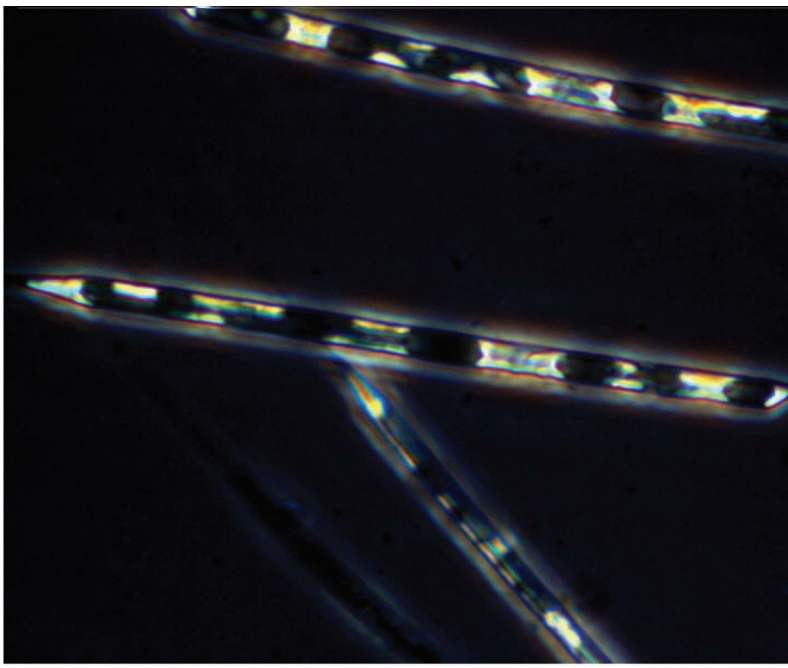
# NPR

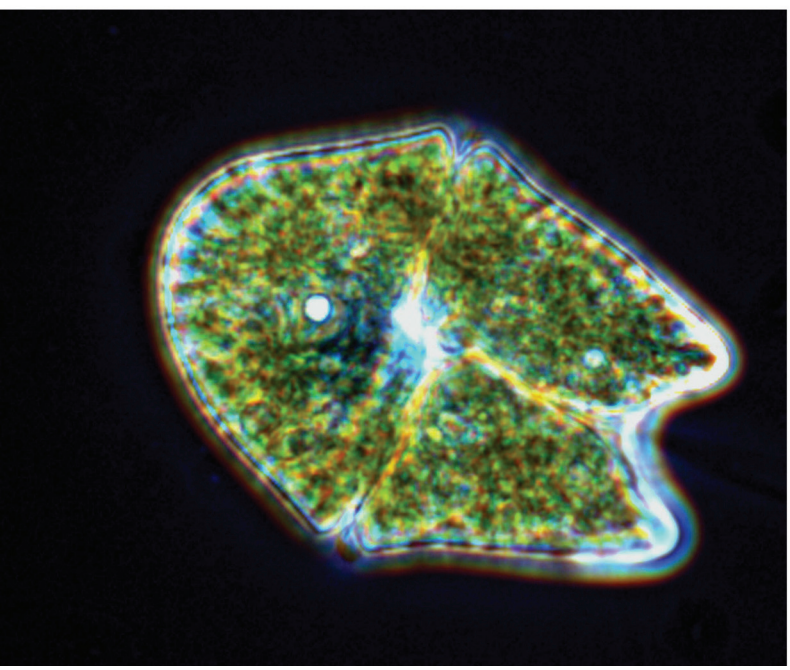
Natural Product Reports

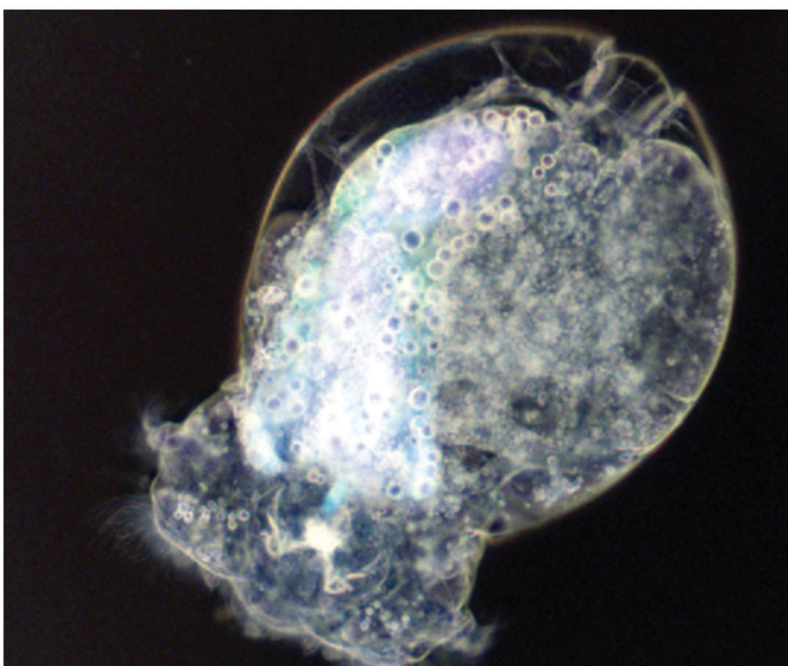
Current developments in natural products chemistry

www.rsc.org/npr

Volume 26 | Number 6 | June 2009 | Pages 717–856






ISSN 0265-0568

RSC Publishing




Chemical Biology
In this issue...


