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NPR

REVIEW

Chemical ecology of the marine plankton

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This review focuses on recent studies on the chemical ecology of planktonic marine ecosystems, with the objective of presenting a comprehensive overview of new findings in the field in the time period covered. In order to highlight the role of chemically mediated interactions in the marine plankton this review has been organized by ecological concepts starting with intraspecific communication, followed by interspecific interactions (including facilitation and mutualism, host-parasite, allelopathy, and predator-prey), and finally the effects of plankton secondary metabolites on community and ecosystem-wide interactions.

1 Introduction

In the previous three years, research in planktonic marine chemical ecology has focused especially on quorum sensing and how it impacts algal-bacterial interactions as well as chemical defense in predator-prey interactions. This review summarizes the new studies in the whole field of chemical ecology of the marine plankton from January 2015 through December 2017. Previous reviews in this series summarized the advances in the field between 2006 and 2008,[1] January 2009 to September 2010,[2] October 2010 to December 2012[3], and January 2013 to December 2014.[4]

Several recent reviews centered on quorum sensing in algal associated bacteria and intraspecific communication among microalgae.[5-7] Zhou and colleagues published a comprehensive review of quorum sensing in marine bacteria with a particular focus on how quorum sensing impacts symbiotic interactions between bacteria and algae.[5] They explored how quorum sensing is involved in modulating behaviors in bacteria that are mutualistic as well as antagonistic to the rest of the microbial community and their algal host. Additionally, they investigated co-evolution between bacteria and algae and the potential role of quorum sensing-induced behaviors to help predict and control harmful algal blooms.[5] Rolland and colleagues focused exclusively on how quorum sensing and quorum quenching (disruption of quorum sensing) are involved in bacterial dynamics in the phycosphere, defined as the microenvironment immediately surrounding phytoplankton cells, dominated by algal exudates.[6] These authors reviewed the types of molecules involved and the ecological role of quorum sensing and quenching among bacteria that live in the phycosphere, commenting on how quorum sensing and quenching are likely important in regulating the relationship between bacteria and algal hosts.[6] In another review, Venuleo and colleagues surveyed how, like bacteria, microalgae extensively participate in intraspecific communication.[7] They explored what consititutes “intraspecific” in these highly variable organisms that can reproduce either sexually and asexually, and the evolution of intraspecific communication. Additionally, they reviewed what is known about the ecological roles of intraspecific communication in many microalgal species.

Other reviews examined the ecology and chemical diversity of harmful algal blooms.[8, 9] Gleason and colleagues discussed interactions between fungi of the phylum Chytridiomycota, or chytrids, and toxic phytoplankton.[8] Additionally, they commented on the paucity of studies regarding the effects of parasitism on toxin production in these

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phytoplankton.[8] Rassmussen and colleagues highlighted the chemical diversity of toxins produced by phytoplankton, particularly dinoflagellates, by reviewing 59 algal toxins.[9] They organized their review by structural class and discussed the algae responsible for toxin production and the mode of action of the toxins, when known.[9] These authors concluded their review with the current state of knowledge for several species of algae previously implicated in large-scale fish kills.[9]

2 Intraspecific signaling

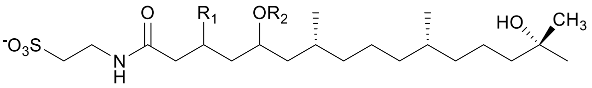
2.1 Reproductive signaling

The existence and involvement of pheromones in copepod reproduction has been established through behavioral assays, but the nature and identities of these cues have remained elusive.[10] Mathematical modeling of copepod mating behavior predicts that males, which correct their courses with high fidelity after initially tracing a female track in the wrong direction, may establish the directionality of a female pheromone trail through detection of a multicomponent signal.[11] In a scenario in which a signal comprised of two components, each differing in its environmental persistence, was compared to a single component signal, a hypothetical male able to sense a ratio between two signal components was more likely to successfully reorient toward the female than a male only able to sense gradients in the absolute concentration of a single chemical.[11] It is possible that the more persistent signal may serve as a "track" while the less persistent signal specifies direction (which way is upstream). It will be necessary to determine whether the findings, in light of assumptions based on a laminar flow environment, are relevant to the natural environment in which female pheromones may take the form of 'clouds' rather than delineated trails.[12] Further examination of the sensory capabilities of male copepods may also provide context to the findings.

Attempts to structurally describe exuded metabolites and pheromones from copepods have been reported for *Temora longicornis* and *Oithona davisae*.[13, 14] Comparisons of male and female exudates were facilitated by continually recirculating live copepod-containing water through extraction media to adsorb diffusible cues.[13, 14] Untargeted metabolomics analyses of *T. longicornis* extracts revealed sex-specific differences in exudate composition and component abundance, and provided proof-of-principle that copepod-specific metabolites, such as copepodamide G (**7**) (which is one of eight compounds that make up a class of copepod-specific compounds), may be captured and measured with this technique.[13] Female *T. longicornis* extracts did not retain the ability of unextracted water exudates to elicit mate-seeking swimming behavior in males, confounding pheromone identification.[13] Similarly, analyses of exuded metabolites from *O. davisae* revealed sex-specific differences, but those extracts, too, did not recapitulate the ability of unmanipulated water exudates to induce mate-seeking swimming behavior in males.[14] The methods used in these studies have the tantalizing potential to expand the understanding of copepod diffusible chemistry and chemical signaling and its influence on plankton ecology, but are hindered by the apparent lability of many copepod pheromones.

In the rotifer *Brachionus manjavacas*, analysis of excreted proteins has yielded new information about the identity of the quorum sensing pheromone 'mixis-inducing protein', responsible for inducing a shift from asexual to sexual reproduction (mixis) in this microscopic animal.[15] Two excreted proteins of molecular weights 39 and 45 kDa made up the majority of a mixture which induced mixis; however, the most promising candidate for the mixis-inducing protein was a 22 kDa protein which, when precipitated out of solution, significantly reduced mixis in female progeny. Initial protein sequencing efforts indicated that the precipitated protein was similar to five known proteins.[15] Additional experiments are needed to determine whether the 22 kDa protein is sufficient for mixis induction and whether other proteins are involved.

Rather than attempt to isolate chemical signals, Basu and colleagues instead characterized transcriptional changes that occur when the diatom *Pseudo-nitzschia multistriata* is exposed to unmanipulated, reproductive chemical cues produced in an experimental setting.[16] The *P. multistriata* life cycle is divided into two phases: vegetative reproduction in which cells divide and become progressively smaller due to the restriction imposed by their rigid silica thecae, and sexual reproduction which occurs when a minimum size threshold is reached.[17] In the sexual reproduction phase, chemical signals trigger cells of opposite mating types to generate genetically diverse zygotes from which cells of maximal size are ultimately formed.[18] The authors found that different *P. multistriata* mating types maintained in contact-free co-culture responded to chemical cues by altering their growth (possibly as a means of synchronization) and initiating transcriptional changes which indicated cells were likely preparing for meiosis and integrating environmental sensing and cell signaling.[16] Differences were observed in the numbers, identities, and magnitudes of regulated genes in each mating type, a possible indication that each produces a different pheromone signal. The work is an elegant approach to investigating the downstream effects of waterborne cues that avoids laborious pheromone isolation and possible complications should the signal be multicomponent, present in a specific ratio, or modified in some way following production. Manipulation of the pathways identified in the study followed by phenotypic evaluation may further clarify the mechanism of pheromone reception by and action on *P. multistriata*.



copepodamides
A (**1**) R_1= -CH_3 R_2= Docosahexaenoyl
B (**2**) R_1= -CH_3 R_2= Eicosapentaenoyl
C (**3**) R_1= -CH_3 R_2= Stearidonoyl
D (**4**) R_1= =CH_2 R_2= Docosahexaenoyl
E (**5**) R_1= =CH_2 R_2= Eicosapentaenoyl
F (**6**) R_1= =CH_2 R_2= Stearidonoyl
G (**7**) R_1= -CH_3 R_2=H
H (**8**) R_1= =CH_2 R_2=H

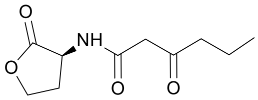
2.2 Quorum sensing

In addition to intraspecific signaling as it relates to reproduction~~,~~ new insights have been gained into the cooperative interaction of quorum sensing, its distribution in phytoplankton, new quorum sensing-regulated activities, and implications for phytoplankton ecology.

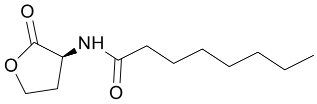
While a subset of marine organisms have been shown to produce self-signaling autoinducer molecules or have been inferred to produce these molecules based on sequence information (e.g., bacteria, rotifers), little was known about the distribution of quorum sensing pathways in the oceans. Classically, bacterial quorum sensing was not thought to play a prominent role in the ocean because the density of bacteria in seawater is usually below the quorum sensing threshold. However, quorum sensing may become relevant in situations where bacteria aggregate on particles or are present during high-density phytoplankton blooms. Examination by Doberva and colleagues of publicly available metagenomic sequence information from the Global Ocean Sampling (GOS) database for the incidence of autoinducer (AI) synthases revealed previously unknown AI sequences.[19] This indicates that quorum sensing is diverse and widespread in the marine environment, often involving uncultivated bacteria.[19] Phylogenetic differences in the distributions of LuxI (AI-1), HdtS (AI-1), LuxS (AI-2), and AinS (AI-1) AI synthases were observed, but evolutionary conclusions are difficult to draw since pathways are known to be transferred horizontally.[19] Because pathway identification was limited to matches from pre-constructed databases, it is likely that quorum sensing diversity and distribution were underestimated and richer than results reflect.

While the work discussed above did not investigate expression of quorum sensing pathways in free-living versus particle-associated bacteria, a separate study of bacterial communication on marine snow directly revealed known autoinducers *N*-(3-oxo-hexanoyl)-L-homoserine lactone (**9**) and *N*-octanoyl-L-homoserine lactone (**10**), indicating that bacterial concentrations on particles can indeed be high enough to facilitate quorum sensing.[20] Of the cultured marine snow isolates, 10 of 53 produced acyl homoserine lactones. The gram-negative alphaproteobacterium *Paracoccus carotinifaciens* produced **9**, the first report of this molecule for this species.[20] The gammaproteobacterium *Pantoea ananatis* was found to produce the L-homoserine lactones *N*-butanoyl (**11**), *N*-hexanoyl (**12**), *N*-decanoyl (**13**), *N*-dodecanoyl (**14**), and *N*-tetradecanoyl-L-homoserine lactone (**15**). Additionally, *P. ananatis* produced five extracellular hydrolytic enzymes, including alkaline phosphatase which was inhibited by the addition of a quorum sensing inhibitor.[20] While the relative importance of alkaline phosphatase to particle remineralization remains to be seen, the findings provide initial support for the idea that quorum sensing is an important contributor to biogeochemical cycling dynamics and supply of organic matter to other residents of the plankton.

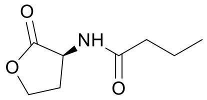
New observations of quorum sensing involvement in organismal defense have also been reported. The pathogenic bacterium *Vibrio cholerae* living in biofilms established on chitinous substrates experienced reduced predation by the flagellate *Rhynchomonas nasuta* than did *V. cholerae* biofilms established on abiotic or non-chitinous substrates.[21] The biofilms formed on chitin exuded ammonium, a toxic byproduct of chitin metabolism, which conferred protection from predation.[21] A quorum sensing mutant with reduced deterrence to *R. nasuta* was found to be impaired in *N*-acetylglucosamine catabolism, chemotaxis, and chitin-regulated pilus synthesis, but interestingly was not impaired in its ability to form a biofilm.[21] Biofilm formation on zooplankton by *V. cholerae* is an important protective mechanism that contributes both to its environmental persistence and periodic outbreaks. Metabolism of chitin appears to augment the protective capacity of *V. cholerae* biofilms through the production of antiprotozoal ammonium, a phenomenon the authors refer to as “metabolite-based grazing resistance” and which they propose may be a common defense property of biofilms.[21] Effects of ammonium on potential hosts were not explored.



