Marine chemical ecology

Valerie J. Paul,* Melany P. Puglisi and Raphael Ritson-Williams

Received (in Cambridge, UK) 6th January 2006, Accepted 1st February 2006
First published as an Advance Article on the web 23rd February 2006
DOI: 10.1039/b404735b

Covering: 2003–2005

This review covers the recent marine chemical ecology literature for phytoplankton, macroalgae, sponges and other benthic invertebrates; 249 references are cited.

1 Introduction

In this report, we review progress over the past two years in the field of marine chemical ecology. Research in this field has continued at a rapid pace since we last reviewed this topic.[1] As the field of chemical ecology matures, research shows that natural products drive complex ecological interactions at all stages of marine plant and animal life histories. Research in marine chemical ecology continues to focus on predator–prey and competitive interactions, settlement cues, and potential defenses against infection by microorganisms. There are many new studies that address chemically mediated interactions between macroorganisms and microorganisms associated on surfaces, adjacent substrates, or in seawater. In addition, a few studies examine chemically mediated interactions between different microorganisms. This review also includes several examples demonstrating that symbionts chemically defend their host against predation.

Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, FL, 34949

Several review articles dealing with various aspects of marine chemical ecology have been published since our last overview of this topic.[1] Pohnert[2] reviewed chemical defense strategies in marine organisms with an emphasis on chemical defense in the plankton and dynamic defenses in benthic organisms, including examples of induced and activated defenses in marine algae. Allelopathic interactions of primary producers in all aquatic ecosystems have been recently reviewed by Gross.[3] For marine systems she discusses allelopathy (including antimicrobial and antifouling activity) in planktonic species and benthic species, including seagrasses, macroalgae, microalgae, and corals.[3] Marine micro- and macroalgae have also been studied as promising resources for antifouling natural products. A recent review of marine natural products that are implicated in antifouling and larval settlement and metamorphosis was published by Fusetani.[4] Another review covers much of the algal antifouling literature and focuses on the published activity of extracts and algal secondary metabolites tested in antibacterial, antifungal, and antialgal assays.[5] Here we report the research in marine chemical ecology that has been published since these reviews.

2 Microorganisms and phytoplankton

2.1 Bacteria

While chemically mediated microbial interactions in the terrestrial environment are well studied, the chemical ecology of marine bacteria is an emerging field. Miao and Qian[6] examined the antibacterial and antifungal activities of 46 strains of fungi isolated from seawater and 19 strains of bacteria isolated from natural biofilms in Hong Kong. Antibacterial activity was prominent in the fungal extracts (32 active extracts), and six strains showed broad spectrum antibacterial activity, inhibiting the growth of at least 15 bacterial strains. Antifungal activities were observed for 11 bacterial cultures. The result of this and other studies of chemically mediated microorganism–microorganism interactions demonstrate that the interactions in the microbial communities are complex and warrant further studies.

Even though biofilms are recognized as important in the establishment of fouling communities, there has been little research on antifouling compounds isolated from the associated microbial community. Burgess *et al.*[7] conducted a screening of bacteria sampled from the surfaces of 30 benthic organisms. Six hundred and fifty bacterial strains were isolated, and forty two of these had

Valerie J. Paul is currently Head Scientist at the Smithsonian Marine Station at Fort Pierce, Florida. She received her BA from the University of California, San Diego in 1979 with majors in Biology and Studies in Chemical Ecology and her PhD in Marine Biology in 1985 from the University of California San Diego, Scripps Institution of Oceanography. Dr Paul joined the faculty of the University of Guam Marine Laboratory in 1985, served as Director of the Laboratory from 1991–1994, and as full Professor from 1993–2002. She was elected a Fellow of the American Association for the Advancement of Science in 1996 and was elected and served as chairperson of the Marine Natural Products Gordon Research Conference in 2000 (vice-chair in 1998). She currently serves on the editorial boards of the journals Coral Reefs and Journal of Natural Products. Valerie's research interests include marine chemical ecology, marine plant–herbivore interactions, coral reef ecology, and marine natural products. She is the author or co-author of over 150 research papers and review articles.

Melany P. Puglisi is currently a postdoctoral fellow at the Shannon Point Marine Center, Western Washington University. She received her BS from Southampton College, Long Island University in 1991 in Chemistry, her MS from the University of Guam Marine Laboratory in Biology in 1995, and her PhD in Pharmacognosy specializing in the field of Marine Chemical Ecology in 2001 from the University of Mississippi, School of Pharmacy. Melany's postdoctoral research at the University of California at San Diego Scripps Institution of Oceanography and Smithsonian Marine Station at Fort Pierce focused on marine microbial chemical defenses of macroalgae. Her current research interest is in chemical defenses of marine plants against co-occurring microorganisms.

Raphael Ritson-Williams is currently working as a technician in the field of marine chemical ecology at the Smithsonian Marine Station at Fort Pierce. He received his BS in Biology from The Evergreen State College in 1998, and his MS in marine biology from the University of Guam in 2002. After Guam he moved to Fort Pierce, Florida to continue his ecological studies of marine natural products with Dr Valerie Paul. His research interests include coral reef biodiversity and natural product diversity especially those compounds that influence evolution and ecology. Recent research projects include the feeding ecology of coral reef herbivores and predators, chemical and aposematic defenses in nudibranchs, the function of tetrodotoxin in marine flatworms, and chemical cues for larval settlement and metamorphosis.

Valerie J. Paul

Melany P. Puglisi

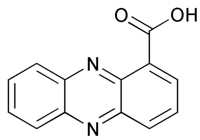
Raphael Ritson-Williams

antibacterial activity against at least one strain of the nine fouling bacteria used in antimicrobial assays. The most potent bacterial isolate was a *Pseudomonas* sp. isolated from the nudibranch *Archidoris pseudoargus.* This bacterium contained five compounds **1–5** that had high antimicrobial activity against the nine bacterial strains, inhibited settlement of barnacle larvae, and reduced *Ulva lactuca* spore settlement and percent cover of germlings. When tested independently, phenazine-1-carboxylic acid **1** had the highest activity against the bacterial strains. This study and similar research with *Vibrio* sp., isolated from the surface of the green alga *Ulva reticulata*,[8] showed that epibiont microorganisms may protect their hosts from fouling organisms.

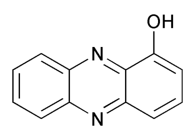
Research on the bacterium *Pseudoalteromonas tunicata* found that its yellow pigment reduced settlement of potential fouling organisms.[9] Using transposon mutagenesis the authors were able to create strains of *P. tunicata* with different combinations of two pigments and showed that only the yellow strain, like the wild type strain, reduced settlement of *Balanus amphitrite*, *Hydroides elegans*, and *Ulva lactuca* and *Polysiphonia* sp. propagules. The use of differential expression allowed manipulation of the natural products without destroying the bacterium of interest.

2.2 Cyanobacteria

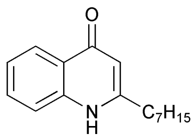
Cyanobacterial blooms are common types of harmful algal blooms that appear to be increasing in frequency worldwide.[10,11] While many cyanobacterial blooms occur in freshwater and will not be discussed here, recent studies have also focused on planktonic blooms in the Baltic Sea. During bloom events, concentrations of cyanobacterial hepatotoxins such as nodularin-R (nodularin) **6**, demethylnodularin-R **7**, and even microcystin-LR **8** have been detected.[12] Karjalainen *et al.* examined the accumulation of nodularin in natural assemblages of zooplankton during blooms of *Nodularia spumigena.*[13] Nodularin was present in relatively low concentrations in common zooplankton species collected during cyanobacterial blooms, although variation in concentrations occurred between species and years. Nodularin concentrations could be detected in copepods as well as in carnivorous zooplankton species, which are major prey items for planktivorous fishes. Not surprisingly, the highest concentrations in zooplankton were observed during cyanobacterial blooms.[13] Nodularin **6** has been found in mussels, fish and seabirds (the common eider *Somateria mollissima*) in the Baltic Sea.[14]



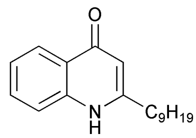
1



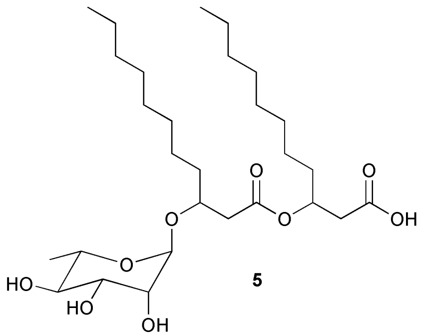
2



3



4



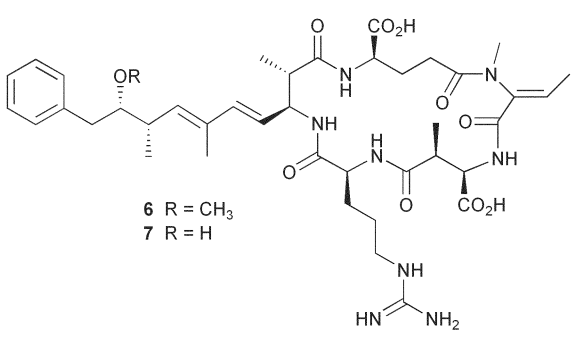
5

Benthic cyanobacteria such as *Lyngbya* spp. continue to intrigue natural products chemists as a rich source of nitrogenous secondary metabolites. To examine genetic, chemical, and morphological diversity, Thacker and Paul[15] compared partial 16S ribosomal DNA sequences among Pacific collections of *Lyngbya* spp. and *Symploca* spp. Genetic divergence did not correlate well with chemical and morphological differences. The authors concluded that chemical variability among species of *Lyngbya* cannot be predicted by 16S rDNA sequence analyses and that other factors including interactions between genotypes and environmental conditions may be important to explain the high degree of chemical variation found in these cyanobacteria.[15]

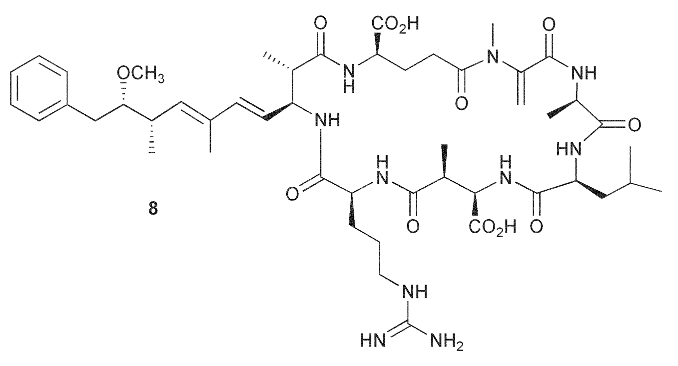
Interactions between generalist and specialist grazers and the benthic cyanobacterium *Lyngbya majuscula* have recently been reviewed.[16] Rabbitfish and sea hares are the grazers most often observed to feed on *Lyngbya.* Capper *et al.*[17] examined the chemical defenses of a bloom of *L. majuscula* from Moreton Bay, Australia, against a variety of consumers that co-occur in Moreton Bay and other allopatric consumers from Guam. Crude extracts containing mainly lyngbyatoxin A, which were incorporated into artificial diets, deterred feeding by all consumers tested in Guam except for the specialist sea hare *Stylocheilus striatus*, which was stimulated to feed by the extract. Australian *S. striatus* showed no preference for control or treated foods. Wild-caught rabbitfish (*Siganus fuscescens*) avoided the *L. majuscula* extract, but captive bred *S. fuscescens* showed no preference between control and treated food.[17] Nuisance blooms of *L. majuscula* in reef habitats can also have negative effects on the settlement of coral larvae,[18] but it is not yet known whether this inhibition is chemically mediated.

2.3 Eukaryotic marine microalgae

During the past few years some excellent studies on the ecology and chemical ecology of toxin-producing marine phytoplankton and other microalgae have been published. These studies have undoubtedly been fueled by interest in harmful algal blooms (HABs) and particularly in understanding bloom dynamics, toxicity, and effects on consumers and competitors. The chemical ecology of eukaryotic marine microalgae was the subject of a recent review by Cembella,[19] which provides a comprehensive overview of marine microalgal toxins. The review includes allelopathic effects and inhibition of grazers by saxitoxins, domoic acid, polyether toxins such as brevetoxins, ciguatoxin and maitotoxin, okadaic acid, and prymnesins and their analogues. Pohnert[2] also covered the topic of chemical defense in the plankton, discussing the few examples that demonstrate a role for microalgal toxins in chemical defense or allelopathic interactions. Both Cembella[19] and Pohnert[2] concluded that we know little about functional roles of secondary metabolites produced by microalgae, especially given the variable results with different phytoplankton and grazer species, or about genetic regulation of the production of these compounds. The topic of allelopathy in phytoplankton, with an emphasis on HAB-forming species, was reviewed by Legrand *et al.*[20] In addition to a thorough review of the literature on this topic, their paper focused on chemical interactions, especially growth inhibition, among competing algae and bacteria and methods of determining allelopathy in phytoplankton. Heil *et al.*[21] reviewed the literature about the dinoflagellate *Prorocentrum minimum*, the cause of harmful algal blooms in estuarine and coastal environments worldwide. This HAB species has expanded its range geographically in past decades and has been increasingly observed to cause deleterious effects. The detrimental effects of toxins produced by *P. minimum* to other organisms and the environment seem to be variable, and the toxins remain to be characterized (see also Wikfors[22]). Heil *et al.*[21] reviewed evidence for toxicity and allelopathic activity of *P. minimum*, and Wikfors[22] reviewed evidence for negative impacts of this microalga on bivalves (clams, oysters, scallops).



6 R = CH_3
7 R = H



8

Studies of the chemical ecology of marine microalgae generally focus on allelopathic effects against competitors or consumers. Microalgal toxins can potentially play a role in these interactions but have rarely been experimentally demonstrated to do so. Several recent studies have examined the allelopathic activities of microalgae and the roles of microalgal toxins in competition among phytoplankton species.

Kubanek *et al.*[23] tested whether the red tide dinoflagellate *Karenia brevis* uses allelopathy to outgrow 12 species of co-occurring phytoplankton in laboratory experiments. Nine of the 12 species were suppressed when grown with *K. brevis* at bloom concentrations. Extra-cellular filtrates and extracts of filtrates inhibited six of the nine species; however, the effects could not be attributed to brevetoxins, which at ecologically relevant concentrations only inhibited one competitor, *Skeletonema costatum.* Allelopathic effects observed in the filtrate experiments may have been due to highly water soluble, volatile, or unstable compounds that were not extracted into the lipophilic extracts and were not identified. *K. brevis* was out-competed by several microalgae in this study, including the dinoflagellate *Prorocentrum minimum* and the diatom *Odontella aurita*, both of which can be abundant during *K. brevis* blooms.[23]

Dinoflagellates of the genus *Alexandrium* were shown to have toxic effects on other dinoflagellates in culture[24] and on natural plankton communities.[25] The effects were not related to levels of paralytic shellfish poisoning (PSP) toxins in *Alexandrium* strains because both toxic and non-PSP producing strains of *Alexandrium* had negative effects on other phytoplankton. *A. tamarense* affected the whole natural phytoplankton community by decreasing overall growth rates (measured by chlorophyll a concentrations) for all phytoplankton organisms except the small (<30 μm) dinoflagellates.[25] Extracellular substances appeared to be responsible for the allelopathic effects,[24] which included immobilization and lysis of other microalgal cells. It is noteworthy that toxins such as the brevetoxins,[23] PSP toxins,[24,25] and domoic acid[26] did not have a negative effect on other microalgal cells in culture. Recently, a role for domoic acid together with copper in iron acquisition in diatoms has been demonstrated, and iron limitation is a likely trigger for toxin production and release.[27]

The bloom-forming planktonic alga *Prymnesium parvum* also uses excreted toxins for food uptake and allelopathy. The toxic properties are due to prymnesins and possibly other compounds, which display haemolytic, ichthyotoxic, and cytotoxic activities. *P. parvum* is photosynthetic but also feeds on microorganisms such as bacteria and protists. Excretion of the toxins in dense cultures of *P. parvum* caused immobilization and lysis of prey cells such as the dinoflagellate *Heterocapsa rotundata.*[28] Cell-free filtrates of *P. parvum* added to different size fractions of a natural Baltic Sea plankton community negatively affected the whole plankton community. Cyanobacteria and dinoflagellates were more resistant than diatoms and ciliates to the toxins, however, all phytoplankton size classes showed reduced chlorophyll a levels and decreased carbon uptake when exposed to the filtrates.[29]

Several studies have evaluated the effects of microalgal toxins on consumers. The negative effects of polyunsaturated aldehydes (PUAs) produced by some species of marine diatoms on copepod reproduction are now well documented.[30,31] These aldehydes, cleaved from fatty acid precursors when diatom cells are damaged, caused developmental arrest and deformed copepod larvae when mothers and their larvae were fed diatom diets that contained the compounds.[31] Several recent reviews provide excellent background on this wound-activated defense and its implications for copepods and marine food webs.[30,32,33] PUAs may also have impacts on the larvae of benthic organisms. The aldehyde 2,4-decadienal reduced survival of the larvae of two benthic polychaetes and two echinoderms depending on the concentration of exposure.[34] The production of PUAs can be strain-specific in diatoms, and thus only certain diatoms might be chemically defended in nature. GC-MS methods have been developed to quantify various polyunsaturated aldehydes in diatom cultures and in natural phytoplankton assemblages.[35] These methods were applied to screen 50 species (71 isolates) of diatoms for polyunsaturated aldehydes.[36] Twenty seven PUA-producers were identified, including major spring-bloom forming diatoms of the genus *Thalassiosira.*[36] In addition to effects on copepods, these polyunsaturated aldehydes may have allelopathic effects on microalgae. Decadienal, one of the common PUAs from diatoms, reduced growth of the diatom *Thalassiosira weissflogii* in a dose- and time-dependent manner. It negatively affected cell membrane integrity, decreased photosynthetic efficiency, and caused cell death.[37] PUAs may be responsible for chemical signaling within phytoplankton communities[37] in addition to their negative effects on invertebrate larvae and copepod grazers.

Another activated defense system in phytoplankton, which is also found in some green macroalgae, is the conversion of dimethylsulfoniopropionate (DMSP) to dimethyl sulfide (DMS) and acrylate.[2] Microzooplankton herbivory is considered to be the key process causing this transformation to occur, however, there have been few studies in natural systems. Archer *et al.*[38] used direct measurements of microzooplankton herbivory on *Phaeocystis* sp. in the North Sea to account for DMS production rates. Estimates of DMSP ingested accounted for the DMS produced by different concentrations of microzooplankton grazing on *Phaeocystis* sp. in bottles.[38] Strom *et al.*[39,40] studied feeding and growth by protists on the DMS-producing microalga *Emiliania huxleyi.* Different strains of *E. huxleyi* had different levels of DMSP-lyase activity, the enzyme responsible for the conversion. Five out of six protist grazer species showed lower feeding rates on strains with high DMSP-lyase activity than on low-lyase strains.[39] DMSP, DMS, and acrylate dissolved in seawater were tested as chemical defenses against protist grazers that were offered *E. huxleyi.*[40] Interestingly, DMSP rather than the activation products DMS and acrylate reduced grazing on *E. huxleyi* by four different species of dinoflagellate grazers.[40]

The red tide dinoflagellate *Karenia brevis* regularly blooms in late summer and fall months. It produces the brevetoxins, which are neurotoxic to mammals and have been implicated in shellfish poisonings and die-offs of finfish, invertebrates, sea turtles and marine mammals.[41] Prince *et al.*[42] studied the copepod *Acartia tonsa* and the fitness effects of feeding on different microalgal diets, including diets rich in *Karenia brevis.* Copepods did not avoid feeding on *K. brevis*, and actually ate more *K. brevis* than the more preferred microalga *Rhodomonas lens* when these were offered as unialgal diets, suggesting possible compensatory feeding. On diets of 93–100% *K. brevis*, copepods experienced decreased survivorship and fecundity per female, but egg hatching rates were the same for all diet regimens. Survivorship of starved copepods was only marginally lower than for copepods fed only *K. brevis*, which was lower than survivorship on all other diets.

