Chemically Mediated Competition and Host–Pathogen Interactions Among Marine Organisms

15

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Contents

Abstract

Competition and pathogenesis are two important ecological processes in marine ecosystems. Competition defines interactions between individuals relative to the acquisition of limiting resources. This process includes the act of fouling, but for the purposes of this chapter, we will focus largely on competitive interactions between macroorganisms. Pathogenesis is the initiation and progression of

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E. Fattorusso, W. H. Gerwick, O. Taglialatela-Scafati (eds.),
Handbook of Marine Natural Products, DOI 10.1007/978-90-481-3834-0_15,
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a disease in a host organism, and it has become recognized as an increasingly important process in marine ecosystems, particularly in the face of accelerating climate change and stress from anthropogenic stressors. There is fairly solid evidence that these processes can be mediated by allelochemicals and antimicrobials, respectively. Yet unequivocal proof that natural products are acting in an ecologically relevant manner, in situ, represents a major experimental challenge for marine ecologists. The goal of this chapter is to (1) provide an overview of these ecological processes, (2) single out particularly successful research techniques relative to these processes, and (3) distinguish marine natural products that have been identified as defenses against competitors and pathogens. This chapter will focus on sessile invertebrates and plants since their mechanisms for defending themselves against competition and pathogenesis are more likely to include the production of natural products. To date, there are several examples of extracts from marine plants and invertebrates that have demonstrated allelopathic and antimicrobial activity using a diversity of experimental methodologies. Highlighted are examples of metabolites shown to play a role in allelopathic interactions with algae, soft corals, and sponges, as well as compounds that display antimicrobial activity in algae, soft corals, and sponges.

15.1 Competition

Competition can be one of the most significant ecological processes in the structure and function of terrestrial and aquatic communities [1]. Competition is typically associated with the acquisition of limiting resources such as space, light, and/or food [2]. In marine communities, competition can be particularly intense between benthic organisms that are attached to the substrate, including algae, sponges, hard and soft corals, and tunicates. Competition has been further characterized as either occurring through contact with another organism (= "direct") or as occurring across a distance (= "indirect") [3]. In marine communities, indirect competition is often mediated through overgrowth/shading [4, 5], but it can also involve the use of toxic natural products [6]. Rice [7] first coined the term "allelopathy" to generically include any instance in which a natural product (= "allelochemical") is released into the environment by an organism, where it either harms or aids another organism in the vicinity (i.e., a potential competitor). While this definition has been further refined with time, a persistent concern is proving whether allelopathy actually occurs in nature [8].

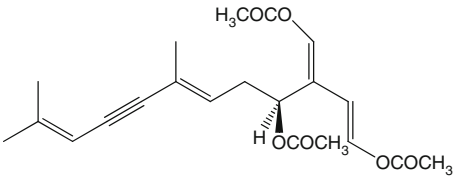
Allelopathic interactions in marine communities include the production of antifoulants that limit overgrowth and thus access to various resources, primarily by microorganisms; this is the subject of a separate chapter in this text (see ▶ Chapter 14). In addition, allelopathic interactions can occur between benthic macroorganisms via direct contact, when the allelochemicals are surface-bound, or via indirect interactions, when the compounds diffuse through the water column to a distant competitor. Despite reasonable field observations supporting potential allelopathic interactions,

there are few unequivocal experimental studies of chemically mediated competition in marine communities and even fewer that have characterized the natural products responsible. Here, we will provide an overview of those studies relative to several important sessile organisms and use the results to comment on the technical challenges of assessing allelopathic interactions in marine communities.

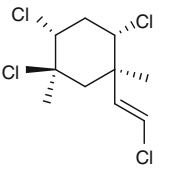
15.1.1 Algae

Macroalgae are important components of most marine ecosystems and are often effective competitors against other organisms. Phase shifts from coral- to algal-dominated communities throughout the Caribbean during the 1980s demonstrate the delicate competitive balance between these key constituents [9, 10]. For example, when the dynamics of space utilization by brown and red seaweeds and the coral *Oculina arbuscula* were examined at 12 reef habitats in North Carolina, across a 25-m-depth gradient, there was a strong negative correlation between algae and coral cover that appeared to be mediated by light preference of these taxa and by algal inhibition of coral recruitment [11]. Manipulative grazer exclusion/nutrient addition experiments further demonstrated that competition with seaweeds, through shading and scour, limits the distribution of corals on these temperate reefs. This study provides an excellent example of the subtle competitive interactions between algae and corals and the complexity of experimental design requisite to tease apart these factors.

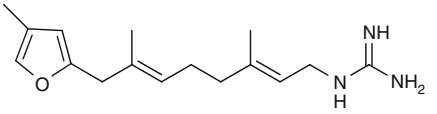
Few studies have provided evidence for allelopathy in macroalgae; three important cases are reviewed here. Kuffner et al. [12] examined larval recruitment of the hard coral *Porites astreoides* and the gorgonian *Briareum asbestinum* in the presence of five species of algae and three species of cyanobacteria. The authors performed field surveys of three reefs in the Florida Keys to ascertain benthic cover of algae, cyanobacteria, sponges, corals, and suitable settlement substrata to assess potential competitors to larval coral recruits. They then constructed replicate larval recruitment chambers [12] that contained conditioned tiles (= control), tiles with an artificial aquarium plant (= mimic), or tiles with one of the algal or cyanobacterial species that were deployed in the field or in an aquarium. One hundred coral larvae were introduced to each chamber and allowed to settle for 4–9 days, at which point the percent survival, percent recruitment, and locations on the tiles were recorded. Their results demonstrated that all but one species of algae caused avoidance behavior or inhibition of recruitment by *P. astreoides* larvae and that *Lyngbya confervoides* and *Dictyota menstrualis* caused significant larval mortality. *Lyngbya majuscula* similarly reduced recruitment and survival of *B. asbestinum* larvae. Recent work has demonstrated that extracts of *D. pulchella* and *D. pinnatifida* affect larval survival of *P. astreoides* (V. Paul, personal communication 2009). While an actual allelochemical compound has not been isolated to date, given field data on the distributions of these algae and cyanobacteria, this research indicates that allelopathy may perpetuate current phase shift



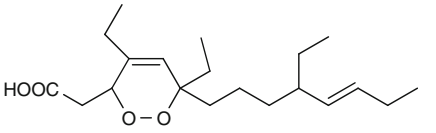
Caulerpenyne (1)



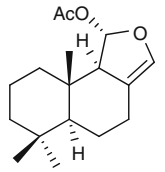
Chloromertensene (2)



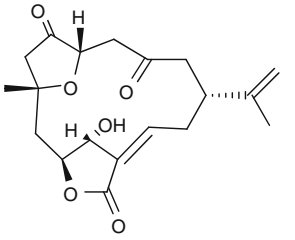
Siphonodictine (3)



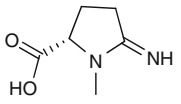
7,8-dihydro-4-ene-plakortide M (4)



7-deacetoxyolepupuane (5)

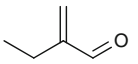


5-episinuleptolide (7)

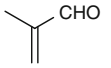


Clionapyrrolidine A (6)

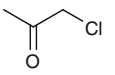
Brevetoxin A R =



Brevetoxin B R =



Brevetoxin C R =



Brevetoxin (8)

Fig. 15.1 Allelopathic natural products isolated from marine organisms

conditions on tropical reefs by inhibiting the ability of corals to settle and become established.

The Australian seaweed *Caulerpa racemosa* var. *cylindracea* has become invasive in the Mediterranean Sea where it has caused significant changes to the benthic communities [13] and physiological stress to the native seagrass *Cymodocea nodosa* [14]. Crude extracts of the invasive alga at 100 ppm and three known pure compounds at 10 and 1 ppm were exposed to leaf fragments of *C. nodosa* for 6 days under controlled laboratory conditions [15]. The leaf fragments were analyzed daily using pulse amplitude modulated (PAM) fluorometry to assess optimal quantum yield (= Fv/Fm), which can be considered a proxy for chlorophyll *a* content [16]. Only the crude extract and caulerpenyne (Fig. 15.1:**1**) at 10 ppm were found to significantly depress quantum yield and therefore photosystem function. While this study provides preliminary evidence for a *C. racemosa* allelochemical that inhibits the native seagrass *C. nodosa*, it suffers from many of the arguments used to criticize allelopathy [8]. Specifically, there is no supporting evidence from field manipulations to demonstrate that an allelochemical was responsible for the stress to *C. nodosa*, and if present, whether the metabolites were waterborne or surficial. There is also no analytical evidence to demonstrate that the compounds were tested at ecologically relevant concentrations; the activity at 10 ppm may be meaningless if caulerpenyne occurs at levels closer to the inactive 1 ppm. Finally, the absence of activity in two other compounds isolated from *C. racemosa*, and the lack of a bioassay-guided isolation approach, does not rule out additional additive and/or synergistic allelopathic compounds.