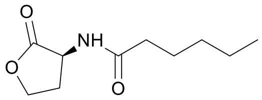
N-(3-oxo-hexanoyl)-L-homoserine lactone (**9**)



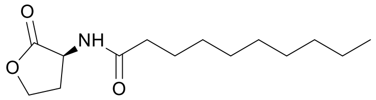
N-octanoyl-L-homoserine lactone (**10**)



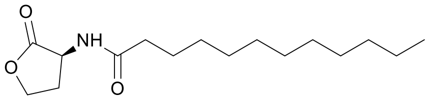
N-butanoyl-L-homoserine lactones (**11**)



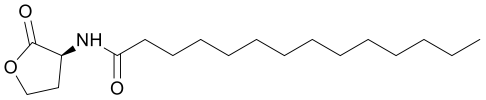
N-hexanoyl-L-homoserine lactones (**12**)



N-decanoyl-L-homoserine lactones (**13**)



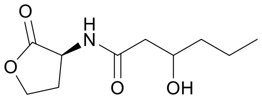
N-dodecanoyl-L-homoserine lactones (**14**)



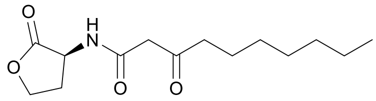
N-tetradecanoyl-L-homoserine lactone (**15**)

In addition to quorum sensing-regulated grazing defense in *V. cholerae*, a recent report details the quorum sensing-dependent mechanism by which another pathogenic marine *Vibrio*, *V. anguillarum*, defends against viral infection.[22] Bacterial mutation of membrane-based phage receptors to prevent viral entry into the cell is a common antiviral defense mechanism recognized as an important driver of bacterial evolution. However, mutation of receptors or transporters important for satisfying the cell’s metabolic demands could bring fitness costs.[22] Non-mutational means of antiphage defense that circumvent or mitigate this liability include aggregate formation, exopolysaccharide production and temporary downregulation of phage receptors.[23-25] *V. anguillarum*, which produces the quorum sensing molecules *N*-(3-hydroxyhexanoyl)-L-homoserine lactone (**16**) and *N*-(3-oxo-decanoyl)-L-homoserine lactone (**17**) and **12** at high densities, protected itself against KVP40 phage infection by alternately forming aggregates and producing exopolysaccharide at low cell densities, as well as temporarily downregulating the phage receptor universal outer membrane protein K at high cell densities.[22] This strategy may allow the bacterium to dynamically respond to differences in phage pressure while minimizing fitness costs (assuming that the magnitude of phage threat correlates with host bacterial density). A quorum sensing mutant locked into a low density phenotype expressed high levels of phage receptor and remained vulnerable to phage infection regardless of culture density.[22] These findings suggest that in certain instances in which phage therapy is being considered, it may be beneficial to combine it with methods to inhibit quorum sensing. This may constrain bacteria to a phenotype that is vulnerable to viral attack and therefore increase the effectiveness of phage therapy.

Because quorum sensing has consequences on marine communities and ecosystems, additional studies on this topic are presented in the bacterial dynamics subsection of the community and ecosystem effects section.



N-(3-hydroxyhexanoyl)-L-homoserine lactone (**16**)



N-(3-oxo-decanoyl)-L-homoserine lactone (**17**)

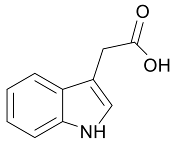
2.3 Other intraspecific signaling

Indole-3-acetic acid (**18**) is a well-known auxin, or plant hormone, that influences plant growth and differentiation and is now appreciated to be important in intraspecific signaling between cell types of the coccolithophore *Emiliana huxleyi*.[26] Coccolith-bearing *E. huxleyi* produce indole-3-acetic acid. This metabolite is then sensed by non-coccolith-bearing, bald cells which respond by undergoing a physiological shift that is characterized by increased quantum yield (an indicator of photosystem II health), a decrease in growth and chlorophyll fluorescence, and an increase in cell size and membrane permeability.[26] This work is the first to report auxin production by a bacteria-free haptophyte, an observation of note as auxin production by algae has been a controversial topic with some attributing the presence of these molecules to production by associated bacteria. Detection in *E. huxleyi* of homologs of tryptophan-dependent indole-3-acetic acid genes from the mustard plant *Arabidopsis thaliana* supported the conclusion that the coccolithophore biosynthesizes the metabolite.[26] The significance of the observed signaling coordination between coccolith-bearing and bald *E. huxleyi* cell types is not clear. Labeeuw and colleagues note that while the two cell types co-occur in blooms, generally coccolith-bearing cells are present at higher densities during a bloom while bald cells increase in number towards the end of a bloom.[26] Further investigation is necessary to establish the benefit of signaling coordination for *E. huxleyi* and its implications for phytoplankton bloom dynamics and biogeochemical cycling.

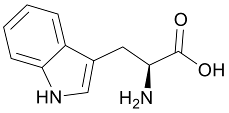
3 Facilitation and mutualism

Several recent studies have focused on chemical cross-talk between species. These interactions include both facilitative and perhaps mutualistic interactions; however true mutualisms between planktonic organisms have rarely been unequivocally demonstrated. Most studies reviewed here focused on the influence of bacteria on the growth or physiology of eukaryotic phytoplankton. Only two studies unambiguously identified the presence of signaling molecules, while several studies infer the presence of a signaling molecule. For the purposes of this review, only papers that demonstrated signaling or cuing functions of nutritional molecules (e.g., amino acids, vitamins) are considered. Thus, studies that established growth effects due to the exchange of dissolved organic nutrients (including vitamins) among organisms are not reviewed here.

Two recent studies have demonstrated a new ecological role for algal-derived tryptophan (**19**) as a biosynthetic substrate that can be used by bacteria to produce the plant hormone indole-3-acetic acid (**18**), which in turn acts as a growth promoter of eukaryotic phytoplankton.[27, 28] In these cases, specific plankton-bacterial relationships were described.[27, 28] For instance, Amin and colleagues showed that **18** produced by the Roseobacter *Sulfitobacter* sp. stimulated the growth of some strains of the diatom *Pseudonitzschia multiseries*.[27] When *Sulfitobacter* sp. grew alone, or with *P. multiseries*, **18** was detected at micromolar concentration ranges demonstrating that bacteria produce and release **18** into the environment. These authors confirmed the ecological relevance of their laboratory findings by demonstrating that **18** was present in field samples at similar concentrations and using metatranscripts for *Sulfitobacter* sp. to identify active **18** biosynthesis pathways in these samples.[27] The authors suggested that *P. multiseries* provides necessary organic carbon to promote bacterial growth as bacterial numbers increased when grown with the *P. multiseries* strain whose growth had been enhanced, while the diatom preferentially utilizes ammonium derived from bacterial metabolism.[27] Additionally, transcriptomic data indicated that *P. multiseries* increased the production of **19** when grown with *Sulfitobacter* sp. Coupling the above data with the observations that extracellular **19** concentrations significantly decreased in the presence of the bacterium suggests that bacteria rapidly take up diatom-produced **19**.[27] The authors speculated that diatom-derived tryptophan served as the biosynthetic precursor for bacterially produced **18**, potentially through several **18** pathways including indole 3-acetonitrile, indole 3-acetamide, and tryptamine pathways, although no isotope tracer studies were confirmed to test this hypothesis.[27]



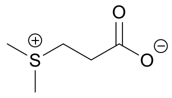
indole-3-acetic acid (**18**)



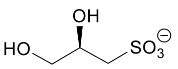
L-Tryptophan (**19**)

The coccolithophorid *Emiliania huxleyi* releases **19** that is used by the alphaproteobacterium *Phaeobacter inhibens* to produce **18**, which in turn stimulates the growth of *E. huxleyi*.[28] *P. inhibens* only grew effectively when with *E. huxleyi*, which provided several nutrients, dimethylsylfonopropionate (DMSP) (**20**), and fixed carbon, that supported bacterial growth.[28] The authors speculated that free-living *P. inhibens* may also use dissolved **20** as a cue to locate *E. huxleyi* cells.[28] *E. huxleyi* grew more rapidly in the presence of the bacterium and additions of **18** to bacteria-free *E. huxleyi* increased its maximum cell density.[28] When *P. inhibens* was fed isotopically labeled **19**, fully labeled **18** was produced, confirming that **19** is used by *P. inhibens* to make **18** and *E. huxleyi* was shown to produce extracellular **19**. Therefore, this work demonstrates that "crosstalk" between these organisms is possible within the phycosphere.[28] Similar to the results of the study by Amin and colleagues,[27] **18** was undetectable in coccolithophore-bacterium co-cultures, indicating a rapid turnover of this molecule among organisms.[28] Considering the taxonomic diversity of the organisms used, these studies provide evidence for the potential of widespread, chemically mediated facultative relationships among eukaryotic phytoplankton and bacterial counterparts.[27, 28]

Other studies have demonstrated the potential for chemical signaling or cuing among eukaryotic phytoplankton and bacteria.[29-31] Authors of these studies inferred chemical cross-talk between organisms by tracking an organism's transcriptomic or metabolomic changes in response to the presence of another species. Durham and colleagues investigated the relationship between the Roseobacter *Rugeria pomeroyi* and the diatom *Thalassiosira pseudonana*.[29] Because bacterial transcriptomes showed increased expression of 2,3-dihydroxypropane-1-sulfonate (**21**) transporters and catabolism genes when in the presence of the diatom, the authors suggested the bacterium provided vitamin B12 to the diatom, and in return the diatom provided **21** to the bacterium. In addition, sulfur metabolism genes of *T. pseudonana* were down-regulated when exposed to the bacterium.[29] While this may signal a tight relationship between *R. pomeroyi* and *T. pseudonana*, the authors state that there is little evidence that the relationship is mediated by non-nutritional molecules, since production of **21** by diatoms did not appear to be stimulated by the bacterium.[29] However, the presence of signaling molecules was not explicitly tested in this study. A more recent study used mutant *R. pomeroyi* to test the role of **21** as a bacterial growth substrate.[30] Bacteria incapable of expressing key **21** transporters were unable to grow when provided with **21** as sole carbon source, and limited growth was observed when this bacterium was grown with *T. pseudonana*.[30] These results support the hypotheses that *T. pseudonana*-produced **21** serves as a carbon source, not as a signaling molecule, for *R. pomeroyi*.



dimethylsulfoniopropionate (**20**)



DHPS (**21**)

Yet, *T. pseudonana* appears to be capable of recognizing *R. pomeroyi*.[31] In the presence of *R. pomeroyi*, *T. pseudonana* regulated expression of genes related to cell signaling and recognition functions, including pathways similar to bacterial recognition pathways found in plants.[31] The authors proposed that these signaling and secondary messenger genes indicate active recognition of the bacterium by the diatom.[31] Presumably, this recognition occurs via dissolved organic compounds, since few *R. pomeroyi* cells were attached to *T. pseudonana* cells and *R. pomeroyi* had upregulated genes for protein excretion/organic molecule excretion.[31] Which signal(s) *T. pseudonana* responds to is as of yet unknown. Future work in this study system to characterize the dissolved organic compounds of the culture medium, as well as more classical conditioned media-based experiments will help to confirm the presence of chemical signaling used by *T. pseudonana* to detect bacteria.

EroS was recently identified as an enzyme exuded by the marine gammaproteobacterium *Vibrio fischeri* which stimulates sexual reproduction and a newly described swarming behavior in the choanoflagellate *Salpingoeca rosetta*.[32] The amino acid sequence of EroS contains a glycosaminoglycan lysase domain, revealing that it acts specifically on chondroitin (a specific glycosaminoglycan). Amino acid substitutions at the catalytic site eliminated the ability of EroS to elicit swarming and mating behaviors in *S. rosetta*, indicating that enzymatic activity of EroS is critical in its function to stimulate mating/swarming.[32] This study demonstrated several previously underappreciated aspects of *S. rosetta* biology: the presence of sulfated glycosaminoglycans in *S. rosetta* membranes, aggregation behavior to increase the rate of sexual reproduction, and the ability of bacteria to influence behaviors and reproduction in *S. rosetta*.[32] Aggregating makes evolutionary sense in the pelagic environment, since cells are likely to be greatly dispersed and finding mates should be challenging. The authors suggest that the ability of *S. rosetta* to respond to the presence of certain bacteria may signify appropriate environments for successful sexual production.[32]

A few recent studies investigated the impacts of eukaryotic phytoplankton on bacterial metabolism or physiology.[30, 33] The alphaproteobacterium *Dinoroseobacter shibae* was found to influence the growth of the dinoflagellate *Prorocentrum minimum*, by shifting from a mutualist, which increases growth, to a pathogen, which promotes rapid decline, during different growth phases of *P. minimum*.[33] Transcriptomics revealed quorum sensing, *CtrA* phosphorelay, and flagella biosynthesis genes were consistently and significantly upregulated after this switch from mutualism to pathogenicity.[33] Various knockout mutants were more inhibitory towards *P. minimum* than wild type bacteria, indicating that these genes modulate the deleterious effects of *D. shibae* on the dinoflagellate. However, two mutants, both lacking a plasmid encoding for type IV secretion systems, had no effect on the growth of *P. minimum*, demonstrating that *D. shibae* contains several genes for pathogenicity.[33] To date, no work has yet been done in this study to isolate or investigate any chemical signals that may turn on the expression of pathogenic genes in *D. shibae*.

The dinoflagellate *Alexandrium tamarense* influences the metabolism of *R. pomeryoi* by causing the bacterium to increase expression of quorum sensing genes.[30] This appears to be a species-specific effect, as the presence of the diatom *T. pseudonana* did not cause differential expression of these quorum sensing genes.[30] *R.*

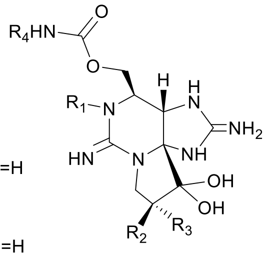
pomeryoi also enhanced expression of genes involved in degradation of **20** in the presence of *A. tamarense*.[30] Landa and colleagues speculated that the presence of **20** (likely produced by *A. tamarense* in the presence of *R. pomeryoi*) served not only as a carbon and sulfur source for the bacterium, but also as a signal to alter *R. pomeryoi* metabolism. In support of this hypothesis *R. pomeryoi* enhanced expression of quorum sensing genes, phosphorelay and flagellum assembly genes as well as gene transfer agent cassette when grown with *A. tamarense*.[30]

Proteomics revealed that compounds exuded by the diatom *Thalassiosira weissflogii* cause changes in the metabolism of the gammaproteobacterium *Marinobacter adhaerens*.[34] Ten bacterial proteins involved in nitrogen metabolism and amino acid transport were differentially expressed in *M. adhaerens* in response to *T. weissflogii*, even though the bacterium was physically separated from the diatom. [34] This lead Stahl and colleagues to conclude that the presence of *T. weissflogii* provides a different nutritional environment for *M. adhaerens*, which is then reflected in the changes in the proteome to accommodate amino acid released by the diatom.[34] The authors did not discuss the potential for cues or signals released by either the bacteria or the diatoms to cause changes in metabolism unrelated to nutrition; however, they noted the insensitivity of their methodological approach indicating that other signaling possibilities cannot be excluded.[34]

These studies, as well as the large body of work showing that dissolved vitamins are readily exchanged among members of plankton (as reviewed by Sañudo-Wilhelmy et al 2014)[35] showcase the myriad enigmatic exchanges of metabolites among planktonic organisms. The identification of such metabolites, whether they are nutritional or for signaling, is challenging due to the rapid turnover of such compounds within the phycosphere, which results in low standing stock concentrations. This type of cross-talk, as evidenced by the large taxonomic and ecological variation in organisms described above, is likely common throughout the pelagic and remains an underexplored area of research. Future studies to assess whether these species-specific relationships constitute true mutualisms are also needed.