Extra-cellular extracts of *K. brevis* did not directly affect *A. tonsa* survivorship or consumption rates when copepods were exposed to extracts while feeding on *Rhodomonas lens.* The authors attributed the negative effects of *Karenia brevis* on the copepods to nutritional inadequacy rather than overt toxicity of the dinoflagellate.[42] A potential mechanism for the nutritional limitation of *K. brevis* was suggested by Giner *et al.*[43] who studied sterol composition of this dinoflagellate. Four novel and two rare sterols were isolated, including gymnodinosterol and brevesterol as the major sterols. The authors suggested these novel sterols might offer protection from predation, but this was not directly tested. They hypothesized that these modified sterols might be of poor nutritional value to marine invertebrates, thus reducing grazing on *K. brevis* and contributing to bloom formation.[43]

A similar study of copepod feeding was conducted with *Acartia tonsa* feeding on the dinoflagellate *Prorocentrum minimum*,[44] which varies in toxicity depending on location and strain.[21,41] *A. tonsa* readily ingested *P. minimum* cells, and egg production rate was positively correlated with ingestion rate. Egg hatching rate was high and not affected by consumption of *P. minimum.* Ingestion, egg production and egg hatching success of females were measured for mixed diets consisting of *P. minimum* and the diatom *Thalossiosira weissflogii.* Diets containing *T. weissflogii* increased egg production, and the authors concluded that *P. minimum* was nutritionally inadequate but that there was no evidence of toxicity to *A. tonsa.*[44] The dinoflagellate *Gyrodinium corsicum* had significant adverse effects on survival of the copepod *Acartia grani* but not on another copepod *Euterpina acutifrons.* PSP toxins were not present in the dinoflagellate; however, aqueous and methanol extracts showed evidence of toxicity in mouse assays, but they were not tested on the copepods.[45] Females of the copepod *Temora stylifera* demonstrated reduced egg production and hatching success when fed uni-algal diets of the dinoflagellate *Alexandrium tamarense.*[46] This strain of *A. tamarense* was not neurotoxic, contained low levels of saxitoxins, and also did not contain polyunsaturated aldehydes; however, extracts blocked fertilization success in sea urchin oocytes, indicating that other unidentified compounds were responsible for the reduced egg production and hatching success observed.[46]

Toxin accumulation by zooplankton grazers was also studied for zooplankton feeding on the dinoflagellate *Alexandrium fundyense*, which contains the paralytic shellfish poisoning (PSP) toxins such as saxitoxin and its analogues.[47] The three studied species of copepods accumulated measurable levels of toxin, but some of the PSP toxins ingested were excreted in fecal pellets, and most of the toxins present in *A. fundyense* ($\geq$90%) were not recovered and could have been released by *Alexandrium* cell breakage during feeding. The copepods *Acartia hudsonica* and *Eurytemora herdmani* consumed different relative amounts of *A. fundyense* cells when offered as mixed diets with *Heterocapsa triquetra*, displaying avoidance of *A. fundyense* cells at higher concentrations. *Centropages hamatus* did not change its feeding behavior and also retained the highest concentrations of toxin during feeding. The authors concluded that copepod grazing primarily disperses PSP toxins into the environment, but even the small amounts accumulated could be passed onto higher trophic levels.[47]

Excellent examples of how PSP toxins (saxitoxin and analogues) can influence consumer–prey interactions with community level implications have been recently reported.[48,49] In these studies of tritrophic level interactions, microalgal toxins that accumulated in prey items influenced feeding behavior of predators such as sea otters[48] and shorebirds.[49] Variable concentrations of PSP toxins in shellfish influenced sea otter foraging behavior and diet as well as prey abundance at a range of sites in the Inside Passage of southeast Alaska. Toxin concentrations influenced when and how otters fed on their preferred prey, the butter clam *Saxidomus giganteus.* At sites where bivalve toxicity was high (>500 µg STX eq 100 g^{-1}), sea otters rarely ate the toxic prey and fed primarily on sea urchins, small clams, and other less toxic and less abundant prey items. At sites with intermediate levels of toxicity (200–500 µg STX eq 100 g^{-1}), sea otters fed selectively, often discarding some prey items, or parts of prey such as clam siphons, which contained higher levels of toxins. Sea otters did not avoid feeding at sites with high prey toxicity, but they did avoid prey items with the highest levels of toxicity; thus, butter clams were larger and more abundant at toxic sites.[48] Kvitek and Bretz[49] also showed that shorebirds avoided foraging on toxic prey. Black oystercatchers (*Haematopus bachmani*) forage on mussels (*Mytilus californianus*) on rocky shores in California. During harmful algal bloom (HAB) events in the late summer months, mussels accumulated PSP toxins rapidly and showed highest levels of toxicity in September. Oystercatchers rejected mussel tissue and switched to less toxic prey such as limpets when PSP toxin levels in mussels were high. On sandy shores, shorebirds such as godwits, sanderlings, whimbrels and willets primarily consumed sand crabs (*Emerita analoga*). When sand crab toxin levels became high during algal bloom events, shorebird abundance decreased on sandy shores and rejection rates of sand crabs increased. Shorebirds were able to detect PSP toxins and avoided lethal concentrations through changes in foraging behavior.[49] Studies with shorebirds and otters demonstrate that HAB toxins can alter upper-level trophic interactions in marine food webs and that PSP toxins can provide prey items with chemical defense against predators.

The transfer of microalgal toxins to upper trophic levels can have unexpected consequences. For example, mass mortalities of marine mammals have been caused by exposure to brevetoxins after a *Karenia brevis* bloom subsided.[50] Manatees were probably exposed by eating seagrass that had high levels of brevetoxins (through active uptake or passive adsorption onto the seagrass), and dolphins died after consumption of contaminated fish, especially menhaden, that had high levels of brevetoxins.[50] Relatively few studies have addressed the fate and effects of natural products through marine food webs.

3 Macroalgae

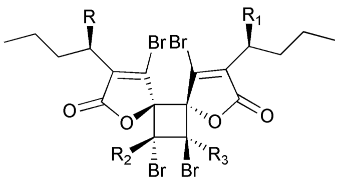
Several useful reviews of natural products and chemical ecology of macroalgae have been recently published. While not directly related to chemical ecology, Smit[51] provided an excellent review of medicinal and pharmaceutical uses of seaweed natural products, which demonstrates the broad range of bioactivities of natural products from macroalgae. La Barre *et al.*[52] discussed modern analytical techniques that might be useful for characterizing and sometimes quantifying chemical defenses in macroalgae. They provide examples in which high field multinuclear and solid-state NMR and mass spectrometry (MS) have been used in metabolic studies of marine plants. The diterpenes in Dictyotacean brown algae from the tropical Atlantic region have been well studied, and the structures and geographical distributions of these compounds were recently reviewed.[53] The authors discuss chemotaxonomy, biogeography, and chemical differentiation of brown algal diterpenes. In addition, they postulate that biosynthetic pathways diverged as Dictyotacean algae moved into different regions of the world's oceans. Amsler and Fairhead[54] present an overview of chemical defenses and other chemically mediated ecological interactions in the brown algae. The authors provide a discussion of the brown algal phlorotannins and colorimetric methods used to measure them, the functions of phlorotannins as both primary and secondary metabolites, and the high spatial and temporal variability observed for concentrations of phlorotannins. They also review non-phlorotannin defenses in brown algae, including terpenes, acetogenins, and related mixed-biosynthetic compounds in Dictyotacean brown algae. Amsler and Fairhead[54] discuss how brown algae can serve as model organisms to test chemical defense theories (induced defenses, carbon–nutrient balance, resource allocation models, and optimal defense theory). This review also encompassed the chemosensory aspects of brown algae from chemo-attraction of brown algal gametes in response to pheromones to chemosensory behaviors of brown algal spores that are attracted by nutrients and other environmental cues.[54]

Several broad surveys of the bioactivity of crude extracts from seaweeds against marine microorganisms and potential fouling organisms have been recently conducted. Twenty one species of algae from the coast of Yucatan, Mexico, were surveyed for antimicrobial activity against pathogenic microbes.[55] Eighteen species were active against the gram-positive bacteria, and almost all extracts inhibited *Bacillus subtilus.* Extracts of *Caulerpa* spp., which are abundant algae along the Yucatan coast, were highly active in the surveys. Extracts of three different parts of the algae (apical, basal, and stolon regions) were evaluated in antimicrobial assays for *C. ashmeadii*, *C. paspaloides*, and *C. prolifera.* In general, extracts from the stolon region had the highest antibacterial activity.[55] In a broad survey of Antarctic macroalgae from three phyla, crude lipophilic and hydrophilic extracts were assayed for their toxicity to diatom cells as a test for possible antifouling activity.[56] Extracts of 16 of the 25 species of algae tested caused significant diatom mortality at natural concentrations. Another study tested the antifouling potential of nine macroalgae species from the west coast of France. Crude extracts were tested against three species of marine fungi, six species of marine bacteria, three species of diatoms, three species of macroalgae, the mussel *Mytilus edulis*, and the barnacle *Balanus amphitrite.*[57] Some of the extracts were seasonally variable with 52% inactive, 31% seasonally active (with highest activity from May to July), and 17% active all year.

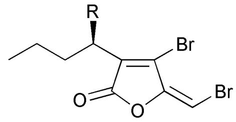
Toxicity of crude extracts of 32 species of Mediterranean seaweeds was examined by Microtox® assay, which analyzes toxicity against the marine bacterium *Vibrio fisheri.*[58] Intra- and inter-specific variation in toxicity was observed, with approximately half of the species demonstrating toxicity. Seasonal variation was found between samples collected in June and November. Overall, toxicity was higher in November, but some species, such as *Lobophora variegata* and *Falkenbergia rufolanosa*, showed higher toxicity in June. Results of the Microtox® assay correlated well with a sea urchin assay, which tested cytotoxic and antimitotic effects of extracts on *Paracentrotus lividus* embryos.[58] While it is difficult to relate these toxicity assays to ecological properties of the seaweeds, the results demonstrate a broad distribution of bioactive compounds in the algae surveyed.

Chemical defense against consumers continues to be an important area of research in chemical ecology. In a recent study, common subtidal macroalgae from the Antarctic Peninsula were evaluated for chemical defenses against the sea star *Odontaster validus*, the rockfish *Notothenia coriiceps*, and the herbivorous amphipod *Gondogeneia antarctica.*[59] Pieces of thalli of 35 species were assessed for palatability in assays with the sea star and rockfish, and those that were unpalatable (over 60% of species tested) were then extracted. Natural concentrations of lipophilic and hydrophilic extracts were tested in artificial diets, which showed that many of these unpalatable algae were chemically defended. At least one extract type of all the ecologically dominant brown algae was unpalatable, except for *Desmerestia antarctica.*[59] Thallus toughness and nutritional quality did not seem to relate to palatability. Another survey of macroalgae from the Antarctic Peninsula examined the nutritional composition (protein, carbon and nitrogen content) of 40 species of algae.[60] Overall protein content was high, and C : N ratio was low relative to temperate and tropical macroalgae, which is probably related to the nutrient-rich waters where these algae reside.[60] Despite nutrient-rich waters, natural products from these unpalatable Antarctic seaweeds generally lack nitrogen.[61] The red alga *Delisea pulchra* yielded new dimeric halogenated furanones, pulchralides A–C **9–11**, and the previously reported furanones fimbrolide **12**, acetoxyfimbrolide **13**, and hydroxyfimbrolide **14**. The red alga *Plocamium cartilagineum* yielded halogenated monoterpenes including anverene **15**, *epi*-plocamene D **16**, and pyranoid **17**. Anverene **15** and *epi*-plocamene D **16** deterred feeding by the amphipod *G. antarctica*, but compound **17** did not; whereas, none of these halogenated monoterpenes inhibited feeding by the sea star *O. validus.*[61] The red alga *Myriogramme smithii* contained simple aromatic compounds such a *p*-hydroxybenzaldehyde and *p*-methoxyphenol, which might be responsible for the unpalatability of this alga. The brown alga *Desmerestia menziesii* contained diterpenes of mixed biogenesis including menzoquinone **18** and hydroquinone **19**. Menzoquinone **18** deterred feeding by the sea star *O. validus* at a concentration three times the isolated concentration.[61] The endemic brown alga *Cystosphaera jacquinotii* contained the novel sterol cystosphaerol **20**. Many of these metabolites were bioactive in antimicrobial assays, but only the few previously mentioned were tested for feeding deterrence against putative consumers.[61]

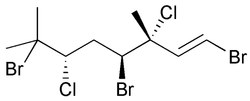
Two non-acidic Antarctic species of *Desmerestia* (*D. anceps* and *D. menziesii*) that were found to be unpalatable[59] were investigated for within-thallus variation in chemical defenses and toughness.[62] Lipophilic and hydrophilic extracts from three different thallus parts (holdfast, primary stem, lateral branches) were incorporated into artificial foods and offered to the sympatric, herbivorous amphipod *Gondogeneia antarctica.* For *D. anceps*, the primary stem extracts were most deterrent, and the primary stem and holdfast were the toughest parts of the thallus. These results were consistent with predictions of optimal defense theory, with the most valuable thallus parts being the most chemically and physically defended. No differences were observed in palatability of extracts among the different thallus parts in *D. menziesii*; however, primary stem and holdfast were once again tougher than lateral branches.[62] The natural products responsible for chemical defense were not explored in this study, but another study looked



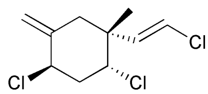
9 R = R_1 = OAc; R_2 = R_3 = H
10 R = R_1 = R_2 = R_3 = H
11 R = OAc, R_1 = R_2 = R_3 = H



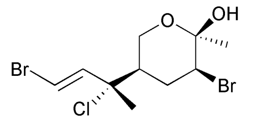
12 R = H
13 R = OAc
14 R = OH



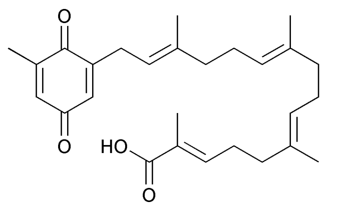
15



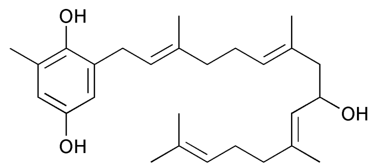
16



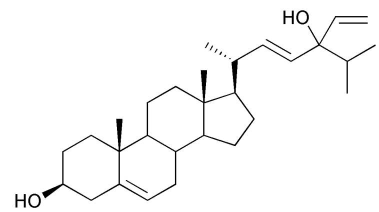
17



18



19

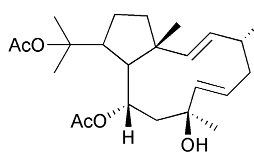


20

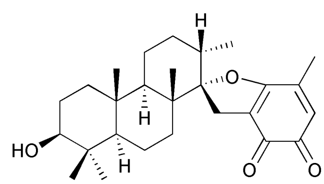
for phlorotannin concentrations in these algae.[63] Phlorotannin concentrations were high in both species of *Desmerestia*; they were also highly variable within individual thalli and at different locations and depths. Phlorotannins were consistently higher in shallow *D. anceps* than in individuals from deeper sites, but this same pattern was not observed for *D. menziesii.*[63] The phlorotannins from *Desmerestia* spp. were not directly tested for their effects on consumers.

Among the unusual chemical defenses found in marine algae, some brown algae of the orders Desmarestiales and Dictyotales are known to contain high concentrations of sulfuric acid within cell vacuoles (pH < 1) (reviewed by Amsler and Fairhead[54]). Pelletreau and Muller-Parker[64] investigated the role of sulfuric acid in *Desmerestia munda* as a defense against herbivory. They found that *D. munda* was a low preference food for the sea urchin *Strongylocentrotus droebachiensis* and that sulfuric acid concentrations added to artificial diets at or below 3.5 pH units inhibited feeding by the sea urchin. A recent survey of 61 species of algae by pH measurements and ion chromatography[65] identified three species within the Desmarestiales and seven species in the Dictyotales that contained high sulfuric acid concentrations. Additionally, some dictyotalean species were found that contain high concentrations of SO_4^{2-} balanced by high concentrations of Mg^{2+}; these were not acidic.[65]

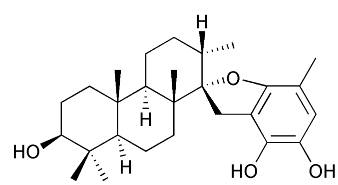
Several brown algal terpenoid compounds function as chemical defenses against herbivores. The crude extract and major diterpene **21** from *Dictyota pfaffii* deterred feeding by the sea urchin *Lytechinus variegatus* and generalist herbivore fishes in field assays, but did not influence feeding by the crab *Pachygrapsus transversus.*[66,67] The brown alga *Stypopodium zonale* produces diterpenes of mixed biogenesis (meroditerpenes) that vary qualitatively and quantitatively depending on where the samples were collected.[68] In an investigation of variation in this alga collected from areas of the Brazilian coast, Soares *et al.*[68] found two distinct chemotypes. Collections from more northern locations contained stypoldione **22** (air-oxidation product of stypotriol **23**) as the major metabolite, and collections from southern locations contained atomaric acid **24** as the major metabolite. Studies of chemical defense by extracts and major metabolites from these two chemotypes showed that



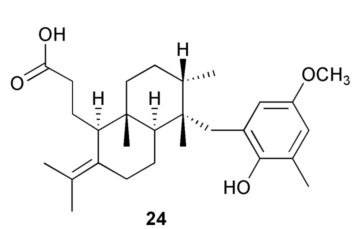
21



22



23



24

extracts from both locations inhibited feeding by the sea urchin *Lytechinus variegatus* and the crab *Pachygrapsus transversus*, but the crude extract containing atomaric acid **24** deterred feeding more than the extract containing stypoldione.[69] Tests with the pure compounds confirmed these results; atomaric acid was a more potent feeding deterrent than stypoldione **22** even though it was present in *S. zonale* at lower concentrations. The deterrent effect of the natural product stypotriol **23** was not tested and compared in these assays.

In an excellent study of geographic and genetic variation in feeding preference of the amphipod *Ampithoe longimana* for species of *Dictyota*, Sotka *et al.*[70] studied eight populations of amphipods from North Carolina to Maine. The authors assayed the feeding preferences of the amphipod for two species of *Dictyota* and two species of red algae (*Hypnea musciformis* and *Gracilaria tikvahiae*), tested the genetic potential for evolution of feeding preferences among full-sib families of one population, and determined the fitness consequences of these feeding preferences. Mitochondrial cytochrome oxidase I (COI) and nuclear ITS-1 genes showed a strong historical separation between northern populations of *A. longimana* in Connecticut and Rhode Island and the other populations further south. Feeding assays showed that amphipod populations sympatric with *Dictyota* had a higher preference for *Dictyota* and lower preference for *Hypnea* relative to other populations. A previous study had demonstrated that a North Carolina population of *A. longimana* sympatric with *Dictyota menstrualis* was more tolerant of *Dictyota* diterpenes than a Connecticut population from outside of the geographic range of *Dictyota* spp.[71] An experiment with full-sib families of amphipods raised in the laboratory on *Enteromorpha* sp. detected significant heritable variation in preferences for *Dictyota.*[72] To determine fitness consequences, juveniles from seven populations were raised for 30 days on *D. menstrualis*, *D. ciliolata* and *Enteromorpha* sp. Reproduction of females from northern populations that did not co-occur with *Dictyota* was lower on the *Dictyota* diet relative to the *Enteromorpha* diet, but this was not the case for the sympatric populations.[70] This is one of very few studies of marine plant–herbivore interactions to investigate the genetic basis for feeding preferences of consumers, although a recent review suggests that other marine invertebrates may also display local adaptation in host use.[72]

Chemical variation in levels of brown algal phlorotannins has been the subject of numerous studies in the past two years. The ease of quantifying this class of compounds by colorimetric assays such as the Folin–Denis or Folin–Ciocalteu assay probably explains why there are so many studies on phlorotannins compared to other macroalgal natural products. Patterns of variation have been explored among different species of brown algae, within species collected at different locations, in different thallus parts, and over different seasons.[63,73–77] In many of these studies, no accompanying measurements of herbivory or feeding deterrence were made. Other researchers examined the palatability of brown algae to herbivores but did not relate that to changes in phlorotannins or other secondary metabolites. For example, several investigations of inducible defenses in brown algae looked at changes in palatability in grazed *versus* ungrazed algae to different consumers.[78,79] One study examined genetic variation for growth and phlorotannin concentrations under different environmental conditions for the brown alga *Fucus vesiculosus.*[80] Growth and phlorotannin concentrations showed both genetic variation and phenotypic plasticity. Heritability for phlorotannin concentration was high and did not differ significantly between environments.[80]

Connan *et al.*[81] surveyed polyphenolic content in eight dominant species of brown algae along the northern coast of Brittany (France) monthly over a 14 month period. Phenolic content varied among species with *Ascophyllum nodosum* and *Fucus vesiculosus* showing highest levels (>5% dry mass). Not unexpectedly, all Fucalean algae showed higher levels than *Laminaria digitata* (<0.5% dry mass), the only member of the Laminariales studied. Seasonal patterns in polyphenolic content were observed, with a summer maximum for the Fucales and a winter maximum for *Laminaria digitata* (although levels were very low for *L. digitata* overall). Stiger *et al.*[74] examined phenolic content in *Turbinaria ornata* and *Sargassum mangarevense*, which are abundant on reef flats around Tahiti. Overall, polyphenolic content in these algae was low, but *T. ornata* had higher levels (approximately 1.2% dry mass) than *S. mangarevense*, and levels were slightly higher in summer than in winter. There was considerable site to site variation in polyphenolic concentrations. Juveniles and recruits had lower concentrations of phenolics than mature thalli.[74]

Several studies examined the effects of solar radiation or UV radiation on phlorotannin concentrations in macroalgae. The effects of UV radiation on phlorotannins and growth in *Fucus gardneri* embryos and juveniles were examined by growing these life history stages of the alga under different UV radiation filter treatments for three weeks.[76] UV-B treatments inhibited and UV-A treatments enhanced the growth of embryos but had no effect on *F. gardneri* juveniles. Phlorotannin concentrations were not influenced by the various UV treatments in either embryos or juveniles. The authors suggested that embryos are negatively affected by UV light but that *F. gardneri* develops a tolerance to UV as the alga matures. Phlorotannin concentrations were not induced by UV light over the time course of the study.[76] A study of the effects of solar radiation on annual and diurnal variation in photosynthetic activity and polyphenolic content of the brown alga *Cystoseira tamariscifolia* indicated that photosynthetic activity and phlorotannin concentrations were very dynamic in this alga.[75] Optimum quantum yield was determined with a portable pulse amplitude modulated (PAM) fluorometer, and this was measured monthly during one year as well as every two hours during the course of one day. There was seasonal variation in phlorotannin concentrations, with highest levels in the summer months. Optimum quantum yield decreased as irradiance increased at noon, possibly indicating photoinhibition. A negative correlation between phlorotannin concentration and irradiance in the summer months was observed, but no relationship was observed in the fall–winter months. Phenolic compounds were exuded from apical portions of the thalli at high irradiances, suggesting that short-term variation in phenolic compounds is controlled by exudation under high irradiances.[75]

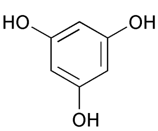
Desiccation stress and herbivory levels on the brown alga *Fucus gardneri* were manipulated in field and outdoor mesocosm experiments during spring and summer months to determine their effects individually and in combination.[82] Field studies used screen awnings to shade the intertidal, which reduced temperatures on sunny days and generally increased relative humidity. Herbivory was manipulated with limpet removal. *F. gardneri* kept under awnings showed enhanced rates of net photosynthesis and survival and grew larger than algae in control areas without awnings.

Phlorotannin concentrations were higher in thalli kept under awnings, but there was no effect of limpet removal. Loss of tissue, increased reproductive output, and increased stress all correlated with lower phlorotannin levels.[82] Similar manipulations of desiccation and herbivory by the isopod *Idotea wosnesenskii* were conducted in mesocosm experiments. The amount of algal tissue lost and growth rates in this experiment were related to levels of herbivory; there was no effect of stress. Concentrations of phlorotannins were again lower in stressed thalli and were not affected by herbivory.[82] In these experiments stress and herbivory seemed to act independently rather than interactively to influence plant growth and chemical defense parameters.