More compelling with respect to allelopathy in algae is the work of de Nys et al. [17]. They observed varying degrees of tissue necrosis in the sponge *Dictyoceratida sp.* the gorgonian *Isis* sp., and the soft corals *Sinularia cruciata*, *S. flexibilis*, *S. polydactyla*, and *Clavularia* sp. near the red alga *Plocamium hamatum* on the Great Barrier Reef, Australia. When in contact with the green alga *Chlorodesmis fastigata*, these species did not exhibit tissue necrosis. To assess potential allelopathic activity of this alga, the authors performed two manipulative field experiments. In the first experiment, they placed *P. hamatum* in contact and noncontact settings (5 and 30 cm distance) with the soft coral *S. cruciata*, and they tagged/monitored unmanipulated field "controls" to rule out handling effects; necrosis was assessed at 24 h, 48 h, and 14 days. The second experiment controlled for mechanical abrasion due to contact, by "painting" the algal metabolite chloromertensene (Fig. 15.1:**2**) onto artificial aquarium plants at a concentration of 2%, which is within the reported range of the metabolite in natural populations [18]. In both cases, contact with the metabolite resulted in tissue necrosis within 48 h, which provided strong evidence for allelopathic inhibition of the soft coral due to the algal monoterpene. However, the authors note that this response was not as extreme as when the soft corals were in contact with the algae, suggesting that there might be other important allelopathic compounds. In contrast to the study by Raniello et al. [15], this study was grounded in solid field observations and manipulative trials. However, the inclusion of a crude extract treatment would have been useful in this study.

15.1.2 Sponges

The functional roles of sponges on reefs, and their numerical dominance, make them some of the most important benthic taxa [19, 20]. In addition, sponges are one of the best-studied models in marine chemical ecology [21], producing a diversity of natural products [22]. Like algae, sponges must protect themselves from competition at the microscale (i.e., epibiosis) [23] and at the macroscale. Although there are more examples of allelopathy by sponges than by algae, many of the methodological challenges faced by phycologists exist for sponge researchers as well. There have been several field studies related to the distributions of marine invertebrates and sponges that demonstrate competitive dominance by chemically defended species [24, 25] or inhibition of settlement and/or metamorphosis near these species [26, 27]. One of the first attempts to address allelopathy on coral reefs focused on the sponge *Aka* (= *Siphonodictyon*) sp. from Palau [28]. This sponge burrows into coral heads leaving only an osculum, surrounded by dead coral tissue, to filter seawater for nutrients. The authors isolated the compound siphonodictine (Fig. 15.1:**3**) from the sponge and tested its effect at 10 ppm and 100 ppm in seawater in laboratory assays with the coral *Acropora formosa*. Within 30 min, they observed toxin-like effects of respiratory depression and tissue necrosis for the low and high doses, respectively. While these data are intriguing from the anecdotal point of view, this study suffers from methodological details. For example, the original field observations were made on the massive coral *Montipora* sp.; staghorn corals like *A. formosa* do not suffer from infestation by this burrowing sponge. Also, ecologically relevant concentrations of the metabolite were never tested, nor were concentrations within sponge mucus, which was identified as the likely "delivery system" of the compound. This is a model species that would benefit from a revised study using a more robust experimental design.

More recently, the crude extracts of 20 Caribbean sponges were tested against 3 species of Caribbean sponges that are fast overgrowers (*Tedania ignis*, *Lissodendoryx isodictialis*, and *Haliclona hogarthi*) and the extract of the North Carolina sponge *Aplysilla longispina* against the colonial tunicate *Diplosoma listerianum* [29]. The authors developed a novel assay approach that placed overgrowth species in the center of a 15 × 15 cm acrylic plate surrounded on opposing sides by either control or extract-treated Phytagel™ (see [29]). This provided the overgrowth sponges with the potential for lateral growth in any direction over a 3-week period, and it allowed the researchers to ascertain whether extract-treated gels were avoided (= allelopathic). Their results showed that only 7 of the 20 Caribbean sponge extracts inhibited overgrowth, while 3 sponges promoted overgrowth, and the rest had no effect. A separate test demonstrated that extracts of a subset of these sponges were stable in the gels for up to 3 weeks, so the authors concluded that their results indicate that allelopathy among sponges may not be widespread. Nonetheless, they do acknowledge that specifics of each of these extracts tested may account for false negatives in their dataset, and this study should be considered a conservative estimate of the degree of allelopathy in Caribbean sponges. This holistic project clearly demonstrates the potential

importance of allelochemical-mediated competition among sponges, and it should prove useful for testing hypotheses based on field observations and relative to the isolation of specific allelopathic compounds.

Porter and Targett [30] focused more specifically on a single species of Caribbean sponge, *Plakortis halichondroides*, and its interaction with the coral *Agaricia lamarcki*, in St. Croix, US Virgin Islands. Field surveys indicated that this sponge is common between 20 and 30 m depth, and when it occurs in contact with a coral, the coral exhibits signs of physiological stress about 40% of the time. Moreover, 14 species of coral, representing 8 of the 9 Caribbean coral families, were overgrown and killed by this sponge. The authors tested 11 hypotheses related to competition-mediated physiological stress as measured through changes in biomass and oxygen flux. They conducted manipulative field experiments including (1) noncontact scenarios, (2) contact with sponge exudate only, (3) direct contact with the sponge for 24 h, and (4) direct contact with the sponge for extended periods. The latter three treatments resulted in a significant depression of zooxanthellae density, chlorophyll *a* concentrations, and nitrogen mass, relative to controls. Likewise, oxygen flux, representative of net photosynthesis, was also depressed under "contact" conditions. This bleaching response approximated the observed responses of natural field interactions, and the fact that a physiological response could be generated by contact with the sponge exudate alone provided compelling evidence for an allelochemical. Interestingly, in a study of sponge-dominated marine caves in the Bahamas, *P. halichondroides* was separated from other species by clear substrate [31]. The water surrounding these sponges contained the metabolite 7,8-dihydro-4-ene-plakortide M (Fig. 15.1:**4**), and at ecologically relevant concentrations, this compound killed cells of a potential sponge competitor *Oscarella* sp. in the laboratory. *Plakortis* spp. produce many compounds of similar structure to **4**, so it is possible that this or a related compound was responsible for coral tissue necrosis in the study by Porter and Targett [30].

To date, the strongest evidence for allelochemical interactions amongst sponges was generated on Guam, USA, where frequent overgrowth of the sponge *Cacospongia* sp., by the sponge *Dysidea* sp., was observed [32]. This overgrowth often resulted in *Cacospongia* sp. tissue necrosis but not reduction in the defensive metabolites of this species. The authors suspended natural concentrations of crude extracts from *Dysidea* sp. in agar solidified onto window screen and cable-tied these strips to branches of the *Cacospongia* colony in the field. Control strips were similarly prepared, but they contained the carrier solvent only, and these were attached to a neighboring branch of the same colony [32]. Approximately 80% of the tissue under the extract-treated agar strips was necrotic after 1 week, while necrosis under control strips was negligible. In a similar assay using the *Dysidea* metabolite 7-deacetoxyolepupuane (Fig. 15.1:**5**) at ecologically relevant concentrations, they also observed a significant treatment response, but the tissue necrosis was only about half that of the crude extract, suggesting that there may be additive effects. The metabolite yields of *Dysidea* in contact with either *Cacospongia* or rock substrate did not differ, indicating that competition does not induce the production of the allelochemical. This study went on to assess the effects of

Cacospongia extracts on *Dysidea* metabolite production, but it found no significant differences between control and treatment groups. It also demonstrated that the allelochemical (Fig. 15.1:**5**) functions as a feeding deterrent compound against the predatory angelfish *Pomacanthus imperator*. This study is a solid model for future studies of allelopathy in marine systems; the use of field observations to generate testable hypotheses has produced a complete picture of the chemical interactions between two competitive species. The sole flaw of this study, the lack of a bioassay-guided approach to isolate additional allelopathic compounds, actually highlights a challenge faced by researchers studying competition in marine ecosystems. These processes tend to be quite slow, so viable bioassays might occur over temporal scales measured in weeks. To sequentially follow multiple chemical fractions, in a replicated manner, could literally take years of fieldwork. Thus, researchers often have to rely on a "best guess" based on the activity of related molecules and/or the concentration relative to the dry mass of the organism. Nonetheless, two recent papers have reduced the time needed to run an allelopathic assay. Sponge allelopathy against the coral *Diploria labyrinthiformis* was assessed using extracts incorporated into Phytagel™ molds [33]. Nine common bioactive Caribbean sponges were extracted, and volumetric equivalents were mixed into the gels and fixed to the surface of corals for 18 h. They used PAM fluorometry to assess impacts to symbiotic zooxanthellae photosystem function, which was indicative of stress. Although allelopathic metabolites were not identified, this process offers promise for future studies assuming the response to the allelochemicals is manifested in zooxanthellae productivity. A similar bioassay procedure was used to assess allelopathy by the burrowing sponge *Cliona tenuis* against the hard coral *Siderastrea siderea* [34]. They were able to follow a bioassay-guided approach to the bioactive compound clionapyrrolidine A (Fig. 15.1: **6**) by limiting exposures to 24 h. While this paper lacked the field detail of the study by Thacker et al. [32], it demonstrates the apparent toxicity of their allelochemical and the potential implications for competition research.