4 Host-parasite interactions

Waterborne cues emitted by predators induce defenses in some marine prey.[36, 37] A pair of recent studies suggest that phytoplankton might respond to chemical cues from parasites in an analogous way, reducing their risk of infection.[38, 39] Lu and colleagues reported that the dinoflagellate *Alexandrium fundyense* detects waterborne cues from the parasitic dinoflagellate *Amoebophrya* sp. resulting in differential gene regulation.[38] When infected by *Amoebophrya* sp. or exposed to its exuded metabolites, *A. fundyense* was found to upregulate its genes associated with energy production, signal transduction, stress, and (putatively) defense. However, there were still distinct differences in gene regulation between the two types of parasite exposure indicating that the molecular mechanisms responsible for detecting and responding to parasitic threat, as well as changes in energy usage and signaling, differ in each case. Production of paralytic shellfish toxins (PSTs) (*e.g.* **22-30**) by *A. fundyense* was not significantly altered by the presence of the parasite or its diffusible metabolites.[38] Overall, this study suggests that recognition of *Amoebophrya* sp. waterborne cues alone might 'prime' a protective, defensive response in the host before encounter.



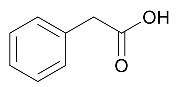
saxitoxin **(22)** R_1=R_2=R_3=R_4=H
neosaxitoxin **(23)** R_1=OH R_2=R_3=R_4=H
GTX-1 **(24)** R_1=OH R_2=H R_3=OSO_3^- R_4=H
GTX-2 **(25)** R_1=R_2=H R_3=OSO_3^- R_4=H
GTX-3 **(26)** R_1=R_3=H R_2=OSO_3^- R_4=H
GTX-4 **(27)** R_1=OH R_2=OSO_3^- R_3=H R_4=H
GTX-5 **(28)** R_1=R_2=R_3=H R_4=SO_3^-
N-sulfocarbamoyl saxitoxin C1 **(29)** R_1=R_2=H R_3=R_4=OSO_3^-
N-sulfocarbamoyl saxitoxin C2 **(30)** R_1=R_3=H R_2=R_4=OSO_3^-

Another dinoflagellate, *Fragilidium duplocampanaeforme,* did not sense the waterborne cues of its parasite; only the physical presence of live zoospores of the parasitic dinoflagellate *Parvilucifera infectans*, and not dead zoospores or zoospore exuded compounds, triggered cells to form temporary, ecdysal cysts.[39] These results contrast with those previously reported for *Alexandrium ostenfeldii* which was observed to form ecdysal cysts in response to waterborne cues from *P. infectans*.[40] More data are needed to understand whether ecdysal cyst formation in diverse dinoflagellates in response to waterborne cues is a common phenomenon affecting plankton community composition and dynamics.

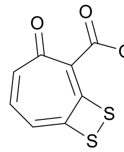
In addition to host sensation of parasites, the reverse case, parasite reception of host exuded metabolites, also occurs. Chytrid zoospores distinguish metabolic nuances in host exudates and increase chemotactic behavior toward attractive diatom hosts.[41] Abiotic factors influenced host attractiveness; extracts from light-stressed diatoms elicited the greatest chemotactic response from zoospores. Scholz and colleagues also attempted to identify differences in broad metabolite classes between species of diatoms within the same genus that differed in their susceptibility to parasite infection.[41] Differences were noted, but due to the experimental setup it was not possible to determine whether the compounds originated from the diatoms or the parasites, nor to draw conclusions about the chemical characteristics of parasite-resistant diatoms.[41] However, both exuded compounds and internal compounds from parasite-resistant diatoms increased protection against parasite infection, hinting at the production of unidentified protective chemical compound(s) by parasite-resistant diatoms.

Once inside its host a parasite may manipulate the host to its own advantage. A recent study by van Tol and colleagues investigated interactions between the flavobacterium *Croceibacter atlanticus* and diatoms.[42] The bacterium is a member of the microbial consortium which attaches to diatom surfaces.[42] Once attached, *C. atlanticus* inhibited the growth of about half of the tested diatoms.[42] Interestingly, the resistant diatoms each still possessed their natural microbial consortia hinting that their microbiome may counteract the negative influence of *C. atlanticus*.[42] When the model diatom *Thalassiosira pseudonana* was infected with *C. atlanticus* it stopped dividing, grew larger, became polyploid, and increased its plasmid number and chlorophyll a abundance.[42] The authors hypothesized that these outcomes benefit the parasitic bacterium by creating a more colonizable surface area and greater excretion of metabolites that aid in growth. The compounds exuded by *C. atlanticus* when grown alone caused no deleterious effects to *T. pseudonana* indicating that either the inhibitor compounds are not constitutively released or that the interaction is not chemically mediated.[42] However, no further steps were taken to identify any induced compound(s) that might be responsible for the growth inhibition of *T. pseudonana* caused by *C. atlanticus*.

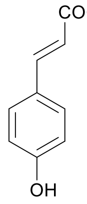
Not all cases of host-parasite interaction are antagonistic at all life stages. The marine Roseobacter *Phaeobacter inhibens* associates with the coccolithophore *Emiliania huxleyi* in a mutualist-to-parasite switch model,[43] similar to its interaction with the dinoflagellate *Prorocentrum minimum*.[33] While acting as mutualists, other *Phaeobacter* spp. have been found to co-opt DMSP (**20**) from *E. huxleyi* to produce phenylacetic acid (**31**), a growth promoter in some algae, and tropodithietic acid (**32**), an antibiotic that protects microalgae from marine pathogens.[44-46] During senescence, the alga produces *p*-coumaric acid (**33**) which triggers *Phaeobacter* spp. to produce algicidal roseobacticides (**34** and **35**), in a switch to parasitism.[47] A recent effort by Wang and colleagues to uncover the roseobacticide biosynthetic gene cluster in *P. inhibens* determined that genes required for biosynthesis of **32** were also required for roseobacticide biosynthesis and that genes from three different loci are needed for roseobacticide production.[43] They also discovered that *P. inhibens* requires two signals, **33** and the QS molecule *N*-(3-hydroxydecanoyl)-L-homoserine lactone (**36**) to switch to parasitism and initiate roseobacticide production. Additionally, the authors uncovered a new biosynthetic mechanism for thiol insertion in the biosynthesis of roseobacticides.[43] The necessity for the dual presence of **33** and **36** for roseobacticide biosynthesis may point to tight regulation of the switch from mutualism to parasitism. For the most part, by utilizing the same biosynthetic enzymes to produce **32, 34** and **35**, a rapid switch from mutualism to parasitism can be accomplished.



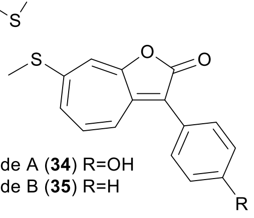
phenylacetic acid (**31**)



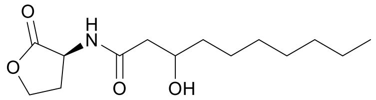
tropodithietic acid (**32**)



p-Coumaric Acid (**33**)

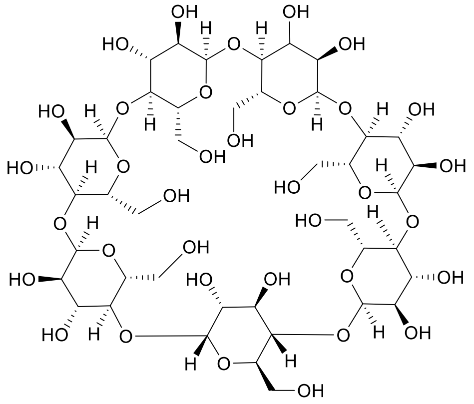


roseobacticide A (**34**) R=OH
roseobacticide B (**35**) R=H

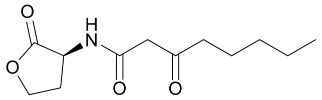


N-(3-hydroxydecanoyl)-L-homoserine lactone (**36**)

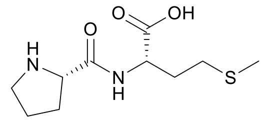
Recently, the algicidal effects of some marine bacteria have been studied in an attempt to better understand algal-bacterial interactions.[48, 49] Many bacteria that are algicidal only appear to be so when at high concentrations.[50-53] Therefore, a recent study by Chi and colleagues was founded on the hypothesis that bacterial quorum sensing is important in controlling algal blooms and more specifically, involved in controlling production of algicidal compounds.[48] The algicidal bacterium *Ponticoccus* sp., isolated from *P. donghaiense*, killed cultures of *P. donghaiense*, *A. tamarense*, and the prymnesiophyte *Phaeocystis globose* when they were in exponential growth phase. When treated with β-cyclodextrin (**37**), a known binder of acyl homoserine lactones, *Ponticoccus* sp. became less algicidal, suggesting that quorum sensing might be at least partially involved in its algicidal activity.[48] The homoserine lactones, *N*-(3-oxo-octanoyl)-L-homoserine lactone (**38**) and *N*-(3-oxo-decanoyl)-L-homoserine lactone (**17**), as well as, the autoinducer synthases (*zlaI* and *zlbI*) and luxR transcriptional regulator genes (*zlaR* and *zlbR*) for were identified as being involved in quorum sensing in *Ponticoccus* sp.[48] However, the algicidal compounds were never identified. Another study characterized prolylmethionine (**39**) and hypoxanthine (**40**) as involved in the algicidal activity of a *Bacillius* sp. against *P. globose*, the alga from which it was isolated.[49] However, *P. globose* only experiences growth inhibition when exposed to **39** and **40** at well above ecologically relevant concentrations.[49]



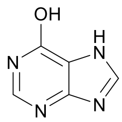
β-cyclodextrin (**37**)



N-3-oxo-octanoyl-L-homoserine lactone (**38**)



prolyl-methionine (**39**)



hypoxanthine (**40**)

5 Allelopathy

Understanding interactions among phytoplankton species is important in order to predict bloom dynamics. For example, not all phytoplankton species that share a fundamental ecological niche co-occur in the field, and for the ones that do, we do not know all the factors that allow for coexistence. In 2009 the rhapidophyte *Chattonella marina* var. marina and the dinoflagellate *Gymnodinium catenatum* co-bloomed in Bahía de La Paz, Gulf of California, but *C. marina* consistently occurred at double or greater concentration relative to *G. catenatum*.[54] Therefore, a study was undertaken by

Fernández-Herrera and colleagues to gain insight into how *C. marina* outcompeted *G. catenatum* during the bloom.[55] *C. marina* was found to cause loss of flagella, nuclear swelling, and cyst formation in *G. catenatum*. Compounds exuded by *C. marina* appeared to account for approximately 50% of the outcome on *G. catenatum*.[55] Additionally, some *G. catenatum* cells lysed after contact with *C. marina* cells. Overall, the study showed that *C. marina* has deleterious effects on *G. catenatum* physiology and growth, but the exuded compounds responsible for the allelopathic portion of these effects were not identified.[55]

Both the dinoflagellate *Alexandrium minutum* and the diatom *Skeletonema costatum* grow off the coast of China, but only *S. costatum* is known to form blooms there.[56] Wang and colleagues found that compounds exuded by *S. costatum* inhibited the growth of *A. minutum*. Yet when *A. minutum* was exposed to 15(S)-hydroxyeicosa-5Z,8Z,11Z,13E,17Z-pentaenoic acid (15(S)-HEPE)(**41**), a known auto-inhibitor of *S. costatum*, it acted as a stimulant at low concentrations and an inhibitor at unnaturally high concentrations.[56] Therefore, **41** was not the allelochemical exuded by *S. costatum* responsible for the observed growth inhibition of *A. minutum* despite being produced. The allelopathy of *S. costatum* against *A. minutum* may explain why only low concentrations of *A. minutum* are reported in the East China Sea. However, the authors of the study warned that this favoring of diatoms could change if coastal pollution continues because eutrophication promotes dominance of dinoflagellates over diatoms in the East China Sea.[56]