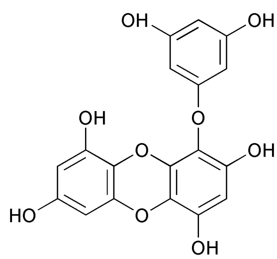
Koivikko *et al.*[83] developed methods to separately examine cell-wall bound phlorotannins and soluble phlorotannins in *Fucus vesiculosus.* They used alkaline degradation of cell-wall bound phlorotannins to release the phenolics for subsequent analysis by the Folin–Ciocalteu assay. Concentrations of cell-wall bound phlorotannins (<1% dry mass) were much lower than soluble phlorotannins (approximately 8% dry mass) in *F. vesiculosus.* Concentrations of both were lower in growing tissue than in non-growing tissue. Nutrient enrichment experiments reduced the concentration of soluble phlorotannins. Phlorotannins were actively exuded into seawater under all nutrient treatments, and exudation rates more than doubled when the alga was grazed by the isopod *Idotea baltica.*[83] Herbivory did not induce increased phlorotannin concentrations in the alga, suggesting that increased exudation was related to compound release rather than compound biosynthesis.

Shibata *et al.*[84] conducted a study examining the localization of phlorotannins in Japanese brown algae (*Eisenia bicyclis*, *Ecklonia cava*, and *E. kurome*). Vanillin–HCl staining and light microscopy were used to observe phlorotannins accumulated in vegetative cells of the outer cortical layer of the thalli. The authors also identified some of the phlorotannin components of the algae by thin layer chromatography and HPLC based on comparisons to standards obtained from prior studies.[85,86] Three species of brown algae contained similar types and amounts of phlorotannins including phloroglucinol **25**, eckol **26**, dieckol **27**, phlorofucofuroeckol A **28**, and 8,8′-bieckol **29**. *E. bicyclis* also had larger amounts of an unidentified tetramer (MW 478). There were no clear seasonal patterns in concentrations of phlorotannins among samples. A prior study had shown that phlorotannin mixtures as well as the polymers dieckol **27**, phlorofucofuroeckol A **28**, and 8,8′-bieckol **29** strongly inhibited digestive enzymes (glycosidases) partially purified from the viscera of the herbivorous turban snail *Turbo cornutus.*[85]

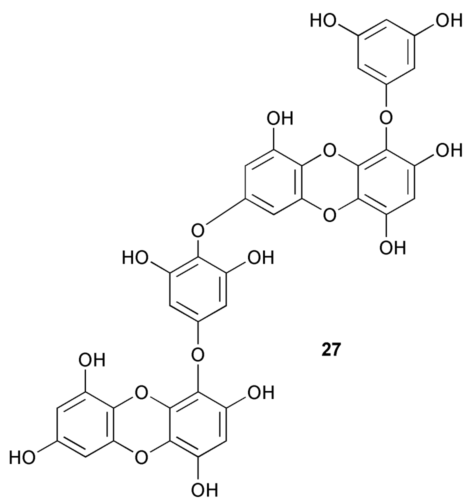
Lüder and Clayton[87] microscopically examined the cellular changes during wound healing after *Ecklonia radiata* was subjected to simulated herbivory. In *E. radiata*, physodes containing polyphenolics are located predominately in the outer epidermal cell layer. The alga was mechanically wounded with a cork borer that created a hole 3 mm in diameter in the center of a piece of a blade. The healing response, including structural changes and distribution of polyphenolics, was examined up to 9 days after wounding. Immediately after wounding new medullary cells formed around the wound site, and within three days physodes containing phlorotannins accumulated around the wound site localized to medullary cells. The authors propose that polyphenolics are needed for wound sealing and wound healing following damage and might also be important in preventing both microbial and herbivore attack on the area of the wound.[87]



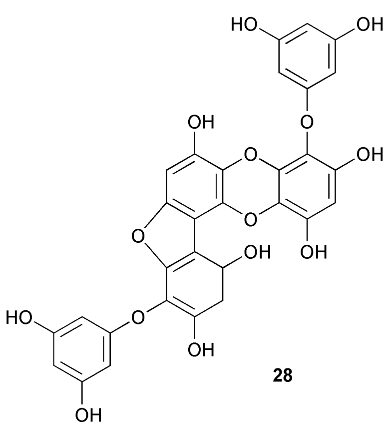
25



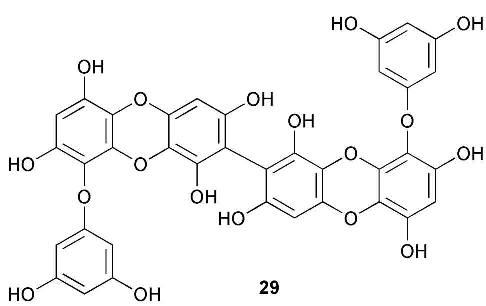
26



27



28



29

Induced defenses in both terrestrial and marine plants can influence the feeding behavior of herbivores. Changes in plant chemistry and palatability can cause herbivores to be more mobile, and thus, damage from grazing would be more dispersed on plants. Borell *et al.*[88] tested these predictions, which they termed the herbivore mobility model, with the brown alga *Ascophyllum nodosum* and the herbivorous snail *Littorina obtusata. A. nodosum* plants of similar size were subjected to three treatments (grazing by *L. obtusata*, simulated grazing with a file, and control) for two weeks. *L. obtusata* individuals were then assayed for their mobility on algae from the three treatments, and dispersal of feeding damage and amount of grazing on algae were also assessed. Snails feeding on algae subjected to prior herbivory moved more than snails on algae subjected to simulated herbivory or control algae. Algae exposed to prior herbivory had less tissue consumed by snails in the feeding assays. Phlorotannin concentrations were highest in previously grazed plants and did not increase in algae in the simulated grazing treatment relative to controls; however, phlorotannins were not tested directly for their deterrent effects on *L. obtusata.* The herbivore mobility model's predictions were supported in this study.[88]

In another study of *Ascophyllum nodosum* and *Littorina obtusata*, Toth *et al.*[89] examined whether inducible defenses (assumed to be phlorotannins) in *A. nodosum* would differ between thallus parts (basal stipes and annual shoots) with different fitness values. Phlorotannin content is highly variable in this alga;[73] basal portions have the highest fitness value and phlorotannin concentration compared to annual shoots and reproductive tissues.[90] Phlorotannin concentrations increased after grazing by *L. obtusata*, and the effect was five times larger in basal stipes than in apical shoots. While juvenile snails grew well on all portions of the alga, whether grazed or ungrazed, adult females produced fewer viable eggs when fed basal compared to apical shoots. Also, the number of non-viable eggs increased when snails fed on previously grazed tissues.[89] The study shows that induced resistance and its variation among algal parts fits the predictions of optimal defense theory and can negatively influence herbivore fitness.

Polyphenolics have been shown to be inducible defenses in the brown algae,[78,89] but not in all cases. Jormalainen *et al.*[91] tested the effects of nutrient enrichment and feeding by two snails on phlorotannin production by *Fucus vesiculosus.* Nutrient enrichment alone increased fouling and decreased phlorotannin levels. Presence of the snail *Theodoxus fluviatilis* induced phlorotannin production; however, this snail does not graze *F. vesiculosus.* The researchers suggested that rather than interpreting this as an induced response, it might instead be the result of altered nutrient uptake because the snails graze hyaline hairs off the surface of the alga, and hyaline hairs are involved in nutrient acquisition.[91] Hemmi *et al.*[77] found no influence of nutrients or herbivory by the herbivorous isopod *Idotea baltica* on phlorotannin levels in *F. vesiculosus* in the northern Baltic Sea. In another study with *F. vesiculosus*, parts of thalli were subjected to simulated grazing (artificial clipping) and enhanced nutrients.[92] Two, ten and thirty eight days after simulated grazing the algal parts were offered to the isopod *I. baltica* to determine preference between clipped and unclipped portions of the thalli. Within two days after clipping an induced resistance to the isopod was observed in both fertilized and unfertilized algae that disappeared after 10–38 days; however, this induced resistance did not relate to increased concentrations of phlorotannins. Nutrient enrichment negatively affected phlorotannin concentrations. The authors concluded that *I. baltica* was not deterred by phlorotannins; but there might be some rapidly induced compounds in *F. vesiculosus* that function as feeding deterrents.[92]

Care must be taken in interpreting the results of the many studies that show a correlation between phlorotannin concentrations and bioactivity in brown algae. Several studies have now shown that compounds other than phlorotannins in brown algae can be responsible for feeding deterrence and other bioactivity.[92–94] In studies of *Fucus vesiculosus*, a polar galactolipid as well as uncharacterized water-soluble compounds were shown to be responsible for deterring consumers.[93,94] Through bioassay-guided fractionation Kubanek *et al.*[94] tried to isolate herbivore deterrent compounds from the aqueous extracts of *F. vesiculosus*, but activity was lost during the isolation process. There was no relationship between phlorotannin decomposition and loss of feeding deterrence. Semi-purified mixtures of phlorotannins did not deter three different herbivores at 3 or 6 times the isolated concentration, but the amphipod *Ampithoe valida* was deterred at 12 times the isolated concentration. Furthermore, juvenile *A. valida* raised for 64 days on artificial diets containing phlorotannins demonstrated enhanced survivorship and growth relative to diets without phlorotannins and ovulated only on the phlorotannin-rich diet. The authors suggest that methods other than measurements of total phlorotannins are needed for understanding chemical defenses of brown algae, because polar, unstable compounds other than phlorotannins may confound interpretation of chemical defenses in this group of algae.[94]

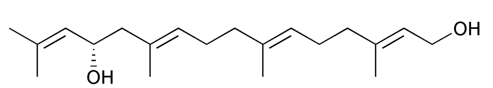
Hemmi and Jormalainen[95] compared geographic variation in nutritional quality (phlorotannin, nitrogen, protein, and sugars) in *Fucus vesiculosus* and compared this with among-population variation in size and fecundity of the herbivorous isopod *Idotea baltica.* Collections of the alga and isopods were made at 12 sites that were 0.5–40 kilometres apart. Algal populations differed in concentrations of phlorotannins, nitrogen, protein and sugars (fucose, mannitol, and melibiose); number of eggs, total egg mass, and egg size of *I. baltica* also differed among collection sites. Size of females positively covaried with phlorotannin concentration in *F. vesiculosus*, and egg size positively covaried with mannitol concentrations. While host plant chemistry was related to variation in herbivore size and reproduction, phlorotannins had a positive rather than negative relationship with herbivore fitness.[95]

Fucus evanescens and *F. vesiculosus* were found to be differentially fouled in the field, and aqueous methanol extracts from both species decreased settlement of barnacle cyprid larvae (*Balanus improvisus*) in the laboratory.[96] When treated with polyvinylpolypyrrolidone, which binds and removes phlorotannins, the *F. vesiculosus* extract lost its activity, but the *F. evanescens* extract continued to inhibit settlement, suggesting compounds other than phlorotannins were active. *Fucus evanescens* also inhibited post settlement survival of barnacles when compared to *F. vesiculosus.*[96] While *Fucus* phlorotannins may have the potential to inhibit barnacle settlement in laboratory assays, this did not explain the lower barnacle settlement on *F. evanescens* observed in the field. Another study of fouling in the brown alga *F. vesiculosus* examined genetic variation in tolerance and resistance to fouling organisms.[97] Thirty algal genotypes grown in the field showed highly variable levels of fouling after 44 days.

Phlorotannin content and biomass of fouling organisms were not correlated. In an aquarium experiment where snails were used to control fouling, algae grew less and phlorotannin content was lower in the fouling (no snails) than in the non-fouling treatment;[97] however, the possible effects of snail grazing on these factors made these results difficult to interpret. Phlorotannins did not seem to explain antifouling activity of brown algae in either of these studies.

Inducible defenses have been observed for other brown algae that are probably not related to concentrations of phlorotannins. A study of the effects of amphipod (*Parhyalella ruffoi*) grazing and UV radiation on two Chilean brown algae, *Glossophora kunthii* and *Macrocystis integrifolia*, demonstrated evidence of inducible defenses in *G. kunthii* but not *M. integrifolia* and no effect of UV radiation on palatability of these two algae.[79] Experiments included acclimation for 12 days without grazers, and non-polar extracts of *G. kunthii* increased in palatability during that time. Addition of grazers for 12 days caused a decrease in palatability of non-polar extracts relative to controls, and then these induced effects disappeared after another 12 days without grazers. Treatments that included the presence of grazers without direct grazing on the alga also induced chemical defense in *G. kunthii*, suggesting a waterborne cue served as a signal for induced defenses. Defenses of different parts of the thalli were compared and apical and basal portions of *G. kunthii* were chemically defended against the amphipod herbivory. Herbivores also avoided the stipes of *M. integrifolia* but this preference was not chemically mediated.[79] A study of inducible defenses in two brown and one red alga from the Kenyan coast also found evidence of induced chemical defenses in response to grazing by the amphipod *Cymadusa filosa.* Nonpolar extracts of grazed *Sargassum asperifolium* and *Cystoseira myrica* were less preferred than those from ungrazed algae in artificial diets.[98] Nonpolar extracts of *Lobophora variegata* from Brazil also suggested an induced chemical defense in response to amphipod grazing.[99]

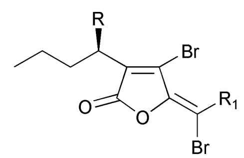
The brown alga *Bifurcaria bifurcata* was examined for seasonal variation of extracts as inhibitors of two marine bacteria and barnacle (*Balanus amphitrite*) settlement.[100] Similar to other algae[57] a seasonal effect of the extracts against all three organisms was observed with the most potent activities during April–June. This increased activity corresponded to the seasonal maximum concentration of the major diterpene eleganediol **30** found in the crude extracts,[101] but individual compounds were not tested for their antifouling activity.[100]



30

Compared to studies with brown algae, and particularly on phlorotannins, only a few recent studies have explored chemical defenses in red and green macroalgae. One red alga (*Hypnea pannosa*) from Kenya was examined for inducible defenses. In tests with live algae, grazed *H. pannosa* was less preferred by the amphipod than ungrazed controls, but this same result was not observed with nonpolar extracts of grazed and control algae.[98] Another study of inducible defenses of four red, four brown, and one green algae from Brazil demonstrated that the live red alga *Pterocladiella capillacea* was less preferred after it was grazed by amphipods than non-grazed controls.[99] Whether these two red algae were defended by an induced chemical defense is not clear, but changes in palatability were observed.[98,99]

Lack of information about heritability of algal secondary metabolites is a gap in our understanding of the variability and evolution of these compounds. The red alga *Delisea pulchra* produces halogenated furanones that vary in concentration spatially and temporally. Wright *et al.*[102] determined the heritability of the four major furanones **31–34** and then determined the effects of different concentrations on feeding by herbivores. Total furanone concentration and the major furanone **33** showed significant genetic variation; however, all four furanones were genetically correlated. All six common herbivores used in feeding assays, except for the gastropod *Phasianotrochus eximius*, consumed *D. pulchra* at lower rates than other macroalgae. The herbivores were usually deterred by extracts and furanone **33** at concentrations that spanned the range of concentrations found in the field, but were occasionally not deterred at the lower end of the concentration range.[102] The authors concluded that herbivores could provide an adequate selective force to act upon this heritability.

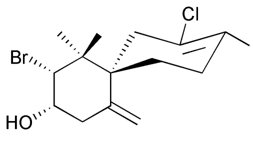


31 R = H; R_1 = Br
32 R = H; R_1 = H
33 R = OAc; R_1 = H
34 R = OH; R_1 = H

The effects of varying levels of grazing by the sea urchin *Heliocidaris erythrogramma* on macroalgal community structure on a temperate Australian reef was studied, and Wright *et al.*[103] found that the chemically defended *Delisea pulchra* had relatively high survivorship when grazed by sea urchins. Grazing by high densities of sea urchins caused the algal community to switch from a mix of foliose algae before grazing, through an intermediate stage where grazer-resistant algae such as *D. pulchra* coexisted with crustose algae, to a community consisting almost entirely of crustose algae.[103]

Host use by the sea urchin *Holopneustes purpurascens* was shown to be chemically mediated and changed from the red alga *Delisea pulchra* to the brown alga *Ecklonia radiata* with ontogeny of the sea urchin. Sea urchin larvae settled and metamorphosed preferentially on *D. pulchra* because of its relatively high concentrations of histamine, which is a settlement cue for larvae of this sea urchin.[104,105] However, the major halogenated furanone **33** in *D. pulchra* deterred feeding by *H. purpurascens*, and juvenile and adult urchins grew better, were more fecund, and had higher survival on *E. radiata* than on *D. pulchra.* Larger sea urchins were found on *E. radiata*, and urchins moved from *D. pulchra* to *E. radiata* habitats at night when predation risk was low from diurnal predators such as fish. Settlement cues and chemical defenses co-occurred in *D. pulchra*, and both played a role in host use by the sea urchin.[104]

The Brazilian red alga *Laurencia obtusa* produces elatol **35** as its major metabolite,[106] a compound previously shown to deter herbivores (reviewed by Paul *et al.*[107]). In this study, both crude algal extracts and elatol at natural concentrations inhibited feeding by two herbivores, the crab *Pachygrapsus transversus* and the



35

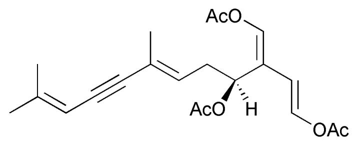
sea urchin *Lytechinus variegatus.*[106] In addition, a crude extract of *L. obtusa* significantly reduced fouling relative to controls after five weeks when incorporated into phytagel™ and placed in the field.[106] Crude extracts also inhibited settlement by mussel larvae (*Perna perna*) in laboratory assays and overall fouling cover in field assays relative to controls or extracts of the brown alga *Stypopodium zonale.*[108] Elatol **35** was found in extracts of the surface of *L. obtusa* dipped in hexane, but it was never tested independently for its antifouling potential.

Several other studies examined antifouling activity in red algae. The red alga *Dilsea carnosa* was observed to have very low fouling cover in the field.[109] However, phytagel™ treated with crude extract was readily fouled in the field. Further field trials of live algae showed that *D. carnosa* is capable of sloughing its outer tissue layer, a mechanical not chemical antifouling strategy.[109] Isethionic acid (2-hydroxyethane sulfonic acid) and floridoside (2-*O*-α-D-galactopyranosylglycerol) were isolated from the invasive red alga *Grateloupia turuturu* collected in France.[110] Isolated compounds were tested individually in the laboratory; isethionic acid and floridoside reduced settlement of *Balanus amphitrite* larvae at concentrations as low as 0.001 mg ml^{-1}, and together the compounds reduced settlement at 0.002 mg ml^{-1}. Isethionic acid was toxic to *B. amphitrite* nauplii while floridoside was not, suggesting that floridoside acts as a chemical cue to reduce barnacle settlement at concentrations that are non-toxic.

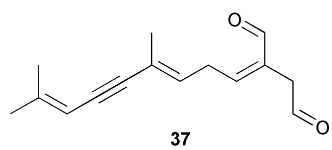
In a comparison of five common red algae from the Swedish west coast for their ability to inhibit bacterial growth and attachment, the red alga *Bonnemaisonia hamifera* was found to be the most active. Extracts of *B. hamifera* inhibited the growth of nine of the eleven bacteria tested.[111] Surface extracts of *B. hamifera* inhibited the growth of three marine bacteria, indicating that the alga had sufficient levels of metabolites on its surface to reduce bacterial growth. Bacterial abundances on field collected algae were much lower on *B. hamifera* than on another red alga *Chondrus crispus.*[111]

Primary and secondary metabolites were measured to determine the cellular processes involved in the colonization of the red alga *Chondrus crispus* by the green algal endophyte *Acrochaete operculata.*[112] Different life stages of *C. crispus* differed in their susceptibility to *A. operculata*; tetrasporophytes were more susceptible than gametophytes, and susceptibility was mediated by different types of carrageenans in the cell wall matrix of *C. crispus.*[112] Contact between *C. crispus* gametophytes and *A. operculata* caused release of L-asparagine by *A. operculata*, which *C. crispus* breaks down to release hydrogen peroxide, ammonium ions, and a carbonyl compound (succinamic acid). Exposure to L-asparagine decreased fouling on *C. crispus* by associated bacteria and *A. operculata* spores.[112] Additionally, H_2O_2 was more toxic to *A. operculata* than to *C. crispus.*

Many recent studies of the chemical ecology of green algae have focused on species of *Caulerpa.* There has been considerable interest in natural products chemistry and chemical defenses in these green algae because many species are highly invasive.[113–115] Studies of the invasive *Caulerpa taxifolia* in the Mediterranean examined the biosynthesis of the major metabolite caulerpenyne **36**[116] and its role in wound healing in the alga.[117] Transformation of caulerpenyne by an esterase to oxytoxin 2 **37** occurs rapidly after injury,[118] and the resulting 1,4-dialdehyde is highly reactive and cannot even be detected four minutes later.[117] The decay kinetics of oxytoxin 2 **37** matched those of the formation of the external wound plug of *C. taxifolia.*[117] Adolph *et al.* conducted experiments that suggested that proteins cross-linking with oxytoxins and similar reactive aldehydes in *C. taxifolia* are essential for wound-plug formation.[117] This suggests an additional function for caulerpenyne in addition to its antimicrobial and feeding deterrent activities.[107] Wound plug formation has been studied for another green alga *Dasycladus vermicularis*,[119] but the role of secondary metabolites has not been demonstrated.