15.1.3 Hard Corals

Scleractinian (= "hard") corals are the primary reef-building components of tropical marine hard-bottom communities, and as such, they are often in contact and potentially in competition with other community constituents. Hard corals are known to utilize overgrowth, extracoelomic digestion, and/or "sweeper tentacles" to gain a competitive edge [4, 35], but at least two studies have addressed potential allelopathy in this group of benthic invertebrates. Competitive hierarchies between hard and soft corals were examined on the reefs of southern Taiwan [36]. The investigation was restricted to the study of natural interactions totaling 1,168 pairwise contacts characterized by either (1) the production of a dead margin, (2) overgrowth without tissue necrosis, or (3) stand-offs which lacked necrosis and overgrowth. Results of this broad field survey indicated that soft corals were typically subordinate to the scleractinians in this competitive network. Nonetheless,

the author noted at least eight interactions that appeared to represent allelopathy, and in all of these cases, soft corals were dominant to their hard-coral competitors. Allelopathic competition in the coral *Tubastraea faulkneri* was also studied on the Great Barrier Reef, Australia [37]. This laboratory-based study assessed the toxicity of *T. faulkneri* extracts against 12 species of scleractinian larvae at standard concentrations ranging from 10 to 500 μg ml^{-1}. These experimental concentrations were estimated to be 100–5,000 times lower than tissue levels. Larval mortality varied between the coral species, and there was no toxicity to conspecifics. Four indole alkaloids were isolated from *T. faulkneri*, but these were not assayed for allelopathic activity. Based on these data, the authors conclude that these compounds are released into the water column to prevent larval recruitment in the vicinity of *T. faulkneri*. While both these studies provide useful preliminary information, neither offers unequivocal proof that hard corals control competitors using allelochemical compounds.

15.1.4 Soft Corals

Like sponges, soft corals have also been the subjects of much interest relative to their chemical ecology, including studies regarding their competitive abilities [38]. Much of that work occurred on the Great Barrier Reef where soft corals are a spatial dominant of the benthic communities [39] and often interact with other soft and hard corals [5]. Some of the earlier experiments that addressed potential allelopathic effects of these soft corals were based on field observations of movement away from a competitor, stunted growth, tissue necrosis, and/or mortality. In some cases, there were responses in the absence of physical contact, suggesting that waterborne allelochemicals may have been involved [40]. However, field manipulations of soft corals into contact interactions suffered from poor experimental design. Specifically, the use of multiple contact treatments against individuals prevents attribution of the observed necrotic effects [41]. More recently, the role of local environmental factors on the competitive ability of two soft corals, *Clavularia inflata* and *Briareum stechei*, and a hard coral, *Acropora longicyathus*, was examined in all pairwise interactions [42]. These manipulative field experiments involved transplanting between an inshore and a midshelf reef, with appropriate back-transplant and nonhandled controls. Competitive endpoints included overgrowth, tissue bleaching and necrosis, and whole colony mortality. Their results at the inshore site gave a dominance pattern of *Briareum* > *Clavularia* > *Acropora*, while the midshelf site exhibited a reversal of dominance between *Clavularia* and *Acropora*. The authors hypothesized that nutrient enrichment, lower light, and predation at the inshore site may be responsible for the soft coral superiority there. While allelopathy was not specifically addressed in this well-designed field study, the responses observed argue for terpene-mediated competitive dominance and for future studies focused on this question. Later experiments by this group were designed to assess the mechanistic processes by which soft coral allelochemicals impact hard coral competitors. Toxic diterpenes were exuded into

the water column by the soft corals *Sinularia flexibilis* and *Lobophytum hedleyi* at concentrations of 5–20 ppm [43]. They found that at concentrations higher than 5 ppm, branchlets of the hard corals *Acropora formosa* and *Porites cylindrica* expelled their zooxanthellae and nematocysts. The authors concluded that these responses would reduce the corals' abilities to obtain nutrients and defend themselves, respectively, thus lowering their fitness in highly competitive environments. While the ecological relevance of the allelochemical concentrations might be questioned, this study nonetheless provides compelling insights into the mechanism of action of putative allelopathic metabolites.

Competition is not limited to interactions between disparate groups of organisms. Competitive dominance of the hybrid soft coral *Sinularia maxima* × *polydactyla* was observed over its parents *S. maxima* and *S. polydactyla* on the reefs of Guam [44]. A significant decline in the parent soft coral populations was noted over a period from 1994 to 2003, associated with an increase in the hybrid population. To determine whether contact-mediated competition might account for the differences in population dynamics, they assessed the outcome of 124 interactions between the hybrid and either parent in the field. After 3 weeks, 80% of these interactions resulted in tissue necrosis on the parent species (i.e., hybrid dominance), while 20% of the interactions were not resolved. A field assay, similar in technique to that described by Pawlik et al. [33], was conducted on these soft corals. However, tissue necrosis was used as an endpoint after 3 weeks of exposure to extract-containing gels. The parent soft corals and the hybrid all produced extracts that exhibited some degree of tissue necrosis to congeners, but not to conspecifics. Necrosis induced by the hybrids was on the order of 40–60% of the exposed tissue, while the parent extracts induced only 5–10% tissue necrosis. The length of this assay made it impractical to conduct a bioassay-guided isolation of the hybrid allelochemical. Nonetheless, recent assays with the isolated metabolite, 5-episinuleptolide (Fig. 15.1:**7**), indicate that this compound is partially responsible for the bioactivity attributed to the hybrid extract (Slattery, 2005).

Like tropical reefs, Antarctic benthic communities are also space limited and therefore highly competitive [45]. The soft coral *Alcyonium paessleri* appears to use contact-mediated allelopathy to compete with at least one space dominant sponge in McMurdo Sound [46]. Replicate soft corals and sponges were cemented individually into small flower pots and then moved to the field in contact and noncontact situations for 1 year. Tissue necrosis was not observed when *A. paessleri* was placed in contact with conspecifics, the soft coral *Clavularia frankliniana*, or four common sponges. However, a fifth sponge, *Mycale acerata*, exhibited significant tissue necrosis when in contact with *A. paessleri*. Interestingly, this sponge was a competitive dominant to the sponge *Kirkpatrickia variolosa* [45], which was a competitive equal to *A. paessleri* [46], suggestive of the competitive hierarchy feedback loops proposed by Jackson and Buss [6]. While a putative allelochemical from *A. paessleri* was not identified, it is possible that the cytotoxic metabolite 22-dehydro-7β-hydroxy-cholesterol (which was a negative olfactory cue for soft-coral predators) might also have allelopathic properties. This compound, along with three other waterborne sterols, was collected in situ near *A. paessleri* colonies using

60 cc syringes [47]. A more elegant allelochemical collection device was developed and tested on soft coral exudates; this apparatus has the potential to further studies of allelopathic compounds released into the water column [48].

15.1.5 Others

Among the most obvious sessile invertebrates on tropical and temperate reefs are the colonial tunicates, and these individuals are often competitive dominants to other groups of benthic organisms [49]. Tunicates typically produce important natural products with defensive properties [22, 50], particularly related to the prevention of surficial fouling [51, 52]. However, most studies associated with tunicate-macroorganism competition seem to indicate that this process is mediated by overgrowth, not allelopathy [53, 54]. On Caribbean coral reefs, the zoanthid *Palythoa caribaeorum* is a dominant member of the benthic community. With some of the fastest growth rates (2.5–4.0 mm/day) recorded for anthozoans, this organism overgrows virtually every other benthic invertebrates, placing itself at the top of the competitive hierarchies in these communities [55]. This species also produces a diversity of natural products, including palytoxin; however, the use of these as allelochemicals has not been evaluated. In contrast, soft bottom communities are often dominated by infaunal worms, including the terebellid polychaete *Thelepus crispus*. This species produces brominated aromatic metabolites that deter the recruits of competitive nereid polychaetes *Nereis vexillosa* [56]. Moreover, these compounds are nonpolar and thus likely remain in the sediment around the polychaete burrows for long periods of time.

Two additional papers demonstrate the complexity of competitive interactions within marine ecosystems. Up to this point, all of the examples of interactions have focused on macroorganisms in benthic communities. While antibiotic-mediated competition between microbes is relatively well accepted [57], the question of competition between microbial and animal decomposers for benthic food falls represents a unique approach to studies of allelopathy [58]. The authors used traps baited with either fresh or microbe-laden (= 48 h colonization in flowing seawater) carrion to assess attractiveness to scavengers; the former bait was 2.6 times more attractive to scavengers. The stone crab preferred the frozen carrion 2.4 times more often than the microbe-laden carrion. In addition, organic extracts of microbe-laden carrion contained noxious extracts that repelled many of the scavengers in the system. While this paper failed to identify the specific metabolites produced by the microbes, this unique field and laboratory study demonstrated a previously underappreciated ecological interaction.

