THE H. T. ODUM SYNTHESIS ESSAY

New Trends in Marine Chemical Ecology

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ABSTRACT: This essay is the outcome of a colloquium convened in November 2005 at the Benthos Laboratory of the Stazione Zoologica Anton Dohrn in Ischia, Italy, on chemical ecology and the role of secondary metabolites in the structuring and functioning of marine biodiversity. The participants of the workshop are part of the European Network of Excellence MarBEF (Marine Biodiversity and Ecosystem Function), a consortium of 56 European marine institutes to integrate and disseminate knowledge and expertise on marine biodiversity. Here we review some of the new trends and emerging topics in marine chemical ecology. The first section deals with microbial chemical interactions. Microbes communicate with each other using diffusible molecules such as N-acylhomoserine lactones (AHL). These are regulators in cell-density-dependent gene regulation (quorum sensing) controlling microbial processes. In chemical interactions with higher organisms, microbes can act either as harmful pathogens that are repelled by the host's chemical defense or as beneficial symbionts. These symbionts are sometimes the true producers of the host's secondary metabolites that have defensive and protective functions for their hosts. We also describe how allelochemicals can shape phytoplankton communities by regulating competition for available resources, and also interactions among individuals of the same species. Compounds such as the diatom-derived unsaturated aldehydes have been demonstrated to act as infochemicals, and they possibly function as a diffusible bloom-termination signal that triggers an active cell death and bloom termination at sea. The same molecules have also been shown to interfere with the reproductive capacity of grazing animals deterring future generations of potential predators. Such compounds differ from those that act as feeding deterrents since they do not target the predator but its offspring. Many of the neurotoxins produced by dinoflagellates act as feeding deterrents, and laboratory experiments have shown that ingestion of these algae by some microzooplankton and macrozooplankton can cause acute responses such as death, incapacitation, altered swimming behavior, and reduced fecundity and egg-hatching success. These effects may rarely occur in nature because of low individual grazing rates on dinoflagellate cells and grazing on other food sources such as microflagellates and diatoms. We also consider the nutritional component of marine plant-herbivore interactions, especially in the plankton, and the information available on the effects of growing conditions of algae on the production of toxic metabolites. Species producing saxitoxins seem to consistently produce the highest amounts of toxins (on a per cell basis) in the exponential phase of growth, and there is a decrease in their production under nitrogen, but not under phosphorus stress, where the production actually increases. We try to explain the circumstances under which organisms defend themselves chemically and argue that the most likely explanatory model for the production of secondary metabolites used for defense in planktonic organisms is the carbon nutrient balance hypothesis, which predicts that most algae produce their toxins mainly under conditions where carbon is in excess and nitrogen (or other nutrients) is limiting. We also discuss chemically mediated macroalgal-herbivore interactions in the benthos and the large variation in concentration of seaweed defense metabolites at different spatial and temporal scales. Seaweeds have been shown to produce a large variety of secondary metabolites with highly variable chemical structures such as terpenoids, acetogenins, amino acid derivates, and polyphenols. Many of these compounds probably have multiple simultaneous functions for the seaweeds and can act as allelopathic, antimicrobial, and antifouling or ultraviolet-screening agents, as well as herbivore deterrents. We also provide examples of interactions between marine benthic invertebrates, especially sponges, molluscs, and cnidarians, that are mediated by specific secondary metabolites and discuss the role of these in shaping benthic communities.

Introduction

Plants and animals in the sea produce a variety of different, often unique, molecules that serve as protection against enemies or that are of vital importance for feeding and reproduction. These chemicals are referred to as secondary metabolites or natural products and are not directly involved in primary metabolism. They differ from the more prevalent macromolecules, such as proteins and

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nucleic acids, that make up the basic machinery of life. Often secondary metabolites constitute a very small fraction of the total biomass of an organism (Cannell 1998), and it is not always clear what biological role these compounds play. In recent years it has become increasingly apparent that secondary metabolites have important ecological functions and may at times contribute as much as primary metabolites to the survival of the producing organism. The science that considers chemical interactions between organisms and their environment is termed chemical ecology, one of the fastest growing and rapidly evolving environmental sub-disciplines.

To date, over 16,000 new compounds have been isolated from sponges, ascidians, soft corals, seaweeds, marine microbes, and many other benthic and pelagic organisms, with more being discovered daily (Bhakuni and Rawat 2005). Some of these products may find important biotechnological applications in biomedical research and in the agriculture, aquaculture, and chemical industries (see Haefner 2003 for recent review on drug development from marine natural products). Aside from their biotechnological applications, secondary metabolites are now believed to be at the basis of ecological specialization by affecting species' distribution patterns and community organization, as well as determining feeding patterns and maintenance of biodiversity through resource and habitat partitioning (McClintock and Baker 2001). The organism has to pay a price for this ecological advantage. The biochemical pathways that generate marine natural products are often complex and it is generally believed that a significant amount of metabolic energy is expended to generate their production that could otherwise have been directed to growth or reproduction (e.g., Cronin 2001). The high energetic cost of producing and maintaining potentially toxic compounds must be compensated for by a defensive benefit to the producing organism. There are many theories as to why marine organisms produce secondary metabolites. Early theories suggested that these were chemical waste products or otherwise functionless metabolites of primary metabolism overflow (see Harper et al. 2001 and Feeney 1992 for reviews on the evolution of chemical ecology). Others later concluded that natural products have evolved under the pressure of natural selection to bind to specific receptors and that they represent ecological responses of organisms to their environment (e.g., Williams et al. 1989). Marine organisms are under intense competitive pressure for space, light, and nutrients. It is not surprising that they have developed a range of defense mechanisms including physical (e.g., tough protective silica surfaces as in the planktonic

diatoms, Hamm et al. 2003) and chemical defenses to ensure survival.

The bulk of research on chemically mediated interactions has focused on predator-prey interactions, especially in the benthic realm, and there is now a considerable amount known about feeding preferences and deterrent molecules in macro-benthic organisms. Much less is known on the chemical ecology of planktonic marine organisms. We also know very little as to why marine invertebrates avoid certain compounds, and few studies have assessed what happens when secondary metabolites are consumed. Few studies have addressed allelopathic interactions in the marine environment and the function of secondary metabolites as defenses against pathogens or other competing plants. In this essay we focus on some of these interactions and several others, especially in regard to some of the newer emerging areas of research in this field.

Several excellent reviews already exist on marine chemical ecology so this paper does not attempt to provide a comprehensive overview, but rather to illustrate some examples of the diversity and importance of chemically mediated interactions involving marine organisms. We focus on a few key topics in this field, such as bacterial quorum sensing and the defensive and protective functions of sponge-associated bacterial interactions, which have indicated the microbial origin of many of these natural products. Another emerging topic we consider is the potential role of secondary metabolites and allelopathy in controlling microalgal biology, species successions during bloom development, and competition and communication within the phytoplankton. In another section of the paper we discuss recent advances in phytoplankton-zooplankton chemical interactions and report on the discovery of diatom metabolites that deter grazers, such as copepods, by reducing their reproductive potential. This model is new for the marine environment where most of the known negative plant-animal interactions are related to feeding deterrence and poisoning and death, but never to reproductive failure, and represents a mechanism allowing phytoplankton blooms to persist when grazing pressure would otherwise have caused them to crash. We also consider the nutritional component of marine plant-herbivore interactions in which we briefly review the information available on the effects of growing conditions of algae on the production of toxic metabolites and discuss theories concerning the circumstances under which organisms defend themselves chemically. We also discuss chemically mediated macroalgal-herbivore interactions and the effects of macroalgal defenses on herbivore fitness and population dynamics, as well

as on ecosystem functioning. We provide selected examples of interactions between marine benthic invertebrates, especially sponges, molluscs, and cnidarians, that are mediated by specific secondary metabolites and discuss the role of these in shaping benthic communities.