36



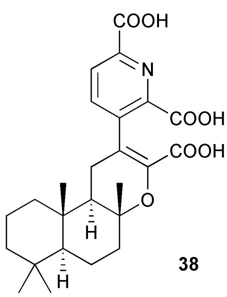
37

Extracts of *Caulerpa prolifera* collected from shallow habitats in Greece were tested for antibacterial and antialgal activities.[120] Extracts contained caulerpenyne esters and exhibited antimicrobial activity toward three of six marine isolates and antialgal activity toward *Phaedactylum tricornutum.*[120] Responses of native herbivores to three species of *Caulerpa* that have recently appeared in southeast Australia were studied by Davis *et al.*[115] The snail *Turbo undulatus* showed the lowest preference for *C. filiformis* of five live algae offered in choice and no-choice assays. Ethanol extracts of *C. filiformis*, *C. taxifolia*, and *C. scalpelliformis* were tested for their effects on feeding by natural assemblages of fish, a sea urchin and molluscs. While few significant results were obtained, some extracts tended to reduce feeding relative to controls. Unfortunately, in this study[115] and the study of *C. prolifera* from Greece,[120] algae were frozen after collection, and green algal metabolites including caulerpenyne **36** decompose quickly under these handling conditions.[121] Good methods of extracting these compounds after shock freezing algae in liquid nitrogen have been developed,[122] and these should be carefully followed by chemists and chemical ecologists working with species of *Caulerpa* and other green algae.

Some green algal water soluble metabolites are potential antifouling compounds; for example, extracts from the green alga *Ulva reticulata* and an epibiont bacterium *Vibrio* sp. inhibited attachment of the polychaete *Hydroides elegans* and the bryozoan *Bugula neritina.*[8] Each polar extract was deactivated with different enzymes and was composed of different carbohydrate monomers, suggesting that the *Vibrio* sp. inhibitory compound(s) were different from *U. reticulata* compound(s).[8] Allelopathic effects of other species of bloom-forming *Ulva* have also been observed. *Ulva pertusa* cultured together with the HAB-forming microalgae *Heterosigma akasiwo* and *Alexandrium tamarense* strongly inhibited the growth of the microalgae.[123] A similar study with *U. pertusa* and *U. linza* co-cultured with the microalga *Prorocentrum micans* also indicated the presence of allelopathic compounds released by *Ulva.* Aqueous and methanol extracts of the *Ulva* spp. strongly inhibited growth of *P. micans.*[124] Aqueous extracts of *Ulva fenestrata* and *Ulvaria obscura* inhibited zygote development of *Fucus gardneri*, growth of epiphytic algae, and development of oyster larvae, also indicating the allelopathic activities of these algae.[125]

Biofilms are a key feature of marine systems that some algal spores use as cues for recruitment into appropriate habitats. Biofilms are implicated in *Enteromorpha* settlement, and a recent study shows that this green alga settles in response to a variety of bacterial strains isolated from natural habitats.[126] Quite a few genetically distinct bacteria stimulated settlement, especially when allowed to develop for 48 h, but not all species had a positive effect; five out of 14 strains of *Pseudoalteromonas* inhibited spore settlement. A possible explanation for this variable settlement response is that *Enteromorpha* spores were found to settle in response to *N*-acylhomoserine lactone (AHL), a bacterial growth promoter.[127] AHL is a compound known for its role in quorum sensing in biofilm bacteria, and by creating mutant bacterial strains the authors were able to show that settlement of *Enteromorpha* only increased in response to the bacterial strains that could produce AHL. Tests with synthetic AHLs showed that settlement activity was lost when the lactone ring structure of AHL was opened.[127]

Our understanding of marine microbe biofilms and diversity has grown in recent years and is revealing complex chemical interactions between microbes and many marine organisms. For instance, microbial compounds not only influence settlement for some marine algae but also mediate the differentiation of cells into a thallus in the green alga *Monostroma oxyspermum.*[128] In the field, *M. oxyspermum* has a leafy morphology similar to *Ulva* spp. but when *M. oxyspermum* was raised in aseptic conditions it grew as unorganized bunches of cells. Thallusin **38**, isolated from an epiphytic marine bacterium, induced differentiation of the algal cells to form a thallus. Indeed when the *M. oxyspermum* was deprived of thallusin the algal thallus began to disintegrate and the alga reverted back to its unorganized state. Thallusin **38** also caused differentiation in other green algae such as *Ulva pertusa* and *Enteromorpha intestinalis.*[128]



38

4 Seagrasses

There are few studies of chemical defenses of seagrasses against herbivores compared to other benthic marine plants. In contrast, plant–pathogen interactions have been addressed more often in seagrasses than algae. This is due to major die-offs of near shore seagrass beds caused by disease. Antimicrobial activities observed in seagrass extracts are attributed to the presence of phenolic compounds.[129] A study of *Thallasia testudinum* demonstrated that the seagrass produces an antibiotic flavone glycoside, luteolin 7-*O*-β-D-glucopyranosyl-2″-sulfate, which inhibits the growth of *Schizochytrium aggregatum* at 1/10th of the natural concentration.[130] More recently, phenolic acids were reported to accumulate above necrotic lesions on blades of *T. testudinum* infected with *Labyrinthula* sp.[131] However, the 'pseudo-induction' of phenolics did not appear to increase resistance of the seagrass to infection. The authors proposed that the increased biosynthesis of phenolics is a response to the excess carbon available in plant tissues because the infection site disrupts plant resource allocation. In a broader study of the antimicrobial activities of the methanol extracts from three cultured and field collected estuarine seagrasses, *Potamogeton pectinatus*, *P. perfoliatus*, and *Ruppia maritima*, crude extracts were reported to inhibit the growth of all 12 strains of gram-positive and three of 12 gram-negative bacteria included in the survey.[132] Interestingly, antimicrobial activity was found in the crude extracts from the natural populations collected in the spring but absent in the extracts from plants collected in the fall. This pattern correlated with new growth in the seagrass shoots and higher nutrient levels in the surrounding waters.

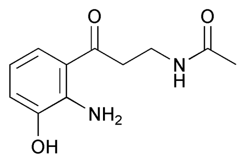
5 Sponges

Sponges (phylum Porifera) continue to be a source of novel and interesting natural products.[133–135] Our knowledge of the ecological roles of these compounds continues to grow as new techniques are developed to understand the complex relationships between sponges and their predators, competitors, microorganisms, and associated invertebrates.[136–138]

Many Antarctic sponges are chemically defended against a range of potential predators,[138] and sponges in northern high latitudes also have chemical defenses. In a series of feeding deterrence assays of crude extracts from 17 benthic organisms from a sub-Arctic fjord, only the sponge *Haliclona viscosa* and the cnidarian *Hormathia nodosa* deterred amphipod feeding.[139] *Haliclona viscosa* also deterred feeding by the starfish *Asterias rubens*, but deterrent compounds were not isolated and characterized.

In tropical regions some sponges are chemically defended while others are not. Walters and Pawlik[140] artificially wounded some Caribbean sponges, and found that those not chemically defended healed at significantly faster rates than the chemically defended species. In addition, morphology was important since tubular shaped individuals healed faster than vasiform shaped individuals of *Niphates digitalis.* The authors proposed that Caribbean reef sponges have adapted to overcome fish predation by investing in the production of chemical defenses or by a rapid wound-healing response after injury.

There are several examples in which one species of sponge produces chemical defenses against multiple predators, competitors, or microorganisms. Compounds from the Antarctic sponge *Isodictya erinacea* deterred the sea star *Perkinaster fuscus*[141] and prevented the molting of the sponge boring amphipod *Orchomene plebs.*[142] Laboratory experiments demonstrated that amphipods fed a continuous diet of erebsinone **39**, isolated from *I. erinacea*, exhibited high levels of premature mortality when they were

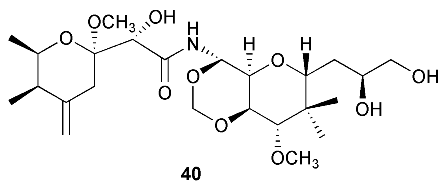


39

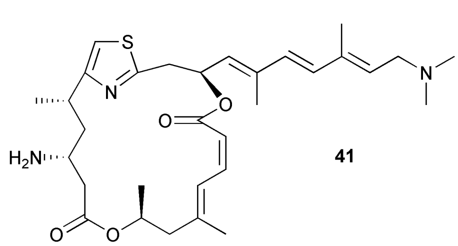
unable to molt.[142] A more recent study of the Brazilian sponge *Geodia corticostylifera* showed that crude extracts are deterrent to generalist fishes, prevent fouling by the mussel *Perna perna*, and act as a chemical cue for colonization by the ophioroid *Ophiactis savignyi.*[136] Unique laboratory experiments conducted in flow-through tanks with sponge mimics constructed of sponge skeleton and coated with phytagel™ alone or containing the crude extract demonstrated that the ophioroid *Ophiactis savignyi* chemically recognized and colonized the mimics containing the sponge extracts.[136]

In past studies, spicules have rarely been shown to be an important structural defense in sponges and are thought, instead, to be important to the structural integrity of the animal. Two recent studies of temperate and subtropical sponges show that sponge spicules can deter fish and invertebrate predators when offered alone or in combination with crude extracts.[137,143] Artificial foods containing spicules from the sponges *Cliona celata* and *Halichondria bowerbanki* and a combination of crude extracts and spicules from the sponges *Microconia prolifera* and *Halichondria bowerbanki* collected from the Long Island Sound were unpalatable to the hermit crab *Pagurus longicarpus.*[143] For *M. prolifera*, the hermit crab was significantly more deterred by the combination of crude extracts and spicules than either offered alone. Their results demonstrate that there is an interaction between the chemical and structural defenses of *M. prolifera* that enhance the feeding deterrence properties of the sponge against *P. longicarpus.* Synergistic interactions between the chemical and structural defenses against the wrasse *Thalassoma bifasciatum* were recently reported for four of eight sponge species surveyed from the Florida Keys and the Bahamas.[137] Synergy was observed in artificial foods with natural concentrations of crude extracts and spicules from *Cinachyrella alloclada*, *Clathria virgultosa*, and *Xestospongia muta*, whereas the combination was only synergistic for *Agelas clathrodes* when the concentration of crude extract was decreased by 3-fold and the spicule concentration was increased by 8-fold of natural concentrations. In addition, the spicules from three other sponges effectively deterred feeding by *T. bifasciatus.* The results of these studies suggest that spicules from some sponges are important structural defenses against different predators.

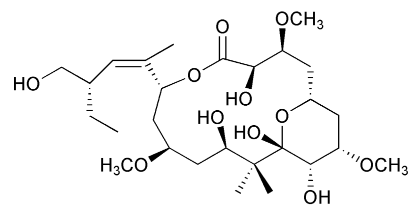
There continue to be few studies of seasonal and temporal variation in the production of chemical defenses by sponges. Two recent studies of the intraspecific variation of secondary metabolites that did not directly address ecological questions could have important ecological implications. In the first study, concentrations of the cytotoxic metabolites mycalamide A **40**, pateamine **41**, and peluroside A **42** were shown to vary both spatially and temporally in individuals of the New Zealand sponge *Mycale hentschelli* from Pelorus Sound and Kapiti Island.[144] Pelorus Sound, a semi-estuarine sheltered area that receives freshwater runoff, and Kabiti Island, a high-energy coastal environment, represent two extremes in marine habitats that are proposed to account for both morphological and chemical differences observed for *M. hentschelli.* Concentrations of mycalamide A **40** were greater in sponges found on the deeper reefs of Kapiti Island, whereas **41** was only present in individuals from Pelorus Sound. Concentrations of **42** varied significantly among individuals at Pelorus Island, but peluroside A **42** was rarely present in samples from Kapiti Island. In addition, metabolite concentrations increased in the spring as the water temperature increased, but declined before peak temperatures in the summer.[144] In a second study, Martí *et al.*[145] demonstrate that the Microtox® assay can be a useful tool for quickly assessing intraspecific variability of avarol **43** and palinurin **44** concentrations in *Dysidea avara* and *Iricinia variabilis* populations, respectively.



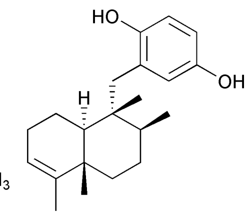
40



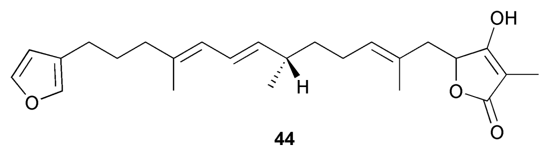
41



42

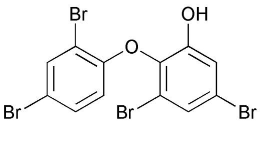


43

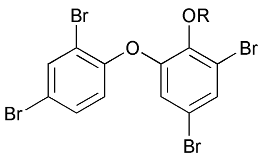


44

The vast and complex nature of metabolites isolated from sponges has raised the question of where the production of secondary metabolites is taking place. Because of the similarity in structures isolated from microorganisms and many sponges, biosynthesis is often suggested to occur in a symbiont. We previously reported several examples of sponge cell localization experiments that suggested secondary metabolites are often localized in bacterial or cyanobacterial cells.[1] Ecological studies of defensive compounds biosynthesized by sponge symbionts are rare. One recent study of *Dysidea granulosa* in Guam by Becerro and Paul[146] used transplant and shading experiments to test the prediction that if the symbiotic cyanobacterium *Oscillatoria spongeliae* is responsible for the production of polybrominated diphenyl ethers **45–47**, changes in the concentrations of polybrominated diphenyl ethers should be positively correlated with changes in *chl* a. While **45** and **47** and *chl* a concentrations were positively correlated in natural populations, the results from the transplant showed a decrease in *chl* a concentrations when sponges were transplanted to a deeper depth while concentrations of **45–47** did not change. Shading experiments showed that concentrations of **45–47** did not change (but tended to decrease) in sponges under all types of treatment plates (black, transparent and UV-filter) while *chl* a concentrations decreased only under the black treatment plate. This study clearly demonstrates that unraveling the complex relationships between symbionts and host sponges is a challenge for chemical ecologists.

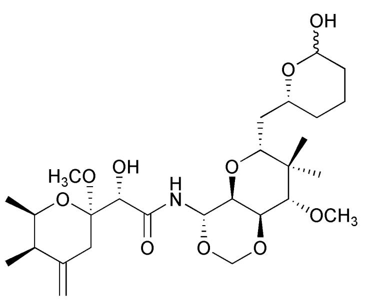


45

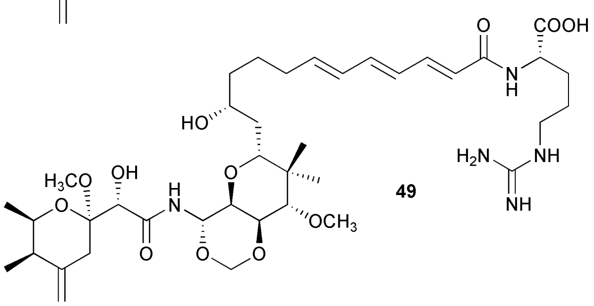


46 R = Me
47 R = H

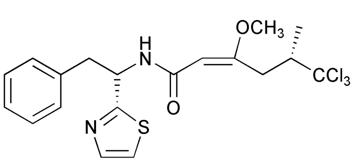
Several recent studies have shown that the biosynthetic genes of some invertebrate secondary metabolites are localized in their symbionts. For example, an uncultured *Pseudomonas* sp. bacterial symbiont of *Theonella swinhoei* contained the PKS genes for the biosynthesis of theopederin **48** and onnamide A **49**.[147] Flatt *et al.*[148] used catalyzed reporter disposition fluorescence *in situ* hybridization (CARD-FISH) to localize the biosynthetic genes for the production of polychlorinated peptides related to barbamide **50** in the cyanobacterial symbiont *Oscillatoria spongeliae* of *Dysidea* (*Lamellodysidea*) *herbacea.* Ridley *et al.*[149] examined chemical variation in four dictyoceratid sponges containing chlorinated amino derivatives or polybrominated diphenyl ethers and related this chemical variation to genetic variation of *O. spongeliae.* They found that only the strain of *O. spongeliae* in the sponge containing chlorinated compounds possessed genes involved in the biosynthesis of these compounds. The gene pathway was absent from *O. spongeliae* in sponges that did not contain the compounds, not a case of selective expression of the metabolites.[149] In another study by Vickery *et al.*,[150] the bacterium *Pseudomonas aeruginosa*, derived from the Antarctic sponge *Isodictya setifera*, produced two phenazine alkaloids **51** and **52** when grown at ambient temperature of 0 °C. Although these studies provide direct evidence that sponge symbionts contain the machinery for biosynthesizing sponge metabolites, they provide little insight into the ecological role of these compounds for the symbiont or the sponge.



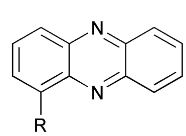
48



49

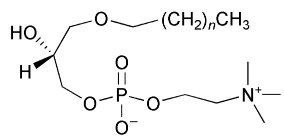


50



51 R = CO_2H
52 R = $CONH_2$

One recent study of *Suberites domuncula* provided evidence that this sponge is responsible for the production of 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine **53** and 1-*O*-octadecyl-*sn*-glycero-3-phosphocholine **54**, active against the bacterial isolate SB1 isolated from *S. domuncula.*[151] Induction experiments exposing the sponge to lipopolysaccharide, an endotoxin from the outer wall of gram-negative bacteria, resulted in higher concentrations of **53** and **54** and increased expression of alkyl-dihydroxyacetonephosphate synthase, a key enzyme in the biosynthetic pathway of this class of compounds that was cloned from the sponge. In another study of *S. domuncula*, Wiens *et al.*[152] were able to localize the major metabolite okadaic acid in the endopinacocytes, the cells lining the canals of the aquiferous system of the sponge, and in cells composing the choanocyte chambers using antibodies specific to okadaic acid. Low concentrations of <100 nM okadaic acid caused an increase in MAP kinase 38, potentially resulting in stimulation of an antibacterial defense; whereas, at high concentrations of >500 nM okadaic acid induced apoptosis.

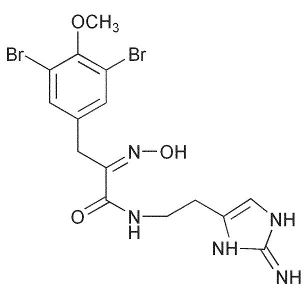


53 n = 14
54 n = 16

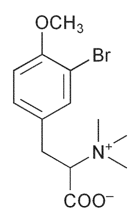
With the growing recognition that some sponge metabolites are produced by symbionts, there is an increased interest in the host-specificity of sponge-associated microbial communities within the tissues and at the sponge surface. Taylor *et al.*[153] proposed that specialist bacteria are more common than generalists in microbial-sponge associations for the temperate Australian sponges *Cymbastela concentrica*, *Callyspongia* sp. and *Stylinos* sp. Further evidence for specific microbial-sponge associations comes from a field transplant experiment with colonies of *Aplysina cavernicola* from the Mediterranean Sea in which the bacterial communities and chemical profiles of sponges transplanted to shallow reefs remained largely unchanged after two months. The small variable fraction of bacteria that did change during this experiment was composed of bacteria common in seawater.[154]

There is growing evidence that interactions between sponges and the bacterial communities with which they are associated are chemically mediated. Crude extracts from sponges promoted the growth of culturable strains of surface-associated bacteria while exhibiting strong antibacterial activities against culturable strains isolated from other species, hard substrates, and seawater. In addition, surface-associated bacterial strains can inhibit the growth of non-surface associated species. In a sponge surface colonization study, Lee and Qian[155] showed that different bacterial communities colonized the surface of surface-sterilized *Mycale adhaerens* colonies and sterile polystyrene dishes after seven days. Although the experimental design did not take into account the physical differences between the sponges and polystyrene dish surfaces, the resulting experiments clearly showed chemically mediated sponge–microbe and microbe–microbe interactions among the isolated strains. In laboratory assays, 50% of the cultured bacterial strains from the polystyrene dish were susceptible to sponge extracts whereas none of the bacteria isolated from the sponge surface was affected by the sponge extracts. In addition, crude extracts from the cultured epibionts from the sponge surface were inhibitory to the isolates from the polystyrene surface. Crude extracts from the sponge *Iricina fusca* from the west coast of India enhanced the growth of bacteria isolated from the surface of sponges.[156] Densities of culturable surface-associated bacteria changed temporally, with the lowest densities in January. Strains of *Bacillus* and *Micrococcus* were common during all sampling months. Crude extracts from *Bacillus* and *Micrococcus* exhibited antibacterial activity against fouling bacteria suggesting that *I. fusca* promoted the growth of bacteria on its surface that may assist the host in the control of epibiosis.[156]

A recent study examined the ability of marine strains of bacteria to attach, swarm, and grow in the presence of sponge extracts.[157] The results demonstrated that crude extracts from eight species of sponges inhibited the attachment of bacterial isolates from sponge surfaces, nearby strata, or adjacent seawater. Extracts from four sponges, *Ailochroia crassa*, *Agelas conifera*, *Amphimedon compressa* and *Aplysina fulva*, completely inhibited swarming for six of the bacterial strains. Other sponge extracts enhanced or had no effect on swarming, but attachment of 23 of the 24 bacterial isolates was inhibited by nearly all of the sponge crude extracts. In contrast, few crude extracts inhibited the growth of the bacterial isolates in standard agar disc assays. In this same study, bioassay-guided fractionation of the sponge *Ailochroia crassa* yielded ianthelline **55**, which inhibited bacterial attachment, and a bromotyrosine derivative **56** that inhibited swarming.



55

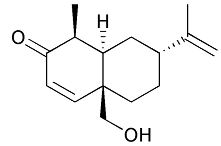


56

Sponge extracts can also inhibit settlement of other fouling organisms. Dobretsov *et al.*[158] showed that conditioned seawater of *Callyspongia pulvinata* decreased the settlement and survival of *Hydroides elegans*, and decreased the density of the diatom *Nitzschia paleacea*, but had no effect on cultured biofilm bacteria. In the field, live sponge decreased the total cover, diversity, and species richness of the fouling community adjacent to the sponge, which the authors attributed to water soluble sponge compound(s). Three Hong Kong sponges, *Haliclona cymaeformis*, *Haliclona* sp., and *Callyspongia* sp., had unique natural bacterial communities.[159] Crude extracts of *Haliclona* sp., and *Callyspongia* sp. incorporated into phytagel™ and left in the field for one week inhibited fouling and changed the bacterial and diatom communities when compared to a solvent control. The methanol extract from *Haliclona* sp. was the most effective at decreasing the richness, diversity, and evenness of the fouling diatom community. After three weeks all six of the sponge extracts (methanol and dichloromethane extracts for each sponge) decreased the mean number of settlers on the gels in the field.[159] In laboratory experiments, compounds isolated from the Mediterranean sponges *Cacospongia scalaris*, *Dysidea* sp., and acetates of compounds from *Ircinia spinosula*, inhibited settlement by cyprids of the barnacle *Balanus amphitrite* but were not toxic to them.[160] A recent review[135] discussed the antifouling potential of derivatives of natural sponge compounds including alkyl amines, isocyano aromatic amines, and *N*-formyl aromatic amines. Synthetic analogues of isocyano compounds inhibited the settlement of *Balanus amphitrite* larvae, but were also toxic to microorganisms.[161] This research has industrial applications but does not consider the ecological functions of these compounds for the sponges.