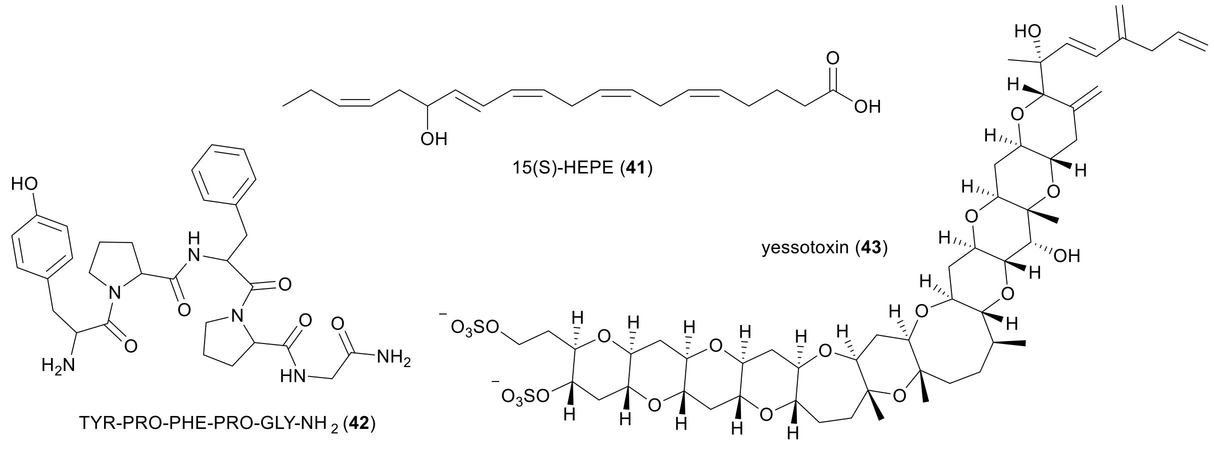
In a pair of studies by Wang and colleagues the diatom *Phaeodactylum tricornutum* was found to be allelopathic against the raphidophyte *Heterosigma akashiwo*.[57, 58] Additionally, using mass spectrometry, an allelopathic peptide with putative primary sequence TYR-PRO-PHE-PRO-GLY-NH_2 (**42**) was identified, although the authors cautioned that further investigation is needed to verify its three dimensional structure.[57] At ecologically relevant concentrations **42** caused increased activity of esterases, cell membrane disruption, and disturbance of cell membrane potential in *H. akashiwo*.[58] None of the deleterious effects lasted the full length of the experiment suggesting that either *H. akashiwo* acclimated to the allelochemical, the *H. akashiwo* cells that survived gave rise to a population that was more resistant to the allelochemical, or that **42** degraded over time.[58]

Allelopathic interactions are not limited to larger phytoplankton. In a recent series of studies Śliwińska-Wilczewska and colleagues explored the effects of the picocyanobacterium *Synechococcus* sp. on the phytoplankton community in the Baltic Sea.[59-61] Compounds exuded by *Synechococcus* sp. inhibited the growth of all the phytoplankton in a natural community, except the cyanobacteria *Nodularia spumigena* and *Gloeocapsa* sp.[59] This caused a shift in the phytoplankton community whereby *Synechococcus* sp. became even more dominant and green algal species decreased in relative abundance.

Allelopathic effects of the same *Synechococcus* sp. were also measured against the filamentous cyanobacteria *Aphanizomenon flos-aquae*, *Nostoc* sp., *Phormidium* sp., and *Rivularia* sp.[60] Compounds exuded from *Synechococcus* sp. inhibited the growth of *Nostoc* sp. and *Phormidium* sp., but enhanced the growth of *A. flos-aquae*. The decrease in growth of *Phormidium* sp. was accompanied by a decrease in chlorophyll a abundance and fluorescence suggesting that its photosynthesis efficiency was reduced by *Synechococcus* sp. exudates. In contrast, in *Nostoc* sp. carotenoids, a class of accessory photosynthetic pigments, increased in the presence of *Synechococcus* sp. exudates despite overall growth inhibition.[60] Therefore, further studies should investigate the mechanism by which *Synechococcus* sp. inhibits the growth of *Nostoc* sp. In an additional study, Śliwińska-Wilczewska and colleagues found that *Synechococcus* sp. was most allelopathic towards the diatom *Navicula perminuta*, as measured by the rate and efficiency of photosynthesis, at optimal growing conditions for *Synechococcus* sp.[61] Unfortunately, the allelochemicals produced by *Synechococcus* sp. were not identified in any of the aforementioned studies.

Allelopathic potency often varies within species.[62-64] Fifteen strains of *Alexandrium tamarense* caused different levels of growth inhibition in *C. marina*, with weakly inhibitory strains themselves strongly suppressed by *C. marina*.[62] The inhibitory effect caused by each *A. tamarense* strain correlated with the lytic activity of exuded compounds greater than 1 kDa. However, lytic activity of exudates were tested using rabbit blood cells and not *C. marina*.[62] Therefore, further studies are needed to explore to what extent exploitation competition, in addition to allelopathy, might be responsible for the decreased growth of *C. marina* when co-cultured with *A. tamarense*.

The lytic effects of six arctic strains of the dinoflagellate *Protoceratium reticulatum* against the cryptomonad *Rhodomonas salina* were tested by Sala-Pérez and colleagues.[63] Lytic activity was



15(S)-HEPE (**41**)

TYR-PRO-PHE-PRO-GLY-NH_2 (**42**)

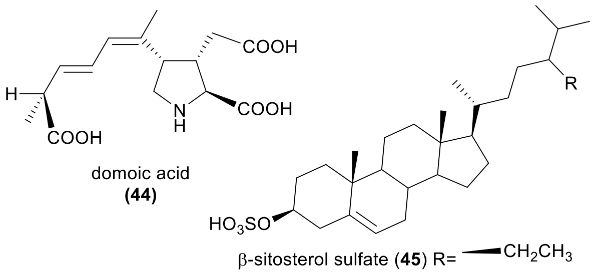
yessotoxin (**43**)

found to be strain- and growth phase-dependent, but independent of temperature. Exudates alone from *P. reticulatum* caused lysis in *R. salina* but did not recapitulate the levels of lysis caused by co-culture, suggesting that the compounds responsible for causing the lysis might be unstable. Additionally, lysis of *R. salina* did not appear to be associated with yessotoxin (**43**).[63]

A study by Xu and colleagues found that the growth of the dinoflagellate *Akashiwo sanguinea* was inhibited by one of two strains of the diatom *Pseudo-nitzschia multiseries*.[64] Compounds exuded in stationary phase by *P. multiseries* strain CLNN16, but not strain CLNN21, inhibited growth in *A. sanguinea*, thereby suggesting that only CLNN16 is allelopathic against *A. sanguinea*. However, no allelochemicals were identified.[64] In another part of this study these authors tested the effects of a strain of *Pseudo-nitzschia pungens* from the South China Sea against *A. sanguinea* and four other co-occurring phytoplankton species (*Prorocentrum minimum*, *Phaeocystis globose*, *C. marina*, and *R. salina*).[64] *P. pungens* inhibited growth of *A. sanguinea*, and to a lesser extent *C. marina* and *R. salina*, in a density-independent manner. Additionally, compounds exuded by lysed cells of both *P. pungens* and *P. multiseries* strain CLNN16 inhibited the growth of *A. sanguinea* and *R. salina*.[64] The authors of the study suggest that both *Pseudo-nitzschia* spp. are allelopathic but that domoic acid (**44**) is not the allelochemical, since the tested strain of *P. pungens* was allelopathic despite not being a known producer of **44**.[64] However, concentrations of **44** were not measured in this study and compounds released only by cell lysis are likely not effective at mediating competition.

Some phytoplankton appear to inhibit their own growth.[65, 66] In a follow-up to their 2013 study,[67] Zhang and colleagues tested the effect of compounds exuded by declining phase *S. costatum* on the interactions between *S. costatum* and the dinoflagellate *Prorocentrum donghaiense* using more field-relevant nutrient concentrations and bacteria free culture conditions.[65] Once again they found that self-inhibition by *S. costatum* is likely what drives the successional bloom of *P. donghaiense* after *S. costatum* in the East China Sea.[65, 67] Despite continued study of this system, additional experiments are still warranted because the self-inhibitory compound(s) produced by *S. costatum* have yet to be identified.

The sterol sulfates, β-sitosterol sulfate (**45**), dihydrobrassicasterol sulfate (**46**), and cholesterol sulfate (**47**)**,** have been found to regulate cell death programming in the diatom *Skeletonema marinoi*.[66] Early exponential phase cultures of *S. marinoi* declined immediate when exposed to their own stationary phase filtrates or the individual compounds **45**, **46**, and **47**. Exposure to **47** alone induced processes associated with cell stress and apoptosis in *S. marinoi*. The role of these compounds in the self-inhibition was further confirmed when interruption of the biosynthesis of **45**, **46**, and **47** resulted in an extended stationary phase and an increase in final cell density of *S. marinoi*.[66]



domoic acid
(**44**)

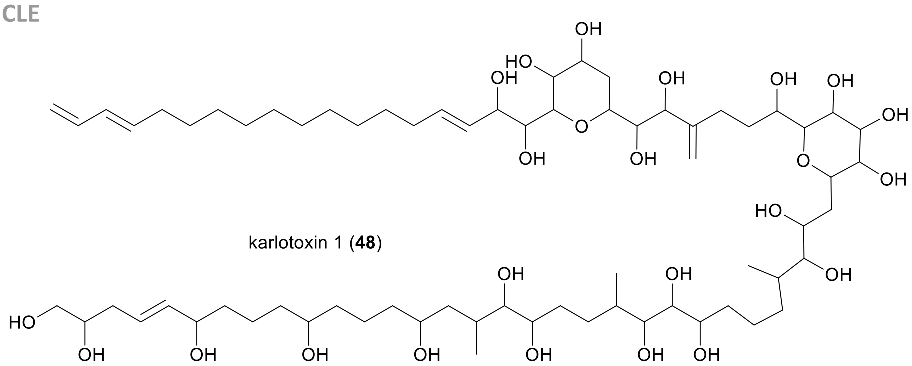
β-sitosterol sulfate (**45**) R= CH_2CH_3
dihydrobrassicasterol sulfate (**46**) R= CH_3
cholesterol sulfate (**47**) R=H

6 Predator-prey interactions

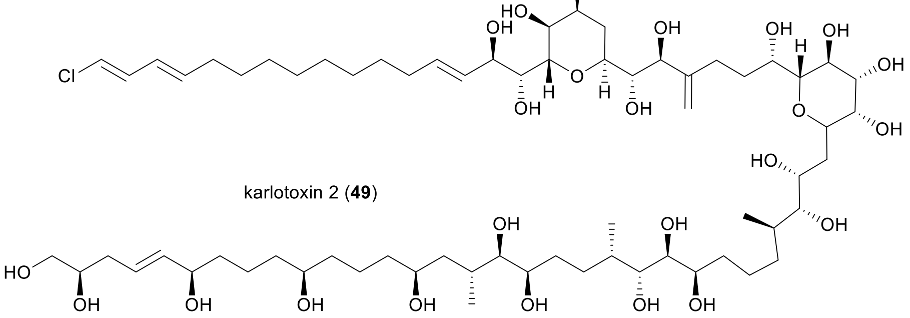
6.1. Chemical defenses

Marine plankton are well-known producers of secondary metabolites hypothesized to act as defenses against predators. Over the past decade, several species of dinoflagellates of the genus *Karlodinium* which produce karlotoxin 1, 2, 8, and 9 (**48-51**),[68] have been implicated in fish kills. These molecules act by binding to cholesterol and disrupting cell membranes (reviewed by Rasmussen and colleagues[9]).[69] Recently, a novel karlotoxin-like molecule was identified by Rasmussen and colleagues from the newly identified ichthyotoxic dinoflagellate species *Karlodinium armiger*.[70] Karmitoxin (**52**) differs from the previously described **48-51** in that it possesses a primary amine at one end. In a rainbow trout gill cell toxicity assay, **52** lysed cells with an LC_{50} value of 125 nM.[70] Additionally, **52** exhibited an LC_{50} of 400 nM for adult female *Acartia tonsa* copepods .[70] The concentrations used to establish LC_{50} values were in the measured range for extracellular **52** from *K. armiger*.[70] However, an ecological assay for planktonic organisms would typically involve exposing copepods to toxins within food particles, not dissolved in seawater. Therefore, it is unclear whether **52** would actually play an important role as a chemical defense against copepods or as an ichthyotoxin in natural populations.

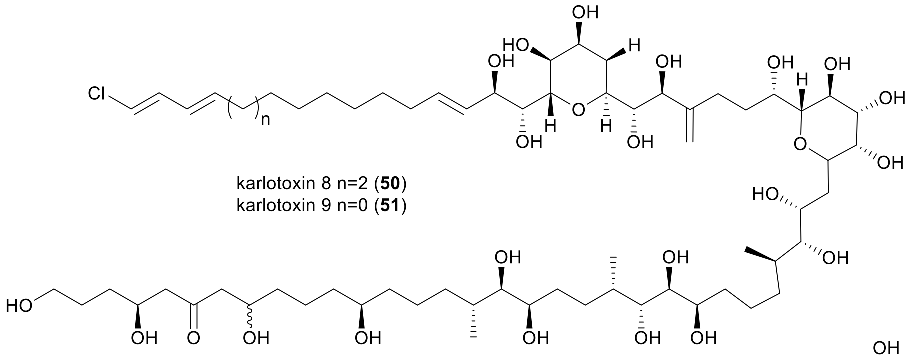
The haptophyte *Prymnesium parvum* has also been implicated in massive fish kills.[71] Recently, Rasmussen and colleagues characterized a new prymnesin that contains a novel ladder-frame polyether backbone.[72] Prymnesin-B1 (**53**) differs from the previously described prymnesin-2 (**54**) in the elimination of rings H and I from the polyether ladder-frame resulting in six fewer carbon atoms in the polyketide backbone, substitution of the α-L-xylofuranose moiety for α-D- galactopyranose, and loss of a chlorine before the terminal alkynyl groups. Additionally, by screening 10 strains of *P. parvum* from all around the world they putatively identified 13 new prymnesins by liquid chromatography high-resolution mass spectrometry, eight of which appear to possess a novel, more heavily chlorinated backbone.[72] By a rainbow trout gill cell assay **53** was found to have an EC_{50} value of 5.98 nM, which makes **53** much less toxic than **54**; therefore, the authors speculated that all prymnesins are ichthyotoxic to varying degrees.[72] Nevertheless, they failed to determine whether the potencies of toxins fall within the expected natural concentrations for prymnesins during a *P. parvum* bloom.