BACTERIAL CELL-TO-CELL COMMUNICATION AND INTERACTIONS WITH EUKARYOTES

Microbes sense their environment via cell-associated and diffusible molecules such as N-acylhomoserine lactones (AHL) that are constantly produced by many bacteria and diffuse through membranes into the surrounding environment. Beyond a certain cell density of the bacterial population (varying between 10^5 and 10^{11} cells ml^{-1}) and corresponding concentrations of AHLs, a threshold or quorum is reached, and expression of target genes is initiated, e.g., the proteins for light emission in luminous bacteria or pathogenic factors that cause disease. The discovery that bacteria communicate with each other using signal molecules has changed our way of perceiving single cell organisms and interspecies communication and information transfer. Quorum sensing typically controls processes, such as swarming (coordinated movement), virulence (coordinated attack), or conjugation (gene transfer between cells), that require high cell densities for success and that are essential for the survival of the producing organisms. For quorum sensing, AHLs are the most intensively studied class of mediators in cell-density-dependent gene regulation (Pappas et al. 2004; Visick and Fuqua 2005; Keller and Surrette 2006) and have been found in bacterial biofilms or on particles and solid surfaces (Gram et al. 2002; Parsek and Fuqua 2004). Surface sensing via AHLs of bacterial biofilms was shown to be the initial step in the settling of the intertidal green macroalga *Ulva*, formerly *Enteromorpha* (Tait et al. 2005; Wheeler et al. 2006), demonstrating for the first time, how marine eukaryotes use signals from prokaryotes.

Eukaryotes have also evolved secondary metabolites with inhibiting activities to avoid pathogenic attacks by the concerted action of many bacteria. The unicellular freshwater alga *Chlamydomonas reinhardtii* produces mimicking, but so far uncharacterized, AHL-interfering metabolites (Teplitski et al. 2004), whereas the red alga *Delisia pulchra* synthesizes halogenated furanones (Manefield et al. 2002) and the North Sea bryozoan *Flustra foliacea* bromo-tryptamine-based alkaloids (Peters et al. 2003). Anti-biofouling compounds may also act on quorum sensing (Rasmussen and Givskov 2006) and a simple screening protocol for antagonists is now available (McLean et al. 2004). AHL-based interactions may have profound effects on biodiversity and

ecosystem functioning. Successfully invading species need to develop resistance to the local population of microbial metabolites. In Tasmania, the introduced toxic dinoflagellate *Gymnodinium catenatum* was found to be more sensitive to algicidal bacteria than indigenous *Gymnodinium* sp. (Skerratt et al. 2002). Future studies will reveal more examples of microbe-eukaryote interactions and their importance for ecosystem functioning.

Marine animals and plants live in close association with microorganisms and their body surfaces are inevitably colonized by epibiotic microbes; some marine animals harbor microorganism within their digestive tracts or even within tissues and cells. Such interactions are complex and reach from harmful diseases to symbioses of mutual benefit (Steinert et al. 2000). Secondary metabolites can act as a defense strategy against unwanted colonization (infection) by microbes. Sessile invertebrates, such as corals, sponges, and ascidians, produce an astonishing variety of antimicrobial compounds (Paul and Puglisi 2004), which help to control surface colonization (Dobretsov et al. 2005; Kelly et al. 2005). Many of these animals, especially sponges, are permanently associated with specific microbes (Hentschel et al. 2003; Taylor et al. 2004), which are obviously not harmed by the antimicrobial secondary metabolites. Associated microorganisms have recently been shown to be involved in the synthesis of numerous metabolites (Hildebrand et al. 2004).

Bacteria associated with marine invertebrates or seaweeds more often show antimicrobial activities than those isolated from seawater and sediment (Zheng et al. 2005). In close associations and symbioses, the actual producer of the secondary metabolite is difficult to distinguish, because most symbiotic microbes are not viable outside their host (Olson et al. 2000). Localization of secondary metabolites to specific cell types within the host is of limited information since the site of synthesis may not be the site of storage. Numerous studies demonstrate secondary metabolite production by symbionts such as the synthesis of the bicyclic glycopeptide theopalauamide by an associated delta-proterobacterium in the sponge *Theonella swinhoei* (Schmidt et al. 2000), the synthesis of bryostatin by bacterial symbionts in the bryozoa *Bugula neritina* (Davidson et al. 2001), or the antimicrobial activity of different bacterial strains isolated from the sponges *Aplysina aerophoba* and *A. cavernicola* (Plate A; Hentschel et al. 2001).

Marine invertebrates have evolved mechanisms that enable them to distinguish between beneficial and detrimental bacteria. Secondary metabolites act as a controlling factor in this host-microbe interaction. Sponge-associated microbial communities most likely represent a mixture of microbes
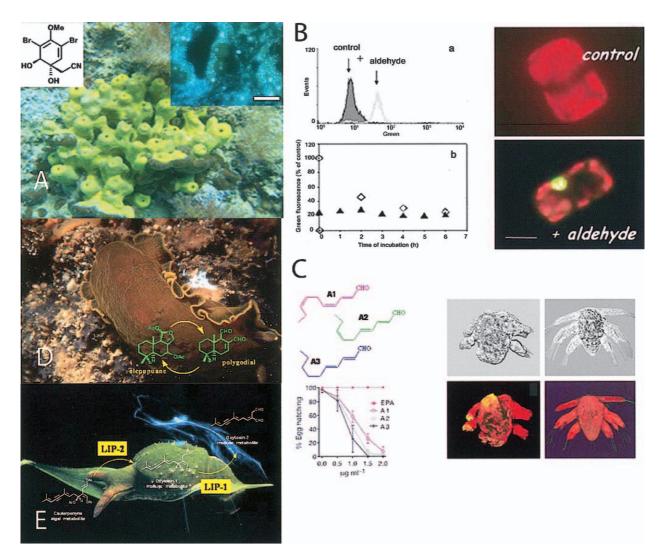
Plate A: The Mediterranean sponge *Aplysina aerophoba* (large image) produces antimicrobial and antitumor compounds. The structure of the brominated alkaloid aeroplysinin-1 (small image left) is given as an example. The tissue of this sponge is densely populated by associated microbes. DAPI staining of a tissue section (small image right; scale bar = 20 μm) visualizes these microbes as small blue dots, in contrast to nuclei of sponge cells, which appear as larger dots. Associated sponge microbes are often involved in the production of bioactive secondary metabolites. B: Diatoms produce unsaturated aldehydes that strongly effect grazer fitness. These compounds are also involved in stress signaling between diatom cells using nitrogen oxide (NO) as a mediator. Panel a: Flow cytometric detection of NO upon exposure to aldehydes in the diatom *Phaeodactylum tricornutum*. Green is green fluorescence from the stain DAF-FM as in Vardi et al. (2006). The signal elicited by aldehydes triggers inhibition of cell growth. Panel b: Decrease in metabolic activity as detected by the enzymatic cleavage of Fluorescein Diacetate. Green is green fluorescence from fluorescein, liberated after cleavage by intracellular esterases. Cell death follows with a mechanism closely resembling apoptosis (photos, upper panel, control, lower panel, TUNEL-positive *Thalassiosira weissflogii* upon exposure to the aldehyde decadienal). Green fluorescence of the nucleus derives from labeling of DNA fragments that result from an active mechanism of cell death. Scale bar = 5 μm. C: Effect of the diatom-derived unsaturated aldehydes 2-trans-4-cis-decatrienal (A1), 2-trans-4-trans-7-cis-decatrienal (A2), and 2-trans-4-trans-decadienal (A3) on copepod egg hatching success (lower left panel) compared to the control fatty acid eicosapentaenoic acid (EPA; from Miralto et al. 1999). Right panel shows abnormal nauplius spawned by a copepod female fed a diet of the aldehyde-producing diatom *Skeletonema costatum* (left) compared to control nauplius spawned by a female fed the dinoflagellate *Prorocentrum minimum* (right). Abnormal nauplius has deformed limbs marked positively for TUNEL staining (yellow) indicating apoptotic dead tissues compared to control (from Ianora et al. 2004). D: Dendrodorid nudibranchs, such as those of the genera *Dendrodoris* or *Doriopsilla*, are defended by the antifeedant dialdehyde, polygodial. This compound is not present in a free form in the mollusc but is released on demand by enzymatic conversion of an inactive tricyclic form named olepupuane. The metabolic equilibrium between polygodial-oleppuane allows control of the concentration of the toxic metabolite in the tissue of the molluscs. Figure provided by Ernesto Mollo. E: The Mediterranean mollusc *Oxynoe olivacea* lives in strict association with algae of the genus *Caulerpa* from which it obtains protection from potential predators. Although the capability of this mollusc to feed upon the alga is very well documented, chemical studies have shown that no caulerpenyne, the major algal secondary metabolite, is detectable in the body of the invertebrate. The mantle and mucus of *O. olivacea* are rich in two aldehydes, oxytoxin-1 and oxytoxin-2, both derived from the algal metabolite by two distinct

acquired from the water column during the host's life time and those that are inherited from the parental generation and that may have accompanied their host over evolutionary times (Hentschel et al. 2002). For different symbiont-bearing marine invertebrates, vertical transmission of symbionts to the offspring has been proven by the presence of symbionts in reproductive cells and larvae (Sipe et al. 2000; Ereskovsky et al. 2005). Recent research suggests that vertical symbiont transmission may be reflected by highly co-evolved host-symbiont associations (Peek et al. 1998).

