)] [[PubMed](#)]
140. Tans, P. An Accounting of the Observed Increase in Oceanic and Atmospheric CO₂ and the Outlook for the Future. *Oceanography* **2009**, *22*, 26–35. [[CrossRef](#)]
141. Hall-Spencer, J.M.; Harvey, B.P. Ocean Acidification Impacts on Coastal Ecosystem Services Due to Habitat Degradation. *Emerg. Top. Life Sci.* **2019**, *3*, 197–206. [[CrossRef](#)]
142. Brown, N.E.M.; Therriault, T.W.; Harley, C.D.G. Field-Based Experimental Acidification Alters Fouling Community Structure and Reduces Diversity. *J. Anim. Ecol.* **2016**, *85*, 1328–1339. [[CrossRef](#)]
143. Speights, C.J.; McCoy, M.W. Range Expansion of a Fouling Species Indirectly Impacts Local Species Interactions. *PeerJ* **2017**, *5*, e3911. [[CrossRef](#)]
144. Dobretsov, S.; Coutinho, R.; Rittschof, D.; Salta, M.; Ragazzola, F.; Hellio, C. The Oceans Are Changing: Impact of Ocean Warming and Acidification on Biofouling Communities. *Biofouling* **2019**, *35*, 585–595. [[CrossRef](#)]
145. Ragazzola, F.; Foster, L.C.; Form, A.; Anderson, P.S.L.; Hansteen, T.H.; Fietzke, J. Ocean Acidification Weakens the Structural Integrity of Coralline Algae. *Glob. Chang. Biol.* **2012**, *18*, 2804–2812. [[CrossRef](#)]
146. Hale, R.; Calosi, P.; Mieszkowska, N.; Widdicombe, S.; McNeill, L. Predicted Levels of Future Ocean Acidification and Temperature Rise Could Alter Community Structure and Biodiversity in Marine Benthic Communities. *Oikos* **2011**, *120*, 661–674. [[CrossRef](#)]
147. Teixidó, N.; Gambi, M.C.; Parravacini, V.; Kroeker, K.; Micheli, F.; Villéger, S.; Ballesteros, E. Functional Biodiversity Loss along Natural CO₂ Gradients. *Nat. Commun.* **2018**, *9*, 5149. [[CrossRef](#)]
148. Zupo, V.; Buia, M.C.; Mazzella, L. A Production Model for *Posidonia oceanica* Based on Temperature. *Estuar. Coast. Shelf Sci.* **1997**, *44*, 483–492. [[CrossRef](#)]
149. Duncan, R.J.; Nielsen, D.A.; Sheehan, C.E.; Deppeler, S.; Hancock, A.M.; Schulz, K.G.; Davidson, A.T.; Petrou, K. Ocean Acidification Alters the Nutritional Value of Antarctic Diatoms. *New Phytol.* **2022**, *233*, 1813–1827. [[CrossRef](#)]
150. Kroeker, K.J.; Kordas, R.L.; Crim, R.; Hendriks, I.E.; Ramajo, L.; Singh, G.S.; Duarte, C.M.; Gattuso, J.-P. Impacts of Ocean Acidification on Marine Organisms: Quantifying Sensitivities and Interaction with Warming. *Glob. Chang. Biol.* **2013**, *19*, 1884–1896. [[CrossRef](#)]
151. Ross, P.M.; Parker, L.; O'Connor, W.A.; Bailey, E.A. The Impact of Ocean Acidification on Reproduction, Early Development and Settlement of Marine Organisms. *Water* **2011**, *3*, 1005–1030. [[CrossRef](#)]
152. Asnicar, D.; Zanovello, L.; Badocco, D.; Munari, M.; Marin, M.G. Different Ecological Histories of Sea Urchins Acclimated to Reduced PH Influence Offspring Response to Multiple Stressors. *Environ. Res.* **2022**, *212*, 113131. [[CrossRef](#)]