Allelochemical-mediated competition within planktonic communities has also been studied [59]. Specifically, the authors studied the dinoflagellate *Karenia brevis*, which is responsible for monospecific blooms that cause red tide conditions in the Gulf of Mexico. They compared the growth of *K. brevis* with 12 co-occurring phytoplankton species under monoculture and mixed culture laboratory conditions. In nine of the mixed culture conditions, *K. brevis* inhibited growth rates relative to

growth in monocultures, while *Rhodomonas lens* growth was enhanced, and two additional species were unaffected. In a follow-up experiment, the extracellular filtrates of *K. brevis* were collected, extracted, and introduced to monocultures of the competitive phytoplankton. Six of the nine species were inhibited by the filtrate and lipophilic extracts of *K. brevis*, supporting an allelopathic mechanism for bloom characteristics. In the final competition experiment, ecologically relevant concentrations of brevetoxin (Fig. 15.1:**8**) were introduced to monocultures of the competitive phytoplankton. This compound only caused weak inhibition in one species, *Skeletonema costatum*, although it did induce autoinhibition in *K. brevis*. This study demonstrated appropriate methodologies for dealing with allelopathy in planktonic organisms, and it provides compelling evidence for multiple allelochemicals within *K. brevis* that likely act somewhat selectively and/or in an additive manner.

15.2 Pathogenesis

In contrast to competition, the importance of pathogenesis as an ecological phenomenon within marine ecosystems has only recently received much attention. In recent decades, reports of diseases affecting populations of marine organisms have increased dramatically worldwide [60–63]. This is especially true for corals, but diseases also threaten algae, seagrasses, sponges, gorgonians, molluscs, crustaceans, sea urchins, fishes, and marine mammals [61, 64]. Not only is the rate at which emerging diseases are being discovered escalating but also the number of host species and geographic ranges of diseases has also been increasing. Diseases affecting natural populations threaten biodiversity, resilience, and the ecological balance of communities, as well as the ecological services they provide to humans [65].

Mass mortalities resulting from infectious disease outbreaks (epizootics) have resulted in major changes in community structure of marine ecosystems in recent years. For example, during the 1980s, a waterborne disease of the long-spined sea urchin *Diadema antillarum* spread rapidly throughout the Caribbean, reducing the abundance of this important herbivore by nearly 99% on most Caribbean reefs [66]. This keystone species served a major ecological role by removing macroalgae from Caribbean reefs, and in its absence, algae overgrew reef surfaces and inhibited the survival and settlement of corals, resulting in a phase shift from coral-dominated to algal-dominated reefs Caribbean-wide [9]. This alteration of community types has remained stable for nearly three decades. Unfortunately, at the time, our knowledge of marine diseases was relatively minimal and the pathogen was never identified, nor were appropriate samples collected and preserved for future analysis.

Why have diseases increased to such unprecedented levels? Increased anthropogenic stress in near-shore environments, overfishing, and environmental conditions associated with global climate change have all been implicated as contributing to increased disease levels [60, 61, 67–69]. It is unknown whether the emergence of these diseases is due to the introduction of novel pathogens [61, 70], changes in virulence or range of existing pathogens [71–73], or changes in host susceptibility

[60, 74]. Overall declines in coral reef communities may also play a role, as reduced species and genetic diversity due to declining population sizes may increase susceptibility to emerging infectious diseases [75]. Understanding diseases in marine ecosystems is increasingly important given that a large proportion of the world's population lives near the coast; this need is particularly acute where societies are closely tied to and depend upon coral reefs for their economic and social well-being.

15.2.1 Coral Diseases

Due to their high profile in the public eye, diseases of scleractinian corals are by far the best described in the marine environment, and new diseases are reported with increasing frequency in an expanding number of hosts on a global scale [63, 76, 77]. Coral disease has emerged as a serious threat to coral reefs worldwide and a major cause of reef deterioration [67, 78]. Although our understanding of coral diseases still lags far behind those of terrestrial wildlife, several lessons can be learned from research accomplishments to date, so we will use this important marine functional group as a case study for disease dynamics and resistance mechanisms.

15.2.1.1 Pathogenesis

Disease transmission, onset, and progression are the result of complex interactions between the host, environment, and pathogen, and all must interact in a precise way for disease to occur. For example, in *Vibrio*-induced coral bleaching, the mere presence of the *Vibrio* bacterium associated with the coral host is not sufficient to cause disease. Adhesion and ingress of the bacterium into the coral also requires elevated seawater temperatures [79]. Thus, understanding coral disease dynamics is difficult due to this complex interplay between both abiotic and biotic interactions. Moreover, just like in human host–pathogen transmission, additional species may act as vectors of disease in marine environments [80].

The frequent association of abundant *Vibrio* spp. with diseased coral tissues has led some researchers to question whether they act as primary pathogens for certain diseases or whether they are more opportunistic, colonizing previously compromised hosts [81, 82]. *Vibrio* spp. are abundant and relatively nonselective, so they could be opportunistic [83], but so far, no evidence directly supports this hypothesis, and there is no evidence from molecular screening studies that *Vibrio* spp. found associated with diseased tissues are pathogenic, or that only compromised hosts are infected.

15.2.1.2 Etiology

Nearly 30 coral diseases have been described from reefs across the world in recent years [77, 78], although only a handful of causes (etiologies) have been elucidated. To date, Koch's postulates have only been fulfilled for five coral diseases (see Table 15.1). Koch's postulates represent the gold standard for the verification of

Table 15.1 Etiology of coral diseases for which Koch's postulates have been fulfilled

Disease	Species affected	Geographic region	Pathogen	Vector	Bioactive extracts/ compounds tested
White band disease-II	*Acropora* spp.	Caribbean	*Vibrio charchariae (=V. harveyi)* [192, 200]	–	–
White plague-II	30+ hard coral	Caribbean	*Aurantimonas coralicida* [201]	–	Pathogen tested against extracts from 18 Caribbean corals ([202], Gochfeld, unpublished data 2009) and three Hawaiian corals [117]
White pox disease	*Acropora palmata*	Caribbean	*Serratia marcescens** [94]	–	Pathogen tested against extracts from 18 Caribbean corals ([202], Gochfeld, unpublished data 2009) and three Hawaiian corals [117], mucus and bacteria from *Acropora palmata* [122]
Aspergillosis	10+ gorgonian	Caribbean	*Aspergillus sydowii* [70, 181]	African dust, or terrestrial runoff [182, 183]	Pathogen tested against extracts from Caribbean gorgonians [135, 136, 185, 188], mild activity against julieannafuran from *Gorgonia ventalina* [71]
Bacterial bleaching	*Oculina patagonica*	Mediterranean	*Vibrio shilonii (=V. shiloi)* [203, 204]	Fireworm *Hermodice carunculata* [205]	Pathogen tested against extracts from 18 Caribbean corals ([202], Gochfeld, unpublished data 2009) and three Hawaiian corals [117]
Bacterial bleaching	*Pocillopora damicornis*	Indo-Pacific	*Vibrio coralliilyticus* [109]	–	Pathogen tested against extracts from 18 Caribbean corals, ([202], Gochfeld, unpublished data 2009) and three Hawaiian corals [117]

(*continued*)

Table 15.1 (continued)

Disease	Species affected	Geographic region	Pathogen	Vector	Bioactive extracts/ compounds tested
White syndrome	20+ hard coral	Indo-Pacific	Two new *Vibrio coralliilyticus* strains + four other *Vibrio* spp.* (Note: different pathogens affected different coral species) [83]	–	–

*Tested at concentrations exceeding those likely to be found in the marine environment

Koch's postulates represent a procedure proposed by Robert Koch in the late 1800s [206] to verify that a presumed microbial pathogen is the actual cause of a particular disease. Under these rules:

- The putative pathogen must always be found associated with a particular disease.
- The putative pathogen must be isolated from the disease state and grown in pure culture under laboratory conditions.
- The pure culture of the putative pathogen must reproduce the disease when inoculated into or onto a healthy organism.
- The putative pathogen must be reisolated from the newly diseased organism and identified as the same putative pathogen.