Microbes play a double role in chemical interactions with higher organisms. They can be harmful pathogens that are repelled by chemical defenses or they may be useful symbionts. Microsymbionts are the secret passengers through evolutionary times, interacting with their hosts by chemical communication, and may in some cases even be the true producers of their host's secondary metabolites.

ALLELOCHEMICALS AND INFOCHEMICALS IN PHYTOPLANKTON

Evidence is accumulating that secondary metabolites in phytoplankton also regulate and control algal biology, species succession, competition, and communication (Legrand et al. 2003). Chemical interactions are very well known and studied in terrestrial ecosystems (Inderjit and Duke 2003), but studies in aquatic systems have been biased by technical difficulties, mainly arising from dilution in the water medium and physical constraints such as viscosity or shear forces (Wolfe 2000). In aquatic systems there is a broader diversity of species and chemical compounds than in terrestrial ecosystems (McClintock and Baker 2001). Nothing is known on quantitative interactions with hydrodynamic, chemical, biological, and molecular factors at different time and space scales. Another drawback is the often missing chemical identification of the compounds produced and of their biosynthetic pathways. As a result of these difficulties, very few ecological models include allelochemistry (An et al. 1993; Mukhopadhyay et al. 1998), but it is evident that such an important process should be included in future models that consider species interactions (Wolfe 2000; Legrand et al. 2003).

The production of allelochemicals confers an adaptive advantage to the producing species, affecting growth and physiological performance of competitor species (Wolfe 2000). Allelochemistry is likely to affect species succession, in addition to traditional abiotic and biotic factors. This has been demonstrated for bacteria (Long and Azam 2001; Kim et al. 1998), cyanobacteria in lakes (Keating 1977, 1978) or at sea (Flores and Wolk 1986; Suikkanen et al. 2004, 2005), dinoflagellates (Sukenik et al. 2002; Tillman and John 2002; Kubanek et al. 2005), and diatoms in coastal environments (Subba Rao et al. 1995). In many of the previous examples, the production of allelochemicals has been shown to be the determinant for bloom development, dynamics, and fate. Rengefors and Legrand (2001) showed an allelopathic interaction between a freshwater dinoflagellate producing an algicidal compound inhibiting the growth of a co-occurring cryptophyte. In the marine environment, the exclusion of other phytoplankton species by the toxic haptophyte *Chrysochromulina polylepis* and its success as a bloom-forming species has been attributed, among other factors, to the production of allelochemicals (Schmidt and Hansen 2001). Another toxic haptophyte, *Prymnesium parvum*, is able to modify the structure of phytoplankton communities by using chemical cues (Fistarol et al. 2003). The toxins used to kill or deter the predators are not always the same molecules that affect competitor species. The allelochemical defense mechanism of *Alexandrium* spp. has been shown to be independent of PSP-toxin content (Tillmann and John 2002). There is no evidence of an allelopathic role for the potent toxin okadaic acid in ciguatera dinoflagellates (Sugg and VanDolah 1999), and domoic acid, which is a toxin released by some diatom species, has been shown to be used by diatoms themselves as a functional component of a high-affinity iron acquisition system (Wells et al. 2005).

A recent line of research is highlighting the role of secondary metabolites as information molecules, used for cell-to-cell communication (Steinke et al. 2002). This is the case for diatom unsaturated aldehydes, which are involved in a stress surveillance mechanism based on fluctuations in calcium and nitric oxide levels (Vardi et al. 2006). According to these authors, when stress conditions during a bloom and cell lysis rates increase, aldehyde concentrations could exceed a certain threshold, and possibly function as a diffusible bloom-termination signal that triggers an active cell death. Diatom-derived aldehydes may also have an allelopathic role, since they have been shown to affect growth and physiological performance of diatoms and other phytoplankton species (Casotti et al.

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hydrolytic enzymes, LIP-1 and LIP-2. The enzymatic transformation of caulerpenyne to oxytoxin-1 and oxytoxin-2 renders oxytoxin-2 1,000 times more potent than caulerpenyne as a fish deterrent. Figure provided by Ernesto Mollo.

2005; Casotti and Ribalet unpublished data), inducing multicellular-like behavior in clonal populations (Plate B). Recently a novel class of oxylipins (oxygenase-mediated oxygenated compounds) based on C_{16} polyunsaturated fatty acids in diatoms has been described (d'Ippolito et al. 2006), suggesting that these new compounds may also be involved in signaling or allelopathy.

Abiotic factors influence the production and accumulation of allelochemicals in freshwater and marine species, by directly affecting their physiological status. Although thorough information is often missing due to the lack of information on the chemical nature and synthetic pathways of many allelochemicals, there are examples of enhancement of their effect upon, for example, nutrient limitation, pH variations, temperature increase, and growth phase. In contrast, the physiological conditions of the target species strongly determine their degree of resistance to allelochemicals. Nutrient-limited cultures of the diatom *Thalassiosira weissflogii* are more sensitive to filtrates of *P. parvum* than nutrient-replete cultures (Fistarol et al. 2005). This suggests that competition in nutrient-limited environments may be dramatically affected by allelochemical production, and that it may represent a determinant in species succession, e.g., at the end of a bloom. Defense responses of target organisms to low doses of chemical cues include sexuality or encystment, as observed in the case of some dinoflagellates (Fistarol et al. 2004).

Chemical cues may also benefit receivers other than those intended and such cues, if beneficial to the receiver, are termed kairomones, while if they are beneficial to both the sender and the receiver are termed synomones (Dicke and Sabelis 1988). The study and the mode of action of such by-products of deterrence are unknown in marine systems, but compounds involved in the dimethyl-sulphonoproprionate (DMSP)-dimethylsulphide (DMS)-acrylate system have potential to be kairomones (Wolfe 2000 and references therein). Tritrophic interactions, in which chemical signals emitted by wounded prey attract their predator's enemies are also known in aquatic systems and are mediated by biogenic volatile compounds (for a review see Steinke et al. 2002). All these mechanisms coexist at sea and represent a key for understanding many processes, from species competition to community composition, to bloom development and dynamics, and even evolution (Lewis 1986).

There is increasing awareness of the importance of allelochemistry in marine ecosystem functioning and biodiversity. Apart from the chemical identification of the molecules involved, future challenges include the identification of biosynthetic pathways in defense mechanisms in phytoplankton and the understanding of the transcriptional changes and signal transduction mechanisms occurring during biotic interactions. In this context, genomic approaches are very promising.

PHYTOPLANKTON-ZOOPLANKTON CHEMICAL INTERACTIONS

In terms of biodiversity, the phytoplankton are among the most diverse organisms in the sea, and due to the complexity of the habitat in which they live, these organisms have evolved some of the most unique metabolites ever isolated in nature. Many of these are potent neurotoxins that can make their way up the marine food chain and are responsible for massive fish kills, both wild and farmed, as well as the deaths of many aquatic birds and mammals, including whales and sea lions (Scholin et al. 2000). In humans, consumption of shellfish containing high levels of toxins can induce at least four types of pathologies: paralytic, neurotoxic, diarrhetic, and amnesic shellfish poisoning. Records of human poisoning by at least two of these syndromes date back hundreds of years. The discovery and characterization of the molecules responsible for this biological activity are quite recent. Okadaic acid was the first toxin isolated from a marine dinoflagellate even though it had previously been found in the sponge *Halichondria okadai*. The toxin was identified from a Tahitian strain of the dinoflagellate *Prorocentrum lima* and a derivative of this toxin, dinophystoxin, was later isolated from temperate species of the dinoflagellate genus *Dinophysis*. Both okadaic acid and dinophystoxin are associated with episodes of diarrhetic shellfish poisoning in humans (see Cembella 2003 and Bhakuni and Rawat 2005 for recent reviews on dinoflagellate toxins).