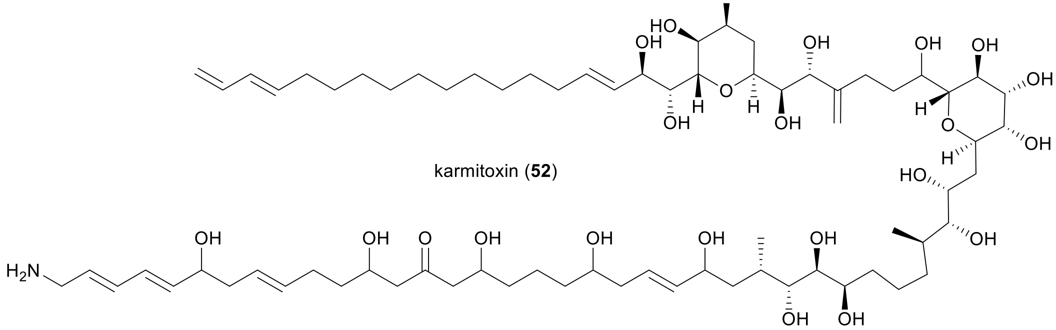
karlotoxin 1 (**48**)



karlotoxin 2 (**49**)



karlotoxin 8 n=2 (**50**)
karlotoxin 9 n=0 (**51**)

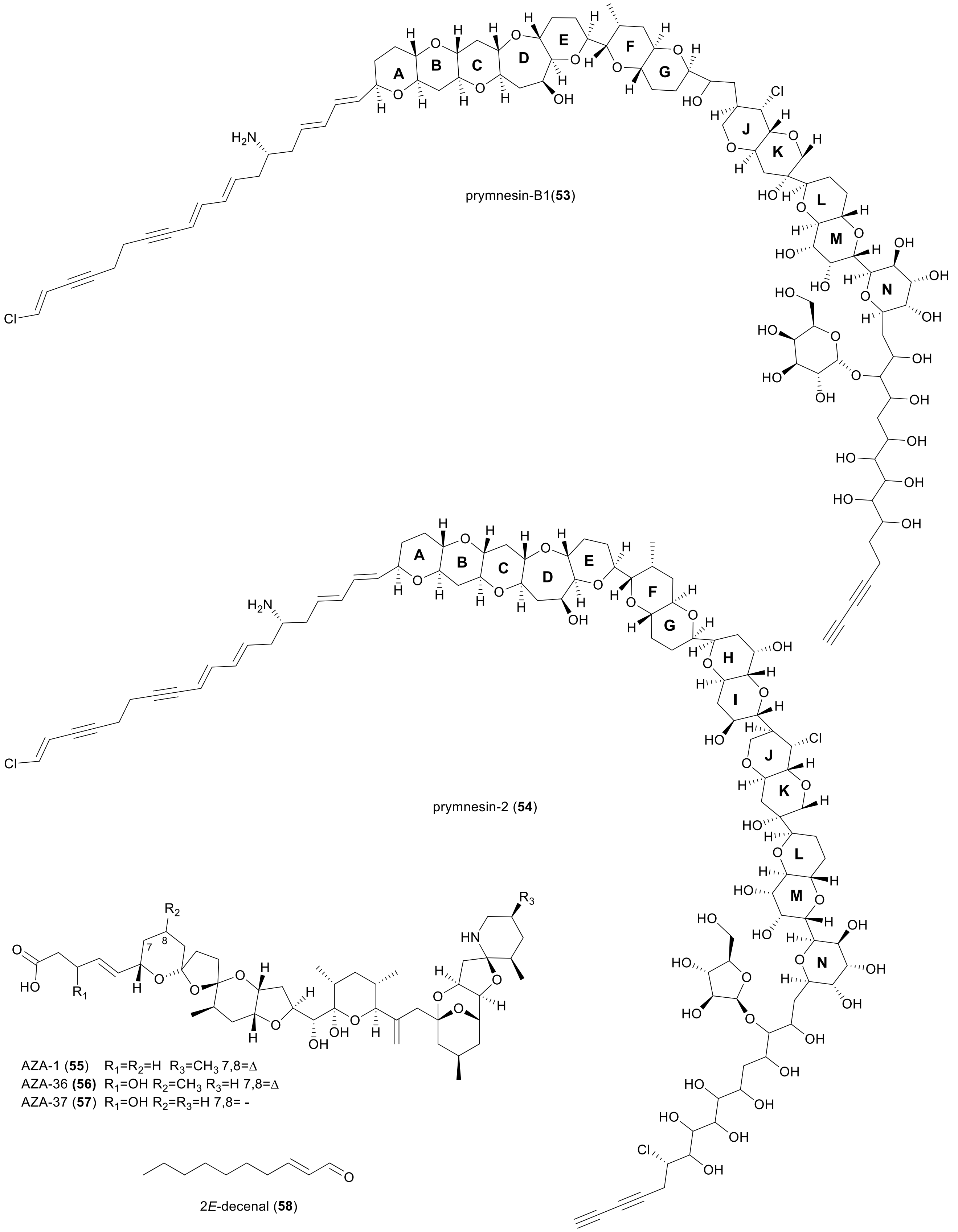


karmitoxin (**52**)

Recently two new toxic azaspiracids (**55** and **56**) were isolated from the dinoflagellate *Azadinium poporum*.[73] These new analogues differ from the previously described **57** by minor alterations to methylation, hydroxylation, and unsaturation patterns. In a cytotoxicity assay with lymphocytes **55** and **56** were much less toxic than **57.**[73] Although the azaspiracids have previously been proposed to function as chemical defenses,[74] this study did not address an ecological function for the novel natural products; further studies should be performed to identify the ecological roles, if any, of azaspiracids in *Azadinium* sp.

A 2015 study on winter phytoplankton blooms in 2004 and 2005 in the northern Adriatic Sea attempted to link diatoms and non-volatile oxylipins, such as 15(S)-HEPE (**41**) and 2*E*-decenal (**58**), to reduced reproductive success in copepods.[75] The study found that mean phytoplankton density during winter blooms increased between 2004 and 2005 driven largely by diatoms, which increased in abundance from 10.3% to 50.1% of the phytoplankton composition. Among the diatoms, *Skeletonema marinoi* was the most dominant species in 2005; whereas in 2004, *Pseudo-nitzschia galaxiae*, *S. marinoi*, and *Chaetoceros* spp. were all close to equally dominant among diatom species.[75] The higher diatom abundance in 2005 was mirrored by higher egg production in copepods *Acartia tonsa* and *Calanus helgolandicus*; yet, hatching success was significantly lower in 2005 than in 2004. Ianora and colleagues suggested that hatching success was compromised because of higher concentrations of oxylipins, particularly non-volatile oxylipins, recorded in 2005, which might have been the result of diatom population booms.[75] A study in 2012 had shown a pattern of downregulation in aldehyde detoxification and apoptosis regulator genes in *C. helgolandicus* in response to a diet of *S. marinoi*, the predominant diatom in the 2005 winter Adriatic Sea blooms, providing further support for the hypothesis that the increase in *S.*

marinoi and oxylipins in 2005 was responsible for deleterious effects on copepods.[76] In 2015, a similar study to the 2012 one was completed exploring the effects of a diet of *S. marinoi* on gene expression in the copepod *Calanus sinicus*.[77] While after the first two days aldehyde detoxification genes, a heat shock protein, and an antioxidant enzyme gene were downregulated in *C. sinicus* fed



prymnesin-B1(**53**)

prymnesin-2 (**54**)

AZA-1 (**55**) R_1=R_2=H R_3=CH_3 7,8=Δ
AZA-36 **(56)** R_1=OH R_2=CH_3 R_3=H 7,8=Δ
AZA-37 (**57**) R_1=OH R_2=R_3=H 7,8= -

2*E*-decenal (**58**)

S. marinoi similar to the response in *C. helgolandicus*, by the fifth day these genes had switched to being upregulated.[77] This suggests that, unlike *C. helgolandicus*[76], *C. sinicus* is capable of restoring initially damaged proteins and activating an antioxidant response.[77] The results of these studies support the generalized hypothesis that oxylipins contribute to the poor food value of diatoms for copepods.

The dinoflagellates *Akashiwo sanguinea* and *Alexandrium catenella* and the raphidophyte *Chattonella marina* negatively impact the growth of the predatory dinoflagellate *Noctiluca scintillans* despite the ability of *N. scintillans* to feed and grow on several other chemically defended dinoflagellates and raphidophytes.[78] When provided with a mixed assemblage diet of *A. catenella*, a producer of PSTs (*e.g.* **22**-**30**), and a non-toxic strain of the raphidophyte *Heterosigma akashiwo*, the growth rate of *N. scintillans* increased with increasing proportion of *H. akashiwo* at a rate greater than expected if toxicity of *A. catenella* was undermining its value as a food.[78, 79] Therefore, it is unlikely that toxicity is responsible for the negative growth rate of *N. scintillans* when feeding on *A. catenella*. In further support of this hypothesis, *H. akashiwo* cultures spiked with compounds exuded by *A. catenella*, and not ones spiked with saxitoxin (**22**), significantly decreased the growth rate of *N. scintillans*.[78] Therefore, there may be unidentified compounds produced, and possibly exuded, by *A. catenella* that either detract from its nutritional value or deter feeding by *N. scintillans* without imposing direct toxicity.

The newly described *Alexandrium pohangense* uses its chemical defense to kill potential protist predators before they even begin grazing.[80] *A. pohangense* forms blooms off the coast of Pohang, South Korea despite having an extremely slow growth rate when growing autotrophically.[81] Consequently, one hypothesis is that *A. pohangense* blooms less by growing and more by avoiding predation, relative to its competitors.[80] In a recent study, pre-bloom concentrations of *A. pohangense* immobilized and then lysed nine different heterotrophic protist predators known to prey upon other chemically defended phytoplankton.[80] Compounds exuded by *A. pohangens* alone killed the majority of protists in a natural assemblage and were nearly as potent at killing the predators as live *A. pohangense*. In conclusion, *A. pohangense* likely forms blooms, at least in part, by killing many would-be protists predators; however, rates of predation on other competitors in a natural assemblage still needs to be measured for comparison and the post-ingestion effects of *A. pohangense* on metazoan predators has yet to be studied.[80] Without these additional studies there is no way of knowing if *A. pohangense* experiences lower predation rates than its competitors. Additionally, future studies should identify the compound(s) responsible for the lytic effects recorded in this study.

Bivalve mollusks are commonly negatively impacted by the chemical composition of phytoplankton. A recent study by Tran and colleagues found that the toxic dinoflagellate *Alexandrium minutum* impacts the daily rhythms of the oyster, *Crassostrea gigas*.[82] Bloom concentrations of *A. minutum* caused loss of the cyclic pattern of expression of *Cgcry*, a circadian rhythm-controlling gene, in *C. gigas*.[82] Similarly, digestive processes and gene expression of genes involved in oxidative stress defense, immunity, and respiration were no longer cyclically regulated when oysters ate *A. minutum*.[82] Moreover, the study found oysters that had accumulated *A. minutum* toxins in their digestive glands remained open throughout the 24 hours, regardless of cycling of the tides or *Cgcry* gene expression, revealing another mechanism by which such compounds can affect grazers.[82] The full consequences of the loss of biological daily rhythms on the long term fitness of oysters have yet to be studied and the authors suggest that the loss of rhythm, and subsequent changes in digestion pattern, may actually protect the oysters from more serious adverse effects when they experience ephemeral blooms of *A. minutum*.[82]

Toxic phytoplankton may also indirectly harm bivalve mollusks by making bivalve mollusks more vulnerable to infection. Several recent studies have suggested that exposure to blooms of toxic *Alexandrium* spp. makes shellfish prone to parasitism [83] and infection.[84] A study by Abi-Khalil and colleagues showed that exposure to toxic *A. catenella* increased the susceptibility of adult *C. gigas* to mortality from infection by the pathogen *Vibrio tasmaniensis*.[84] Additionally, the authors found an association between *V. tasmaniensis* infection, mortality, and toxin (i.e., GTX-2 (**25**) and GTX-C2 (**30**)) accumulation in adult oysters.[84] In contrast, *C. gigas* larvae did not experience increased susceptibility of infection by *Vibrio* sp when to fed *A. catenella*.[85] However, larvae did experience an increased immune response, but this was unrelated to accumulation of PSTs (*e.g.* **22**-**30**).[85] On the other hand, other recent studies have shown that **22** induces apoptosis of immune cells in *C. gigas*[86] and decreases phagocytosis, increases reactive oxygen species production,[87] and causes an upregulation of immune pattern recognition receptor genes and their signaling pathways in the Chilean mussel, *Mytilus chilensis*.[88] Unfortunately, these three studies[86-88] involved either bathing the immune cells in dissolved **22** (or other related toxins (**23**-**30**)) or directly injecting **22** into mussel muscle, which is not how mussels would be exposed in under natural conditions.