0265-0568(2009)26:6;1-J

# Chemical ecology of the marine plankton

**Kelsey L. Poulson, R. Drew Sieg and Julia Kubanek***

*Received 30th January 2009*
*First published as an Advance Article on the web 13th March 2009*
**DOI: 10.1039/b806214p**

Covering: 2006 to 2008 inclusive

This report summarizes recent research advancements in the chemical ecology of pelagic (open water) marine ecosystems. With the goal of providing a comprehensive overview of new knowledge in this field, we have reviewed the recent literature, and have organized it by type of ecological interaction, starting with studies on chemically-mediated intra-species communication, followed by inter-species interactions, and leading up to ecosystem-level effects of plankton secondary metabolites.



## 1 Introduction

Major recent research foci of pelagic marine chemical ecology have been on allelopathic effects in competition, the role of algal toxins in predator–prey interactions, and community and ecosystem-level effects including bioaccumulation and transfer of toxins within food webs. There have also been new insights into host–parasite interactions, advances in chemically-mediated mate identification and tracking, and intraspecific signaling, particularly among diatoms using polyunsaturated aldehydes (PUAs).

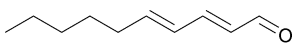
Before delving into the primary literature, it is worthwhile to point out some relevant recent review articles. For a review on advances in chemical ecology of the marine benthos (bottom-dwelling organisms), see Paul and Ritson-Williams.[1] Cell–cell communication, allelopathic interactions, and phytoplankton–zooplankton interactions, as well as new advances in the chemical ecology of the benthos, were reviewed by Ianora *et al.*[2] The function of chemical signals in both marine and freshwater pelagic systems, as well as their ability to structure interspecific associations, was well covered in a review by Pohnert *et al.*[3] A comprehensive, taxonomically-organized review of Antarctic marine chemical ecology, including molecular structures and known ecological functions, was recently published by Avila *et al.*[4] A review focusing on the ecological roles of volatile organic compounds in freshwater and marine systems was published by Fink.[5] A book chapter on allelopathic interactions in pelagic and benthic communities was written by Granéli and Pavia.[6] A general review of phytoplankton allelopathy, particularly on abiotic and biotic factors that can alter allelopathic interactions with an emphasis on the effects of eutrophication, was provided by Granéli *et al.*[7] In a separate review, Granéli[8] discussed how allelopathy is used by the toxic haptophyte *Prymnesium parvum*. The effects of Baltic Sea cyanobacterial toxins on multiple ecological scales were reviewed by Karjalainen *et al.*[9] A thorough book chapter on toxic diatoms was written by Trainer *et al.*[10] covering the mechanism of action and physiological effects of the diatom toxin domoic acid, as well as general taxonomy and physiology of diatoms responsible for domoic acid production. This review[10] also discussed oceanographic factors that favor harmful diatom bloom formation. A short review on cellular signaling among diatoms by Vardi[11] discussed how PUAs are perceived by marine diatoms, and the effects PUAs have on diatom populations. A general overview of grazing pressures faced by *Phaeocystis* spp., including chemically-mediated predator–prey interactions, has been written by Nejstgaard *et al.*[12] A book chapter by Kubanek and Snell[13] reviewed quorum sensing among rotifers as a means to switch from asexual to sexual reproductive strategies for these zooplankters. Although it does not focus specifically on pelagic chemical ecology, a review on dynamic scaling by Zimmer and Zimmer[14] discussed the proper means to assess the ecological relevance of chemical cues in ecology studies.

## 2 Intraspecific signaling

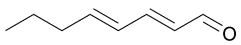
Polyunsaturated aldehydes (PUAs) such as (2*E*,4*E*)-decadienal (**1**), (2*E*,4*E*)-octadienal (**2**), and (2*E*,4*E*)-heptadienal (**3**) are produced by a variety of diatoms[15] and other phytoplankton taxa.[16] PUAs have been implicated in a range of functions, including intraspecific signaling and programmed cell death,[11] anti-grazing defenses,[17] allelopathy,[16] and bacteria–phytoplankton interactions.[18] In diatoms, PUAs are produced by the

*School of Biology and School of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332-0230, USA; Tel: +1 (404) 894-8424; Fax: +1 (404) 385-4440. E-mail: julia.kubanek@biology.gatech.edu*

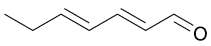
breakdown of unsaturated fatty acids in response to mechanical stress.[19]



(2*E*,4*E*)-Decadienal
(**1**)



(2*E*,4*E*)-Octadienal
(**2**)



(2*E*,4*E*)-Heptadienal
(**3**)