153. Foo, S.A.; Byrne, M. Marine Gametes in a Changing Ocean: Impacts of Climate Change Stressors on Fecundity and the Egg. *Mar. Environ. Res.* **2017**, *128*, 12–24. [[CrossRef](#)]
154. Milazzo, M.; Cattano, C.; Alonzo, S.H.; Foggo, A.; Gristina, M.; Rodolfo-Metalpa, R.; Sinopoli, M.; Spatafora, D.; Stiver, K.A.; Hall-Spencer, J.M. Ocean Acidification Affects Fish Spawning but Not Paternity at CO₂ Seeps. *Proc. R. Soc. B Biol. Sci.* **2016**, *283*, 20161021. [[CrossRef](#)]
155. Martin, S.; Gattuso, J.-P. Response of Mediterranean Coralline Algae to Ocean Acidification and Elevated Temperature. *Glob. Chang. Biol.* **2009**, *15*, 2089–2100. [[CrossRef](#)]
156. Ragazzola, F.; Marchini, A.; Adani, M.; Bordone, A.; Castelli, A.; Cerrati, G.; Kolzenburg, R.; Langeneck, J.; di Marzo, C.; Nannini, M.; et al. An Intertidal Life: Combined Effects of Acidification and Winter Heatwaves on a Coralline Alga (*Ellisolandia elongata*) and Its Associated Invertebrate Community. *Mar. Environ. Res.* **2021**, *169*, 105342. [[CrossRef](#)]
157. Langer, G.; Nehrke, G.; Probert, I.; Ly, J.; Ziveri, P. Strain-Specific Responses of *Emiliania huxleyi* to Changing Seawater Carbonate Chemistry. *Biogeosciences* **2009**, *6*, 2637–2646. [[CrossRef](#)]
158. Albright, R.; Takeshita, Y.; Koweek, D.A.; Ninokawa, A.; Wolfe, K.; Rivlin, T.; Nebuchina, Y.; Young, J.; Caldeira, K. Carbon Dioxide Addition to Coral Reef Waters Suppresses Net Community Calcification. *Nature* **2018**, *555*, 516–519. [[CrossRef](#)] [[PubMed](#)]
159. Hall-Spencer, J.M.; Rodolfo-Metalpa, R.; Martin, S.; Ransome, E.; Fine, M.; Turner, S.M.; Rowley, S.J.; Tedesco, D.; Buia, M.C. Volcanic Carbon Dioxide Vents Show Ecosystem Effects of Ocean Acidification. *Nature* **2008**, *454*, 96–99. [[CrossRef](#)] [[PubMed](#)]
160. Porzio, L.; Garrard, S.L.; Buia, M.C. The Effect of Ocean Acidification on Early Algal Colonization Stages at Natural CO₂ Vents. *Mar. Biol.* **2013**, *160*, 2247–2259. [[CrossRef](#)]
161. Porzio, L.; Buia, M.C.; Hall-Spencer, J.M. Effects of Ocean Acidification on Macroalgal Communities. *J. Exp. Mar. Biol. Ecol.* **2011**, *400*, 278–287. [[CrossRef](#)]
162. Vizzini, S.; Martínez-Crego, B.; Andolina, C.; Massa-Gallucci, A.; Connell, S.D.; Gambi, M.C. Ocean Acidification as a Driver of Community Simplification via the Collapse of Higher-Order and Rise of Lower-Order Consumers. *Sci. Rep.* **2017**, *7*, 4018. [[CrossRef](#)]
163. Agostini, S.; Harvey, B.P.; Wada, S.; Kon, K.; Milazzo, M.; Inaba, K.; Hall-Spencer, J.M. Ocean Acidification Drives Community Shifts towards Simplified Non-Calcified Habitats in a Subtropical–temperate Transition Zone. *Sci. Rep.* **2018**, *8*, 11354. [[CrossRef](#)]

164. Harvey, B.P.; Kon, K.; Agostini, S.; Wada, S.; Hall-Spencer, J.M. Ocean Acidification Locks Algal Communities in a Species-Poor Early Successional Stage. *Glob. Chang. Biol.* **2021**, *27*, 2174–2187. [[CrossRef](#)]