6 Cnidarians

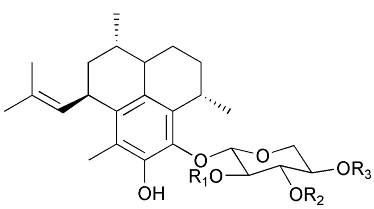
Studies of the chemical ecology of Alcyonarians (Octocorallia), especially the Alcyonacea (soft corals) and the Gorgonacea (gorgonian corals) have addressed predator defense, antibacterial defenses, and antifungal defenses against *Aspergillus sydowii.* Recent studies of crude extracts and pure compounds from gorgonian corals show that these serve multiple ecological roles. For example, *n*-hexane extracts from *Stereonephthya* aff. *curvata*, an exotic soft coral introduced to the Arraial do Cabo region in Brazil about 10 years ago, deterred fish feeding and through contact caused necrosis in the tissues of the Brazilian endemic soft coral *Phyllogorgia dilatata* within three weeks. The authors proposed that its chemical defenses promoted the spread of this invasive species.[162] The sesquiterpene ainigmaptilone A **57**, isolated from the Antarctic gorgonian coral *Ainigmaptilon antarcticus*, also had multiple ecological roles; it deterred predation by the sea star *Odantaster validus* and inhibited the growth of sympatric Antarctic bacteria and diatoms.[163]



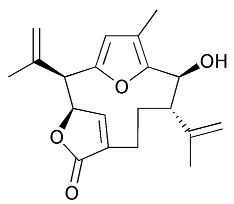
57

Aspergillosis, a disease caused by the fungal pathogen *Aspergillus sydowii*, continues to infect *Gorgonia ventalina* populations in the Caribbean. *Gorgonia* spp. are also threatened with overgrowth by other invertebrates including the hydrozoan *Millepora alcicornis.* Short-term induction experiments with *G. ventalina* demonstrated that tissue necrosis results when healthy *G. ventalina* is placed in contact with diseased *G. ventalina* infected with aspergillosis or live *M. alcicornis.*[164] In addition, there was an increase in the number of purple sclerites at the wound site. The antifungal activities of the non-polar crude extracts against *A. sydowii* remained unchanged throughout the experiment suggesting that natural products are not induced defenses to these potential threats.[164] In longer term experiments with *G. ventalina* and *G. flabellum*, mechanical damage caused by scraping with a dive knife and cable ties attached to the sea fans did not induce purpling, suggesting a recognition system that signals the induction of purpling in response to infection and invading biotic agents. In addition, most colonies of *G. ventalina* and *G. flabellum* put in contact with diseased *G. ventalina* infected with aspergillosis or live *M. alcicornis* recovered after seven months, suggesting that the induction of the purpling response at the infected regions is a successful defense facilitating the recovery and survival of *Gorgonia* sea fans.[164]

Relatively few studies have addressed the potential of octocoral symbionts as the source of bioactive secondary metabolites. Two recent studies of the Caribbean gorgonians *Pseudopterogorgia bipinnata* and *P. elisabethae* provide evidence that some diterpenes may be produced by the cellular symbionts in the genus *Symbiodinium.*[165,166] Biosynthetic studies of the Caribbean gorgonians *P. elisabethae* and *P. bipinnata* suggest that isolated dinoflagellate symbionts produce pseudopterosins A **58** and D **59** and kallolide A **60**, respectively.[165,166] Further, high concentrations of pseudopterosins were positively correlated with a greater abundance of the symbiont *Symbiodinium* sp. at the tips of *P. elisabethae* colonies.[165] In another recent study of *P. elisabethae*, Puyana *et al.*[167] investigated the intraspecific variation of pseudopterosins in sea fans from the archipelago of San Andres and Provencia in the southwestern Caribbean. Sea fans from Provencia were chemically rich in pseudopterosins **61–69** and seco-pseudopterosin **70**, whereas the sea fans from San Andres contained only low concentrations of these compounds. The ecological implications of these results have not been explored.



58 $R_1 = R_2 = R_3 = H$
59 $R_3 = Ac$, $R_1 = R_2 = H$

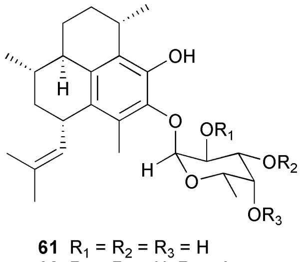


60

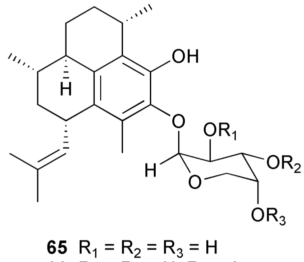
Symbionts may provide important chemical defenses for their host, but the mechanisms of acquisition and maintenance of symbionts are still poorly understood. Recent research on the soft coral *Sinularia lochmodes* found that the lectin SLL-2 may be used to selectively uptake specific symbionts.[168] The lectin was found on the surface of *Symbiodinium* cells released from this soft coral. Three cultured strains of *Symbiodinium* exposed to SSL-2 in the laboratory at less than natural concentrations had reduced motility similar to that exhibited by natural symbiotic zooxanthellae. Growth was not affected in two strains but was inhibited in one strain. When non-symbiotic dinoflagellates and algae were tested, SLL-2 had no effect on the motility and growth of the dinoflagellate *Alexandrium minutum* but caused significant mortality for *Gymnodinium catenatum* and *Prorocentrum micans.* The chlorophyte *Tetraselmis* sp. also lost cell motility and reduced growth suggesting that this lectin not only modifies the physiology of symbiotic zooxanthellae but may also be a chemical mechanism for selecting the appropriate symbiont.

The antimicrobial properties of the eggs of 11 species of scleractinian corals were evaluated by testing extracts for their ability to deter surface attachment and inhibit growth of two laboratory bacteria and 92 marine bacterial isolates.[169] Antimicrobial activity was found in eggs of only one species of coral, *Montipora digitata*, which has been previously reported to produce montiporic acids[170] and other cytotoxic diacetylenes.[171]

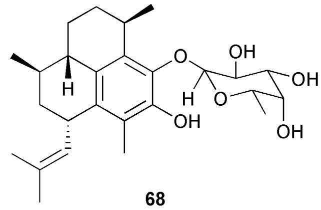
Chemical cues are often used by cnidarians for recruitment into an appropriate habitat. Recent research on the larvae of the stony corals *Acropora tenuis* and *A. millepora* showed that these larvae selectively settled in response to crustose coralline algae.[172] The coral larvae had the highest settlement in response to *Titanoderma prototypum*, which also caused the lowest coral post-settlement mortality of the algae tested. Methanol extracts of *T. prototypum* and *Hydrolithon reinboldii* both induced high levels of metamorphosis at low concentrations.[172]



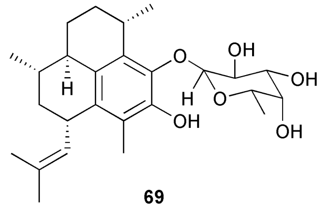
61 $R_1 = R_2 = R_3 = H$
62 $R_1 = R_2 = H$, $R_3 = Ac$
63 $R_1 = R_3 = H$, $R_2 = Ac$
64 $R_2 = R_3 = H$, $R_1 = Ac$



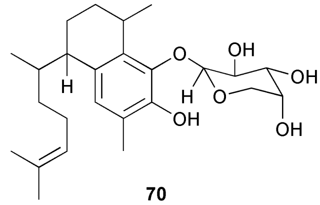
65 $R_1 = R_2 = R_3 = H$
66 $R_1 = R_2 = H$, $R_3 = Ac$
67 $R_1 = R_3 = H$, $R_2 = Ac$



68



69



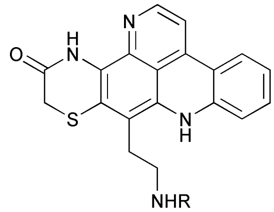
70

7 Ascidians (tunicates)

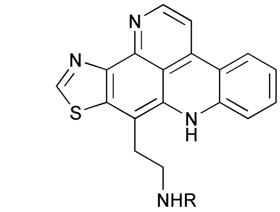
The ascidians (Ascidiacea, Tunicata) are rich sources of bioactive secondary metabolites[133,134] that can deter invertebrate and fish predators and inhibit the growth of microorganisms.[173–175] Fresh animal tissue and lipophilic crude extracts from the Antarctic ascidian *Distaplia cylindrica* deterred predation by the sea star *Odantaster validus.*[173] Strong acids concentrated at the surface of the tunic also deterred *O. validus*, suggesting that this tunicate employs multiple defense strategies. Hydrophilic and the lipophilic crude extracts of *D. cylindrica* caused mortality in a chain-forming diatom demonstrating the potential of the crude extracts for controlling surface fouling. The congeneric ascidian *Distaplia nathensis* also has antifouling extracts; its water soluble extract inhibited the growth of all 14 tested bacteria and attachment of the mussel *Perna indica.*[176] Extracts of three other ascidian species, from seagrass habitats in the Caribbean, did not deter bacterial attachment but did inhibit growth of some bacterial strains and settlement of cyprid barnacle larvae (*Balanus amphitrite*).[177] In the field none of the ascidian extracts inhibited polychaete attachment; however, methanol extracts of *A. stellatum* and *B. planus* inhibited bryozoan attachment, and chloroform and methanol extracts of these two ascidians inhibited settlement by barnacles.[177]

A broad survey of the chemical defenses of the adults and larvae of six colonial ascidians from the Mediterranean showed that defense strategies of tunicates can vary significantly among species.[174] Overall, the tunic material from the adults was less palatable than zooids (thorax and abdomen) when offered to fish and crustaceans, and unpalatability was found in all species in at least one assay. Crude extracts from the tunic of two of the six species tested significantly deterred feeding by hermit crabs. The authors suggested that predators may be deterred by a combination of factors including chemical defenses and low caloric content, digestibility, and pH. Overall, ascidians that produced large larvae in low numbers had larvae that were better chemically defended against fish or invertebrates than ascidians that produced smaller and more numerous larvae.[174] These results suggest that chemical defenses are more prominent in the larvae of invertebrates with low fecundity.

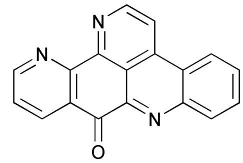
Few studies have assessed chemical variation in ascidians. López-Legentil *et al.*[178] surveyed the major alkaloids in Mediterranean specimens of purple, blue, green, and brown morphs of *Cystodes dellechiajei.* They identified two distinct chemotypes. The purple morph of *C. dellechiajei* contained the pyridoacridines shermilamine B **71** and kuanoniamine D **72** in the tunic and their deacetylated forms **73** and **74** in the zooids while the blue and green morphs contained the C9-unsubstituted pyrridoacridines, ascididemin **75** and 11-hydroxyascididemin **76** in the tunic and zooids. The brown morph only contained minor concentrations of ascididemin. Further studies of the mitochondrial DNA of the different color morphs of *C. dellechiajei* showed little correlation between the chemotypes, morphotypes (spicules), and genotypes[179] with one exception. There was an association between the color of the purple morph and the pyridoacridines, which are purple under the acidic conditions found in the tunic of *Cystodytes.*[178] Crude extracts and ascididemin **75**, the major metabolite from the blue morph of *Cystodes* from the Mediterranean, deterred fish feeding in field and laboratory feeding assays, but were palatable to the sea urchin *Diadema savignyi.* Tunic acidity and spicules from *Cystodes* spp. did not deter predators.[175] Indirect evidence from energy-dispersive X-ray analysis indicated that pyridoacridine alkaloids present in *Cystodes dellechiajei* were accumulated in granules of the pigment cells in the purple morph. Turon *et al.*[180] proposed that the biosynthesis of the alkaloids occurs in the ascidian because sulfur signals corresponding to shermilamine B or kuanoniamine D or their deacetylated derivatives were not found in the bacterial cells sparsely distributed throughout the tunic and the zooids.



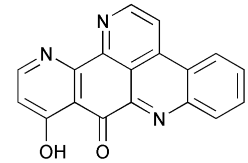
71 R = $COCH_3$
73 R = H



72 R = $COCH_3$
74 R = H

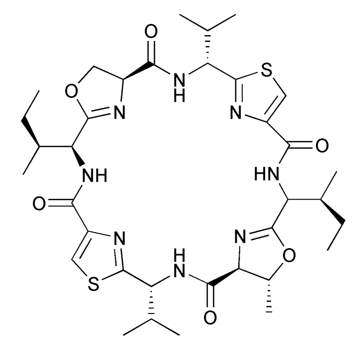


75

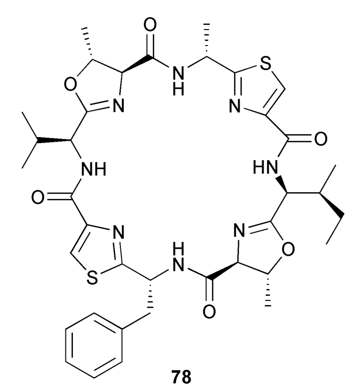


76

Until recently, the origin of metabolites in *Lissoclinum* sp. has been the subject of some debate. Schmidt *et al.*[181] reported that the biosynthetic genes for patellamides A **77** and C **78**, isolated from *Lissoclinum patella*, occur in the cyanobacterial symbiont *Prochloron didemni.* The function of the biosynthetic genes was



77



78

confirmed by heterologous expression of the whole pathway in *Escherichia coli.* Long *et al.*[182] also expressed genes for patellamide biosynthesis in *E. coli* by "shotgun" cloning. Their study utilized a universal expression system in tandem with direct chemical analysis to identify clones that produced patellamides. In another study, *Prochloron* spp. symbionts of five didemnin ascidians from Okinawa, Japan have been implicated in the production of UV absorbing mycosporine-like amino acids (MAAs) that accumulate in the tunic of the transparent animals and protect them from harmful UV exposure.[183] Of the species surveyed, MAAs were only detected in tunicates containing *Prochloron* symbionts.

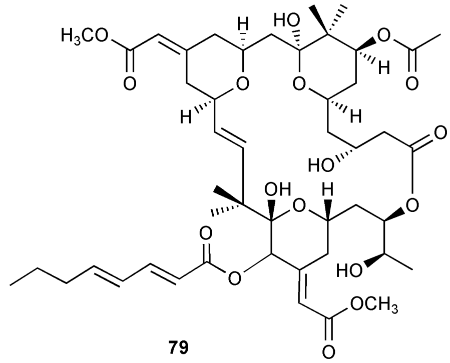
Chemical alarm cues are known from many marine organisms but have rarely been observed in ascidians. A recent study measured the electrophysiological responses associated with arrested cilliary activity in the branchial chamber of the ascidian *Clavelina huntsmani.*[184] *C. huntsmani* responded to water soluble compounds released from conspecifics by stopping water flow through its branchial chamber, but did not respond to conspecific water after it was boiled, water soluble cues from *Corella willmeriana*, or cues from the mussel *Mytilus californianus.* The ascidian *Corella willmeriana* did not respond to conspecific cues or those from *C. huntsmani.* The author suggested that arrested water movement through the ascidian is an alarm response to conspecific cues.

8 Bryozoans

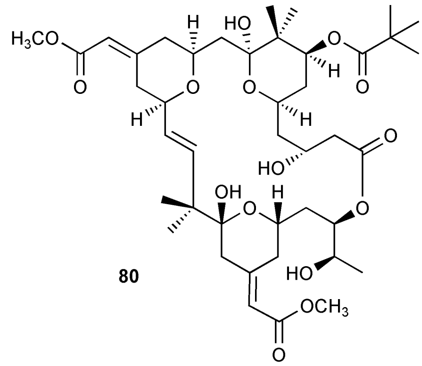
The production of bryostatins by the bacterial symbiont *Endobugula sertula* of *Bugula neritina* is one of the first examples in which an invertebrate symbiont was demonstrated to biosynthesize secondary metabolites found in an invertebrate.[185,186] Recent studies of *Bugula neritina* by Lopanik *et al.*[186,187] demonstrated that three bryostatins protect the larvae of *B. neritina* from North Carolina from predation by fish. Crude extracts prepared from *B. neritina* larvae deterred feeding by pinfish but crude extracts from the adults were palatable. When the larvae were raised without their symbionts they were also palatable. Bioassay-guided fractionation of the active extracts resulted in the isolation of three ichthyodeterrent compounds, bryostatins 1 **79**, 10 **80** and the new bryostatin derivative, bryostatin 20 **81**.[187] These three compounds were present in larvae with symbionts but absent in larvae without symbionts, and interestingly they were absent in *B. neritina* larvae from Delaware.[186] Using 16S rDNA specific primers for the symbiont *E. sertula*, the authors found that the variable presence of bryostatins in *B. neritina* larvae correlated to the presence or absence of the bacterial symbiont. This study illustrates the complex interactions between symbiont and host, and the importance of secondary metabolites in defense for marine larvae.

Bryostatins related to those isolated from *Bugula neritina* are reported from *B. simplex.* A closely related bacterial symbiont to *Endobugula sertula* was characterized from the pallial sinuses of *B. simplex* larvae. The larval symbiont, *Endobugula glebosa*, is consistently and uniquely associated with *B. simplex* exhibiting an ecological and evolutionary relationship similar to that of the invertebrate–symbiont relationship between *B. neritina* and *Endobugula sertula.*[188]

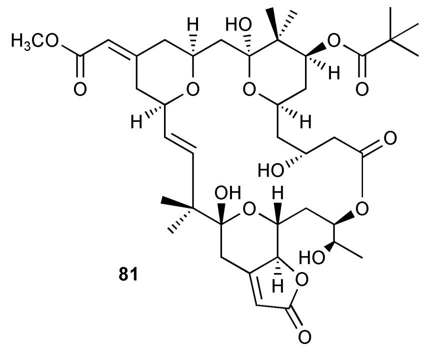
The roles of chemical cues for settlement of bryozoan larvae have recently been studied. Bacterial and diatom biofilms were



79



80



81

used to assess settlement cues for *B. neritina.*[189] Individual bacteria species had no effect or a negative effect on the number of *B. neritina* settlers, and settlement was negatively associated with the density of the bacterium *Pseudoalteromonas* sp. The diatom *Amphora cofeaeformis* induced higher rates of settlement than any other individual diatom.

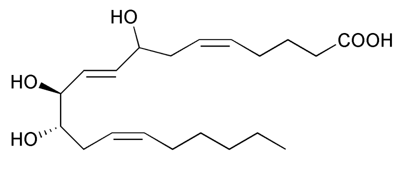
Chemical cues not only determine where bryozoans settle but are also implicated in inducible structural defenses of the bryozoan *Membranipora membranacea.*[190] Waterborne cues from fourteen species of nudibranchs were tested for their ability to induce spines that inhibit nudibranch feeding. The nudibranch conditioned seawater (water that held test species for six hours) was incubated with *M. membranacea* for three days, and the growth of spines was scored after five days. Four nudibranch species that were known to feed on *M. membranacea* and four nudibranchs that do not feed on *M. membranacea* induced spine formation, although the induction of spine formation was variable between trials for all nudibranch species except for the *M. membranacea* specialist *Doridella steinbergae. Doridella steinbergae* egg masses and juveniles also induced spine growth. Interestingly there was no phylogenetic pattern for the nudibranch species that induced spine formation.

9 Crustaceans

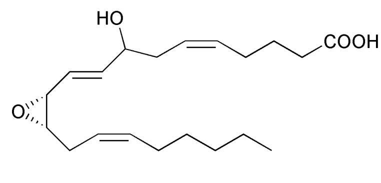
Chemical defenses in crustaceans are not well studied. The isopod *Santia* sp. from Papua New Guinea is host to a complex episymbiotic community that is dominated by bacteria and small unicellular *Synechococcus*-type cyanobacteria.[191] In the absence of symbionts the isopod was palatable to reef fishes, but crude extracts prepared from the isopods with the episymbionts were unpalatable, demonstrating that these microbial symbionts provided a chemical defense for the isopod. Observation of newly emerged juveniles climbing on the mother and inoculating themselves with her episymbionts suggests that the chemically deterrent symbionts are vertically transferred from the mother to the offspring.

While chemical defenses in crustaceans are rarely studied, settlement and chemical cues are relatively well studied. A recent review by Rittschof and Cohen[192] discusses the structure–activity relationship of peptides for chemical signaling in crustaceans. Barnacles are often used as assay organisms to test antifouling natural products in a variety of organisms,[110,161,177] thus understanding their positive settlement cues has ecological and industrial significance. Settlement plates with a smooth surface texture and the amount of time left in the field increased settlement of *Balanus improvisus* larvae. Recruitment was unaffected by crude extract from conspecific adults.[193] Surface texture was shown to be important for barnacle cyprid behavior as they tended to swim faster, more continuously and more randomly when offered a textured surface compared to a smooth surface. The presence of an adult extract also decreased the velocity and randomness of the swimming cyprids. In flume experiments cyprids were more likely to remain on smooth surfaces and leave rough surfaces.