Satisfaction of Koch's postulates is difficult under the best of circumstances, but particularly so when the host is a marine organism since (1) less than 1% of known marine microbes have been successfully cultured under laboratory conditions. For the most part, we do not understand the specific biotic and abiotic conditions to which these organisms are exposed, and these conditions have rarely been successfully duplicated in the laboratory. (2) For a particular microorganism to cause pathogenesis to a particular host, additional factors may be required, such as the presence of specific other microorganisms (e.g., as in the polymicrobial black band disease), aspects of seawater chemistry, temperature, light, or metabolites produced by the host, symbiont, or microbial associates. (3) In most cases, even those for which Koch's postulates have been performed, the natural mode of infection of a pathogen is not known; hence, exposing the intact healthy host to a slurry of bacteria in an aquarium may have little relevance to the real world. (4) Likewise, the concentration of pathogen used for inoculation in laboratory experiments may be far greater than a host would ever be exposed to in nature. (5) Pathogenesis may only occur when a host's immune system is already compromised by other pathogens or other types of environmental stressors, following predation or another type of injury, or when the host's physiological condition is more susceptible (e.g., during periods of rapid growth or reproduction). (6) Natural transmission of the pathogen may require an intermediate host or vector rather than through the water column. (7) Reisolation of the pathogen from the site of the new infection cannot necessarily prove whether the pathogen was not there prior to inoculation or occurred in the surrounding water. (8) Marine invertebrates, particularly sponges and corals, are relatively simple organisms. The signs or symptoms that can be seen (at least by the human eye) are relatively few but may in fact represent responses to many different types of conditions, pathogens, or other stressors. Therefore, even if a putative pathogen is identified using Koch's postulates, it does not mean that only that pathogen is responsible for a particular response. More than one pathogen may cause the same response in a given host. (9) Through host–environment interactions, it is highly likely that pathogens may cause different signs or symptoms in different individual hosts or in different species of hosts.

disease etiology; however, fulfilling Koch's postulates requires the ability to culture the pathogen in the laboratory, which may not be possible for many marine diseases [84]. Several other hurdles in the characterization of disease etiologies exist [85]. For example, corals can only exhibit a limited range of symptoms that a human observer can visually identify; therefore, it is likely that multiple types of etiologies may result in the same signs in a coral. The converse is also a problem; any given pathogen or abiotic stressor may cause multiple signs in corals. This could vary depending upon the coral host genotype, zooxanthellae clade, associated microbial community or interactions among any of these factors.

In the past five years, numerous new coral diseases have been described, particularly from the Indo-Pacific region, and our knowledge of their etiologies has advanced rapidly. Three new diseases were reported from the Great Barrier Reef, Australia [86]: skeletal eroding band caused by the protozoan, *Halofolliculina corallasia*; a ciliate disease called brown band disease; and white syndrome, a collective term for disease resulting in tissue loss. White syndrome infections in certain coral species in Palau, the Great Barrier Reef, and the Marshall Islands were found to be caused by six different strains of pathogenic *Vibrio* spp. [82]; these pathogens were shown to cause infection and were identified at the site of infection using molecular methods.

Other etiologies are more difficult to confirm. Dark spot syndrome (DSS) in corals from American Samoa and Hawaii was found to be associated with endolithic fungi [87]. This was also found to be the case for DSS in the Caribbean coral *Siderastrea siderea* but other unidentified endolithic organisms were found associated with DSS in *Agaricia* sp. [88]. Due to the endolithic nature of these fungi, fulfilling Koch's postulates is unlikely. A new bacterial strain *Thallasomonas loyaeana*, isolated from white plague in *Favia favus* from the Red Sea, was found to infect the coral in laboratory experiments, but only in the presence of a filterable factor in the water, and reisolation of the pathogen was not performed [89, 90]. Black band disease (BBD), the first coral disease reported, consists of a polymicrobial consortium, the components of which may vary with environmental conditions [91]; due to this variability, it will likely be impossible to fulfill Koch's postulates for all possible combinations.

15.2.1.3 Geographic Variability

In the Caribbean, coral disease has been implicated as a major factor contributing to the decline of coral reefs, resulting in apparent ecological phase shifts from coral- to algal-dominated ecosystems [9, 76]. An outbreak of white band disease in the 1980s killed acroporid corals throughout the Caribbean [92, 93], and an outbreak of white pox disease in the Florida Keys reduced the cover of the dominant *Acropora palmata* by over 70% between 1996 and 2002 [94]. Until recently, Indo-Pacific corals were believed to be relatively immune to disease; however, it is now apparent that coral diseases are major problems on Indo-Pacific reefs as well [77]. Although coral disease research in the Indo-Pacific is in its infancy, there are clear signs that diseases are emerging as a serious threat to these reefs. For example, a substantial increase in white syndrome has been observed on the Great

Barrier Reef [86]. Coral diseases have also been reported from Philippines [95], American Samoa [96], Marshall Islands [83], Marianas Islands [97], main Hawaiian Islands [98], and even from comparatively pristine areas such as the Northwestern Hawaiian Islands [99] and Palmyra Atoll [100].

15.2.1.4 Environmental Stressors

Natural and anthropogenic stressors have been implicated in the increased incidence and prevalence of marine diseases. Climate change, particularly elevated seawater temperature, may influence pathogenesis, either by reducing coral resistance or by increasing the growth and virulence of coral pathogens [60, 68, 72, 101]. For example, there is a significant relationship between the frequency of warm temperature anomalies and outbreaks of white syndrome on the Great Barrier Reef [102], and an outbreak of white plague following a thermally induced bleaching event on reefs in the US Virgin Islands caused an average of 51.5% loss in coral cover [103]. BBD also appears to be affected by water temperature, with distinct seasonal increases in disease prevalence on both Caribbean and Indo-Pacific reefs [104, 105]. Bacterial bleaching of *Oculina patagonica* is restricted to summer temperatures [106]. However, not all coral diseases are affected by thermal stress. For example, although there was seasonality in the prevalence of DSS in the Bahamas, this seasonality was not directly related to seawater temperature [107]. Nonetheless, the virulence of certain pathogens does increase at higher temperatures [72, 108, 109], and elevated temperature as expected with climate change predictions may facilitate the expansion of a pathogen's ecological niche [73].

Anthropogenic impacts on coastal areas are increasingly reducing the water quality on coral reefs, with evidence of direct negative impacts on coral health and of interacting effects with pathogens. Experimental studies have demonstrated that nutrient enrichment significantly increases coral mortality and the severity of yellow band disease [110], as well as the rate of progression of BBD [111], and addition of dissolved organic carbon caused coral pathologies similar to band diseases [112]. Correlative data suggest that disease prevalence increases with exposure to anthropogenic stressors, including nutrients [74, 113]. Both BBD and white plague type II were significantly more prevalent on reefs closest to sewage effluent in the Virgin Islands [114]. However, the high prevalence of coral diseases at relatively pristine sites [99, 100, 107, 115, 116] argues against a direct role of human disturbance in all diseases. In addition to nutrients, sediment from terrestrial runoff may serve as a potential reservoir for opportunistic pathogens of corals [77].

Sewage can also bring human enteric pathogens into the sea [94]. These organisms can survive in the ocean, have been isolated from the surfaces of corals, and should therefore be considered potential pathogens to marine organisms. Protection from these potential pathogens may be particularly important in nearshore habitats that might be more susceptible to leaching or spills. In assays testing the antibacterial activity of three Hawaiian corals, the highest levels of inhibitory activity were against the human enteric bacteria *Serratia marcescens*, *Clostridium perfringens*, and *Yersinia enterocolitica* [117]. *Clostridium perfringens* and

other enteric bacteria have been found within the surface mucus layers of several species of corals in the Florida Keys [118]. Since coral diseases have typically been reported more frequently from reefs with higher levels of human activities, these fecal pathogens may represent risk factors associated with coral disease.

15.2.1.5 Susceptibility

Differential susceptibility to disease has consistently been found among coral genera. In the Caribbean, the genus *Acropora* is selectively targeted by certain diseases [76], and in the Indo-Pacific, disease prevalence appears to be greatest among acroporids, poritids, and pocilloporids [86, 95, 99]. Intraspecific variability has also been observed, with diseased colonies found immediately adjacent to unaffected conspecifics [107, 117]. The fact that affected genera, species, or colonies (individuals) occur adjacent to unaffected ones indicates a high degree of variability in resistance to infection. Whether they originate from the host coral, zooxanthellae symbionts, or microbial associates, differences in levels of defenses within and between coral species and colonies and from different locations may help explain patterns of disease occurrence on reefs.

15.2.1.6 Resistance

Corals employ a suite of defense mechanisms to rid themselves of sediment, settling organisms, and potential pathogens, including mucus, phagocytic cells, enzymes and other proteins that are upregulated in response to various stressors, and antimicrobial chemical defenses [64, 76, 117, 119, 120]. Differences in types and levels of defense vary among genera, species, and even at the level of the individual colony [117]. These differences might enable particular populations, species, or genotypes to have an advantage over others in resisting invasion by pathogens [121]. To date, few studies have specifically examined mechanisms of defense against pathogens, yet an understanding of mechanisms of disease resistance in corals is essential to our understanding of patterns of disease prevalence and virulence.

It has been suggested that coral mucus–associated bacteria may act as a first line of defense in the protection of corals and may help prevent infection by occupying niches on the coral surface and tissues and/or by producing antimicrobial compounds, possibly as allelopathic defenses against potential competitors [120, 122–124]. These natural microbial communities preferentially utilize carbon sources in the mucus for their own metabolism [125]. Natural coral-associated bacterial communities have been shown to change in response to environmental stress, including pathogenesis. For example, there was a shift in the mucus-associated bacterial community on *Acropora palmata* following bleaching; there was a decline in the natural complement of bacteria, many of which exhibited antibacterial activity and an increase in *Vibrio* spp. [122]. It was concluded that this could indicate an environmentally induced shift away from beneficial bacteria in the healthy host, and that variability in the protective qualities of coral mucus could allow the emergence of disease. In addition, chemical compounds produced by corals or their microbial

associates can also function as attractants to certain bacteria [117], which may represent a mechanism by which pathogenesis is initiated or by which the natural coral-associated microbial community can be maintained.