165. Peña, V.; Harvey, B.P.; Agostini, S.; Porzio, L.; Milazzo, M.; Horta, P.; Le Gall, L.; Hall-Spencer, J.M. Major Loss of Coralline Algal Diversity in Response to Ocean Acidification. *Glob. Chang. Biol.* **2021**, *27*, 4785–4798. [[CrossRef](#)]
166. Short, J.; Kendrick, G.A.; Falter, J.; McCulloch, M.T. Interactions between Filamentous Turf Algae and Coralline Algae Are Modified under Ocean Acidification. *J. Exp. Mar. Biol. Ecol.* **2014**, *456*, 70–77. [[CrossRef](#)]
167. Smith, J.N.; Mongin, M.; Thompson, A.; Jonker, M.J.; De'ath, G.; Fabricius, K.E. Shifts in Coralline Algae, Macroalgae, and Coral Juveniles in the Great Barrier Reef Associated with Present-Day Ocean Acidification. *Glob. Chang. Biol.* **2020**, *26*, 2149–2160. [[CrossRef](#)]
168. Schwartz, W. J. D. Milliman (Editor), Marine Carbonates (Recent Sedimentary Carbonates, Part I). XV, 375 S., 94 Abb., 80 Tab., 39 Taf. Berlin–Heidelberg–New York 1974: Springer-Verlag. DM 66,00. *Z. Allg. Mikrobiol.* **1976**, *16*, 242. [[CrossRef](#)]
169. Donnarumma, L.; Lombardi, C.; Cocito, S.; Gambi, M.C. Settlement Pattern of *Posidonia oceanica* along a Gradient of Ocean Acidification: An Approach with Mimics. *Mediterr. Mar. Sci.* **2014**, *15*, 498–509. [[CrossRef](#)]
170. Casola, E.; Scardi, M.; Mazzella, L.; Fresi, E. Structure of the Epiphytic Community of *Posidonia oceanica* Leaves in a Shallow Meadow. *Mar. Ecol.* **1987**, *8*, 285–296. [[CrossRef](#)]
171. Mecca, S.; Casoli, E.; Ardizzone, G.; Gambi, M.C. Effects of Ocean Acidification on Phenology and Epiphytes of the Seagrass *Posidonia oceanica* at Two CO₂ Vent Systems of Ischia (Italy). *Mediterr. Mar. Sci.* **2020**, *21*, 70–83. [[CrossRef](#)]
172. Martin, S.; Rodolfo-Metalpa, R.; Ransome, E.; Rowley, S.; Buia, M.-C.; Gattuso, J.-P.; Hall-Spencer, J. Effects of Naturally Acidified Seawater on Seagrass Calcareous Epibionts. *Biol. Lett.* **2008**, *4*, 689–692. [[CrossRef](#)]
173. Mirasole, A.; Badalamenti, F.; Di Franco, A.; Gambi, M.C.; Teixidó, N. Boosted Fish Abundance Associated with *Posidonia oceanica* Meadows in Temperate Shallow CO₂ Vents. *Sci. Total Environ.* **2021**, *771*, 145438. [[CrossRef](#)]
174. Torstensson, A.; Chierici, M.; Wulff, A. The Influence of Increased Temperature and Carbon Dioxide Levels on the Benthic/Sea Ice Diatom *Navicula directa*. *Polar Biol.* **2012**, *35*, 205–214. [[CrossRef](#)]
175. Tortell, P.D.; Rau, G.H.; Morel, F.M.M. Inorganic Carbon Acquisition in Coastal Pacific Phytoplankton Communities. *Limnol. Oceanogr.* **2000**, *45*, 1485–1500. [[CrossRef](#)]
176. Hopkinson, B.M.; Dupont, C.L.; Allen, A.E.; Morel, F.M.M. Efficiency of the CO₂-Concentrating Mechanism of Diatoms. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 3830–3837. [[CrossRef](#)]
177. Kjørboe, T. Turbulence, Phytoplankton Cell Size, and the Structure of Pelagic Food Webs. In *Advances in Marine Biology*; Blaxter, J.H.S., Southward, A.J., Eds.; Academic Press: Cambridge, MA, USA, 1993; Volume 29, pp. 1–72.