The brevetoxins are a family of at least 9 compounds that are sodium channel activators. They cause repetitive depolarization of nerve membranes with an increase in the influx of sodium ions that ultimately deplete cellular reserves of acetylcholine at the synapses. Another group of toxins, the saxitoxins, include at least a dozen compounds that cause the opposite effect to the brevetoxins. They bind to the sodium channels and specifically block sodium permeability of the nerve membrane, ultimately causing paralysis and respiratory failure in humans. The differences in structure of the various saxitoxins alter the rates at which they bind to and depart from the binding site on the sodium channel. Yessotoxin, isolated from the dinoflagellate *Ptychodiscus brevis* ($=$ *Gymnodinium breve*), partially resembles the brevetoxins in structure and toxicity. Ciguatera poisoning produces various symptoms such as cardiovascular, gastrointestinal, sensory, and motor disturbances. No

effective drug is currently known for therapy. The toxin is produced by a benthic dinoflagellate, *Gambierdiscus toxicus,* and is transmitted to fish along the marine food chain. *G. toxicus* also produces a more polar toxin, maitoxin, which together with ciguatoxin, are probably the most potent neurotoxins ever isolated from marine organisms.

The putative function of dinoflagellate toxins is often assumed to be chemical defense but although acute responses such as death, incapacitation, altered swimming behavior, and reduced fecundity and egg-hatching success have been reported after ingestion of highly toxic algae by microzooplankton and macrozooplankton in laboratory experiments (reviewed by Turner et al. 1998), these effects may rarely occur in nature because of low individual grazing rates on dinoflagellate cells and grazing on other food sources such as microflagellates and diatoms (Turner and Borkman 2005).

There is also high species-specific variability in the effects on grazing and selectivity of consumers, with effects ranging from severe physical incapacitation and death in some species (Turriff et al. 1995) to no apparent physiological effects in others (Teegarden and Cembella 1996). This variability indicates that some species are more resistant to these compounds and may have evolved counterdefenses and detoxification mechanisms in the continuing arms race between plant defenses and animal responses. Colin and Dam (2003, 2005) have recently shown that when two geographically distant populations of the copepod *Acartia hudsonica* were reared on the toxic dinoflagellate *Alexandrium fundyense,* the one that had not experienced recurrent blooms of the toxic algae had lower somatic growth, size at maturity, egg production, and survival, compared to the other population that showed no effects on these life history parameters. Some copepod species also seem capable of concentrating toxins in their body tissues (Tester et al. 2000; Doucette et al. 2005), as occurs in bivalve molluscs, and ingested toxins may then act as defenses to deter predation by fish and other zooplanktivorous consumers (Ianora et al. 2004b). Many benthic invertebrates are capable of sequestering compounds from the food they consume and using them as defensive molecules against predators (Cimino and Ghiselin 2001). There is no reason why this should not also occur in the plankton.

Another major algal class in freshwater and marine environments is the diatoms, with over 1,600 recognized species. Traditionally, diatoms have been considered an optimum food for zooplankton larval growth and the transfer of energy through the food chain to top carnivores. Until recently, diatoms were not known to produce toxins, but in 1987 there was an unprecedented

episode of human shellfish poisoning that caused three deaths and 107 cases of gastrointestinal and neurological problems due to consumption of mussels from Prince Edward Island in Canada. The causative agent responsible for these disorders was domoic acid produced by the diatom *Pseudonitzschia australis.* The toxicity of domoic acid is due to the fact that it mimics the excitatory activity of the neurotransmitter L-glutamic acid inducing destructive neuronal depolarization and successive degeneration of the hippocampus of the brain. In severe cases of this pathology, known as amnesic shellfish poisoning, victims show permanent loss of recent memory. Domoic acid has not been shown to induce negative effects on planktonic organisms that consume *P. australis* and the natural function of domoic acid remains elusive (Wells et al. 2005).

Diatoms also produce another interesting class of molecules that induce abortions or congenital malformations in the animals that ingest them. These teratogenic compounds, belonging to the complex class of oxylipins, including polyunsaturated aldehydes, are produced by the diatom cells from precursor membrane-bound lipids (Pohnert 2000; d'Ippolito et al. 2004; Cutignano et al. 2006). By definition, teratogens are substances that induce structural malformations in the offspring of organisms exposed to them during gestation. The structural malformations that can occur include fetal growth retardation, embryo and fetal mortality, and functional impairment due to malformed limbs or organs. Such compounds were unknown in marine plants even though they are rather common in higher terrestrial plants.

Diatom-derived unsaturated aldehydes were first isolated by Miralto et al. (1999) who showed, in vitro, that they reduced copepod hatching success, cleavage of sea urchin embryos, and proliferation of human adenocarcinoma cells. The same authors showed that diatoms also modified hatching success in the field in February 1997 and 1998 during two major diatom blooms in the North Adriatic Sea. Egg viability in these periods was only 12% and 24%, respectively, of the total number of eggs produced, compared to 90% after the bloom in June. Deleterious effects on reproductive processes have since then been demonstrated in other organisms such as echinoderms and polychaetes (e.g., Caldwell et al. 2002), ascidians (Tosti et al. 2003), cladocerans (Carotenuto et al. 2005), and molluscs (Adolph et al. 2003). Toxic effects have also been demonstrated on diatom cells themselves (Casotti et al. 2005; Vardi et al. 2006). Tosti et al. (2003) have shown that decadienal inhibited the fertilization current that is generated in oocytes in *Ciona intestinalis* upon interaction with the spermatozoan; this inhibition was dose-dependent and accompa-

nied by inhibition of the voltage-gated calcium current activity of the plasma membrane. Decadie-nal affected actin reorganization, which is responsible for the segregation of cell lineages leading to altered regulatory mechanisms during mitotic cell cycle progression. Recent studies indicate that diatom-derived aldehydes are also apoptogenic inducers through the activation of specific caspases that lead to the enzymatic breakdown of DNA (Romano et al. 2003). Aldehydes may be sequestered during oocyte development and be passed maternally to the embryo, or may act directly on embryos. By whichever route, the timing of reproduction in relation to toxic diatom abundance will have important consequences for invertebrate recruitment (Ianora et al. 2003; Caldwell et al. 2004). This biological model is new for the marine environment since most of the known negative plant-animal interactions are related to feeding deterrence and poisoning or death, but never to reproductive failure.

The discovery of an enzyme cascade leading to the production of volatile biologically active oxylipins in marine phytoplankton is rather new (see review by Pohnert 2004, 2005) even though the oxidative cleavage of fatty acids to form similar defensive compounds is well known in higher terrestrial plants (Blée 1998) and freshwater microalgae (Juttner and Durst 1997). When diatoms are crushed, as occurs during grazing by copepods, lipolytic enzymes cleave membrane-bound lipids to liberate eicosanoic (C_{20}) and hexadecanoic (C_{16}) fatty acids that are then oxidized to yield C_7, C_8, or C_{10} polyunsaturated aldehydes (d'Ippolito et al. 2004; Cutignano et al. 2006). Pohnert (2000) termed this a wound-activated defense, similar to higher terrestrial plants triggered by grazing to avoid self-toxicity (Casotti et al. 2005).

Miralto et al. (1999) suggested that diatom aldehydes and other metabolites with cytotoxic effects produced by marine plants may act as birth control compounds that interfere with the reproductive capacity of grazing animals deterring future generations of potential predators (Plate C; Ianora et al. 2004a). Although the effect of such toxins is less catastrophic than others that induce acute poisoning episodes leading to death of predatory animals, they are nonetheless insidious occurring through abortions, birth defects, poor development rates, and high mortality. The end result is that the toxins, whether straightforward or indirect, are assumed to eliminate a predator with no co-evolutionary relationship.

The production of teratogens in diatoms poses interesting questions on their ecological role. The most likely explanation for the production of these compounds is that they evolved to repel grazers. In this case, the mechanism of chemical defense functions by reducing grazing effects of subsequent generations of copepods. Such compounds differ from those that act as feeding deterrents, the purpose of which is not to intoxicate the predator but discourage further consumption, or those that lead to physical incapacitation such as paralysis and death of the predator. Cembella (2003, p. 425) describes metabolites such as diatom-derived aldehydes as stealth compounds of low acute toxicity to adult predators that lead to postdigestive reduction in fecundity or depressed viability of gametes, which he terms the ''kill the children'' selection. This type of feeding deterrence would not protect the individual ingested cells but the community as a whole and the defense compounds would not target the predator but its offspring. In the end, grazing pressure would be reduced allowing blooms to persist when grazing pressure would otherwise have caused them to crash.