Chemical defenses in sessile soft-bodied organisms provide protection from predators, competitors, pathogens, and fouling organisms [126–129] and are necessary for the survival of these species. Site-specific variability in chemical defenses may result from a variety of abiotic and biotic factors, including light [130, 131], temperature [132], predation pressure [133, 134], competition [125], or pathogens [71, 117, 135, 136]. By comparison with the soft-bodied invertebrates, there have been fewer investigations of chemical defenses in scleractinian corals. This is likely due to a combination of conservation concerns, since most corals are listed as threatened or endangered species in many countries, and the technical difficulties of working with scleractinian corals. The presence of antimicrobial chemical defenses has been proposed as a potential mechanism of disease resistance in scleractinian corals [76, 119], but few studies have tested this directly.

In one of the earliest studies on coral chemical defenses, antimicrobial activity was documented in aqueous extracts of at least one colony from 37 of 55 species of Australian corals [137]. In another study on Australian corals, extracts from 100 coral species inhibited the growth of a marine cyanobacterium, and extracts from eight species inhibited the growth of marine bacteria [138]. These studies used the standard disk diffusion assay to assess the presence of antimicrobial activity. In this assay, a lawn of the test microbe is grown on an agar plate, and extract is added to a paper disk, which is placed on the lawn. Antimicrobial activity is observed as a zone of inhibition surrounding the disk in which microbial growth was inhibited. Although still widely used, and one of the only assay options for extracts with limited solubility in aqueous growth media, this assay has limitations. First, it requires that the extract be able to diffuse out of the disk and into the growth media at a rate that is uniform and relevant to microbial growth. Second, assay duration needs to be carefully considered since initial inhibition may be masked by later growth. Third, the concentration of extract added to the disks likely has little relevance to the concentration found in the organisms themselves. These two studies also assessed antimicrobial activity using a single standard concentration of extract, but neither compared this to the concentration of extracts in the corals themselves.

Coral eggs have also been evaluated for the production of antimicrobial chemical defenses, but extracts of eggs from only one out of 11 coral species inhibited growth of only three out of 94 bacterial strains [139]. The Indo-Pacific coral, *Pocillopora damicornis*, rapidly (1 min) produced antibacterial activity in response to mechanical stress [140]. They added cultures of *Vibrio coralliilyticus*, the pathogen that causes bacterial bleaching in *P. damicornis*, to seawater in which a coral had been stressed, and the released antibacterial activity in the seawater killed the pathogen. Of the several bacterial strains tested, the greatest antibacterial activity was observed against this natural pathogen. The Caribbean coral *Siderastrea siderea* also exhibits antibacterial activity against ecologically relevant marine bacteria using the disk diffusion assay [107]. More recently, a liquid growth assay was used to demonstrate that aqueous extracts from several species of

Caribbean corals, and three species of Hawaiian corals exhibit antibacterial activity against a panel of bacteria including known coral pathogens, potential pathogens from human waste, and bacteria isolated from the surfaces of Hawaiian corals [117]. This assay was performed in 96-well plate format, and natural concentrations of extracts were added to wells of bacterial cultures to assess their ability to inhibit bacterial growth. To date, the only natural products isolated from hard corals known to have antibacterial activity to potential ecologically relevant bacteria (i.e., anthropogenic *Escherichia coli*) are montiporic acid A and B [141]. Interestingly, the former compound performs multiple ecological roles, also acting as a feeding attractant to the coral specialist mollusc *Drupella* sp. [142].

Selectivity is a hallmark of antimicrobial activity in corals [107, 117, 137, 140]. This selectivity may be adaptive if it facilitates the development or maintenance of the coral's natural microbial community. Instead, selection to inhibit only those organisms that might be detrimental to coral health should be favored. The presence of antimicrobial activity in various coral extracts suggests that corals may be able to regulate, to some extent, the microbial communities that are associated with their surfaces.

Antibacterial activity also varies by coral condition. Concentrations of specific chemical components in extracts from *Siderastrea siderea* vary with tissue condition (healthy colonies vs. healthy and diseased tissues on colonies with DSS), reflecting changes in levels of infection of individual colonies [107]. When healthy tissues on colonies of *Montipora capitata* affected by *Montipora* white syndrome (MWS) were compared to those from their nearest healthy neighbors (controls), control extracts exhibited significantly greater antibacterial activity against *Yersinia enterocolitica*, and there was a trend toward this pattern against six other bacterial strains, suggesting that higher levels of antibacterial activity in control corals may protect them from pathogens and may help explain why these colonies remain disease free on a reef with other infected colonies [117]. Within colonies affected by MWS, diseased tissues had significantly greater antibacterial activity against *Aurantimonas coralicida*, and there was a trend toward this pattern against five other bacterial strains. This could result from induced defenses in diseased tissues in response to pathogen challenge.

15.2.2 Algae/Seagrass Diseases

Seagrass pathogenesis causes dramatic and long-lasting effects within regional ecosystems. An outbreak of eelgrass wasting disease nearly eradicated populations of the eelgrass *Zostera marina* from North America and Europe in the 1930s, with periodic recurrences in the intervening decades [143]. The zoosporic fungus *Labyrinthula zosterae* was identified as the etiologic agent of this disease [144], and an unidentified congener has been attributed to the mortality of the seagrass *Thalassia testudinum* [145]. *Labyrinthula* spp. are ubiquitous in coastal marine habitats, but only periodically do they become pathogenic, and the reasons for this are not clear, but they may be associated with other environmental stressors reducing overall seagrass health, and therefore resistance.

Marine algae are also susceptible to disease. Red spot disease affecting the commercially important Japanese kelp *Laminaria japonica* is the result of a bacterial infection, although the identity of the pathogen remains in question [146]. The kelp *Nereocystis luetkeana* suffers from a bacterial infection known as white rot disease, caused by *Acinetobacter* sp. [147]. "Raisin disease" in *Sargassum* spp. is caused by a fungal infection of *Lindra thalassiae* [148], and other fungal infections have afflicted the commercially cultivated red algae *Porphyra* spp. in Japan [149]. Coralline algae are also susceptible to disease. The most common, coralline lethal orange disease, affects a diversity of crustose coralline algal species worldwide; to date, the bacterial pathogen has not been identified [150]. A coralline white band syndrome also affects coralline algae in the Caribbean, causing extensive mortality [151].

Antimicrobial activity is widespread in marine plants. Using a combination of disk diffusion assays and liquid growth assays to test extracts from 49 Caribbean marine algae and three seagrasses for antimicrobial effects against five ecologically relevant marine microbes, 90% of extracts were active against at least one microorganism, and 77% of extracts were active against two or more [152]. In the same assays, comparable levels of activity were observed among 54 species of Indo-Pacific marine algae and two species of seagrasses [153]. A more targeted assessment of antifungal activity in seagrasses from Florida demonstrated that four out of five species selectively inhibited the growth of fungi, including the seagrass pathogen *Lindra thalassiae* [154]. In addition to identifying antifungal activity in whole tissues and organic extracts, this study demonstrated that oxidative stress signaling pathways are also incorporated into the seagrasses' defense against fungal infection [154].

Phenolic metabolites (including condensed tannins) represent the major group of secondary metabolites in seagrasses, and their proposed ecological activities include antipredator and antimicrobial defenses [155]. Infection by the pathogen *Labyrinthula zosterae* induces an increase in phenolic production in *Zostera marina*, even in areas distant from the site of infection, suggesting that the induced response is systemic [156]. In particular, caffeic acid (Fig. 15.2: **9**) increases in response to infection with *L. zosterae* and exhibits antimicrobial activity against the pathogen [157]. Gallic acid (Fig. 15.2: **10**) concentration increased by 250% following infection by *L. zosterae*, but its ability to inhibit the growth of the pathogen has not yet been established [156]. In the seagrass *Thalassia testudinum*, there is also evidence that phenolic levels increase in blades infected with the pathogen *Labyrinthula* sp.; however, this does not appear to be induced by the infection per se. Instead, this was considered pseudoinduction, a result of compounds accumulating in a localized region of the blade as the lesion inhibited the flow of resources within the blade [145]. Thus, unlike induced responses to pathogenesis, changes in levels of phenolics in *T. testudinum* in response to infection are unlikely to be adaptive [145]. In addition to phenolics, *T. testudinum* produces the flavone glycoside 7-O-beta-D-glucopyranosyl-2"-sulfate (Fig. 15.2:**11**), which provides antifungal protection [158].