The barnacle *Balanus amphitrite* preferentially settles in response to marine biofilms, and current research focuses on teasing apart the multiple factors that influence the rates of settlement. Qian *et al.*[194] found distinct biofilm communities at high-, mid- and sub-tidal heights but the barnacle cyprids preferred the biofilm from a mid-intertidal height. This selection was not correlated to biofilm biomass or abundance but was likely related to bacterial composition of the biofilm. Hung *et al.*[195] found that ultraviolet light did not affect bacterial density in biofilms but did decrease the percentage of respiring bacterial cells as the dose of UV light increased. Even so, this did not have an effect on the rates of settlement of *B. amphitrite* cyprids. Individual diatom species were tested to determine if they induced *B. amphitrite* metamorphosis.[196] Biofilm extracellular polymeric substances (EPS), but not free EPS, induced the same rates of larval settlement as the positive control of conspecific adult extract. In five axenic diatom cultures at three different densities, the species and age of the diatom influenced barnacle settlement. In two non-axenic diatom cultures only the biofilm age increased the metamorphosis rates of *B. amphitrite.*[196] The bacterium *Pseudomonas aeruginosa*, isolated from the shell surface of *B. amphitrite*, also induced metamorphosis in *B. amphitrite* cyprids, the rates of which varied under different salinity and temperature treatments.[197] When offered together the surface bound components and the culture supernatant of *P. aeruginosa* doubled the metamorphosis of barnacle cyprids, but the surface-bound components alone inhibited metamorphosis. The media on which *P. aeruginosa* was raised also had an influence on cyprid metamorphosis with culture supernatants from a semi-solid culture showing the highest rates of metamorphosis. Semi-solid bacterial cultures showed higher protein content than that of the other cultures, which were mostly composed of carbohydrates. FTIR analysis showed that the semi-solid cultures contained compounds with peaks characteristic of ketones, however these were not tested for their influence on barnacle metamorphosis. The eicosanoid hepoxilins and trioxlins were examined for their roles in egg hatching and settlement of the barnacles *Balanus amphitrite* and *Elminius modestus.*[198] Trioxilin A_3 **82** did not induce egg hatching in *E. modestus* but the unstable epoxide precursor hepoxilin A_3 **83** did. None of the compounds tested induced settlement in *B. amphitrite* cyprids.



82



83

Other barnacles, especially parasitic species, use chemical cues to find the appropriate host or habitat for settlement. The parasitic rhizocephalan barnacle *Loxothylacus texanus* settles on the external carapace of the blue crab *Callinectes sapidus.*[199] Barnacle larvae settled less when proteins and carbohydrates were removed, but settled more when lipids were removed from the exoskeleton of *Callinectes sapidus.* These cues were associated with the epicuticle layer of *C. sapidus* exoskeletons, not biofilms on the carapace. Larvae of another parasitic barnacle, *Heterosaccus dollfusi*, modified their swimming behavior towards the host (direction and velocity) in the presence of waterborne metabolite(s) from the host crab *Charybdis longicollis.*[200] Swimming behavior of nauplius larvae of the commensal barnacle *Trevathana dentata* was also modified by water soluble cues from the host coral *Cyphastrea chalcidicum.*[201]

Crabs also use chemical cues to find appropriate habitats for settlement. In the laboratory and the field, megalopae of the crab *Callinectes sapidus* preferred live eelgrass habitat over oysters and mud.[202] More first stage juvenile crabs were recovered from live oyster habitat (*Crassostrea virginica*) than eelgrass habitat; however, in the field both megalopae and the first stage juveniles settled in *Zostera marina* habitats. Another study found that current speeds and waterborne cues were important for swimming orientation and behavior of *C. sapidus* megalopae.[203] At high current speeds in flume studies, *C. sapidus* intermolt megalopae were most often found at the top of the water column swimming at faster speeds.[203] This was not true for premolt megalopae which had a constant proportion of megalopae oriented upstream irrespective of current speed in offshore water, but increased their swimming upstream in the presence of *Zostera marina* conditioned water. At lower current speeds, more premolt megalopae of *C. sapidus* oriented themselves upstream in the presence of *Zostera marina* and *Spartina alterniflora* conditioned water when compared to offshore water. The megalopae may perceive predators by waterborne cues as the presence of crab *Uca pugilator* and grass shrimp *Palaemonetes pugio* conditioned water added to the *Z. marina* water resulted in less megalopae orienting upstream, but water from the crab *Panopeus herbstii* had no effect. The study showed that larvae could detect and respond both to positive and negative environmental odors.[203]

Megalopae larvae of the crab *Sesarma curacaoense* rely on conspecific water soluble chemical cues for fundamental physiological and ecological processes including faster development, less mortality and metamorphosis.[204] Although quite variable, interspecific cues from *S. rectum* also increased the rate of development and the percentage of metamorphosis suggesting that *Sesarma* spp. produce chemically similar waterborne cues. Other grapsid crabs including *Armases miersii* and *Chasmagnathus granulata* did not speed up development or increase the percentage of metamorphosis in *S. curacaoense* larvae. In a set of field cage experiments megalopae of the fiddler crab *Uca minax* were suspended over a marsh and at increasing distances from the marsh into a river site.[205] After 12 days, molting response was greatest in the marsh site with or without marsh sediment and lowest at the river site, which was not different from the filtered seawater control. A regression analysis showed that with increasing distance from the marsh, molting rates decreased. Chemical cues for molting of fiddler crab megalopae originate in marshes and decline in effectiveness a short distance away from marsh habitats.[205]

Chemical cues aid crustaceans in finding the appropriate habitat and in finding a mate. A water soluble female pheromone trail changed the swimming direction and pattern of male copepods (*Centropages typicus*).[206] As the female trail aged (from 0–30 seconds) fewer trails were found and the males increasingly swam in the wrong direction. Male copepods (*Tigriopus japonicus*) engage in pre-copulatory mate guarding to enhance their reproductive success. Males use surface proteins similar to α_2-macroglobulin to recognize females; the actual proteins were partially sequenced by mass spectrometry.[207] Males of the Chinese mitten crab *Eriocheir sinensis* did not use waterborne chemical cues to find females, but instead mate recognition occurred after physical contact.[208]

10 Molluscs

Natural products influence the ecology of marine molluscs at all life history stages. They function as chemical defenses and in recruitment and fertilization success. The chemical defenses of sacoglossan opisthobranchs have recently been reviewed by Marín and Ros.[211]

A recent study of the sea hare *Aplysia californica* represents the first neurophysiological study to demonstrate that chemical defenses disrupt the sensory responses of a predator.[209] *A. californica* releases secretions when attacked by the spiny lobster *Panulirus interruptus* that induced feeding and grooming behaviors, and in some cases caused the lobsters to perform a defensive tail flip, allowing 60–67% of sea hares used in this study to escape predation. Millimolar concentrations of amino acids in the ink-opaline secretion induce "phagomimicry" where the secreted amino acids mimic the stimulatory properties of food to divert predators.[209] *P. interruptus* individuals were observed digging and grabbing in the area where the ink-opaline was released into the seawater, thus distracting the predator while the sea hare escaped. Ink-opaline is a sticky substance that can also coat the spiny lobster's sensory and feeding appendages, which causes confusion in sensory messages and interferes with the ability of lobsters to attack their prey.

The feeding selectivity of marine consumers on benthic cyanobacteria has been rarely studied but has the potential to control large blooms. Feeding assays with the opisthobranchs *Stylocheilus striatus* and *Bursatella leachii* showed that they prefer the cyanobacterium *Lyngbya majuscula* over diverse macroalgae.[212] Even though *S. striatus* consumed more *L. majuscula* during the first eight days of the experiment, *B. leachii* had a higher conversion efficiency (algal mass to body mass) and grew to a larger size. To understand the fate of cyanobacterial toxins in these opisthobranchs, Capper *et al.*[213] described the different mechanisms for toxin allocation and excretion in *Stylocheilus striatus*, *Bursatella leachii* and *Diniatys dentifer. S. striatus* accumulated more of both lyngbyatoxin A and debromoaplysiatoxin in its digestive gland than in its head and foot. *B. leachii* only accumulated lyngbyatoxin A in its digestive gland and trace amounts in its head and foot. *D. dentifer* had trace amounts of lyngbyatoxin A in its digestive gland and variable concentrations of debromoaplysiatoxin in its head and foot. When the whole animal was compared to its excretions, *S. striatus* accumulated high concentrations of lyngbyatoxin A in its body (3.94 mg kg^{-1}) and excreted low concentrations in its ink (0.12 mg kg^{-1}) and its fecal matter (0.56 mg kg^{-1}). *B. leachii* showed the opposite trend with the lowest concentrations of lyngbyatoxin A in its body (2.24 mg kg^{-1}) and higher concentrations in its ink (5.41 mg kg^{-1}) and fecal matter (6.71 mg kg^{-1}). This study illustrates that consumers are not necessarily deterred by toxins and can have different strategies for accumulation and detoxification of secondary metabolites.

Recruitment of gastropod larvae is a complex process that is often mediated by chemical cues. Larvae of the Australian blacklip abalone *Haliotis rubra* settled in response to a crustose coralline alga (*Amphiroa anceps*) and a green alga (*Ulva australis*), but not the methanol extracts of these algae or to biofilms associated with the surfaces of the algae.[214] In contrast, the nudibranch *Phestilla sibogae* metamorphoses in response to a water soluble cue from *Porites* spp. corals. Hadfield and Koehl[215] found that *P. sibogae* larvae stopped swimming and sank toward the bottom of a flume tank when exposed to waterborne cues from *Porites compressa* (obtained by soaking healthy *P. compressa* branches in seawater), but resumed swimming when not exposed to the coral water. This ability of larvae to sink in response to dissolved settlement cues could enhance their transport to a suitable habitat for settlement and metamorphosis. Behavioral responses to chemical cues from prey or host species were also recently found for host specific barnacles,[199–201] and are important mechanisms for finding the right habitats for recruitment.

Marine gastropods also used chemical cues to avoid predators. The snail *Tegula funebralis* exhibited a flight response to water soluble cues released during feeding by a crab predator.[216] The snails responded to ground up conspecifics and crabs actively feeding on conspecifics but not to crabs feeding on other species of snails or crabs not feeding. A study of the Antarctic limpet *Nacella concinna* reported evasive behavior in response to physical contact or crude extracts from a predatory sea star.[217] *N. concinna* displays evasion responses including extension of pallial tentacles, raising its shell in a mushroom-like fashion, rotation, and flight in response to the hydrophilic crude extracts from the sea star *Neosmilaster gergianus.* No evidence that waterborne chemical cues alerted the limpet to the presence of the predatory sea star was found because evasive behaviors were only observed when limpets made physical contact with *N. gergianus.*[217]

Inducible defenses in the form of a thicker shell have been described for some molluscs. In a field experiment where crab populations were manipulated, multiple factors interacted to influence the shell thickness of *Littorina subrotundata.*[210] In general, snails collected adjacent to crab shelters (higher densities of the predatory crab *Hemigrapsus nudus*) had greater shell mass, but the results were only significant for two of the six collection days. To determine whether the presence of predators was the most important factor, snails from the field were brought into the laboratory and raised in the presence of waterborne cues from *H. nudus* feeding on *L. subrotundata.* Collection location and the presence of waterborne cues from the feeding crab significantly increased *L. subrotundata* shell thickness.[210] An induced defense was also found in the green mussel *Perna viridis* in response to crab and snail predators.[218] Exposure to waterborne cues from the portunid crab *Thalamita danae* and the muricid snail *Thais clavigera* induced greater shell width, height and lip thickness of *Perna viridis* when compared to the control. When *Thais clavigera* individuals were offered a choice, they ate less of the mussels from the snail treatment than from the control group. *Thalamita danae* ate the same number of mussels from each treatment, but there was an increase in the shell breaking time of *Perna viridis* from the crab treatment compared to the control treatment.

The release of tryptophan plays a key role in gamete fertilization for the red abalone *Haliotis rufescens.*[219] In a well designed series of experiments, sperm attraction to the egg was lost when the treatment was flooded with exogenous tryptophan and with the addition of the enzyme tryptophanase. Sperm swam faster towards the egg at distances of 50 and 100 μm but not >150 μm from the egg, probably related to a concentration gradient of tryptophan released from the egg. This research shows that sperm chemotaxis may be an important mechanism for fertilization success of mass spawning benthic organisms.

11 Polychaetes

There are few reports investigating the chemical defenses of marine worms. Chemical defenses of invertebrates from deep-sea hydrothermal vents or cold-seep communities have not been studied. In a survey of the palatability of deep-sea invertebrates against shallow water consumers, the sharp setae from the polychaete *Archinome rosacea* deterred feeding by the shore crab *Pachygrapsus crassipes.*[220] Crude extracts from parts of three out of ten polychaetes and two bivalves deterred feeding by different consumers including *P. crassipes*, the lesser blue crab *Callinectes similes*, and the fish *Fundulus heteroclitus* and *Leiostomus xanthurus.* The presence of chemoautotrophic bacteria may contribute to the production of chemical defenses in deep-sea invertebrates. This study demonstrates that organisms from extreme environments may produce chemical defenses, but further studies need to be conducted with predators from deep-sea hydrothermal vents or cold-seep communities.

The recruitment of the polychaete *Hydroides elegans* is relatively well studied because it is a common fouling organism. Understanding the positive cues for settlement has increased along with our understanding of marine biofilms. Individual diatoms isolated from marine biofilms induced high settlement rates of *H. elegans* larvae, but their associated bacteria did not affect settlement.[221] Inductive strains comprised seven different diatom genera. Two individual diatom strains, *Achnanthes* sp. and *Nitzschia constricta* were density dependent inducers of *H. elegans* settlement at the same rates as natural marine biofilms.[222] Further work showed that the >100 kDa fractions of the extracellular polymers from *Achnanthes* sp. and *Nitzschia constricta* induced significant larval settlement.[223] Another study of the specific *H. elegans* larval metamorphosis cue examined a suite of amino acids.[224] Nine amino acids were toxic to the larvae and eight induced abnormal metamorphosis. Three other amino acids (asparagine, aspartic acid and glutamic acid) induced significant rates of normal metamorphosis but never at the same rate as the positive biofilm control.

Chemical cues as pheromones are well known in terrestrial organisms and were recently implicated in reproductive isolation for the polychaete *Neanthes acuminata.*[225] Initial experiments showed that exposure of male *N. acuminata* to females from another population initiated higher levels of aggressive behavior. Only when exposed to females from their own populations did the males show little aggressive behavior. Further experimentation showed that conditioned seawater from all populations caused similar aggressive behavior as did the live worms, suggesting that waterborne compound(s) were responsible for the observed behavior.

12 Echinoderms

Echinoderms are reported to produce chemical defenses against predators although recent studies are rare. The juveniles and embryos from two brooding sea stars and an isopod deterred feeding by the sea star *Odontaster validus.*[226] Unpalatable organic extracts from embryos of the sea star *Lyasterias perrieri* and juveniles of the isopod *Glyptonotus antarcticus* provide evidence that they are chemically defended.

Echinoderm natural products can have antifouling activity as illustrated by two studies that used spores from the brown alga *Hincksia irregularis* as a model system for testing antifouling potential. The aqueous extracts of the sea stars *Astropecten articulatus* and *Luidia clathrata* and the brittle star *Astrocyclus caecilia* had little effect on spore settlement, but all extracts inhibited short and long term germination when tested at below natural concentrations.[227] Extracts from all three of these echinoderms also changed the direction and speed of the swimming behavior of *H. irregularis* spores.[228] These studies showed that these extracts can inhibit settlement of *H. irregularis*, but their ecological activity in the field against a suite of fouling organisms remains to be confirmed.

Few settlement cues for echinoderms have been isolated and characterized. Previous research on the urchin *Holopneustes purpurascens* had attributed settlement to an isethionic acid and floridoside complex.[229] Through bioassay-guided fractionation and the use of cation exchange chromatography the settlement cue was isolated and identified as histamine.[105] Synthetic histamine induced similar rates of urchin settlement as isolated natural histamine. Histamine is found in especially high concentrations (although quite variable) in the alga *Delisea pulchra* onto which *H. purpurascens* recruits in the field.

Chemical cues serve as alarm cues for some sea urchins. In y-maze experiments, juvenile *Strongylocentrotus franciscanus* urchins were attracted to adult conspecifics when the predator *Pycnopodia helianthoides* was upstream of the adult.[230] The only significant choice (other than the positive control of kelp) for the juveniles was when the adult was downstream of the *P. helianthoides*, suggesting that the adults produce a secondary chemical cue in response to primary cues emitted by the predator, since there was no significant response when the adults were upstream of the predator. This treatment also caused the juveniles to orient upstream more often but did not change their crawling speed. The authors consider this an alarm response, suggesting that the juveniles were better protected when they were adjacent to adults, a distribution pattern observed in the field.

Water soluble feeding cues were implicated in another y-maze experiment, which tested the chemotactic response of *Pycnopodia helianthoides* to its prey the butter clam *Saxidomus giganteus*.[231] Even though there was little reaction to whole live clams when compared to a shell control, this sea star detected and reached damaged clams faster than live clams in a y-maze. In field experiments with damaged *S. giganteus* suspended above the benthos, *P. helianthoides* only approached its prey when it was down current of a damaged clam. When *P. helianthoides* was placed up current of the damaged *S. giganteus*, it did not approach the prey; the response was similar to the control of empty clam shells, which did not induce movement. In a field manipulation that directed sea stars through a corridor, 13 of 15 sea stars passed over live clams to reach damaged clams. In a treatment of damaged clams 14 of 15 sea stars reached the damaged clams, and in the live clam treatment no sea stars reached the live clams.[231] The study showed that waterborne cues emitted by damaged prey changed the foraging behavior of a key predator.

Water soluble cues may function in competitive interactions by inducing a negative effect on the feeding rates of sea stars.[232] In laboratory experiments the feeding rates of the sea stars *Leptasterias polaris* and *Asterias vulgaris* increased when presented with conspecifics and were reduced in the presence of the other species, although feeding rates were also found to be temperature dependent. The feeding rate of both sea stars was reduced in the presence of water soluble cues from the other species feeding. In the field, combinations of sea stars were placed in 2 m diameter circular plots with a 0.2 m^2 bed of mussels. *L. polaris* reduced its feeding and had a higher proportion of sea stars leave the plot when introduced with *A. vulgaris* than when alone, however it took seven days for this trend to develop. *A. vulgaris* did not change its feeding behavior when introduced alone or with *L. polaris*.

13 Other invertebrates

A recent study looked at the waterborne cues involved in settlement of the actinotroch larva of *Phoronis pallida*.[233] Adult *P. pallida* are consistently found embedded in the burrow walls of the mud shrimp *Upogebia pugettensis*. Seawater conditioned with *U. pugettensis*, burrow walls, and *Upogebia* gut tissue modified the behavior of *P. pallida* larvae by increasing their swimming velocity and benthic probing behavior. The *U. pugettensis* conditioned seawater treated with arginase and lipase eliminated *P. pallida* probing behavior by the larvae.

Chemical defenses were found in the Antarctic brachiopod *Liothyrella uva*.[234] Tissues and crude extracts from the exposed pedicle of Antarctic brachiopod *Liothyrella uva* were unpalatable to the sea star *Odontaster validus* and the fish *Notothenia coriiceps*. In addition, crude extracts of the lophophore, intestine, and stomach were effective growth inhibitors of several strains of Antarctic bacteria suggesting that *L. uva* produces multiple chemical defenses against predators and pathogens.

14 Vertebrates

14.1 Fish

Six species of coral dwelling gobies from the genus *Gobiodon* were found to contain toxins in their excreted mucus.[235] Each goby species elicited a different response in a loss of equilibrium bioassay with the planktivorous cardinal fish *Apogon fragilis*. The wrasse *Thalassoma lunare* ate less of the food with skin secretions of *Gobiodon okinawae* added. The rockcod *Cephalopholis cyanostigma* also ate fewer pieces of food when whole *G. erythrospilus* was concealed within the food. The bioassays used ecologically relevant fish predators, but further chemical characterization is necessary to determine what compounds are deterrent and whether the different species of *Gobiodon* have the same toxic compounds. Many marine natural products have multiple ecological roles, and the skin toxins of *Gobiodon* spp. may inhibit parasitism by gnathiid isopods.[236] Even though just as many parasites attached to three *Gobiodon* spp. as the control fish *Paragobiodon xanthosomus*, the parasites preferentially settled on the body of *P. xanthosomus*, but only on the fins of *Gobiodon* spp. Since there are no toxin glands in the fins of *Gobiodon* spp. the authors attributed the differential distribution of the parasites to the skin toxin of *Gobiodon* spp., however, they did not test alternate explanations for the observed patterns.

Chemical cues are used by fish to locate food. For example, a recent study compared the responses of three fish species, Pacific halibut, walleye pollock and sablefish, to squid extract (squid mantle soaked in water).[237] All three species exhibited increased movement in response to squid extract. Sablefish were the most responsive to squid extract, with Pacific halibut being the least sensitive, although to some extent this is a reflection of different swimming behavior measured for each fish species. The size of the fish was found to be important as larger sablefish were more active than smaller sablefish, however this could be a reflection of the different behavioral measures of swimming activity *versus* turning rates.

Chemical cues function as fish alarm signals, and a recent example is the coral goby *Asterropteryx semipunctatus*, which modified its swimming and feeding behavior when exposed to conspecific skin extracts.[238] In an interesting learning experiment, fish were exposed to the conspecific alarm cue along with a neutral cue (extract from the damsel fish *Acanthochromis polyacanthus*), and after three days the fish responded the same to the neutral cue as to one exposure to the alarm cue, showing that the fish had learned to associate an alarm signal with the previously neutral cue. Studies of fish deterrence and chemical signaling remain limited by a poor understanding of the natural product chemistry responsible for the observed fish behavior.

Chemical cues affect many facets of fish ecology at different life history stages including larval recruitment. A physiological experiment using *Pomacentrus nagasakiensis* showed that larval and post-settlement juvenile fish have a neurobiological response to sound and olfactory conspecific cues.[239] In a broad survey of 18 reef fish species, 13 species used conspecific cues to settle, 10 species used visual cues, 10 species used chemical cues, and three species used mechanical cues such as sounds or vibrations.[240] In a more in depth study, Lecchini *et al.*[241] used the coral reef fish *Chromis viridis* to determine which cues drive recruitment patterns. They found that *C. viridis* responded to visual and acoustic/vibratory cues from a distance less than 75 cm but responded to olfactory cues from less than 375 cm. A major peak was separated by HPLC from ethyl acetate extractions of *C. viridis* conditioned seawater that caused 83% of the larvae to swim towards the compound(s) in a compartment within an aquarium.