Only one other example has been reported of an activated enzyme-cleavage mechanism of defense in the plankton whereby DMSP is cleaved into the gas DMS and the feeding deterrent acrylate in the bloom-forming coccolithophorid, Emiliana huxleyi. In feeding assays with a protistan grazer, Wolfe et al. (1997) showed selective feeding on a strain with low DMPS activity compared to one with higher activity. DMS and acrylate are also produced in another bloom-forming alga, Phaeocystis, which is thought to be a poor food source for a variety of zooplankton grazers. Turner et al. (2002) concluded that although copepods feed well upon Phaeocystis, the resulting poor fecundity on this diet may inhibit copepod population increases during blooms, contributing to the perpetuation of blooms. Phaeocystis has recently been reported to also produce the polyunsaturated aldehyde decadienal (Hansen et al. 2004) indicating that nondiatom marine phytoplankton are capable of producing unsaturated aldehydes as well. The raphidophycean Chattonella marina, one of the most noxious red tide phytoplankton species, has also been shown to use nitric oxide to induce fish death through excess mucous production in the gills leading to tissue hypoxia (Kim et al. 2006). Studies on chemical interactions in the plankton are still in their infancy but there remains great scope for research into the effects of toxins on gamete, embryonic, and larval development of herbivorous grazers, and understanding why zooplankton avoid consuming certain metabolites and what happens when they do.

THE NUTRITIONAL COMPONENT OF MARINE PLANT-HERBIVORE INTERACTIONS

Many theories exist concerning the circumstances under which organisms defend themselves chemi-

cally. These have been summarized for seaweeds and marine invertebrates in an excellent review by Cronin (2001) and in a more general context by Stamp (2003). In this section we briefly review the information available on the effects of growing conditions of algae on the production of toxins, and try to identify the most likely explanatory model for the production of secondary metabolites used for defense in planktonic organisms or more specifically in the microalgal-herbivore interactions discussed above.

If a trait is to be selected for, the benefits of this trait in terms of fitness gain should be more than the costs incurred to display this trait. Whereas earlier theories suggested that secondary metabolites were essentially waste overflow products, it is now well accepted that secondary metabolites have a function and that their production comes with a cost; energy or resources that are used for secondary metabolites cannot be used for growth or reproduction. Direct evidence for these costs is scarce (Cronin 2001), which might be due to the fact that it is difficult to establish the exact currency with which these costs can be measured. Diverse measures as biomass allocation to various tissues, amounts of limiting resources to various processes, or competitive abilities of organisms with different allocation patterns have been used.

A fundamental question in chemical ecology concerns the circumstances under which an organism invests in defense structures or metabolites and several different explanatory models have been proposed. The plant apparency model (PAM; Feeney 1976; Rhoades 1979) bases its predictions on the apparency of plants: large, common or predictable (both spatially and temporally) plants cannot hide from predators. It is reasonably certain that they will come under attack and should invest in defense chemicals. Those that are less common or less predictable can escape predation through this lack of exposure and should be able to get away without heavy investment in chemical defense structures.

The resource availability model (RAM; Coley et al. 1985) predicts that those species that have evolved in a nutrient-rich environment are inherently fast growing species, which should invest minimally in defenses because they can easily replace lost tissue. Those species that evolved in a low-resource environment should be slower growers and invest more in defense mechanisms. Bryant et al. (1983) and Tuomi et al. (1988) developed the carbon-nutrient balance hypothesis (CNBH) that focuses more on ecological time scales rather than the evolutionary time scales invoked by the RAM, and predicts that under light limiting conditions plants should invest in growth and reproduction, whereas

under conditions of nutrient limitation (and hence an excess in photosynthetic products) low-nutrient (carbon rich) secondary metabolites should be produced. Organisms that experience other forms of stress (temperature, ultraviolet [UV]) should in general be less able to acquire resources. Based on this, the environmental stress theory (EST) predicts that under stressful conditions the levels of chemical defense compounds should be lower and the organisms more vulnerable to predation (Rhoades 1979).

According to the growth differentiation balance hypothesis (GDBH) a trade-off exists between resources allocated to differentiation processes (production of chemical defenses, cell specialization) and growth, whereby differentiation occurs only after growth (Loomis 1953; Herms and Mattson 1992). This means that young actively growing tissue should contain low levels of secondary metabolites, as the growing process takes precedence over specialization or, on another level, that actively growing juveniles should be less defended than adults because the adults have entered a phase of specialization. The optimal defense theory (ODT; McKey 1974; Feeny 1975; Rhoades and Cates 1976; Rhoades 1979) predicts that younger individuals and younger parts of the organisms should have higher levels of defense as these are under higher risk—as they are usually more nutritious—of being preyed upon. This is based on the assertion that organisms should defend themselves in such a way that they maximize their fitness, i.e., reacting to the risk of predation, or defend their different tissues in direct relation to the vulnerability or value of the tissue. The different theories, which are all based on sound scientific work (see Cronin 2001 and Stamp 2003, and references therein), make completely different predictions. This is partly due to the fact that they were developed for different organisms because the researchers who formulated them worked with different organismal interactions.

Which models do we expect to yield correct predictions for microalgae in the pelagic realm? Many of the substances produced by microalgae have very limited effects on the copepods that eat them, but for the sake of argument here, we will assume that the secondary metabolites produced by diatoms, haptophytes, dinophytes, and other algae are chemical defense substances with the purpose to harm their predators. Many of the predictions of the ODT and GDBH relate to modular organisms that can invest differentially in different organs. This is not possible in unicellular organisms, but if we translate the predictions of these two most widely accepted models, the ODT would predict that fast growing populations, i.e., pre-bloom conditions,

should mainly consist of cells that have high amounts of chemical defenses, but on the other hand predation rates are higher in late bloom conditions, and as a result one would expect higher defense levels under these conditions. With the help of the ODT it is difficult to make a prediction. If we assume that cells in different phases of the bloom are of different life stages, then the GDBH predicts that mature populations (late-bloom conditions) should be most heavily defended, but it is difficult to envisage how this differentiation between growth and differentiation could occur in unicellular organisms.

Many of the toxins produced by marine microalgae contain no nitrogen, except for PSP toxins (saxitoxin contains 33% nitrogen on a molecular basis, and has been previously invoked as a nitrogen store, Dale and Yentsch 1978). The CNBH would predict that most algae should produce their toxins mainly under conditions where carbon is in excess and nitrogen (or other nutrients) is limiting, except for those algae producing PSP toxins. The EST predicts that under nutrient-stress acquisition of all resources is more difficult and there is a lower production of algal toxins. The PAM would predict that those species that form large, predictable blooms are more likely to have developed chemical defense systems, as they are likely to have specialized predators, and a bloom is bound to be found. Since the predation risk for individual algae within blooms is probably lower, due to the safety in numbers effect, we cannot make a proper prediction of what should happen using the PAM. It is also unclear what the exact predictions of the RAM are, since solid information as to where microalgae evolved is lacking. If the present location is a good proxy for this, one would expect that algae in coastal (more productive) regions produce lower amounts of toxins, and those that mainly occur in the open ocean should invest more in their chemical defense.

There is information available concerning the conditions under which microalgae produce toxins in the laboratory. Species producing saxitoxins seem to consistently produce the highest amounts of toxins (on a per cell basis) in the exponential phase of growth, and there is a decrease in their production under nitrogen (Parkhill and Cembella 1999; Velzeboer et al. 2001; Lippemeier et al. 2003; Poulton et al. 2005), but not under phosphorus stress, where the production of saxitoxins actually increases (Boyer et al. 1987; Anderson et al. 1990). The haemolytic activity of the haptophytes *Chrysochromulina* and *Prymnesium* increases both under phosphorus and nitrogen limitation (Johansson and Granéli 1999; Granéli and Johansson 2003), and the dinophysistoxins in *P. lima* are also higher

under nitrogen limitation (McLachlan et al. 1994). Domoic acid, which contains some nitrogen, is produced in higher quantities under phosphorus limitation, but not under nitrogen limitation (Pan et al. 1996, 1998). Nothing is known about the influence of growing conditions of other diatom species producing unsaturated aldehydes that halt cell proliferation. Where nutrient limitation consistently causes an increase in the concentrations of defense chemicals, unless the substances contain substantial amounts of the limiting nutrient, one would expect that aldehyde production should be highest in nutrient-limited cells as well. Since the precursors of unsaturated aldehydes are highly unsaturated fatty acids, the concentration of which tends to decrease in algae under nutrient limitation (Boersma 2000), the opposite may be true.

The data from field studies are much less conclusive, possibly because much of the predictive capacity focuses on the occurrence of blooms of potentially toxic species and not on the circumstances in which these species actually produce more or less secondary metabolites. Often the algae that produce toxins bloom fairly close to the coast, with relatively high nutrient levels, which is not the prediction of the RAM, although in many cases the producers of defense molecules are not the fastest growers. Similar trophic conditions do not necessarily lead to similar blooms (Luckas et al. 2005). The algae that produce toxins are certainly often not the dominating species, and it is difficult to invoke PAM as the most likely model, with the possible exception of the largely single species blooms in the Northern Adriatic (Miralto et al. 1999, 2003) or *Alexandrium* in some parts of the North Sea (Gerdts personal communication).