178. Zunino, S.; Canu, D.M.; Zupo, V.; Solidoro, C. Direct and Indirect Impacts of Marine Acidification on the Ecosystem Services Provided by Coralligenous Reefs and Seagrass Systems. *Glob. Ecol. Conserv.* **2019**, *18*, e00625. [[CrossRef](#)]
179. Chapin, F.S.; Zavaleta, E.S.; Eviner, V.T.; Naylor, R.L.; Vitousek, P.M.; Reynolds, H.L.; Hooper, D.U.; Lavorel, S.; Sala, O.E.; Hobbie, S.E.; et al. Consequences of Changing Biodiversity. *Nature* **2000**, *405*, 234–242. [[CrossRef](#)] [[PubMed](#)]
180. Wahl, M.; Shahnaz, L.; Dobretsov, S.; Saha, M.; Symanowski, F.; David, K.; Lachnit, T.; Vasel, M.; Weinberger, F. Ecology of Antifouling Resistance in the Bladder Wrack *Fucus vesiculosus*: Patterns of Microfouling and Antimicrobial Protection; *Mar. Ecol. Prog. Ser.* **2010**, *411*, 33–48. [[CrossRef](#)]
181. Beltrand, M.; Dineen, A.S.; Hitzeroth, C.; Baum, B.; de Cerff, C.; de Vos, C.; Lewis, J.; Zarouf, S.; Pillay, D. Warming Effects on Two Autogenic Engineers (*Zostera capensis* and *Gracilaria gracilis*): Consequences for Macrofaunal Assemblages and Benthic Heterogeneity in Intertidal Sandflat Ecosystems. *Estuaries Coasts* **2022**, *45*, 247–259. [[CrossRef](#)]
182. Marbà, N.; Duarte, C.M. Mediterranean Warming Triggers Seagrass (*Posidonia oceanica*) Shoot Mortality. *Glob. Chang. Biol.* **2010**, *16*, 2366–2375. [[CrossRef](#)]
183. Lewandowska, A.M.; Boyce, D.G.; Hofmann, M.; Matthiessen, B.; Sommer, U.; Worm, B. Effects of Sea Surface Warming on Marine Plankton. *Ecol. Lett.* **2014**, *17*, 614–623. [[CrossRef](#)]
184. Li, W.K.W.; McLaughlin, F.A.; Lovejoy, C.; Carmack, E.C. Smallest Algae Thrive as the Arctic Ocean Freshens. *Science* **2009**, *326*, 539. [[CrossRef](#)]
185. Morán, X.A.G.; López-Urrutia, Á.; Calvo-Díaz, A.; Li, W.K.W. Increasing Importance of Small Phytoplankton in a Warmer Ocean. *Glob. Chang. Biol.* **2010**, *16*, 1137–1144. [[CrossRef](#)]
186. Li, C.; Meng, Y.; He, C.; Chan, V.B.S.; Yao, H.; Thiyagarajan, V. Mechanical Robustness of the Calcareous Tubeworm *Hydroides elegans*: Warming Mitigates the Adverse Effects of Ocean Acidification. *Biofouling* **2016**, *32*, 191–204. [[CrossRef](#)]
187. Whalan, S.; Webster, N.S. Sponge Larval Settlement Cues: The Role of Microbial Biofilms in a Warming Ocean. *Sci. Rep.* **2014**, *4*, 4072. [[CrossRef](#)]
188. Short, J.; Foster, T.; Falter, J.; Kendrick, G.A.; McCulloch, M.T. Crustose Coralline Algal Growth, Calcification and Mortality Following a Marine Heatwave in Western Australia. *Cont. Shelf Res.* **2015**, *106*, 38–44. [[CrossRef](#)]
189. McPherson, M.L.; Finger, D.J.I.; Houskeeper, H.F.; Bell, T.W.; Carr, M.H.; Rogers-Bennett, L.; Kudela, R.M. Large-Scale Shift in the Structure of a Kelp Forest Ecosystem Co-Occurs with an Epizootic and Marine Heatwave. *Commun. Biol.* **2021**, *4*, 298. [[CrossRef](#)]
190. Li, X.; Roevros, N.; Dehairs, F.; Chou, L. Biological Responses of the Marine Diatom *Chaetoceros socialis* to Changing Environmental Conditions: A Laboratory Experiment. *PLoS ONE* **2017**, *12*, e0188615. [[CrossRef](#)]
191. Kelaher, B.P.; Mamo, L.T.; Provost, E.; Litchfield, S.G.; Giles, A.; Butcherine, P. Influence of Ocean Warming and Acidification on Habitat-Forming Coralline Algae and Their Associated Molluscan Assemblages. *Glob. Ecol. Conserv.* **2022**, *35*, e02081. [[CrossRef](#)]