Adult fish also use chemical cues to home into their habitat. In a series of experiments on the black rockfish *Sebastes inermis*, olfactory cues were more important for homing behavior than visual cues.[242] In experiments where the fish were blinded, a total of six of seven blind and six of seven control fish returned to their territories. The time taken to return was not different between the blind and control fish. Only two out of six fish with their olfactory pits blocked (with petroleum jelly) returned to their home site, and the time required to return was longer.[242] Contrary to this, pelagic fish did not appear to use olfactory cues to find fish aggregation devices (FADs) in Australia, and instead are thought to use sound or vibrations to cue into FADs.[243]

14.2 Birds

Recent research has illustrated the importance of chemical cues in marine birds. Feathers were collected from male and female crested auklets, *Aethia cristatella*, and through chemical extraction and GC-MS analysis were found to contain twenty volatile compounds.[244] The concentration of nine of these compounds was variable between summer months (breeding season) and winter months. When tested for attraction quality in a modified y-maze, the whole feather and a combination of *cis*-4-decenal and octanal caused significant attraction, while controls of amyl acetate and mammalian musk did not cause any attraction. The volatile compounds of the crested auklet may serve as a cue for sexual selection, and they may also improve bird fitness by serving an anti-parasite role. Octanal and hexanal, which are known from insects as potent invertebrate repellents, were also found in odors from the crested auklet.[245] Octanal and decanal from *Aethia cristatella* feathers were found to repel tick ectoparasties.[246] In a concentration gradient of crested auklet odorant in ethanol, duration of tick attachment was significantly reduced compared to an ethanol control at 100% and 10%, but not at 1% of natural concentration. Ticks exposed to octanal became moribund in less than 1 hour and none recovered. Lice also became moribund within seconds of exposure to octanal and (*Z*)-4-decenal.[246] However when directly tested against lice, auklet feathers did not reduce survival, and in the field, crested auklets had significantly more lice than the least auklet *A. pusilla*.[247] The synthetic analogues of *A. cristatella* compounds were extremely deterrent compared to the ethanol control when tested against mosquitoes; however, the experiments were not ecologically relevant as they measured the number of mosquito landings on a human hand with the compounds on it.[248]

15 Conclusions

As the discipline of marine chemical ecology grows we are gaining a better understanding of the diversity and range of ecological functions of secondary metabolites. The fact that natural products influence fundamental ecological processes such as predator–prey interactions, trophic cascades, competition, prey capture, reproduction and larval recruitment has become well established in ecological theory. Recent advances in molecular identification of microbes are revealing their importance as symbionts as well as pathogens and their influence on ecological processes. Molecular techniques have revealed that some symbiotic microbes are responsible for the production of secondary metabolites that serve fundamental roles for their hosts.

The discipline of marine chemical ecology continues to be limited by a poor understanding of the processes that control chemical diversity and variation. Even though concentrations of natural products can be highly variable, our understanding of the physical and biological (especially genetic heritability) factors that influence secondary metabolite production remains limited. The phenotypic and genetic variability in the physiology of consumers to overcome chemical defenses is another important aspect of chemical ecology that remains poorly researched. While we were able to expand the scope of this review to include more taxonomic groups including microbes, worms, echinoderms, crustaceans, and fish, many of the secondary metabolites that function as chemical cues for reproductive and behavioral processes for these groups remain unidentified. As researchers develop better techniques for bioassay-guided fractionation and chemical characterization of small amounts of compounds we expect more natural products involved in chemical signaling to be characterized. Basic research such as identifying and characterizing waterborne compounds is advancing as analytical chemistry techniques improve.

Perhaps some of the most exciting and novel recent advances in marine natural products are in isolating and describing biosynthetic genes that are responsible for producing secondary metabolites. These techniques can be applied to ecological questions about localization of compound production within organisms and environmental regulation of natural product biosynthesis. These studies have the potential to answer fundamental evolutionary and ecological questions such as which organisms in complex symbioses are producing secondary metabolites and the effects of genetic mutations on the structure and evolution of novel compounds. Advances in other fields such as genomics, proteomics and metabolomics[249] also have potential applications in chemical ecology.

Interdisciplinary approaches are key to further advances in marine chemical ecology. Continued collaborations between microbiologists, molecular biologists, physiologists, biochemists, cellular biologists, natural product chemists and ecologists will allow the field of chemical ecology to mature into a discipline that is capable of describing many of the basic processes that explain patterns of population, behavioral and community ecology.

16 Acknowledgements

We would like to thank all of the authors who provided us with their reprints and papers that were in press. Special thanks also to Commissioning Editor Marcel Jaspars, Greg Cronin and Clifford Ross for their helpful comments that improved the clarity of this manuscript. Recent research in our laboratory on algal chemical defenses has been supported by the Smithsonian Marine Science Network, and our work on settlement cues for marine invertebrate larvae has been supported by NSF (OCE 9911867 and OCE 0317707). M. P. P. was supported by the Florida Center of Excellence in Biomedical and Marine Biotechnology. This is contribution #635 of the Smithsonian Marine Station at Fort Pierce and P200603 from the Florida Center of Excellence in Biomedical and Marine Biotechnology.

17 References

1 V. J. Paul and M. Puglisi, *Nat. Prod. Rep.*, 2004, **21**, 189–209.
2 G. Pohnert, *Top. Curr. Chem.*, 2004, **239**, 179–219.
3 E. M. Gross, *Crit. Rev. Plant Sci.*, 2003, **22**, 313–339.
4 N. Fusetani, *Nat. Prod. Rep.*, 2004, **21**, 94–104.
5 P. Bhadury and P. C. Wright, *Planta*, 2004, **219**, 561–578.
6 L. Miao and P.-Y. Qian, *Aquat. Microb. Ecol.*, 2005, **38**, 231–238.
7 J. G. Burgess, K. G. Boyd, E. Armstrong, Z. Jlang, L. Yan, M. Berggren, U. May, T. Piscane, A. Granmo and D. R. Adams, *Biofouling*, 2003, **19**, 197–205.
8 T. Harder, S. Dobretsov and P.-Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2004, **274**, 133–141.
9 S. Egan, S. James, C. Holmstrom and S. Kjelleberg, *Environ. Microbiol.*, 2002, **4**, 433–442.
10 A. J. Watkinson, J. M. O'Neil and W. C. Dennison, *Harmful Algae*, 2005, **4**, 697–715.
11 V. J. Paul, R. W. Thacker, K. Banks and S. Golubic, *Coral Reefs*, 2005, **24**, 693–697.
12 K. M. Karlsson, H. Kankaanpää, M. Huttunen and J. Meriluoto, *Harmful Algae*, 2005, **4**, 163–166.
13 M. Karjalainen, B. Kozlowsky-Suzuki, M. Lehtiniemi, J. Engström-Öst, H. Kankaanpää and M. Viitasalo, *Mar. Biol.*, 2006, **148**, 683–691.
14 V. O. Sipiä, K. A. Karlsson, J. A. O. Meriluoto and H. T. Kankaanpää, *Environ. Toxicol. Chem.*, 2004, **23**, 1256–1260.
15 R. W. Thacker and V. J. Paul, *Appl. Environ. Microbiol.*, 2004, **70**, 3305–3312.
16 A. Capper, I. R. Tibbetts, J. M. O'Neil and G. R. Shaw, in *Harmful Algae 2002*, ed. K. A. Steidinger, J. H. Landsberg, C. R. Tomas and G. A. Vargo, Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, 2004, pp. 461–464.
17 A. Capper, E. Cruz-Rivera, V. J. Paul and I. R. Tibbetts, *Hydrobiologia*, 2006, **553**, 319–326.
18 I. B. Kuffner and V. J. Paul, *Coral Reefs*, 2004, **23**, 455–458.
19 A. D. Cembella, *Phycologia*, 2003, **42**, 420–447.
20 C. Legrand, K. Rengefors, G. O. Fistarol and E. Graneli, *Phycologia*, 2003, **42**, 406–419.
21 C. A. Heil, P. M. Glibert and C. Fan, *Harmful Algae*, 2005, **4**, 449–470.
22 G. H. Wikfors, *Harmful Algae*, 2005, **4**, 585–592.
23 J. Kubanek, M. Hicks, J. Naar and T. A. Villareal, *Limnol. Oceanogr.*, 2005, **50**, 883–895.
24 U. Tillmann and U. John, *Mar. Ecol.: Prog. Ser.*, 2002, **230**, 47–58.
25 G. O. Fistarol, C. Legrand, E. Selander, C. Hummert, W. Stolte and E. Granéli, *Aquat. Microb. Ecol.*, 2004, **35**, 45–56.
26 N. Lundholm, P. J. Hansen and Y. Kotaki, *Mar. Ecol.: Prog. Ser.*, 2005, **288**, 21–33.
27 M. L. Wells, C. G. Trick, W. P. Cochlan, M. P. Hughes and V. L. Trainer, *Limnol. Oceanogr.*, 2005, **50**, 1908–1917.
28 A. Skovgaard and P. J. Hansen, *Limnol. Oceanogr.*, 2003, **48**, 1161–1166.
29 G. O. Fistarol, C. Legrand and E. Granéli, *Mar. Ecol.: Prog. Ser.*, 2003, **255**, 115–125.
30 G. Pohnert, *ChemBioChem*, 2005, **6**, 946–959.
31 A. Ianora, A. Miralto, S. A. Poulet, Y. Carotenuto, I. Buttino, G. Romano, R. Casotti, G. Pohnert, T. Wichard, L. Colucci-D'Amato, G. Terrazzano and V. Smetacek, *Nature*, 2004, **429**, 403–407.
32 A. Ianora, S. A. Poulet and A. Miralto, *Phycologia*, 2003, **42**, 351–363.
33 G. A. Paffenhöfer, A. Ianora, A. Miralto, J. T. Turner, G. S. Kleppel, M. R. d'Alcalà, R. Casotti, G. S. Caldwell, G. Pohnert, A. Fontana, D. Müller-Navarra, S. Jonasdottir, V. Armbrust, U. Båmstedt, S. Ban, M. G. Bentley, M. Boersma, M. Bundy, I. Buttino, A. Calbet, F. Carlotti, Y. Carotenuto, G. d'Ippolito, B. Frost, C. Guisande, W. Lampert, R. F. Lee, S. Mazza, M. G. Mazzocchi, J. C. Nejstgaard, S. A. Poulet, G. Romano, V. Smetacek, S. Uye, S. Wakeham, S. Watson and T. Wichard, *Mar. Ecol.: Prog. Ser.*, 2005, **286**, 293–305.
34 G. S. Caldwell, C. Lewis, P. J. W. Olive and M. G. Bentley, *Mar. Environ. Res.*, 2005, **59**, 405–417.
35 T. Wichard, S. A. Poulet and G. Pohnert, *J. Chromatogr., B*, 2005, **814**, 155–161.
36 T. Wichard, S. A. Poulet, C. Halsband-Lenk, A. Albaina, R. Harris, D. Liu and G. Pohnert, *J. Chem. Ecol.*, 2005, **31**, 949–958.
37 R. Casotti, S. Mazza, C. Brunet, V. Vantrepotte, A. Ianora and A. Miralto, *J. Phycol.*, 2005, **41**, 7–20.
38 S. D. Archer, C. E. Stelfox-Widdicombe, G. Malin and R. H. Burkill, *J. Plankton Res.*, 2003, **25**, 235–242.
39 S. Strom, G. Wolfe, J. Holmes, H. Stecher, C. Shimeneck, S. Lambert and E. Moreno, *Limnol. Oceanogr.*, 2003, **48**, 217–229.
40 S. Strom, G. Wolfe, A. Slajer, S. Lambert and J. Clough, *Limnol. Oceanogr.*, 2003, **48**, 230–237.
41 J. H. Landsberg, *Rev. Fish. Sci.*, 2002, **10**, 113–390.
42 E. K. Prince, L. Lettieri, K. McCurdy and J. Kubanek, *Oecologia*, 2006, in press, DOI: 10.1007/s00442-00005-00274-00442.
43 J. Giner, J. A. Faraldos and G. L. Boyer, *J. Phycol.*, 2003, **39**, 315–319.
44 H. G. Dam and S. P. Colin, *Harmful Algae*, 2005, **4**, 575–584.
45 R. M. da Costa, J. Franco, E. Caho and F. Fernández, *J. Exp. Mar. Biol. Ecol.*, 2005, **322**, 177–183.
46 A. Ianora, J. T. Turner, F. Esposito, Y. Carotenuto, G. d'Ippolito, G. Romano, A. Fontana, C. Guisande and A. Miralto, *Mar. Ecol.: Prog. Ser.*, 2004, **280**, 199–210.
47 G. J. Teegarden, A. D. Cembella, C. L. Capuano, S. H. Barron and E. G. Durbin, *J. Plankton Res.*, 2003, **25**, 429–443.
48 R. Kvitek and C. Bretz, *Mar. Ecol.: Prog. Ser.*, 2004, **271**, 233–243.
49 R. Kvitek and C. Bretz, *Mar. Ecol.: Prog. Ser.*, 2005, **293**, 303–309.
50 L. J. Flewelling, J. P. Naar, J. P. Abbott, D. G. Baden, N. B. Barros, G. D. Bossart, M.-Y. D. Bottein, D. G. Hammond, E. M. Haubold, C. A. Heil, M. S. Henry, H. M. Jacocks, T. A. Leighfield, R. H. Pierce, T. D. Pitchford, S. A. Rommel, P. S. Scott, K. A. Steidinger, E. W. Truby, F. M. V. Dolah and J. H. Landsberg, *Nature*, 2005, **435**, 755–756.
51 A. J. Smit, *J. Appl. Phycol.*, 2004, **16**, 245–262.
52 S. L. La Barre, F. Weinberger, N. Kervarec and P. Potin, *Phytochem. Rev.*, 2004, **3**, 371–379.
53 M. A. Vallim, J. C. De Paula, R. C. Pereira and V. L. Teixeira, *Biochem. Syst. Ecol.*, 2005, **33**, 1–16.
54 C. D. Amsler and V. A. Fairhead, *Adv. Bot. Res.*, 2005, **43**, 1–91.
55 Y. Freile-Pelegrín and J. L. Morales, *Bot. Mar.*, 2004, **47**, 140–146.
56 C. D. Amsler, I. N. Okogbue, D. M. Landry, M. O. Amsler, J. B. McClintock and B. J. Baker, *Bot. Mar.*, 2005, **48**, 318–322.
57 C. Hellio, J.-P. Maréchal, B. Véron, G. Bremer, A. Clare and Y. L. Gal, *Mar. Biotechnol.*, 2004, **6**, 67–82.
58 R. Martí, M. J. Uriz and X. Turon, *Mar. Ecol.: Prog. Ser.*, 2004, **282**, 73–85.
59 C. D. Amsler, K. Iken, J. B. McClintock, M. O. Amsler, K. J. Peters, J. M. Hubbard, F. B. Furrow and B. J. Baker, *Mar. Ecol.: Prog. Ser.*, 2005, **294**, 141–159.

60 K. J. Peters, C. D. Amsler, M. Amsler, J. B. McClintock, R. B. Dunbar and B. J. Baker, *Phycologia*, 2005, **44**, 453–463.
61 S. Ankisetty, S. Nandiraju, H. Win, Y. C. Park, C. D. Amsler, J. B. McClintock, J. A. Baker, T. K. Diyabalanage, A. Pasaribu, M. P. Singh, W. M. Maiese, R. D. Walsh, M. J. Zaworotko and B. J. Baker, *J. Nat. Prod.*, 2004, **67**, 1295–1302.
62 V. A. Fairhead, C. D. Amsler, J. B. McClintock and B. J. Baker, *J. Exp. Mar. Biol. Ecol.*, 2005, **322**, 1–12.
63 V. A. Fairhead, C. D. Amsler, J. B. McClintock and B. J. Baker, *Polar Biol.*, 2005, **28**, 680–686.
64 K. N. Pelletreau and G. Muller-Parker, *Mar. Biol.*, 2002, **141**, 1–9.
65 H. Sasaki, H. Kataoka, A. Murakami and H. Kawai, *Hydrobiologia*, 2004, **512**, 255–262.
66 J. P. Barbosa, V. L. Teixeira, R. Villaça, R. C. Pereira, J. L. Abrantes and I. C. P. d. P. Frugulhetti, *Biochem. Syst. Ecol.*, 2003, **31**, 1451–1453.
67 J. P. Barbosa, V. L. Teixeira and R. G. Pereira, *Bot. Mar.*, 2004, **47**, 147–151.
68 A. R. Soares, V. L. Teixeira, R. C. Pereira and R. Villaça, *Biochem. Syst. Ecol.*, 2003, **31**, 1347–1350.
69 R. C. Pereira, A. R. Soares, V. L. Teixeira, R. Villaça and B. A. P. d. Gama, *Bot. Mar.*, 2004, **47**, 202–208.
70 E. E. Sotka, J. P. Wares and M. E. Hay, *Evolution*, 2003, **57**, 2262–2276.
71 E. E. Sotka and M. E. Hay, *Ecology*, 2002, **83**, 2721–2735.
72 E. E. Sotka, *Ecology Lett.*, 2005, **8**, 448–459.
73 H. Pavia, G. B. Toth, A. Lindgren and P. Åberg, *Phycologia*, 2003, **42**, 378–383.
74 V. Stiger, E. Deslandes and C. E. Payri, *Bot. Mar.*, 2004, **47**, 402–409.
75 R. T. Abdala-Díaz, A. Cabello-Pasini, E. Pérez-Rodríguez, R. M. C. Álvarez and F. L. Figueroa, *Mar. Biol.*, 2006, **148**, 459–465.
76 B. E. Henry and K. V. Alstyne, *J. Phycol.*, 2004, **40**, 527–533.
77 A. Hemmi, A. Mäkinen, V. Jormalainen and T. Honkanen, *Aquat. Ecol.*, 2005, **39**, 201–211.
78 S. Rohde, M. Molis and M. Wahl, *J. Ecol.*, 2004, **92**, 1011–1018.
79 E. Macaya, E. Rothäusler, M. Thiel, M. Molis and M. Wahl, *J. Exp. Mar. Biol. Ecol.*, 2005, **325**, 214–227.
80 V. Jormalainen and T. Honkanen, *J. Evol. Biol.*, 2004, **17**, 807–820.
81 S. Connan, F. Goulard, V. Stiger, E. Deslandes and E. A. Gall, *Bot. Mar.*, 2004, **47**, 410–416.
82 M. N. Dethier, S. L. Williams and A. Freeman, *Ecol. Monogr.*, 2005, **75**, 403–418.
83 R. Koivikko, J. Loponen, T. Honkanen and V. Jormalainen, *J. Chem. Ecol.*, 2005, **31**, 195–212.
84 T. Shibata, S. Kawaguchi, Y. Hama, M. Inagaki, K. Yamaguchi and T. Nakamura, *J. Appl. Phycol.*, 2004, **16**, 291–296.
85 T. Shibata, K. Yamaguchi, K. Nagayama, S. Kawaguchi and T. Nakamura, *Eur. J. Phycol.*, 2002, **37**, 493–500.
86 T. Nakamura, K. Nagayama, K. Uchida and R. Tanaka, *Fish. Sci.*, 1996, **62**, 923–926.
87 U. H. Lüder and M. N. Clayton, *Planta*, 2004, **218**, 928–937.
88 E. M. Borell, A. Foggo and R. A. Coleman, *Oecologia*, 2004, **140**, 328–334.
89 G. Toth, O. Langhamer and H. Pavia, *Ecology*, 2005, **86**, 612–618.
90 H. Pavia, G. B. Toth and P. Åberg, *Ecology*, 2002, **83**, 891–897.
91 V. Jormalainen, T. Honkanen, R. Koivikko and J. Eränen, *Oikos*, 2003, **103**, 640–650.
92 A. Hemmi, T. Honkanen and V. Jormalainen, *Mar. Ecol.: Prog. Ser.*, 2004, **273**, 109–120.
93 M. S. Deal, M. E. Hay, D. Wilson and W. Fenical, *Oecologia*, 2003, **136**, 107–114.
94 J. Kubanek, S. E. Lester, W. Fenical and M. E. Hay, *Mar. Ecol.: Prog. Ser.*, 2004, **277**, 79–93.
95 A. Hemmi and V. Jormalainen, *Mar. Biol.*, 2004, **145**, 759–768.
96 S. Wilkstrom and H. Pavia, *Oecologia*, 2004, **138**, 223–230.
97 T. Honkanen and V. Jormalainen, *Oecologia*, 2005, **144**, 196–205.
98 J. Ceh, M. Molis, T. M. Dzeha and M. Wahl, *J. Phycol.*, 2005, **41**, 726–731.
99 K. Weidner, B. G. Lages, B. A. da Gama, M. Molis, M. Wahl and R. C. Pereira, *Mar. Ecol.: Prog. Ser.*, 2004, **283**, 113–125.
100 J. Maréchal, G. Culioli, C. Hellio, H. Thomas-Guyon, M. Callow, A. Clare and A. Ortalo-Magné, *J. Exp. Mar. Biol. Ecol.*, 2004, **313**, 47–62.
101 G. Culioli, A. Ortalo-Magné, M. Richou, R. Valls and L. Piovetti, *Biochem. Syst. Ecol.*, 2002, **30**, 61–64.
102 J. T. Wright, R. d. Nys, A. G. Poore and P. D. Steinberg, *Ecology*, 2004, **85**, 2946–2959.
103 J. T. Wright, S. A. Dworjanyn, C. N. Rogers, P. D. Steinberg, J. E. Williamson and A. G. B. Poore, *Mar. Ecol.: Prog. Ser.*, 2005, **298**, 143–156.
104 J. E. Williamson, D. G. Carson, R. de Nys and P. D. Steinberg, *Ecology*, 2004, **85**, 1355–1371.
105 R. L. Swanson, J. E. Williamson, R. de Nys, N. Kumar, M. P. Bucknall and P. D. Steinberg, *Biol. Bull.*, 2004, **206**, 161–172.
106 R. C. Pereira, B. A. da Gama, V. L. Teixeira and Y. Yoneshigue-Valentin, *Braz. J. Biol.*, 2003, **63**, 665–672.
107 V. Paul, E. Cruz-Rivera and R. W. Thacker, in *Marine chemical ecology*, eds. J. B. McClintock and B. J. Baker, CRC Press, Boca Raton, 2001, pp. 227–265.
108 B. A. P. Da Gama, R. C. Pereira, A. R. Soares, V. L. Teixeira and Y. Yoneshigue-Valentin, *Biofouling*, 2003, **19**, 161–169.
109 G. M. Nylund and H. Pavia, *Mar. Ecol.: Prog. Ser.*, 2005, **299**, 111–121.
110 C. Hellio, C. Simon-Colin, A. Clare and E. Deslandes, *Biofouling*, 2004, **20**, 139–145.
111 G. M. Nylund, G. Cervin, M. Hermansson and H. Pavia, *Mar. Ecol.: Prog. Ser.*, 2005, **302**, 27–36.
112 G. P. Weinberger, G. Pohnert, M.-L. Berndt, K. Bouarab, B. Kloareg and P. Potin, *J. Exp. Bot.*, 2005, **56**, 1317–1326.
113 A. Meinesz, T. Belsher, T. Thibaut, B. Antolic, K. B. Mustapha, C.-F. Boudouresque, D. Chiaverini, F. Cinelli, J.-M. Cottalorda, A. Djellouli, A. E. Abed, C. Orestano, A. M. Grau, L. Ivesa, A. Jaklin, H. Langar, E. Massulti-Pascual, A. Peirano, L. Tunesi, J. de Vaugelas, N. Zavodnik and A. Zuljevic, *Biol. Invasions*, 2001, **3**, 201–210.
114 O. Jousson, J. Pawlowski, L. Zaninetti, F. W. Zechman, F. Dini, G. D. Guiseppe, R. Woodfield and A. Meinesz, *Nature*, 2000, **408**, 157–158.
115 A. R. Davis, K. Benkendorff and D. W. Ward, *Mar. Biol.*, 2005, **146**, 859–868.
116 G. Pohnert and V. Jung, *Org. Lett.*, 2003, **5**, 5091–5093.
117 S. Adolph, V. Jung, J. Rattke and G. Pohnert, *Angew. Chem., Int. Ed.*, 2005, **44**, 2–4.
118 V. Jung and G. Pohnert, *Tetrahedron*, 2001, **57**, 7169–7172.
119 C. Ross, V. Vreeland, J. H. Waite and R. S. Jacobs, *J. Phycol.*, 2005, **41**, 46–54.
120 V. Smyrniotopoulos, D. Abatis, L. Tziveleka, C. Tsitsimpikou, V. Roussis, A. Loukis and C. Vagias, *J. Nat. Prod.*, 2003, **66**, 21–24.
121 V. J. Paul and W. Fenical, in *Bioorganic marine chemistry*, ed. P. J. Scheuer, Springer-Verlag, Berlin, 1987, vol. 1, pp. 1–29.
122 V. Jung, T. Thibaut, A. Meinesz and G. Pohnert, *J. Chem. Ecol.*, 2002, **28**, 2091–2105.
123 Q. Jin and S. Dong, *J. Exp. Mar. Biol. Ecol.*, 2003, **293**, 41–55.
124 Q. Jin, S. Dong and C. Wang, *Eur. J. Phycol.*, 2005, **40**, 31–37.
125 T. A. Nelson, D. J. Lee and B. C. Smith, *J. Phycol.*, 2003, **39**, 874–879.
126 P. Patel, M. E. Callow, I. Joint and J. A. Callow, *Environ. Microbiol.*, 2003, **5**, 338–349.
127 I. Joint, K. Tait, M. E. Callow, J. A. Callow, D. Milton, P. Williams and M. Cámara, *Science*, 2002, **298**, 1207.
128 Y. Matsuo, H. Imagawa, M. Nishizawa and Y. Shizuri, *Science*, 2005, **307**, 1598.
129 T. M. Arnold and N. M. Targett, *J. Chem. Ecol.*, 2002, **28**, 1919–1934.
130 P. R. Jensen, K. M. Jenkins, D. Porter and W. Fenical, *Appl. Environ. Microbiol.*, 1998, **64**, 1490–1496.
131 L. Steele, M. Caldwell, A. Boettcher and T. Arnold, *Mar. Ecol.: Prog. Ser.*, 2005, **303**, 123–131.
132 P. J. Bushmann and M. S. Ailstock, *J. Exp. Mar. Biol. Ecol.*, 2006, in press, DOI: 10.1016/j.jembe.2005.1010.1005.
133 J. W. Blunt, B. R. Copp, M. H. Munro, P. T. Northcote and M. R. Prinsep, *Nat. Prod. Rep.*, 2004, **21**, 1–49.
134 J. W. Blunt, B. R. Copp, M. H. Munro, P. T. Northcote and M. R. Prinsep, *Nat. Prod. Rep.*, 2005, **22**, 15–61.
135 J. C. Braekman and D. Daloze, *Phytochem. Rev.*, 2004, **3**, 275–283.
136 E. E. G. Clavico, G. Muricy, B. A. P. da Gama, D. Batista, C. R. R. Ventura and R. C. Pereira, *Mar. Biol.*, 2006, **148**, 479–488.
137 A. C. Jones, J. E. Blum and J. R. Pawlik, *J. Exp. Mar. Biol. Ecol.*, 2005, **322**, 67–81.
138 J. B. McClintock, C. D. Amsler, B. J. Baker and R. W. M. van Soest, *Integr. Comp. Biol.*, 2005, **45**, 359–368.
139 H. Lippert, K. Iken, C. Volk, M. Köck and E. Rachor, *J. Exp. Mar. Biol. Ecol.*, 2004, **310**, 131–146.