The CNBH explains the observed results fairly well, with the possible exception of the production of nitrogen-based substances under nitrogen limitation. We argue that based on the information available this hypothesis is the most accurate for microalgae. If we consider the (over)simplification that nitrogen drives bloom dynamics in the sea, then non-nitrogen compounds are preferably produced in late bloom conditions, whereas nitrogen containing compounds should be produced earlier in the bloom (Klöpper et al. 2003; Doucette et al. 2005; Poulton et al. 2005).

Having established what nutrition does to the production of chemical defense metabolites in algae, another question of interest is what nutrition does to the consumers when they are confronted with these substances. Do well-fed consumers have a higher resistance to these chemicals because they are able to allocate more resources into detoxification mechanisms? Although this is a well studied

subject for other systems (see section below), this question is almost impossible to answer for micro-algal-zooplankton interactions because these interactions in the pelagic zone are virtually unknown. One of the main problems is that in many cases the exact target species of the chemicals under consideration is unclear (Turner and Tester 1997). We do know that within-species differences exist in resistance to toxic algae (e.g., Colin and Dam 2005), but these differences have mainly been attributed to genetic differentiation even though copepods are capable of acclimating to consuming toxic *Alexandrium.* Since detoxification or storage of deleterious compounds is costly, animals that are well fed should have a higher resistance to chemical defense metabolites present in their food, but we are not aware of any studies that have specifically tested this hypothesis. In feeding deterrent studies, Cronin and Hay (1996) observed that hunger decreased the susceptibility to toxic substances, and that starved animals feed more than fed animals. Experimental setups to separate hunger effects from toxic effects are not easy, but if we are to differentiate between the two, ways to circumvent these problems must be found, possibly by directly measuring those substances that are involved in the detoxification of the chemicals produced by the microalgae.

CHEMICALLY MEDIATED MACROALGAL-HERBIVORE INTERACTIONS IN THE BENTHOS

A large number and variety of herbivores, ranging from highly mobile macrograzers (e.g., mammals, fish, sea urchins, and large crustaceans and gastropods) to smaller, more sedentary mesograzers (e.g., small gastropods, amphipods, isopods, and polychaetes), consume an equally large number and variety of marine macroalgae (green, red, and brown seaweeds; Hay and Steinberg 1992; Paul et al. 2001). In contrast to terrestrial herbivores, most marine herbivores are generalist grazers that consume many different seaweeds, although some herbivore species can be highly specialized on one or a few algal species (Hay 1992). Grazing pressure is highly dependent on the specific seaweed and herbivore involved in the interaction, but is generally considered to be higher in tropical coral reefs than in temperate habitats (Hay and Fenical 1988; Hay and Steinberg 1992; Paul et al. 2001). Large mobile grazers, such as fish, crabs, and sea urchins, are generally considered to have a more drastic negative effect on seaweed production and fitness than mesograzers. Due to their ability to rapidly consume large amounts of algal tissues, they are hypothesized to select for constitutive or activated defenses (i.e., defenses that are produced and present continuously within the plants; Hay 1996;

Paul and Puglisi 2004). Mesograzers use plants both as food and habitat, and they consume individual algae in small bites over a more extended period of time. It has been hypothesized that mesograzers may select for inducible rather than constitutive defenses (i.e., defenses that are produced in response to specific environmental cues; Hay 1996).

Seaweeds have evolved an array of strategies to cope with herbivory, including tolerance through compensatory growth, escape through spatial, temporal, or associational refuges, and structural, morphological, or chemical defenses (Hay and Fenical 1988; Duffy and Hay 1990). Several of these strategies may be used simultaneously by seaweeds in order to reduce herbivory. Seaweeds, like terrestrial plants, produce a large variety of secondary metabolites with highly variable chemical structures, e.g., terpenoids, acetogenins, amino acid derivates, and polyphenols (reviewed in McClintock and Baker 2001). Apparent differences in the secondary chemistry of seaweeds and terrestrial plants include the relative scarcity of nitrogen-containing algal metabolites and the higher proportion of halogenated compounds in seaweeds, probably reflecting relative differences in availability of nitrogen and halides such as bromine and chlorine in terrestrial versus marine systems. Although the majority of algal secondary metabolites have not been bioassayed, there is growing evidence that many of these compounds can deter marine herbivores. Some, if not most, of the herbivore deterrent compounds probably have multiple simultaneous functions for the seaweeds as secondary metabolites, and can function as allelopathic, antimicrobial, and antifouling or UV-screening agents, and as herbivore deterrents, and may also have multiple simultaneous functions (Hay and Fenical 1988; Potin et al. 2002; Amsler and Fairhead 2006). There is a large variation in the effectiveness of different compounds towards different herbivore species, and the function of different compounds is often poorly correlated to chemical structure (Duffy and Hay 1990; Hay and Steinberg 1992; Paul et al. 2001; Paul and Puglisi 2004). The chemical structure of seaweed defense metabolites is highly variable, but most isolated compounds are lipid soluble with some exceptions, e.g., phlorotannins and coumarins.

There is a large variation in concentration of seaweed defense metabolites at different spatial (i.e., within and between individuals, populations, and species) and temporal scales (Hay and Fenical 1988; Van Alstyne et al. 2001; Paul and Puglisi 2004). Several theoretical models that were initially formulated to explain the large variation in terrestrial plant secondary metabolite concentrations have also been applied to marine plant-herbivore

interactions (e.g., Hay and Steinberg 1992; Cronin 2001; Arnold and Targett 2002; Paul and Puglisi 2004). Some of these models, such as the RAM and PAM, make predictions about interspecific differences in chemical defences (see previous section for definitions of these models). Much of the earlier work on patterns of algal chemical defences were focused on differences among species and higher-order taxa, as well as on biogeographic comparisons (see Hay and Steinberg 1992; Hay 1996; Van Alstyne et al. 2001). More recently there has been in shift in the focus of research on algal chemical defenses towards intraspecific variation and dynamic responses. The empirical evidence for costs of chemical defenses in macroalgae is scarce, but have been indicated in a few studies based on phenotypic correlations in brown seaweeds (Pavia et al. 1999; Jormalainen and Honkanen 2004), and in a recent study based on experimental manipulation of defense production in the red seaweed *D. pulchra* (Pavia et al. 1999; Jormalainen and Honkanen 2004; Dworjanyn et al. 2006).

During the last decade, the number of laboratory experiments showing herbivore-induced chemical resistance in seaweeds, primarily brown seaweeds, has increased markedly (e.g., Cronin and Hay 1996; Pavia and Toth 2000; Sotka et al. 2002; Taylor et al. 2002; Toth et al. 2005), implying that inducible defense productions can be an important strategy to cope with herbivory in marine macroalgae. So far, all examples of inducible chemical defense responses of seaweeds in response to natural herbivore attacks come from experiments with mesoherbivores, in accordance with the hypothesis suggested by Hay (1996).

In comparison to terrestrial plant-herbivore systems, very little is known about exogenous and endogenous processes, e.g., the spread and perception of eliciting signals, behind herbivore induced chemical defence production in seaweeds. The mechanisms and processes behind inducible defense production, as well as the more rapid activated defense responses (e.g., Paul and Van Alstyne 1992; Jung and Pohnert 2001; Van Alstyne and Houser 2003), will most likely be an important focus in future studies of chemically mediated macroalgal-herbivore interactions. Like diatoms, recent results indicate the importance of oxylipin pathways in the regulation of seaweed induced defenses (Bouarab et al. 2004; Pohnert 2004). The volatile methyl ester of the plant defense hormone jasmonate (MeJA) has been shown to trigger the accumulation of phlorotannins in the common rockweed *Fucus vesiculosus* when exposed at low tide. The timing and magnitude of this increase are similar to those induced by herbivores in brown algal fucoids suggesting that analogs of cyclopentena(o)ne structures may play

a role in the development of antiherbivore responses in *Fucus* tissues, including those responses involving interplant communication (Arnold et al. 2001). A strong effort is required toward the identification of these chemical signals or of toxic molecules. Marine macroalgae produce a number of oxylipins (Gerwick et al. 1999), some of them belonging to the prostaglandin and leukotriene series, and it has been suggested that they may play important detrimental roles in herbivore fitness (Bouarab et al. 2004). The by-products of the biogenesis of fatty-acid-derived C8 and C11 hydrocarbons and sulfated C11 compounds, which compose the sexual pheromones of marine heterokont algae, have been shown to also play an important role as chemical defenses against herbivores. Studies on the brown alga *Dictyopteris* spp. have shown that 9-oxo nonadienoic acid deters amphipod grazers (Schnitzler et al. 2001). In brown algal kelps, it has also been shown that *Laminaria* spp. synthesize polyunsaturated aldehydes of similar structure and using closely related biosynthetic pathways as diatoms (Boonprab et al. 2003). In response to wounding, the red alga *Gracilaria chilensis* releases free fatty acids as well as the hydroxylated eicosanoids and this liberation of oxylipins was shown to be part of the defense of *G. chilensis* against epiphytism (Lion et al. 2006). Given the importance of the effect of mechanical wounding on the induction of defense responses during insect feeding in higher plants (Mithöfer et al. 2005), such a response to grazers in seaweeds clearly needs additional careful investigations.