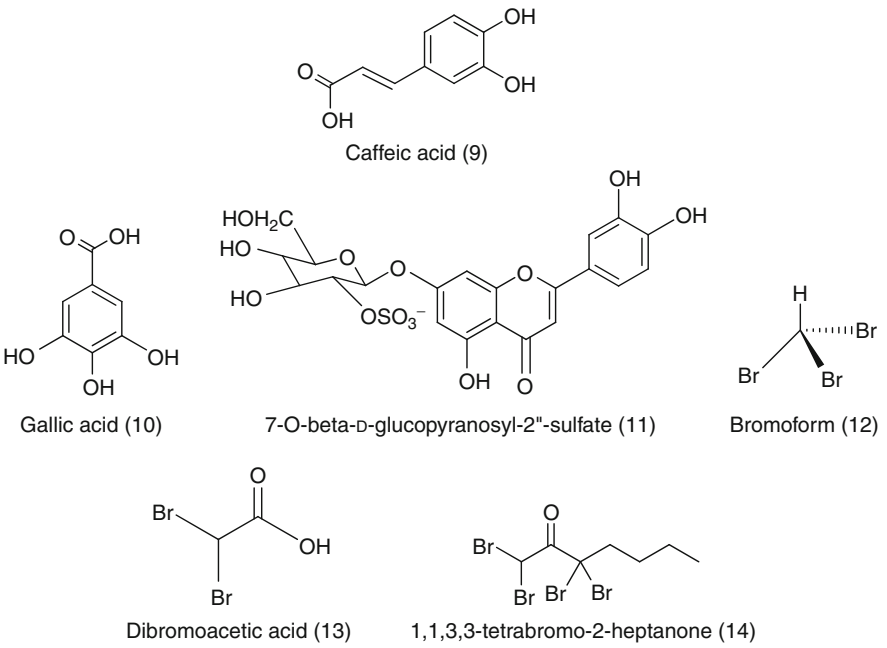


Fig. 15.2 Antimicrobial natural products isolated from marine algae and seagrasses

The red alga *Asparagopsis armata* exhibits antibacterial activity against ecologically relevant *Vibrio* spp. and biomedical bacteria [159]. In this species, the antibacterial compounds are bromoform (Fig. 15.2:**12**) and dibromoacetic acid (Fig. 15.2:**13**), both of which are released into the water column and may thereby interact with bacteria prior to settlement [159]. When *A. armata* is cultured in media without bromine, higher densities of epiphytic bacteria grew on algae that did not produce these brominated compounds. The low level of fouling observed on the Swedish red alga *Bonnemaisonia hamifera* is due to the antibacterial compound 1,1,3,3-tetrabromo-2-heptanone (Fig. 15.2: **14**), a poly-halogenated 2-heptanone [160]. This compound was tested at natural surface concentrations against 18 bacteria isolated from red algae and exhibited selective antibacterial activity. In addition, when applied to gels and deployed in the sea, natural surface concentrations of this compound reduced overall bacterial growth relative to controls [160]. The red alga *Delisea pulchra* produces halogenated furanones, which, at natural concentrations, prevents surface bacterial colonization by inhibiting bacterial quorum sensing [161].

15.2.3 Sponge Diseases

In contrast to corals, diseases of sponges were documented as early as the 1930s, when disease outbreaks devastated the commercial bath sponge industry in the

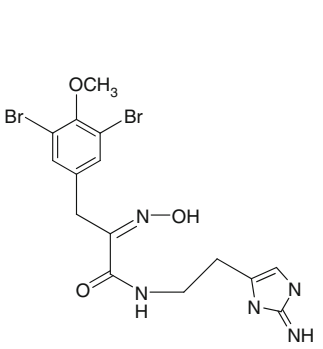
Caribbean and later in the Mediterranean [162]. In the past few years, reports of sponge diseases have reemerged across the globe. In only a few cases have the etiologic agents been identified. Tissue necrosis in *Geodia papyracea* from Belize resulted from the sponge's own symbiotic cyanobacteria multiplying faster than the host could control their numbers under thermal stress [163]. A spongin-boring alpha-proteobacterium was identified as the etiologic agent of disease in *Rhopaloeides odorabile* from the Great Barrier Reef through the fulfillment of Koch's postulates [164]. Various other bacteria [165], including those that specifically attack structural spongin fibers [166], fungi [167], and cyanobacteria [168], have also been postulated as sponge pathogens, although these have not been confirmed. Recently, the disease *Aplysina* red band syndrome (ARBS) was described from the Bahamas, where it affected approximately 10% of *Aplysina* spp. rope sponges [168]. This disease manifests as a red band composed of filamentous cyanobacteria surrounding a necrotic lesion that becomes colonized with algae. ARBS is easily transmitted via sponge–sponge contact. ARBS reduces sponge growth and weakens the sponge skeleton, which often breaks at the site of the lesion.

Sponges produce a diversity of secondary metabolites that play important roles in their survival [32, 127, 169] and provide an impressive source of pharmacologically active molecules [170–172]. Some of these compounds are believed to be produced de novo by the sponges themselves, while others are now recognized to be products of microbial symbionts [173]. Sponges naturally host diverse microbial communities, consisting of symbionts, opportunists, and pathogens [174]. Most of the early studies on sponge antimicrobials used biomedical models (i.e., human pathogens) to assess potential antimicrobial activity of sponge extracts or metabolites, and numerous compounds with pharmaceutical interest have been identified. More recently, assays evaluating potential ecological roles have been developed, and these have used microorganisms isolated from marine sources, usually the sponge itself, the water column, or the surfaces of nearby biotic or abiotic substrata. Clearly, these assays have more relevance in terms of the efficacy of antimicrobial activity against potential pathogens to which the sponge would naturally be exposed. The greatest number of studies has assessed the antifouling effects of sponge metabolites (discussed in ▶ Chapter 14), but the prevention of microbial fouling of the sponge surface is of course relevant to the protection of the sponge from pathogenesis as well.

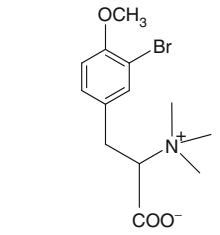
Antimicrobial activity of sponge extracts is widespread, both taxonomically and geographically [175]. Fewer studies have characterized individual metabolites that act to inhibit marine microbes specifically. Extracts and pure compounds from several species of Caribbean sponges were assessed for their ability to inhibit attachment of *Vibrio harveyi*, a ubiquitous marine bacterium, to agar blocks [176]. Since attachment is a primary step in settlement, this process is critical to both fouling and pathogenesis. Eighty-one percent of the sponges tested, and six compounds isolated from four different sponge species inhibited *V. harveyi* attachment at natural or lower concentrations [176]. Extracts from a subset of these sponge species were also tested for their effects on the growth and attachment of

24 marine bacterial isolates from the sponges' natural habitat [169]. Using the disk diffusion assay, only four of these extracts inhibited microbial growth. One isolate from the surface of the sponge *Agelas conifera* exhibited increased attachment but reduced growth when exposed to the extract from that sponge [169]. Using bioassay-guided isolation of the extract from *Aiolochroia crassa*, ianthellin (Fig. 15.3: **15**) was identified as a metabolite that inhibited attachment, while a bromotyrosine derivative (Fig. 15.3: **16**) inhibited bacterial swarming, a mechanism by which bacteria colonize a surface [169]. In a study of Red Sea sponges, the disk diffusion assay was used to characterize the effects of sponge extracts on the growth of several ecologically relevant bacteria [177]. Eight of the 11 sponge extracts inhibited bacterial growth, and bioassay-guided isolation of the active compounds from *Amphimedon viridis*, the sponge with the greatest activity, yielded a mixture of two pyridinium alkaloids, halitoxin (Fig. 15.3: **17**) and amphitoxin (Fig. 15.3: **18**). This mixture exhibited selective activity against seawater isolates, whereas isolates from the sponge itself were not inhibited. Interestingly, the surface of *A. viridis* appeared to be free of bacterial fouling, suggesting that bacteria in the water column are unable to colonize the surface of *A. viridis*, whereas bacteria associated with the sponge may be actively selected by the fact that they are immune to the sponge's chemical defenses. Following wounding, an activated defense in *Aplysina aerophoba* catalyzes the production of the antibacterial compounds aeroplysinin-1 (Fig. 15.3: **19**) and dienone (Fig. 15.3: **20**) to concentrations known to be active; this response might protect injured cells that may have an increased probability of penetration by potential pathogens [178].

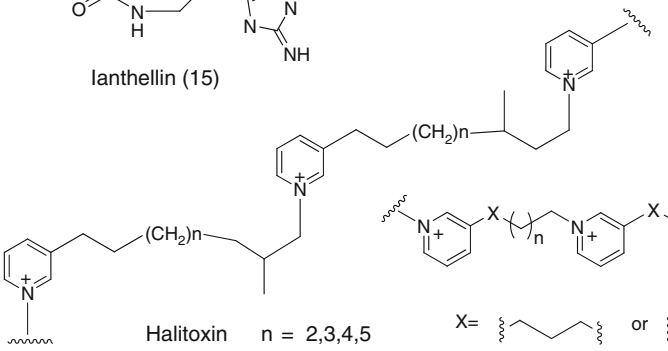
The location of metabolites within the sponge may provide clues as to their potential utility as antimicrobial defenses under natural conditions, as well as to their biosynthetic origin. In one of the few studies to address these issues, a variety of techniques were used to demonstrate that oroidin (Fig. 15.3: **21**) and sceptrin (Fig. 15.3: **22**) were affiliated with sponge rather than bacterial cells in *Agelas conifera* and indicated that sponge spherulous cells were responsible for production of these metabolites [179]. Natural concentrations of extracts caused polyp retraction in the coral *Madracis mirabilis*, and the pure metabolites exhibited antimicrobial activity and feeding deterrence [179]. Tissue wounding increased the concentration of these metabolites, both of which were also found to be released into the water column. The antimicrobial brominated compounds aerothionin (Fig. 15.3: **23**) and homoaerothionin (Fig. 15.3: **24**) in *Aplysina fistularis* are also exuded into the water column and are believed to play a role in inhibiting surface fouling [180]. The ability of antimicrobial compounds to be released into the water column in the vicinity of the sponge surface, or at least to be bioavailable on the sponge surface, is likely crucial to their efficacy in preventing pathogenesis. Novel methods to measure these compounds, which likely occur in very dilute concentrations, are clearly needed. Solid-phase microextraction (SPME) fibers may provide one method to detect minute concentrations of specific types of metabolites over very fine spatial scales.