Shellfish larvae may be especially vulnerable to phytoplankton compounds. Sperm and oocytes exposed to the dinoflagellate *Karenia brevis* just prior to fertilization experienced decreased fertilization success and increased mortality.[89] Additionally, long term exposure to live *K. brevis* or its exuded compounds caused increased mortality in all larvae, whether they were initially exposed pre- or post-fertilization.[89] Even after the removal of *K. brevis*, larvae exposed to high concentrations of *K. brevis* cells continued to experience higher rates of mortality and lower growth rates indicating *K. brevis* can have deleterious effects on oyster larvae long after it is gone.[89] Because these effects were also experienced by larvae which were only exposed to exuded compounds, the source of the deleterious effects is likely chemical in nature; but, the compound(s) responsible were not investigated in this study[89] and therefore are a source of future studies.

Some algae modulate their own toxin production in response to chemical cues from predators. In a study by Tammilehto and colleagues, the diatom *Pseudo-nitzschia seriata* increased its production of domoic acid (**44**) when in the presence of two different species of copepods, *Calanus hyperboreus* and *C. finmarchicus* that were feeding on other *P. seriata* cells, separated by a permeable membrane.[90] Unlike toxin production, chain length in *P. seriata* was only affected when in direct contact with grazers, in which case *P. seriata* maintained a small proportion of large (four cell) chains whereas without the grazer *P. seriata* increased the proportion of large chains over time.[90] Lysed conspecific cells caused no changes in *P. seriata*, thereby indicating that the copepods and not the dead conspecifics were responsible for the chemical cue that resulted in the more defensive behaviors.[90] In a follow-up study by Harðardóttir and colleagues copepodites (sexually immature copepods) induced toxin production in *P. seriata* and the previously assumed non-toxic *Pseudo-nitzschia obtusa*.[91] Despite *P. seriata* having overall higher toxin production than its congener, both *Pseudo-nitzschia* spp. were eaten in equal proportion by the copepodites, which were found to bioaccumulate **44**.[91] This suggests that copepodites are as resistant to **44** as their adult counterparts and brings into question if **44** is a chemical defense against *Calanus* copepods at all. On one hand, *Pseudo-nitzschia* spp. increased production of **44** in response to the copepods; however, it does not appear to be toxic to various life stages of *C. finmarchicus*.[90, 91] One hypothesis is that **44** was once an effective deterrent against grazing by *Calanus* copepods but the copepods have evolved resistance to the toxin. Alternatively, the nutritional benefits of *Pseudo-nitzschia* spp. may outweigh the deleterious effects of **44**.

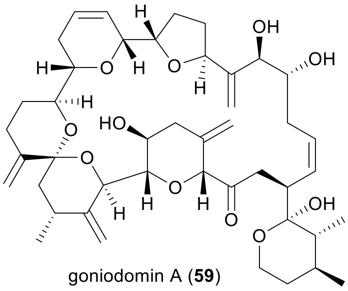
The toxic dinoflagellate *Alexandrium fundyense* increased its production of PSTs (**22**-**30**) when exposed to copepods with which it has historically co-occurred; whereas with other copepods it requires a feeding cue before toxin induction.[92, 93] The greatest increase in toxin production in *A. fundyense*, in most cases, was when it was exposed to a predator that was feeding or had recently eaten *A. fundyense*.[92, 93] As part of the study, the authors attempted to decouple the response to a predator from the response to an injured conspecifics by measuring toxin induction in *A. fundyense* in the presence of lysed conspecifics and congenerics in the absence of predators.[92] While not as dramatic as the response to the feeding predator cues, there was an increase in toxin production in response to lysed algal cells alone indicating the *A. fundyense* was likely responding to some sort of cue from the damaged conspecifics in addition to the predator cues.[92]

Phytoplankton also modulate toxin production based on the type of predators. Senft-Batoh and colleagues hypothesized that, because PSTs (*e.g.* **22**-**30**) act by blocking voltage-gated sodium channels, if *A. fundyense* produces PSTs as an inducible chemical defense they will only increase production of PSTs in the presence of metazoan grazers, which have at least perfunctory nervous systems, but not in the presence of protist grazers.[93] In support of their hypothesis, *A. fundyense* increased its toxin production in response to seven different metazoan predators from across three phyla, but not in response to either of two protist predators, each from a different phylum.[93] Additionally, the magnitude of toxin induction showed a positive asymptotic relationship with the total number of algal cells ingested by predators.[93] Overall, the study suggests that grazer-induced production of PSTs in *A. fundyense* is phylum- and species-specific and that the induction becomes greater when the proximity of the grazer and the likelihood of being eaten increases. As an opportunity for future investigation, we don't yet know the identity of the chemical cues from ciliate predators which elicit induction of toxin production in *A. fundyense*.[93]

In 2015, a study identified copepodamides (**1-8**) as predatory cues produced by copepods which induce toxin production in *Alexandrium minutum*.[94] Copepodamides are polar lipids whose backbone is likely biosynthesized by attachment of taurine via an amide bond to a hydroxylated phytanic acid analog, to which is appended an unsaturated fatty acid moiety. The copepodamides induced up to a 20-fold increase in toxin production at pico- to nanomolar concentrations.[94] Additionally, the study showed copepodamides to be active at ecologically relevant concentrations by measuring the exudation rate of copepodamides from field-collected copepods and by measuring copepodamide concentrations in field samples.[94]

Chemical defenses utilized by phytoplankton are typically not effective against all potential predators. Often there are predators sensitive to a chemical defense which experience deleterious effects, whereas others are partially or wholly resistant. The copepod *C. finmarchicus* is sensitive to *A. fundyense* toxicity, producing fewer eggs when fed a diet including *A. fundyense*.[95] However, reduced egg production may be due to nutritional inadequacy of *A. fundyense* rather than toxicity. Other copepods such as *Acartia hudsonica* appear to have evolved partial resistance to *Alexandrium* spp. toxicity.[96] The initial hypothesis was that *A. hudsonica* mitigated *Alexandrium fundyense* toxicity via a mutant sodium channel; however, the mutant isoform was found to be just as sensitive as the wild-type.[96] Therefore, later studies suggested that it is the ratio of the isoforms that is important for mitigating toxicity.[97] In contrast, more recent studies have found that there is no difference in the ratio of wild-type to mutant isoforms in populations more frequently exposed to *Alexandrium* blooms.[98, 99] Consequently, a recent study by Finiguerra and colleagues found that, even when fed a sole diet of *Alexandrium fundyense*, the mutant isoform conferred no advantage.[100] Additionally, expression of the mutant isoform appeared to be disadvantageous for *Acartia hudsonica*, causing decreased growth in individuals who predominantly express it when eating a diet without *A. fundyense*.[100] Therefore, the mechanism of resistance of *Acartia hudsonica* to the toxicity of *A. fundyense* still requires further investigation. Roncalli and colleagues found that adult *Calanus finmarchicus* copepods upregulated genes involved in digestive processes when eating a diet enriched in *A. fundyense*, possibly in an attempt to limit toxin absorption.[99] Nonetheless, in this study toxin accumulation or biotransformation in the copepods was not measured so this hypothesis cannot yet be verified.

In order to avoid the deleterious effects of consuming chemically defended *Alexandrium* spp., the copepod *Temora longicornis* changed its feeding behavior in a species- and strain-specific way.[101] The copepods exhibited normal feeding behavior in response to the nontoxic dinoflagellate *Protoceratium reticulatum* and a strain of *Alexandrium tamarense* which displays lytic activity and produces large quantities of PSTs (*e.g.* **22**-**30**). The copepods also consumed prey at a normal rate in response to *Alexandrium pseudogonyaulax* which produces goniodomin A (**59**) and to a strain of *A. tamarense* that is lytic and produces only low quantities of PSTs.[101] However, copepods subsequently rejected most cells after an hour of exposure to *A. pseudogonyaula* and regurgitated most of the *A. tamarense* cells. In contrast to all the other *Alexandrium* spp. treatments, the copepods reduced their rate of feeding in response to a non-lytic strain of *A. tamarense* that produces moderate concentrations of PSTs.[101] Since the least favored prey was neither the most toxic nor lytic, the copepods are likely using another cue to assess food quality.[101] These different feeding behaviors affect predator-prey dynamics and may also have drastic impacts on apparent competition dynamics among the phytoplankton. Prey that are captured and rejected may experience an advantage over competitors whereas prey that cause a decrease in copepod feeding behavior provide equal predation relief to their competitors and therefore lose their competitive advantage.[101]



goniodomin A (**59**)

The copepod *Acartia tonsa* avoids thin layers of *K. brevis* exudates in order to avoid negative effects of consuming or exposure to *K. brevis*.[102] In a recent study by True and colleagues *A. tonsa* swimming behaviors were quantified after exposure to a distinct layer of cell-free exudates from various concentrations of *K. brevis*. As concentration of *K. brevis* cells used to make the exudates increased, *A. tonsa* increasingly avoided the exudate layer.[102] This avoidance response[102] is starkly different from an attraction response in *A. tonsa* which was observed previously as a result of contact with a layer of exudates from the more palatable green alga *Tetraselmis* sp.[103] This suggests that exudates alone are enough for *A. tonsa* to distinguish preferable and non-preferable prey sources and cause them to change their swimming and foraging behavior. Avoidance of *K. brevis* exudates allows *A. tonsa* to minimize time wasted potentially capturing less favorable prey.[102] For *K. brevis* this avoidance could cause a positive feed-back loop as higher concentrations of exuded compounds trigger stronger avoidance which reduces grazing on the dinoflagellate, allowing the bloom to grow. The authors of the study warned that *A. tonsa* individuals in this particular study were from Georgia, not the Florida gulf coast where *K. brevis* predominantly blooms, and therefore represent naïve predators which may be more sensitive to the deleterious effects of *K. brevis*.[102] In spite of this, they postulated that the interaction is still relevant when considering the expansion of bloom-forming species distributions due to climate change.[102]

6.2 Prey capture and predator avoidance

The detection of suitable food is imperative for the survival of predators. For nearly 40 years scientists have assumed that current-feeding copepods detect non-motile prey using long-range chemoreception. However, in a recent study Gonçalves and Kiørboe challenged this view and suggested instead that the copepods use near-field chemoreception.[104] Through critical analysis of foundational studies and with the addition of their own data they showed that prey need to be within a few prey lengths of the setae of a copepod in order to elicit a capture response. At these distances diffusion through the thick boundary layer encompassing the setae would take longer than most reported beat cycles for current-feeding copepods.[104] Furthermore, using models Gonçalves and Kiørboe showed that previously reported distances for prey detection falls within the limits of fluid mechanical signal and detection for the copepods. Finally, they argued that the rate of leakage from an individual phytoplankton cell is not high enough to result in a detectable concentration of solutes outside the chemical boundary layer of the cell.[104] In 2016 Paffenhöfer and Jiang responded to the study[105] and suggested that Gonçalves and Kiørboe[104] used too low of a leakage rate in their calculations. Moreover, they argued that phytoplankton cells might go through short bursts of intense leakages that result in concentrations of solutes inside their boundary layer building up high enough for copepod detection.[105] Paffenhöfer and Jiang therefore concluded that it is in fact possible for copepods to use long-range chemoreception and disputed that without long-range chemoreception it would be very difficult for copepods which use feeding-currents to survive in environments with lots of particulates and low food concentrations.[105] In a rebuttal Kiørboe and colleagues used new reasoning and evidence to demonstrate that mechanoreception is sufficient for copepods to detect adequate amounts of food to survive even in environments with low concentrations of food.[106] In part Kiørboe and colleagues argued that Paffenhöfer and Jiang[105] incorrectly assumed that the forces of the feeding current perfectly balance gravity so that the copepods hover as they feed;[106] however, a previous study showed that feeding currents are often so strong that copepods are driven through the water.[107] Additionally, Kiørboe and colleagues reported observations that even at realistically low phytoplankton concentrations copepods detect prey at distances consistent with mechanoreception.[106] Finally, they argued that intense bursts of leakage by phytoplankton should be rare and therefore only a few phytoplankton cells at any given time would be detectable to copepods if the burst are necessary for the use of chemoreception for prey detection.[106] This represents a partial paradigm shift in our understanding of how current-feeding copepods interact with their phytoplankton prey, whereby the copepods may first sense potential prey using mechanoreception of hydrodynamic cues, followed by assessment of palatability via contact chemoreception. Overall, this novel perspective necessitates re-evaluation of conclusions from former studies.