To address these challenges will require more extended collaborations between ecologists, chemists, physiologists, and molecular biologists, as well as stronger initial focus on a few selected model species of seaweeds and marine herbivores. In parallel to these mechanistic studies there is also an obvious need for a better understanding of the effects of macroalgal defenses on herbivore fitness (e.g., Toth et al. 2005) and population dynamics, as well as on ecosystem functioning. This will probably require increased ambitions to conduct ecologically relevant manipulative experiments in natural seaweed populations, as well as collaboration between marine chemical ecologists and ecological modellers.

CHEMICALLY-MEDIATED INTERACTIONS IN BENTHIC INVERTEBRATES

Secondary metabolites are widespread among invertebrates and understanding their functional roles in the producing organism has been under intense study in recent times. Herein we report selected examples of interactions between marine benthic invertebrates, especially sponges, molluscs,

and cnidarians that are mediated by specific secondary metabolites. The topic is discussed in a number of excellent review papers (e.g., Scheuer 1990; Herring 1979; Proksch and Ebel 1998; Kajiwara 1999; Paul and Puglisi 2004) and is the subject matter of several outstanding books (e.g., Paul 1992; McClintock and Baker 2001). For more information, the reader is invited to consult the series ''Marine Natural Products'' (Faulkner 2002; Blunt et al. 2005) for the chemistry discussed in this section.

The hypothesis that sessile or slow-moving organisms, without obvious escape mechanisms and physical protection, are likely to be chemically defended has recently been explored with greater frequency in the marine environment. Of these organisms, opisthobranch molluscs appear to be particularly well endowed with secondary metabolites (Cimino et al. 1999, 2001). In these gastropods, the reduction of the physical protection offered by the shell is compensated by the development of complex strategies of defense (survival) that include use of chemicals. In some orders (e.g., Nudibranchia), the shell is completely absent and these species show high specialized behaviors. Opisthobranchs occupy many ecological niches and can be either herbivorous or carnivorous, being able to feed upon sponges, algae, hydroids, bryozoans, tunicates, and soft corals. The trophic relationships of these molluscs are documented by molecular markers that allow for the study of many community interactions even in the absence of direct observation.

Terpenes are the largely predominant chemical class in trophic interactions between sponges and opisthobarnchs (Cimino et al. 1999). In some cases, the specificity of feeding behaviors is so high that some authors have proposed the metabolite content as another parameter to consider in the taxonomy of the species. This happens with the four major genera of the class Chromodorididae (Nudibranchia). Tracing the terpenoid content in samples from 19 sites along the Pacific coast of North America, the nudibranch *Cadlina luteomarginanata* has been shown to feed on sponges belonging mainly to the genera *Aplysilla* and *Pleraplysilla* (Kubanek et al. 2000). Studies with samples collected all over the world, from the Arctic to Antarctica, have also shown a similar specialization in many other chromodoridids. *Hypselodoris* lives in close association with sponges, like *Dysidea*, that contains furanosesquiterpenes, *Glossodoris* with sponges, like *Spongia*, that contain sesterterpenes, and *Chromodoris* with sponges, like *Dendrilla* and *Aplysilla*, that contain diterpenes.

The trophic interdependence and transfer of dietary metabolites from sponges to nudibranchs have been rigorously shown by laboratory experiments with *Hypselodoris picta* (ex webbi) and the sponge *Dysidea fragilis*. In aquaria, the nudibranchs were able to recognize and feed upon the sponge, in spite of the fact that the sponge contained metabolites absent in the original diet of the mollusc. The transfer of furodisynin, the major sponge chemical deterrent from *Dysidea* sp. to the defensive organs of *H. picta* was shown by chemical analysis of the metabolite content before and after feeding in aquaria. The role of most of these metabolites is not known, although it is generally accepted that they are chemical deterrents that thwart predation. Several furanosesquiterpenes (e.g., longifolin or furodisyn) are observed to be toxic to fish at concentrations below 10 ppm, whereas Chromodoris-derived furanoditerpenoids (e.g., aplysillin) are feeding deterrents below 1 ppm against generalist predators, such as fish and sea stars.

In some cases opisthobranchs are not only capable of accumulating dietary molecules but also transform or even produce chemical mediators de novo. The behavior of the Doridid nudibranchs of the genera *Dendrodoris*, namely *D. limbata* and *D. grandiflora*, and *Doriopsilla*, namely *D. pelseneri* and *D. areolata*, is unique in that they have acquired the capability of biosynthesizing defensive terpenes that are also present in sponges (Fontana et al. 2000, 2003; Gavagnin et al. 2001). An instructive example of this activity is found in the nudibranch *D. areolata*. This mollusc biosynthesizes an array of sesquiterpenoids related to drimane and ent-pallescensin A, which are suggested to play different ecophysiological functions. Biochemically, these molecules have been reported from sponges of the genus *Dysidea*. This finding represents an unusual case of apparent evolutionary convergence, which is even more singular since the nudibranch and the sponge may represent a prey-predator pair. The different metabolites of *Doriopsilla* derive by branching the biosynthetic pathway from a common precursor, allowing for the development of chemical diversity at a minimum cost. If one accepts that secondary metabolites are produced from universally present precursors by specific enzymes that probably arose by primary metabolism, biosynthesis in *D. areolata* may reflect two basic aspects of the biogenesis of terpenes in marine organisms: distinct carbon skeletons might derive from similar enzymes acting on slightly different substrates, and chemical similarities amongst taxonomically nonrelated organisms (e.g., *Dysidea* and *Doriopsilla*) may be enzyme derived from either gene lateral transfer or variation of genes originally coding for primary metabolism.

The origin of this competence is rather controversial and, at present, is interpreted by two

hypotheses that involve lateral gene transfer or retrobiosynthesis (Cimino and Ghiselin 1999). In either case, the de novo biosynthesis of the defensive molecules is regarded as an evolutionary advantage, since the capability of producing the allomones emancipates the organism from the availability of a specific food.

The defensive strategy of *Dendrodoris* and *Doriopsilla* is rather complex and relies on the ultimate production of polygodial, a dialdehyde compound that is toxic at very low concentrations. The dialdehyde moiety of polygodial, such as the related groups occurring in many other deterrent molecules isolated from molluscs (crispatenine from *Elysia crispate*, onchidal from pulmonates of the genera *Onchidella*, chlorodesmin from *Cyerce nigricans*) and sponges (see below), is strongly electrophilic and tends to react immediately with any nucleophile, such the amine groups of proteins, present in the environment. For this reason, the defensive dialdehyde of *Dendrodoris* and *Doriopsilla* is stored in an inactive form from which it can be readily released on demand by action of specific lipases present in the mollusc's tissues (Plate D). An identical strategy has also been documented for the defensive role of halimedatetracetate in the mollusc *Bosellia mimetica* and the alga *Halimeda tuna*, as well as oxytoxins in some opisthobranchs belonging to the order Sacoglossa (for a review see Cimino et al. 1999). It has been shown that *Oxynoe olivacea*, a green sacoglossan that lives camouflaged upon algae of the genus *Caulerpa*, is able to transform the major algal metabolite, caulerpenyne, to oxytoxins by hydrolysis of the acetyl groups. The process, that increases the toxicity of the algal metabolite 100 times, is due to two esterases, named Lip-1 and Lip-2, that have been functionally characterized in enzymatic preparations of the mollusc (Plate E; Cutignano et al. 2004a). A similar mechanism has also been proposed as origin of the allelopathic activity associated to the strong invasivity of caulerpacean algae (Jung and Pohnert 2001). The lipolytic activity of algal preparations appears significantly lower than that shown by the mollusc's enzymes (Cutignano et al. 2004b).