Ianthellin (15)



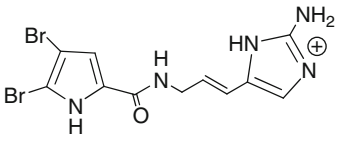
Bromotyrosine derivative (16)



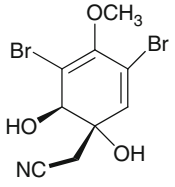
Halitoxin n = 2,3,4,5
Amphitoxin n = 5

Halitoxin (17)

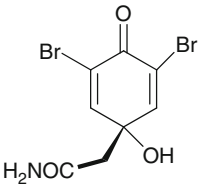
Amphitoxin (18)



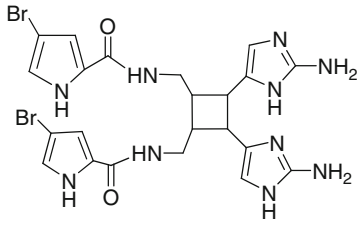
Oroidin (21)



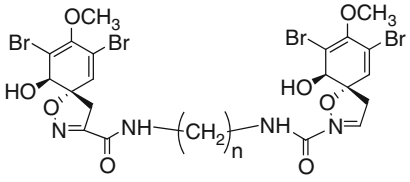
Aeroplysinin-1 (19)



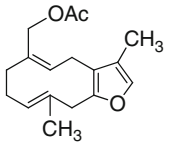
Dienone (20)



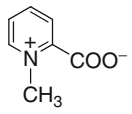
Sceptrin (22)



Aerothionin (23) n = 4
Homoaerothionin (24) n = 5



Julieannafuran (25)



Homarine (26)

Fig. 15.3 Antimicrobial natural products isolated from marine invertebrates

15.2.4 Soft Coral/Gorgonian Diseases

One of the most extensively studied marine diseases is Aspergillosis, a disease affecting Caribbean gorgonian corals. Aspergillosis is caused by the terrestrial fungus *Aspergillus sydowii* [181], which finds its way onto Caribbean reefs via dust storms from the Sahara Desert and in sediment that runs off of land as a result of human activity [182, 183]. A Caribbean-wide outbreak of Aspergillosis resulted in extensive mortality of the sea fans *Gorgonia* spp. [183, 184]. Both waterborne and physical transmission of Aspergillosis have been demonstrated [183]. Secondary metabolite production by *A. sydowii* isolated from diseased sea fans may be associated with pathogenicity, but this has not yet been verified [183].

Temperature increases activation of certain host immune responses (e.g., oxidative enzymes and cellular immunity), suggesting that gorgonians may increase resistance to disease when faced with warming climatic conditions [68]. However, elevated temperature has been shown to reduce the efficacy of chemical defenses against the fungal pathogen *A. sydowii* [185], suggesting perhaps a trade-off in immune responses under different environmental conditions. Reduced variability in levels of disease resistance on reefs with higher levels of Aspergillosis suggests selection for disease resistance [136], and it has been proposed that resistance in the remaining population of sea fans following the Caribbean-wide Aspergillosis epidemic may explain the recent decline in prevalence of this disease [186].

Extracts from many Caribbean gorgonian species have antimicrobial activity [187], and several inhibit spore germination of *Aspergillus sydowii* [135, 188]. The immune system of the Caribbean Sea fan, *Gorgonia ventalina*, has been studied extensively, and antifungal compounds are one of several immune mechanisms employed in response to Aspergillosis infection. These antifungal compounds are found in higher concentrations in infected sea fans and may be induced by infections with Aspergillosis [135, 136, 189]. Differences in levels of antifungal chemical defenses have been linked to both interspecific [190] and intraspecific [191] variability in susceptibility to Aspergillosis. Intraspecific variability in antifungal activity occurs at the level of the individual sea fan, with elevated levels of antifungal activity in the vicinity of the lesion itself [135], as well as among reefs, which may explain some of the geographic variability observed in the prevalence of this disease [136, 191]. To date, there have not been any bioassay-guided attempts to identify antifungal compounds. In contrast with changes in antifungal activity, there was an indirect effect of pathogenesis on chemical defense: infected sea fans produced significantly reduced concentrations of the feeding-deterrent compound julieannafuran (Fig. 15.3: **25**), thereby increasing the susceptibility of diseased corals to predation [71]. Bacteria isolated from the surfaces of gorgonians also produce antibacterial defenses [192] and could thereby be participating in the host's defense against pathogenesis.

Alcyonacean soft coral extracts from the Antarctic [193], Red Sea [194], and Indo-Pacific [195] also exhibit widespread antimicrobial activity. Some of this activity is associated with waterborne molecules released from the surface of the coral that inhibit bacterial attachment [47, 195]; one such molecule is the highly polar homarine

(Fig. 15.3:**26**; [47]). These studies used a variety of methods to obtain the waterborne molecules, including the use of syringes to sample water at different distances from soft corals in situ [47], and "conditioning" seawater in the laboratory by placing soft corals into individual containers and sampling the water therein [195].

15.2.5 Tunicate Diseases

Of the invertebrates, tunicates possess the most complex cell-based immune system, while still maintaining a cell-free immune response, which includes the production of antibacterial peptides and other antimicrobial secondary metabolites [64, 196]. Like other sessile marine organisms, the antifouling properties of tunicate extracts and secondary metabolites have been widely studied [51, 197]. With regards to antimicrobial activity that might aid in preventing pathogenesis, tunicate compounds have been evaluated predominantly against potential human pathogens, and a diversity of proteins and other metabolites has been considered as viable leads for pharmaceutical research (e.g., [172]). For example, when extracts from the tunicate *Ecteinascidia turbinata* were injected into the American eel, *Anguilla rostrata*, suffering from a bacterial infection, the extract increased antibody titers and survival [198], confirming a diversity of mechanisms of action for tunicate antibacterial defenses. In a rare example of ecological relevance, the chemical inhibition of bacterial settlement was compared with antibacterial toxicity in Mediterranean tunicates, and it was concluded that while these two processes were likely complementary, they were controlled by different metabolites [199].

15.3 Conclusions

Marine communities worldwide face unprecedented variation due, in part, to climate change and anthropogenic stressors. While the importance of predation to the structure and function of these communities has long been accepted, it is increasingly clear that competition and pathogenesis will play significant roles in the near future. Reduced biodiversity in marine communities will likely lead to greater competition for limiting resources; this should favor selection of species with allelopathic compounds. These compounds might act to prevent fouling organisms or macroorganisms from overgrowing surface tissues via contact-mediated ("direct") processes. Alternatively, the more proactive ("indirect") approach of releasing allelopathic compounds into the water column where they can act at a distance will also be beneficial. The significant impact of pathogenesis on marine communities has been recognized for at least three decades. In that time, more species have been affected, with increasing frequency, and over broader spatial scales. Antimicrobial compounds often represent the most targeted defense system of the organisms that produce them as they often exhibit selective activity against particular species. These disease resistance mechanisms exhibit similar variability among individuals within a population, as innate immunity does for human hosts exposed to pathogenic microbes.

To date, there is good evidence to support allelopathy and antimicrobial activity in marine algae and invertebrates. More challenging is the use of bioassay-guided isolation of the putative marine natural products involved in competitive and pathogenic interactions. Competitive processes are often characterized by interactions that occur over spatial scales of weeks to months, too long and costly for bioassay-guided procedures. However, recent elegant experimental designs with algae, sponges, and soft corals have led to the identification of allelopathic compounds of ecological relevance. In contrast, whereas pathogenic interactions might occur over a time span of several hours, many marine microbes are at present "unculturable," and for those, performing ecologically relevant antimicrobial assays in a laboratory setting will be impossible. Nonetheless, the molecular biology revolution has provided important insights into putative pathogens and mechanisms of infectivity. There have also been some successes isolating bioactive antimicrobial natural products from seagrasses, algae, sponges, and soft corals. Future studies of competition and pathogenesis in marine communities should focus on the role of bioactive natural products and include the experimental lessons reviewed here.

Acknowledgments Thanks to S. Ankisetty and H.N. Kamel for the help with locating references and drawing structures for this chapter. Funding was provided by NOAA/NIUST and NSF to M. Slattery and D.J. Gochfeld. For this support, we are grateful.

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