Volatile organic compounds released by wounded phytoplankton may act as chemical cues which zooplankton, such as copepods, can use to easily find prey. In a well-controlled and innovative study, Maibam and colleagues assessed the effects of volatile organic compounds from three diatoms (*Pseudonitzschia delicatissima*, *Chaetoceros affinis*, and *Skeletonema marinoi*), and a dinoflagellate (*Prorocentrum minutum*) on the behavior of the copepod *Centropages typicus* both at current and future ocean pH conditions.[108] The copepods clearly recognized the volatile organic compounds; however, for three of four species tested the response of the copepods to the volatile organic compounds was inconsistent with what would be expected if they were used as prey cues.[108] Additionally, the behavior by copepods in response to volatile organic compounds from dinoflagellates suggests that pH may affect either the chemical structure of some volatile organic compounds or chemoreception of these molecules by *C. typicus*.[108]

In some cases their surface chemistry may help prey avoid being captured by predators. Pelagic bacterial community composition changes after being filtered by either benthic and pelagic tunicates.[109] A study by Dadon-Pilosof and colleagues revealed that retention of bacteria by tunicates may be less size- and shape-dependent and more surface chemistry-dependent than previously thought.[109] The tunicates had some of the a lowest retention efficiencies for the SAR11 clade of Alphaproteobacteria despite them being similar in size to other bacteria and beads.[109] Therefore, the authors of the study proposed that the “stickiness” of the bacteria, or their ability to adhere to the hydrophobic mucous net of the tunicates, explains the retention efficiency of different groups of bacteria. In support of this hypothesis, bacteria in the SAR11 clade that possessed more hydrophilic cell surfaces experienced low retention efficiency by the tunicates; however, this was not the case for all the bacteria tested, as Flavobacteriaceae NS5 and SAR116 bacteria both had highly hydrophobic cell surfaces and experienced low retention efficiency by the tunicates.[109] Therefore, the hydrophobicity of the surface of bacteria could play a role in how some, but not all, bacteria, like the SAR11 clade, escape predation by filter feeders.

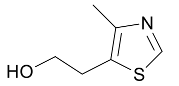
While surface chemistry might reduce predation for some organisms, certain proteins on the surface of cyanobacterium *Synechococcus* sp. increase its susceptibility of predation.[110] The glycoprotein SwmA as well as the large surface protein SwmB are required for motility in this strain.[110-112] Additionally, SwmA has previously been implicated in the formation of the S layer around the cell which protects and stabilizes the cell.[113] Therefore, SwmA and SwmB were hypothesized to protect *Synechococcus* sp. against ciliate and nanoflagellate predators.[110] Nonetheless, when Strom and colleagues tested this hypothesis they found that there was a lower predation rate on mutants lacking SwmA mutant than either the wild-type or mutants lacking SwmB.[110] This study indicates that SwmA and the presence of the S layer actually increased predation rate, contrary to the original hypothesis.

Just as predators use chemical cues to find their prey, some prey use chemical cues from predators to avoid predation. However, little is known about how organisms integrate visual and chemical cues to avoid predation. Crab larvae are a particularly good model organism to study the integration of visual and chemical cues because they tend to live at the edge of their photo detection capabilities and one species, *Rhithropanopeus harrisii*, has previously been shown to increase the frequency of swimming descents in the presence of fish kairomones.[114] In a study by Charpentier and Cohen, the effect of fish exudates on crab visual acuity was compared between larvae of *R. harrisii*, an exclusively estuarine crab[115] which likely experiences higher predation pressure[116], and larvae of *Hemigrapsus sanguineus*, an intertidal crab that spends a portion of its larval phase in the pelagic zone to escape predation.[117, 118] In response to fish mucus, *R. harrisii* larvae showed a less dramatic increase in defensive behavior and immediate physiological response but showed long-term phenotypic plasticity in the form of changes in eye structure; whereas, *H. sanguineus* larvae experienced more drastic increases in defensive behavior and retinal response, which would greater assist it in avoiding visual predators only in the short-term.[118] This study was successful in uncovering how crab larvae integrate both chemical and visual cues when assessing predation pressure; nevertheless, future studies should ensure that both sets of cues, particularly the chemical cues, are provided to the study organisms at realistic concentrations. In another study Charpentier and colleagues showed that, a more advanced larval stage, called zoea, of these same crab species experienced increased spine length in response to fish mucus.[119] The estuarine dwelling *R. harrisii* zoea is shorter in body length but has longer spines than the transitory *H. sanguineus* zoea. During the study, *R. harrisii* zoea exposed to fish mucus for their entire development were consumed less by the fish *Fundulus heteroclitus* and their mean spine length was longer than zoea exposed to fish mucus for only a couple hours.[119] This indicates that longer spines in *R. harrisii* zoea reduces predation by gape-limited predators. Overall, these two studies demonstrate that crab zoea use multiple forms of inducible defenses (behavioral, physiological, and morphological) to avoid predation.[118, 119]

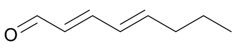
Like zooplankton, phytoplankton use chemical cues of predators to induce defenses and avoid predation. In a study by Lindström and colleagues, the dinoflagellates *Lingulodinium polyedra* and *Alexandrium tamarense* increased the intensity of their bioluminescence in response to copepodamides (**1-8**), the previously described compounds exuded by predatory copepods.[120] The greatest increase in light intensity in both species was in response to copepodamides combined with mechanostimulation, which mimics how algae might experience attack by copepods.[120] In contrast to previous studies with live copepods,[121, 122] copepodamides did not induce changes in cell size or swimming behavior in either dinoflagellate.[120] While the study demonstrates that bioluminescence intensity is induced by predator chemical cues further work is needed to show that the increased bioluminescence results in lower grazing rates on *L. polyedra* and *A. tamarense* due to release from predation mediated by a tritrophic interaction.

In a pair of studies Wohlrab and colleagues analyzed how gene expression[123] and phenotype[124] varied between two strains of *A. fundyense* with respect to predator response. One strain formed larger chains, produced less PSTs (**22-27**), and was lytic against the cryptomonad *Rhodomonas* sp. (hereby called the lytic strain) and the other strain formed some small chains, produced high levels of PSTs, but was non-lytic (hereby called the non-lytic strain).[123, 124] Many genes which were more highly expressed in the lytic strain belonged to proteins known to be involved in secondary metabolism; yet these genes were not differentially expressed in either strain in response to chemical cues from a caged grazing predator.[123] Exposure of the lytic strain of *A. fundyense* to a caged dinoflagellate predator, *Polykrikos kofoidii*, grazing on the non-lytic conspecific strain did not result in upregulation of secondary metabolism genes that were differentially expressed between the two strains.[123] Therefore, the authors suggest that secondary metabolism genes that were more highly expressed in the lytic strain in the absence of the predator are either not involved in the production of the lytic compound(s) or production of the lytic compounds is not an inducible anti-predatory trait.[123] However, assays have not yet been carried out to directly assess if lytic capability changes in this environment. Additionally, it would be valuable to test whether induction of the lytic compound(s) is strain-specific. Furthermore, chemical characterization of the lytic compound(s) may determine which genes are involved in their production. In contrast to the lytic strain, when the non-lytic strain of *A. fundyense* was exposed to grazing caged *P. kofoidii*, the non-lytic strain increased expression of genes for proteins involved in isoprenoid and polyketide biosynthesis[123] suggesting that the non-lytic strain may possess an as of yet undescribed, inducible chemical defense. The differences in gene expression in response to the predator were not as strong as the initial differences between the two strains.[123] When these same strains were exposed to caged or uncaged copepod predators (*Centropages typicus*), both strains shifted toward smaller cell size and shorter chains.[124] Direct grazing resulted in increased production of PSTs in both strains but only the lytic strain increased production of PSTs in response to chemical cues from copepods alone.[124] Wohlrab and colleagues argued that in the absence of predation the non-lytic strain is already in a more defended phenotype based on the lower grazing rates on the non-lytic strain compared to the lytic strain at the start of the copepod grazing experiment; therefore, the non-lytic strain does not need to change as much to defend itself.[124] On the other hand, the lytic strain is more vulnerable to some predators; however, once there are grazer cues the lytic strain drastically changes its phenotype to a more defended state which results in lower grazing rates.[124] In general, this resulted in the lytic strain more closely resembling the non-lytic strain.[124] These two studies[123, 124] highlight the complexity and specificity of chemical interactions within phytoplankton species and between phytoplankton and their predators.

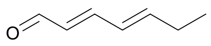
In a partner study to a 2015 study which investigated how variability in calcification and DMSP (**20**) production in the coccolithophore *Emiliania huxleyi* strains affect predation by several microzooplankton,[125] Poulson-Ellestad and colleagues found that the metabolites exuded by four different strains of *E. huxleyi* were affected by grazing by the dinoflagellate *Oxyrrhis marina*.[126] *O. marina* grazing coefficient, a function of grazing rate adjusted for prey and predator concentrations, and growth rate were variable across the four strains, independent of calcification. Using metabolomics, the authors discovered substantial variability in the metabolites exuded by *E. huxleyi* strains with less than 25% similarity between any two strains that had been exposed to *O. marina* grazing. Additionally, no single metabolite changed the same way in all of the strains.[126] Nevertheless, the concentration of 4-methyl-5-thiazoleethanol (**60**), a metabolite produced by both *O. marina* and *E. huxleyi*, positively correlated with the grazing coefficient of *O. marina* on three of the four strains of *E. huxleyi*.[126] However, its concentration was variable across the strains with regard to ingestion and growth rate of *O. marina* suggesting the change in concentration of **60** in response to grazing pressure was not uniform across the three strains.[126] Overall, this study illuminates how chemically different strains of the same species can be and how even their response to predation may not unify their metabolism. Differences in metabolism and response to predation is strain-specific and important to keep in mind as we work to better understand the chemical interactions involved in predator-prey interactions, especially when it comes to studying chemical defenses and predation cues.



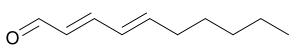
4-methyl-5-thiazoleethanol (**60**)



(2*E*,4*E*)-2,4-octadienal (**61**)



(2*E*,4*E*)-2,4-heptadienal (**62**)



(2*E*,4*E*)-2,4-decadienal (**63**)

7 Community and ecosystem effects

7.1 Community and ecosystem effects

The allelopathic effects of diatom-produced secondary metabolites on multiple pelagic trophic levels are not well understood. It has been suggested that diatom polyunsaturated aldehyde (PUA) production functions mainly to deter microzooplankton herbivory[127] and suppress the growth of competitors.[128] However, a more recent study did not observe growth suppression of competing phytoplankton in the presence of diatom-produced PUAs. In a tritrophic system where copepods eat both microzooplankton and phytoplankton, Franzè and colleagues found evidence that PUAs reduce ciliate microzooplankton grazing while increasing predation by the copepod *Acartia tonsa*.[129] This suggests a cost-benefit tradeoff for diatoms; however the study did not determine whether the effect on copepods was general or species-specific. While PUA treatments contained natural ratios and concentrations, only (2*E*,4*E*)-2,4-octadienal (**61**) and (2*E*,4*E*)-2,4-heptadienal (**62**) were used to create these conditions, excluding many other PUAs that have previously been associated with diatoms.[129] Further experiments that utilize a more comprehensive mixture of PUAs may reveal whether different classes of bloom-associated metabolites will produce similar trophic cascade effects.

In communities living on particulate matter in the open ocean, PUAs were found to affect bacteria that metabolize particulate organic carbon derived from phytoplankton.[130] Bacterial metabolic activity increased at PUA concentrations of 1 and 10 µM as was measured by respiration, cellular growth, hydrolysis of lipids, and acid phosphatase activity associated with the release of inorganic phosphorus into the water column. In contrast, high concentrations of PUAs, near 100 µM, were inhibitory and caused a shift in the composition of the microbial community.[130] Naturally occurring PUAs (**61**, **62**, and (2*E*,4*E*)-2,4-decadienal (**63**)) that diffused from naturally acquired particulate organic carbon were measured at micromolar concentrations.[130] These concentrations are much higher than the previously reported picomolar concentrations in the open ocean,[131, 132] but the observed changes in bacterial response may indicate that sinking organic particles act as PUA hotspots. These results also suggest that PUAs modulate the remineralization of particulate organic carbon at shallow ocean depths which could reduce the ocean's efficiency as a carbon sink.

Mechanisms for how domoic acid (**44**) is transferred from the plankton to the benthos are understudied, and Schnetzer and colleagues suggest that exposure to **44** produced by the diatom *Pseudo-nitschia australis* could be high for particle-associated microbes associated with marine snow.[133] Under colder, darker conditions, decomposition of marine snow contributed to an increase in dissolved **44**, but the acute toxicity and consequences of chronic exposure for organisms associated with these degrading aggregates was not tested.[133] Diatom toxins such as **44** have also been found to bioaccumulate in eukaryotic organisms with the potential to affect growth, reproduction, and behavior, thus having a large effect on food web dynamics. High concentrations of **44** associated with *Pseudo-nitzschia* spp. blooms were reported during the 2015 *Eubalaena australis* whale calving season in Gulfo Nuevo, Argentina.[134] Copepods were the most abundant mesozooplankton in the study area and the authors suggested that they were the primary vector for transferring **44** to *E. australis*. However, other mesozooplankton could not be excluded as the primary vector since rates of **44** bioaccumulation and detoxification in different species were not determined.[134] In another study it was suggested that Scottish harbor seal (*Phoca vitulina*) populations may be negatively affected by **44** associated with *Pseudo-nitzschia* spp. blooms.[135] A correlation was observed between **44** in *P. vitulina* urine and their decline in population on the east coast of Scotland. Additionally, *P. vitulina* from the eastern and northern Scottish coasts were more likely to show **44** urine contamination than those found along the west coast.[135] Detection of **44** and PSTs (*e.g.* **22**-**30**) in fecal samples suggested that seals are exposed to these toxins through several fish including plaice, dab, and cod.[135] Fish collected in the same area contained detectable levels of both types of toxins making them plausible vectors for transferring toxins to seals.[135]

Bioaccumulation of dinoflagellate toxins in turtles and shellfish continues to be a problem along the Florida gulf coast where toxic *Karenia brevis* blooms occur. One study showed a correlation between total tumor score and accumulation of brevetoxins (**64-67**) in green sea turtles (*Chelonia mydas*), a species known to suffer from fibropapillomatosis.[136] However, plasma concentrations of brevetoxins measured in green and Kemp's ridley sea turtles in this study were the lowest recorded, which may indicate that detoxification pathways are upregulated or that brevetoxins do not bioaccumulate in the plasma of all turtle species. Perrault and colleagues suggested that abundance of brevetoxins in plasma was low because sampled turtles were exposed to the end of a bloom. In another study, the invasive green mussel *Perna viridis* retained brevetoxins at high concentrations after a *K. brevis* bloom.[137] The detoxification of this mussel was slower than that of native oysters *Crassostrea virginica* and clams *Mercenaria mercenaria* posing a risk for the transfer of *K. brevis* toxins to higher trophic levels.[137] Previous studies investigated how the New Zealand green-lipped mussel, *Perna canaliculus,* accumulated brevetoxins from a *Karenia selliformis* bloom,[138-140] but McFarland and colleagues are the first to study brevetoxins accumulation in *P. viridis*.