Sponges are the richest source of secondary metabolites isolated from marine organisms (Faulkner 2002; Blunt et al. 2005). Although this is in part due to the major attention that several research groups have paid to this phylum, the chemical diversity known in porifera is the consequence of the extraordinary ability of these organisms to elaborate chemical metabolites that are thought to function as feeding deterrents, inhibitors of fouling or infectious organisms, mediators of predation, and spatial competition. There is a general agreement that sponges, as sessile soft-bodied animals, use chemical means to control predator-prey relationships and the stabilization of marine populations. Sponge-derived secondary metabolites are suggested to mediate allelopathy, antiovergrowth, and UV protection, but the commonly studied roles are those of antifouling and predator deterrence (Walter and Pawlik 2005). Several studies carried out on different species from tropical, temperate, and cold waters, have placed in evidence the deterrent activity of crude extracts of sponges (Pawlik et al. 1995). Only in a few cases has the antifeeding mechanism been associated to discrete compounds. The unusual macrolide dehydrohalichondramide, found in the nudibranch *Hexabranchus sanguineus* (Spanish dancer), is the major deterrent compound of the sponge *Halichondria* sp. (Pawlik et al. 1988). In addition to those discussed above for Opisthobranchs, other potential defensive allomones include terpenes, for example scalaranes (e.g., scalaradial) and other sesterterpenes (e.g., variabilin), macrolides and other polyketides, derivatized amino acids, and pyrroles, these last compounds were recently characterized as defending products of marine worms (Kicklighter et al. 2003).

The deterrent properties of sponge-derived secondary metabolites are species specific, although tropical and temperate sponges show a comparable deterrence to global, sympatric, and allopatric predators (Becerro et al. 2003). Particularly interesting are the results on the Antarctic sponge *Latrunculi apicalis*. This cold-adapted porifera is rarely preyed upon by sea stars, which are the dominant spongivores in Antarctic benthic communities. The sponge contains alkaloids, namely dischorhabidins (e.g., dischorhabidin-G) that elicit a tube foot retraction response in the sea star *Perkinaster focus* (Yang et al. 1995; Furrow et al. 2003). Defensive alkaloids are apparently localized in the external part of the sponge body (dischorhabidins-G was found within 2 mm from the sponge surface), preserving the most vulnerable tissues, where the likelihood of predation from sea stars is highest.

Investigations on the Caribbean sponges *Erylus formosa* and *Ectyoplasia ferox* showed that multiple ecological functions are associated with a single chemical component, namely triterpene glycosides. Tests at different concentrations show that these molecules were able to deter predation, microbial attachment, and fouling of invertebrates and algae (Kubanek et al. 2000, 2002). The ecological activity was strongly dependent on the molecular structure. The metabolites are apparently restricted to the sponge surface and the biological effect is through direct contact with the sponge rather than by waterborne interactions. This strategy aims to minimize

the loss of compounds into seawater and to increase the efficiency of the chemical defense. The triterpene glycosides have been described in several other sponges and sea cucumbers (Faulkner 2002; Blunt et al. 2005) where presumably they play the same defensive role. They are also structurally related to saponins, toxic molecules with a steroid core that have been typically reported in sea stars.

With the exception of relatively small-scale and localized predation by certain specialists, soft corals are rarely exploited as a food source. Randall (1967) reported that the gut contents of only 11 of 212 species of reef fish contained gorgonians. Evidence of chemical defense in these organisms is abundant. O'Neal and Pawlik (2002) surveyed 32 species of Caribbean gorgonians and determined that all of them (100%) yielded predation-deterrent crude organic extracts. Analogously to Kelman et al. (1999), the same authors also concluded that secondary metabolites, rather than physical protection (sclerites), are the primary means of defense against fish and other generalist predators (O'Neal and Pawlik 2002). Like in sponges, evidence regarding the benefits of specific compounds are rather few and concern only very few cases, such as the furanocembranolide 11β,12β-epoxypukalide (Epifanio et al. 2000), renillafoulins (Keifer et al. 1986), flexibilide, and lophotoxin 1 (Epifanio et al. 2000).

In addition to the allomonal roles of the chemicals discussed above, secondary metabolites also function in carrying chemical messages (pheromones) within the marine benthic biota as in the case of the sperm-release pheromone (uric acid) of the marine polychaete *Platynereis dumerilii* (Zeeck et al. 1998), the egg release pheromone L-Ovothiol A in *P. dumerilii* (Rohl et al. 1999), and sex pheromones (ceramides) of the hair crab *Erimacrus isenbeckii* (Asai et al. 2000). Peptides have also been involved in interspecies communication (Rittschof and Cohen 2004), like in the case of the attracting pheromone that stimulates male attraction in *Aplysia.* A particularly outstanding example of chemical communication based on low weight compounds is encountered within the cephalaspidean mollusc of the genera *Navanax* and *Haminoea* (for a review see Cimino et al. 2001). This latter group of invertebrates embraces shelled opisthobranchs that biosynthesize (Cutignano et al. 2003, 2004a) a series of oxygenated 3-alkylpyridine derivatives, generically named haminols differing only in the position and number of double bonds at the C-2 position of the linear chain (Spinella et al. 1993; Marin et al. 1999). When molested, the molluscs release a white mucus containing haminols, which serve as alarm pheromones inducing an escape reaction in conspecifics (Cimino et al. 1991;

Spinella et al. 1993). A similar escape reaction by different species has been ascribed to imbricatine, an unusual amino acid derivative produced by the sea star *Dermasterias imbricate* that induces a swimming reaction in actinias of the genus *Stomphia* (Elliott et al. 1989).

Concluding Remarks

Marine chemical ecology is a young science that requires the collaborative effort of biologists, ecologists, and chemists. Identifying the compounds responsible for mediating feeding, reproduction, and behavioral interactions is only the first step in understanding the ecological relevance of a compound. These effects then need to be translated from laboratory assays to their natural context in order to provide the ultimate test and major challenge for field ecologists. An increased understanding of chemical defenses will be achieved when we know how ecologically realistic doses of these metabolites affect growth, reproduction, and survivorship of consumers. In the long run, such studies will lead to a better understanding of how these compounds can help regulate ecosystem functionality by underpinning the chemical and molecular processes that are crucial for the fitness and survival of the producing organisms. If we can understand the natural function of these compounds, we can develop new strategies for the correct management and protection of these potentially important natural resources for the future, and find new biotechnological applications for these products in our day-to-day lives.

To address these issues, novel and ecologically relevant methods need to be developed and applied to investigations of allelopathy, antipredation, antifouling, antimicrobial, and other possible functions of secondary metabolites. The problem is that often the natural concentrations of a compound are not known. Most feeding deterrents have been isolated and identified by natural products chemists looking for unusual compounds, and most chemical studies do not provide information on the yield of these compounds after extraction. It is difficult for ecologists conducting bioassay experiments to know the natural concentrations of the metabolites to be tested. At the same time, ecologists often assume that chemistry plays a specific role in structuring the marine biota, or in direct interactions of predators and prey or of competitors, without a clear idea of the molecules or biosynthetic pathways really involved in these processes. To complicate matters even further is the fact that there are geographical variations in the concentration of natural products and not only does the number and percentage of organisms that produce toxins follow a longitudinal gradient but the concentration of toxins also

increase (Sennett 2001). Notwithstanding, much progress has been made in recent years in designing ecologically relevant bioassays with natural concentrations of a compound and in understanding the biosynthetic pathways involved in their production.

There is a need to compare metabolites from microbes to microalgae and macroalgae, and from mesozooplankton to benthic macroinvertebrates in terms of structure, function, and biosynthetic pathways. Compounds with different biological activities, from those with feeding deterrent properties to those that induce developmental arrest and growth reduction or toxicity and death, should be investigated and compared to determine the diversity of natural products and importance of secondary metabolites in the evolution of biodiversity. Large databases of natural products exist but because of the economic interests involved, access is very expensive in contrast to free access to taxonomic data. There is now an increased awareness that compounds and compound classes previously thought to be exclusive of only certain taxa are more and more frequently found in phylogenetically and ecologically distant organisms.

There is a need to chart out research plans to address open questions and hypotheses on chemical interactions in the plankton and benthic realms. Progress will be enhanced if good communication and collaborative efforts can be established. Only through interactive collaboration among a broad interdisciplinary community of scientists will enough information be gathered to stimulate new ideas for future research.

ACKNOWLEDGMENTS

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