140 K. D. Walters and J. R. Pawlik, *Integr. Comp. Biol.*, 2005, **45**, 352–358.
141 B. H. Moon, B. J. Baker and J. B. McClintock, *J. Nat. Prod.*, 1998, **61**, 116–118.
142 B. Moon, Y. C. Park, J. B. McClintock and B. J. Baker, *Tetrahedron*, 2000, **56**, 9057–9062.
143 M. S. Hill, N. A. Lopez and K. A. Young, *Mar. Ecol.: Prog. Ser.*, 2005, **291**, 93–102.
144 M. Page, L. West, P. Northcote, C. Battershill and M. Kelly, *J. Chem. Ecol.*, 2005, **31**, 1161–1174.
145 R. Martí, A. Fontana, M. J. Uriz and G. Cimino, *J. Chem. Ecol.*, 2003, **29**, 1307–1318.
146 M. A. Becerro and V. J. Paul, *Mar. Ecol.: Prog. Ser.*, 2004, **280**, 115–128.
147 J. Piel, D. Hui, G. Wen, D. Butzke, M. Platzer, N. Fusetani and S. Matsunaga, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 16222–16227.
148 P. M. Flatt, J. T. Gautschi, R. W. Thacker, M. Musafija-Girt, P. Crews and W. H. Gerwick, *Mar. Biol.*, 2005, **147**, 761–774.
149 C. P. Ridley, P. R. Bergquist, M. K. Harper, D. J. Faulkner, J. Hooper and M. G. Haygood, *Chem. Biol.*, 2005, **12**, 397–406.
150 C. L. M. Vickery, C. D. Amsler and J. B. McClintock, *Antarctic J. U. S.*, 2004, **33**, 47–50.
151 W. E. Müller, M. Klemt, N. L. Thakur, H. C. Schröder, A. Aiello, M. D'Esposito, M. Menna and E. Fattorusso, *Mar. Biol.*, 2004, **144**, 19–29.
152 M. Wiens, B. Luckas, F. Brümmer, M. Shokry, A. Ammar, R. Steffen, R. Batel, B. Diehl-Seifert, H. C. Schröder and W. E. Müller, *Mar. Biol.*, 2003, **142**, 213–223.
153 M. W. Taylor, P. J. Schupp, I. Dahillöf, S. Kjelleberg and P. D. Steinberg, *Environ. Microbiol.*, 2004, **6**, 121–130.
154 C. Thoms, M. Horn, M. Wagner, U. Hentschel and P. Proksch, *Mar. Biol.*, 2003, **142**, 685–692.
155 O. O. Lee and P.-Y. Qian, *Aquat. Microb. Ecol.*, 2004, **34**, 11–21.
156 N. L. Thakur, A. C. Anil and W. E. Müller, *Aquat. Microb. Ecol.*, 2004, **37**, 295–304.
157 S. R. Kelly, E. Garo, P. R. Jensen, W. Fenical and J. R. Pawlik, *Aquat. Microb. Ecol.*, 2005, **40**, 191–203.
158 S. Dobretsov, H.-U. Dahms and P.-Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2004, **271**, 133–146.
159 S. Dobretsov, H.-U. Dahms, M. Y. Tsoi and P. Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2005, **297**, 119–129.
160 C. Hellio, M. Tsoukatou, J.-P. Maréchal, N. Aldred, C. Beaupoil, A. S. Clare, C. Vagias and V. Roussis, *Mar. Biotechnol.*, 2005, **7**, 297–305.
161 Y. Kitano, Y. Nogata, K. Shinshima, E. Yoshimura, K. Chiba, M. Tada and I. Sakaguchi, *Biofouling*, 2004, **20**, 93–100.
162 B. G. Lages, B. G. Fleury, C. E. L. Ferreira and R. C. Pereira, *J. Exp. Mar. Biol. Ecol.*, 2006, **328**, 127–135.
163 K. Iken and B. J. Baker, *J. Nat. Prod.*, 2003, **66**, 888–890.
164 A. P. Alker, K. Kim, D. H. Dube and C. D. Harvell, *Coral Reefs*, 2004, **23**, 397–405.
165 L. Mydlarz, R. S. Jacobs, J. Boehnlein and R. G. Kerr, *Chem. Biol.*, 2003, **10**, 1051–1056.
166 J. Boehnlein, L. Santiago-Vázquez and R. Kerr, *Mar. Ecol.: Prog. Ser.*, 2005, **303**, 105–111.
167 M. Puyana, G. Narvaez, A. Paz, O. Osorno and C. Duque, *J. Chem. Ecol.*, 2004, **30**, 1183–1201.
168 K. Koike, M. Jimbo, R. Sakai, M. Kaeriyama, K. Muramoto, T. Ogata, T. Maruyama and H. Kamiya, *Biol. Bull.*, 2004, **207**, 80–86.
169 C. P. Marquis, A. H. Baird, R. de Nys, C. Holmström and N. Koziumi, *Coral Reefs*, 2005, **24**, 248–253.
170 N. Fusetani, T. Toyoda, N. Asai, S. Matsunaga and T. Maruyama, *J. Nat. Prod.*, 1996, **59**, 796–797.
171 N. Alam, B. H. Bae, J. Hong, C. O. Lee, K. S. Im and J. H. Jung, *J. Nat. Prod.*, 2001, **64**, 1059–1063.
172 L. Harrington, K. Fabricius, G. De'ath and A. Negri, *Ecology*, 2004, **85**, 3428–3437.
173 J. B. McClintock, M. O. Amsler, C. D. Amsler, K. J. Southworth, C. Petrie and B. J. Baker, *Mar. Biol.*, 2004, **145**, 885–894.
174 I. Tarjuelo, S. López-Legentil, M. Codina and X. Turon, *Mar. Ecol.: Prog. Ser.*, 2002, **235**, 103–115.
175 S. López-Legentil, X. Turon and P. Schupp, *J. Exp. Mar. Biol. Ecol.*, 2006, in press, DOI: 10.1016/j.jembe.2005.1011.1002.
176 A. Murugan and M. S. Ramasamy, *Indian J. Mar. Sci.*, 2003, **32**, 162–164.
177 P. J. Bryan, J. B. McClintock, M. Slattery and D. P. Rittschof, *Biofouling*, 2003, **19**, 235–245.
178 S. López-Legentil, R. Dieckmann, N. Bontemps-Subielos, X. Turon and B. Banaigs, *Biochem. Syst. Ecol.*, 2005, **33**, 1107–1119.
179 S. López-Legentil and X. Turon, *Zool. Scr.*, 2005, **34**, 3–14.
180 X. Turon, S. López-Legentil and B. Banaigs, *Invertebr. Biol.*, 2005, **124**, 355–369.
181 E. W. Schmidt, J. T. Nelson, D. A. Rasko, S. Sudek, J. A. Eisen, M. G. Haygood and J. Ravel, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 7315–7320.
182 P. F. Long, W. C. Dunlap, C. N. Battershill and M. Jaspars, *ChemBioChem*, 2005, **6**, 1760–1765.
183 E. Hirose, K. Ohtsuka, M. Ishikura and T. Maruyama, *J. Mar. Biol. Assoc. U. K.*, 2004, **84**, 789–794.
184 N. Pelletier, *Mar. Biol.*, 2004, **145**, 1159–1165.
185 M. Hildebrand, L. E. Waggoner, G. E. Lim, K. H. Sharp, C. P. Ridley and M. G. Haygood, *Nat. Prod. Rep.*, 2004, **21**, 122–142.
186 N. Lopanik, N. Lindquist and N. Targett, *Oecologia*, 2004, **139**, 131–139.
187 N. Lopanik, K. Gustafson and N. Lindquist, *J. Nat. Prod.*, 2004, **67**, 1412–1414.
188 G. E. Lim and M. G. Haygood, *Appl. Environ. Microbiol.*, 2004, **70**, 4921–4929.
189 H.-U. Dahms, S. Dobretsov and P.-Y. Qian, *J. Exp. Mar. Biol. Ecol.*, 2004, **313**, 191–209.
190 E. Iyengar and C. Harvell, *Mar. Ecol.: Prog. Ser.*, 2002, **225**, 205–218.
191 N. Lindquist, P. Barber and J. B. Weisz, *Proc. R. Soc. London, Ser. B*, 2005, **272**, 1209–1216.
192 D. Rittschof and J. H. Cohen, *Peptides*, 2004, **25**, 1503–1516.
193 K. Berntsson, P. Jonsson, A. Larsson and S. Holdt, *Mar. Ecol.: Prog. Ser.*, 2004, **275**, 199–210.
194 P.-Y. Qian, V. Thiyagarajan, S. Lau and S. Cheung, *Aquat. Microb. Ecol.*, 2003, **33**, 225–237.
195 O. S. Hung, L. A. Gosselin, V. Thiyagarajan, R. S. Wu and P.-Y. Qian, *J. Exp. Mar. Biol. Ecol.*, 2005, **323**, 16–26.
196 J. S. Patil and A. C. Anil, *Mar. Ecol.: Prog. Ser.*, 2005, **301**, 231–245.
197 L. Khandeparker, A. C. Anil and S. Raghukumar, *Aquat. Microb. Ecol.*, 2002, **28**, 37–54.
198 C. L. Vogan, B. H. Maskrey, G. W. Taylor, S. Henry, C. R. Pace-Asciak, A. S. Clare and A. F. Rowley, *J. Exp. Mar. Biol. Ecol.*, 2003, **206**, 3219–3226.
199 E. J. Boone, A. A. Boettcher, T. D. Sherman and J. J. O'Brien, *Mar. Ecol.: Prog. Ser.*, 2003, **252**, 187–197.
200 Z. Pasternak, B. Blasius and A. Abelson, *J. Plankton Res.*, 2004, **26**, 487–493.
201 Z. Pasternak, B. Blasius, Y. Achituv and A. Abelson, *Proc. R. Soc. London, Ser. B*, 2004, **271**, 1745–1750.
202 J. van Montfrans, C. Ryer and R. Orth, *Mar. Ecol.: Prog. Ser.*, 2003, **260**, 209–217.
203 R. B. Forward, Jr., R. A. Tankersley, K. A. Smith and J. M. Welch, *Mar. Biol.*, 2003, **142**, 747–756.
204 P. Gebauer, K. Paschke and K. Anger, *J. Exp. Mar. Biol. Ecol.*, 2002, **268**, 1–12.
205 N. O'Connor and M. Judge, *Mar. Ecol.: Prog. Ser.*, 2004, **282**, 229–236.
206 E. Bagøien and T. Kiørboe, *Mar. Ecol.: Prog. Ser.*, 2005, **300**, 105–115.
207 J. H. Ting and T. W. Snell, *Mar. Biol.*, 2003, **143**, 1–8.
208 L. M. Herborg, M. G. Bentley, A. S. Clare and K. S. Last, *J. Exp. Mar. Biol. Ecol.*, 2006, **329**, 1–10.
209 C. E. Kicklighter, S. Shabani, P. M. Johnson and C. D. Derby, *Curr. Biol.*, 2005, **15**, 549–554.
210 B. Dalziel and E. G. Boulding, *J. Exp. Mar. Biol. Ecol.*, 2005, **317**, 25–35.
211 A. Marín and J. Ros, *Sci. Mar.*, 2004, **68**, 227–241.
212 A. Capper, I. R. Tibbetts, J. M. O'Neil and G. R. Shaw, *J. Exp. Mar. Biol. Ecol.*, 2006, in press, DOI: 10.1016/j.jembe.2005.1010.1009.
213 A. Capper, I. R. Tibbetts, J. M. O'Neil and G. R. Shaw, *J. Chem. Ecol.*, 2005, **31**, 1595–1606.
214 M. J. Huggett, R. de Nys, J. E. Williamson, M. Heasman and P. D. Steinberg, *Mar. Biol.*, 2005, **147**, 1155–1163.
215 M. G. Hadfield and M. A. R. Koehl, *Biol. Bull.*, 2004, **207**, 28–43.
216 H. P. Jacobsen and O. B. Stabell, *Oikos*, 2004, **104**, 43–50.
217 A. R. Mahon, C. D. Amsler, J. B. McClintock and B. J. Baker, *Polar Biol.*, 2002, **25**, 469–473.
218 S. G. Cheung, S. Lam, Q. F. Gao, K. K. Mak and P. K. Shin, *Mar. Biol.*, 2004, **144**, 675–684.

219 J. A. Riffell, P. J. Krug and R. K. Zimmer, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 4501–4506.
220 C. E. Kicklighter, C. R. Fisher and M. E. Hay, *Mar. Ecol.: Prog. Ser.*, 2004, **275**, 11–19.
221 T. Harder, C. Lam and P.-Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2002, **229**, 105–112.
222 C. Lam, T. Harder and P.-Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2003, **263**, 83–92.
223 C. Lam, T. Harder and P.-Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2005, **286**, 145–154.
224 T. Jin and P.-Y. Qian, *Mar. Ecol.: Prog. Ser.*, 2004, **267**, 209–218.
225 R. Sutton, E. Bolton, H. D. Bartels-Hardege, M. Eswards, D. J. Reish and J. D. Hardege, *J. Chem. Ecol.*, 2005, **31**, 1865–1876.
226 J. B. McClintock, A. R. Mahon, K. J. Peters, C. D. Amsler and B. J. Baker, *Antarctic Sci.*, 2003, **15**, 339–344.
227 S. P. Greer, K. B. Iken, J. B. McClintock and C. D. Amsler, *Biofouling*, 2003, **19**, 315–326.
228 K. Iken, S. P. Greer, C. D. Amsler and J. B. McClintock, *Biofouling*, 2003, **19**, 327–334.
229 J. E. Williamson, R. d. Nys, N. Kumar, D. G. Carson and P. D. Steinberg, *Biol. Bull.*, 2000, **198**, 332–345.
230 M. Nishazaki and J. Ackerman, *Limnol. Oceanogr.*, 2005, **50**, 354–362.
231 R. Brewer and B. Konar, *Mar. Biol.*, 2005, **147**, 789–795.
232 C. Gaymer, J. Himmelman and L. Johnson, *Mar. Ecol.: Prog. Ser.*, 2002, **232**, 149–162.
233 S. Santagata, *Biol. Bull.*, 2004, **207**, 103–115.
234 A. R. Mahon, C. D. Amsler, J. B. McClintock, M. O. Amsler and B. J. Baker, *J. Exp. Mar. Biol. Ecol.*, 2003, **290**, 197–210.
235 M. Schubert, P. Munday, M. J. Caley, G. P. Jones and L. E. Llewellyn, *Environ. Biol. Fishes*, 2003, **67**, 359–367.
236 P. L. Munday, M. Schubert, J. A. Baggio, G. P. Jones, M. J. Caley and A. S. Grutter, *J. Fish Biol.*, 2003, **62**, 976–981.
237 M. W. Davis, M. L. Spencer and M. L. Ottmar, *J. Exp. Mar. Biol. Ecol.*, 2006, **328**, 1–9.
238 J. K. Larson and M. I. McCormick, *Anim. Behav.*, 2005, **69**, 51–57.
239 K. J. Wright, D. M. Higgs, A. J. Belanger and J. M. Leis, *Mar. Biol.*, 2005, **147**, 1425–1434.
240 D. Lecchini, S. Planes and R. Galzin, *Behav. Ecol. Sociobiol.*, 2005, **58**, 18–26.
241 D. Lecchini, J. Shima, B. Banaigs and R. Galzin, *Oecologia*, 2005, **143**, 326–334.
242 H. Mitamura, N. Arai, W. Sakamoto, Y. Mitsunaga, H. Tanaka, Y. Mukai, K. Nakamura, M. Sasaki and Y. Yoneda, *J. Exp. Mar. Biol. Ecol.*, 2005, **322**, 123–134.
243 T. Dempster and M. Kingsford, *Mar. Ecol.: Prog. Ser.*, 2003, **258**, 213–222.
244 J. C. Hagelin, I. L. Jones and L. E. L. Rasmussen, *Proc. R. Soc. London, Ser. B*, 2003, **270**, 1323–1329.
245 H. D. Douglas, III, J. E. Co, T. H. Jones and W. E. Conner, *Naturwissenschaften*, 2001, **88**, 330–332.
246 H. D. Douglas, III, J. E. Co, T. H. Jones and W. E. Conner, *J. Chem. Ecol.*, 2004, **30**, 1921–1935.
247 H. D. Douglas, III, J. R. Malenke and D. H. Clayton, *J. Ornithol.*, 2005, **146**, 111–115.
248 H. D. Douglas III, J. E. Co, T. H. Jones, W. E. Conner and J. F. Day, *J. Med. Entomol.*, 2005, **42**, 647–651.
249 S. Rochfort, *J. Nat. Prod.*, 2005, **68**, 1813–1820.