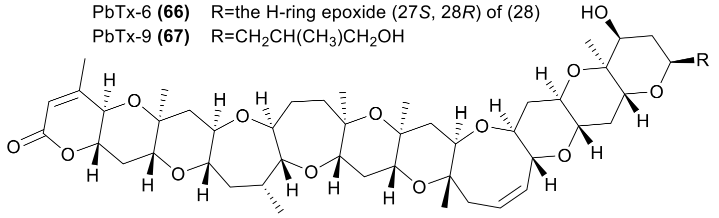
Another investigation reported that feeding on PSTs (e.g. **22**-**30**) and bioactive extracellular compound (BEC) producing *Alexandrium minutum* strains had strain-specific effects on the escape response of juvenile *Pecten maximus* scallops to a predatory starfish.[141] After feeding on the BEC-producing strain scallops reacted more slowly, whereas, after feeding on the strain which produces PSTs scallops had normal reaction times but still failed to escape.[141] This is in agreement with previous findings that clams exposed to PST-producing *A. tamarense* experienced muscle paralysis from bioaccumulated PSTs which made them more vulnerable to predators. [142] On the other hand, the scallops exposed to the BEC-producing strain experienced the greatest reduction in filtration rate.[141] This illuminates the need to characterize the bioactive extracellular compounds of *A. minutum*. Additionally, these results suggest that both classes of compounds produced by *A. minutum* reduce behavioral responses of *P. maximus*, although the effects of PSTs and BEC strains do not appear to be chronic.[141] Most importantly, this study highlights the necessity of not assuming that the known toxins, in this case the PSTs, of prey are responsible for all deleterious effects on predators.[141]

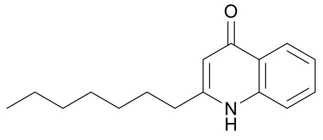
In addition, a recent study by Toth and colleagues presented evidence that teredinid shipworm larvae find wood (*Picea abies*) using waterborne chemical cues.[143] Field experiments showed shipworm larvae to prefer nets with wood over nets without wood. Future experiments could test larval attraction to extracts of multiple types and sizes of wood. Maximum and average larval swimming speeds were also determined;[143] however, this part of the study used veliger blue mussel (*Mytilus edulis*) larvae instead of shipworms. Therefore, it will be important to determine whether teredinid and veliger larvae swim faster when exposed to wood-based chemical attractants.

7.2 Bacterial dynamics

Several studies reported how bacterial quorum sensing molecules affect bacteria-phytoplankton community dynamics. Harvey and colleagues found that *Pseudoalteromonas piscicida* produces the autoinducer precursor 2-heptyl-4-quinolone (**68**) which, at nanomolar concentrations, stops growth in the coccolithophore *Emiliania huxleyi*.[144] Since **68** alone did not recapitulate the rapid mortality caused by the whole exudate of *P. piscicida* it is likely that a mixture of compounds kills coccolithophores as seen under natural conditions. While *P. piscicida* concentrations in these experiments were above those found in nature, the authors suggest that their experimental design mimics the natural environment of

Type II brevetoxins
PbTx-2 (**64**) R=$CH_2C(=CH_2)CHO$
PbTx-3 (**65**) R=$CH_2C(=CH_2)CH_2OH$
PbTx-6 (**66**) R=the H-ring epoxide (27*S*, 28*R*) of (28)
PbTx-9 (**67**) R=$CH_2CH(CH_3)CH_2OH$





2-heptyl-4-quinolone (**68**)

bacteria co-occurring with algae.[144] In addition, **68** had no effect on microalgae *Dunaliella tertiolecta* or *Phaeodactylum tricornutus* which suggests that differences in growth suppression among various phytoplankton species have the potential to alter community dynamics.[144] A next logical step would be to investigate the effect of **68** in microcosm experiments involving *P. piscicida* and multiple species of phytoplankton.

Acyl homoserine lactones, a class of quorum sensing autoinducers, were found to affect hydrolytic enzyme activity of particle-associated bacteria.[145] Acyl homoserine lactones variably inhibited or stimulated phosphatase, aminopeptidase, and lipase activities in samples from different study sites which shows that additional unknown factors are involved in enzymatic regulation of collected bacteria.[145] Quorum sensing autoinducers may be important for regulation of particulate organic carbon degradation, and ultimately carbon flux and biological pump efficiency, but the relationship remains unclear.

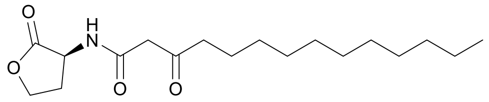
In a study by Johnson and colleagues, the bacterium *Ruegeria pomeroyi* was found to increase production of the quorum sensing autoinducer *N*-(3-oxo-tetradecanoyl)-L-homoserine lactone (**69**) when grown on algal-derived DMSP (**20**).[146] However, when grown on propionate (**70**) which represents a portion of the structure of **20**, production of autoinducers did not increase indicating that **20** functions as signal in addition to being a food source. Therefore, production of **20** by other organisms has the potential to alter inter- and intra-species bacterial communication by upregulating production of signaling molecules.[146]

Bacteria associated with microalgae may play an important role in protecting aquaculture against pathogenic organisms. *Bacillus* sp. isolated from the microalga *Chaetoceros muelleri* degrades *N*-hexanoyl-L-homoserine lactone (**12**) and *N*-(3-hydroxybutanoyl)-L-homoserine lactone (**71**).[147] The latter is produced by pathogenic *Vibrio campbellii* and its removal by *Bacillus* sp. increases the survival of prawn (*Macrobrachium rosenbergii*) larvae raised in aquaculture.[147] The strategy of using *Bacillus* sp. to remove *Vibrio* quorum sensing autoinducers could be extended to benefit other animals raised in the aquaculture industry as an alternative to using antibiotics.

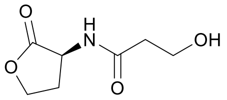
There has been some evidence that intraspecific and interspecific competition among bacteria may be facilitated by algal blooms. A bacteria resource utilization model based on spatiotemporal response to lysed diatoms predicted that competition favors chemotaxis under bloom conditions.[148] While the model simplified dissolved organic matter to be a single substance, different diffusivity values predicted that motile bacteria's exposure to slowly diffusing molecules does not increase during lysis of a diatom cell even though they are the main consumers of dissolved organic matter.[148] In an additional study, unique microbiomes were identified for harmful algal bloom-associated dinoflagellates *Alexandrium fundyense* and *Dinophysis acuminata*.[149] Exposure to exuded compounds of both algae resulted in specific bacterial profiles where growth was either suppressed or promoted.[149] However, the chemical components of the exudates have not been reported. While both types of algae were associated with microbiomes dominated by Flavobacteria, the assumption that allelochemicals suppress growth of competing bacteria is only one possible hypothesis and further work should be done to determine the chemicals that allow Flavobacteria to dominate microbial communities during harmful algal blooms.[149]

Bacterial communities associated with marine diatoms may play an important role in degrading aromatic hydrocarbons in crude oil and facilitating the production of marine oil snow. Mishamandani and colleagues found that there are transitions in dominance of bacterial community members associated with *Skeletonema costatum* when exposed to crude oil.[150] Specifically, an increase in *Methylophaga* population size was observed followed by increases in several species of hydrocarbonoclastic bacteria. These microbial communities were found to be capable of degrading polycyclic hydrocarbons naphthalene (**72**), phenanthrene (**73**), and some additional alkylated naphthalene derivatives.[150]

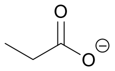
Additionally, PUAs were shown to be more toxic to marine bacteria at higher concentrations and toxicity of these molecules increased with chain length for 2*E*,4*E/Z*-dienals (*e.g.*, **61**-**63**).[151] The degree of saturation of membrane phospholipid fatty acids in the bacteria increased with increasing concentration of PUA exposure and decreasing cell growth.[151] However, the PUA concentrations used for these experiments[151] were much higher than previously measured during a *Skeletonema marinoi* bloom in the Adriatic Sea[131] and even higher than localized concentrations associated with marine particles in another report discussed earlier.[130] If PUAs are truly toxic for specific species of marine bacteria at natural concentrations, then these compounds have the potential to disrupt and alter natural microbial communities.



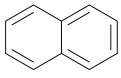
N-(3-oxo-tetradecanoyl)-L-homoserine lactone (**69**)



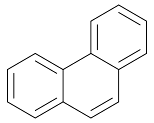
N-hydroxybutanoyl-L-homoserine lactone (**71**)



propionate (**70**)



naphthalene (**72**)



phenanthrene (**73**)

7.3 Effects of global change

Zooplankton preferences with respect to salinity, pH, and predator waterborne chemical cues were studied using larvae of the polychaete *Platynereis dumerilii* and the copepod *Euterpina acutifrons* as models.[152] A microfluidics chip designed for these experiments served as a useful instrument for observing zooplankton larval behaviors under various conditions and has potential as a tool for studying ocean acidification.[152] *P. dumerilii* preferred pH ranges close to neutral and a salinity range comparable to and slightly above that of the open ocean.[152] Ramanathan and colleagues suggested that zooplankton larvae use high salinity as a proxy for nutrient richness. *E. acutifrons* had a slightly higher pH preference, just above neutral, which could have negative implications as ocean pH continues to decrease due to climate change.[152] Both zooplankton preferred natural sea water over waterborne chemical cues from sea bass, a predator.[152] In addition, *P. dumerilii* larvae preferred extracts of *Dunaliella* microalgae over *Isochrysis,* and when ciliated cells found in the larval foregut were ablated, the larval reaction time to the extract slowed.[152] One possibility is that these cells act as chemoreceptors and allow the larvae to detect potential food sources. Interactions between receptors in the larval foregut and metabolites released by algal prey is an area that should be further explored.

A study by Hattenrath-Lehmann and colleagues explored how increased levels of carbon dioxide impact the growth and toxicity of *Alexandrium fundyense*.[153] A strain from Northport Bay, New York, (USA) upon being treated with increasing CO_2 levels, displayed higher growth rates and toxicity attributed to increased concentrations of particularly toxic diastereomers GTX-1 (**24**) and -4 (**27**).[153, 154] However, toxicity of another strain, from the Bay of Fundy, Canada located about 700 km away, did not change with increasing carbon dioxide.[153] The results from this study suggest that increasing acidification of our oceans could facilitate *A. fundyense* harmful algal bloom formation and make blooms more toxic, but not in a way predictable across genetically diverse populations.

8 Conclusions

The chemical ecology of the marine plankton continues to be an active field of research. Greater attention has been aimed at understanding the influence of bacteria on phytoplankton natural product biosynthesis, induction, and metabolism. Recent work has focused on the effects of quorum sensing of marine planktonic bacteria especially with respect to bacterial-algal relationships and community level interactions. Additionally, much attention has been devoted to phytoplankton chemical defenses, particularly PSTs (*e.g.* **22**-**30**); however, there continues to be problems with assumptions being made that known phytoplankton “toxins” function as chemical defenses without explicitly demonstrating that they serve defensive functions. In comparison to the previous review[4] there have been more recent studies on intraspecific signaling and fewer studies on allelopathic interactions. However, there continues to be a relative scarcity of studies on chemical cues involved in parasitic interactions.

Full characterization and isolation of the compounds responsible for chemically mediated pelagic interactions remains problematic, evidenced by the small number of novel natural products published from these study systems during the reporting period. Small quantities, diffusion, and instability of many of these compounds has made it difficult to isolate individual compounds but continued advancements in techniques and instrumentation support improvements in the field. The use of -omics (metabolomics, proteomics, and transcriptomics) are revealing candidate signaling molecules and the mechanisms of biological response to many chemical signals and cues. In fact, a recent review by Kuhlisch and Pohnert highlighted how metabolomics has successfully been applied in many chemical ecology studies.[155] Along with discerning the environmental impacts of algal toxins in their ecological communites, more studies could be undertaken to predict how they might influence interactions in other communites as many of the harmful algal bloom species expand and shift their niches due to climate change.[156] As the field advances scientists also need to persist in the hard work of using ecologically relevant approaches while studying allelopathy and chemical defenses, with consideration of natural concentrations verified from field samples, and testing defenses using appropriate ecological targets and compound dispersal methods. Albeit less studied than terrestrial or marine benthic systems, chemically mediated interactions are clearly important planktonic ecosystems and therefore represent a rich source of novel natural products yet to be discovered

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