192. Lawrence, C.M.; Bolton, J.J. Experimental Effects of Warming and Epiphyte Grazing on the Ecophysiology of Two Seagrass Morphotypes. *J. Exp. Mar. Biol. Ecol.* **2023**, *558*, 151834. [[CrossRef](#)]
193. Smale, D.; Wernberg, T. Short-Term In Situ Warming Influences Early Development of Sessile Assemblages. *Mar. Ecol. Prog. Ser.* **2012**, *453*, 129–136. [[CrossRef](#)]
194. Savva, I.; Bennett, S.; Roca, G.; Jordà, G.; Marbà, N. Thermal Tolerance of Mediterranean Marine Macrophytes: Vulnerability to Global Warming. *Ecol. Evol.* **2018**, *8*, 12032–12043. [[CrossRef](#)]
195. Ontoria, Y.; Cuesta-Gracia, A.; Ruiz, J.M.; Romero, J.; Pérez, M. The Negative Effects of Short-Term Extreme Thermal Events on the Seagrass *Posidonia oceanica* Are Exacerbated by Ammonium Additions. *PLoS ONE* **2019**, *14*, e0222798. [[CrossRef](#)]
196. Burrows, M.T.; Schoeman, D.S.; Buckley, L.B.; Moore, P.; Poloczanska, E.S.; Brander, K.M.; Brown, C.; Bruno, J.F.; Duarte, C.M.; Halpern, B.S.; et al. The Pace of Shifting Climate in Marine and Terrestrial Ecosystems. *Science* **2011**, *334*, 652–655. [[CrossRef](#)]
197. Richardson, C.; Kennedy, H.; Duarte, C.; Kennedy, P.; Proud, S. Age and Growth of the Fan Mussel *Pinna nobilis* from South-East Spanish Mediterranean Seagrass (*Posidonia oceanica*) Meadows. *Mar. Biol.* **1999**, *133*, 205–212. [[CrossRef](#)]
198. Steinacher, M.; Joos, F.; Frölicher, T.L.; Bopp, L.; Cadule, P.; Cocco, V.; Doney, S.C.; Gehlen, M.; Lindsay, K.; Moore, J.K.; et al. Projected 21st Century Decrease in Marine Productivity: A Multi-Model Analysis. *Biogeosciences* **2010**, *7*, 979–1005. [[CrossRef](#)]
199. Hofmann, M.; Worm, B.; Rahmstorf, S.; Schellnhuber, H.J. Declining Ocean Chlorophyll under Unabated Anthropogenic CO₂ Emissions. *Environ. Res. Lett.* **2011**, *6*, 034035. [[CrossRef](#)]
200. Sommer, U.; Aberle, N.; Lengfellner, K.; Lewandowska, A. The Baltic Sea Spring Phytoplankton Bloom in a Changing Climate: An Experimental Approach. *Mar. Biol.* **2012**, *159*, 2479–2490. [[CrossRef](#)]
201. Sommer, U.; Sommer, F. Cladocerans versus Copepods: The Cause of Contrasting Top-down Controls on Freshwater and Marine Phytoplankton. *Oecologia* **2006**, *147*, 183–194. [[CrossRef](#)]
202. Stibor, H.; Vadstein, O.; Diehl, S.; Gelzleichter, A.; Hansen, T.; Hantzschke, F.; Katechakis, A.; Lippert, B.; Løseth, K.; Peters, C.; et al. Copepods Act as a Switch between Alternative Trophic Cascades in Marine Pelagic Food Webs. *Ecol. Lett.* **2004**, *7*, 321–328. [[CrossRef](#)]
203. Swain, G.; Anil, A.C.; Baier, R.E.; Chia, F.; Conte, E.; Cook, A.; Hadfield, M.; Haslbeck, E.; Holm, E.; Kavanagh, C.; et al. Biofouling and Barnacle Adhesion Data for Fouling-release Coatings Subjected to Static Immersion at Seven Marine Sites. *Biofouling* **2000**, *16*, 331–344. [[CrossRef](#)]
204. Khosravi, M.; Nasrolahi, A.; Shokri, M.R.; Dobretsov, S.; Pansch, C. Impact of Warming on Biofouling Communities in the Northern Persian Gulf. *J. Therm. Biol.* **2019**, *85*, 102403. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.