(2*E*,4*E*/*Z*)-Decadienal (**1**) affects neighboring conspecificis when released by wounded diatoms *Thalassiosira weissflogii* and *Phaeodactylum tricornutum.*[15] Perception of **1** at the cell surface of diatoms starts a signaling cascade, building up intracellular calcium and nitric oxide production *via* nitric oxide synthase-like activity, which can lead to cell death. The production of nitric oxide was found to be rapid (occurring within five minutes) and dependent on the concentration of **1** in aqueous medium.[15] Cell death rates were also high: *P. tricornutum* exposed to 66 μM **1** suffered 90% mortality within four hours. Treatment of cells with a nitric oxide donor (sodium nitroprusside or diethylamine nitric oxide) increased cell death proportionally with nitric oxide accumulation, whereas a nitric oxide synthase inhibitor (*NG*-monomethyl-L-arginine) depressed PUA-dependent cell death.[15] Pre-conditioning *P. tricornutum* cells with low concentrations of **1** (0.66 μM) increased recovery potential as well as nitric oxide production relative to cells that were not pre-treated, when the same cells were later exposed to 13 μM **1**. Pre-treated cultures also underwent a six-fold increase in cell density compared to non-pre-treated cells after transfer into media lacking **1**. This suggests that nitric oxide build-up is harmful but diatoms can acclimate to exposure. Interspecific variation in production or susceptibility to PUAs may be involved in bloom succession, or may act as cues for environmental stress.[15] Vardi *et al.*[20] characterized a protein belonging to the conserved YqeH subfamily involved in nitric oxide production. Over-expression of the YqeH synthesis gene (PtNOA) in *P. tricornutum* led to increased nitric oxide production and decreased growth, as well as lowered photosynthetic efficiency compared to wild-type controls.[20] Concentrations of **1** that were sub-lethal to wild-type cells caused depressed growth in cells that over-expressed PtNOA largely through decreased photosystem II efficiency, suggesting that these mutants were hypersensitive to **1** exposure.[20] Mutants over-expressing PtNOA increased expression of metacaspases but reduced expression of superoxide dismutase (MnSOD) which, coupled with the involvement of nitric oxide, suggests that PtNOA expression is related to programmed cell death.[20]

PUAs may be released by non-wounded diatom cells to communicate with conspecifics.[21] When monitored throughout

**Kelsey L. Poulson**

*Kelsey L. Poulson received her B.S. in Biological Sciences and Biochemistry from the University of Wisconsin-Milwaukee, USA, in 2007. She is currently a Ph.D. student in Dr. Julia Kubanek's laboratory at Georgia Tech, where she studies marine plankton chemical ecology.*

**R. Drew Sieg**

*R. Drew Sieg received his B.S. in Biology from the University of Richmond, USA, in 2007. He is also a current Ph.D. student in Dr. Kubanek's laboratory, studying the ecology of chemically-mediated interactions in the plankton community.*

**Julia Kubanek**

*Julia Kubanek received her B.Sc. in Chemistry from Queen's University, Canada, in 1991 and then traveled for two years, working on dive boats on the Great Barrier Reef and dreaming about combining her chemistry training with marine research. She completed her Ph.D. on marine natural product chemistry at the University of British Columbia in 1998, and went on to postdoctoral research at the Scripps Institution of Oceanography and the University of North Carolina at Wilmington. In 2001, she joined the faculty at Georgia Tech, where she is now Associate Professor of Biology and Chemistry. She is an author of approximately 50 research articles on plankton chemical ecology, coral reef chemical ecology, and marine natural product discovery and biosynthesis.*

their growth cycle, cultures of the diatom *Skeletonema marinoi* only released PUAs during late stationary phase but well before cell lysis was prominent. When PUAs were added to cultures during late stationary phase at ecologically-relevant (nanomolar) concentrations, *S. marinoi* cells experienced a temporary increase in growth, followed by a dramatic decline in cell densities.[21] PUAs added at nanomolar concentrations to exponential or early stationary growth phases did not affect *S. marinoi* growth, although micromolar concentrations caused a significant decrease in growth during these two phases.[21] *S. marinoi* cells that were previously exposed to low PUA concentrations did not respond to later PUA exposure. PUAs could act as sub-lethal signals because they are released into the environment, their presence in the environment is ephemeral, and they only affect diatoms during specific growth periods.[21] Although it is not yet entirely clear what message(s) are being relayed by these compounds, it has been proposed that as diatom cells sense deteriorating environmental conditions, PUAs are released to signal for organized bloom termination,[22] and similar signaling processes have been hypothesized for other phytoplankton groups.[23] However, the evolution of this strategy is counter-intuitive, requiring group selection arguments that are typically rejected unless cooperating organisms have a high degree of genetic relatedness. Genetic studies of bloom population structure should help shed light on this matter.

The effects of filtrates from the haptophyte *Prymnesium parvum* can also lead to self-imposed cell death. Olli and Trunov[24] found that *P. parvum* filtrates are toxic to less dense cultures of the same species. However, cells were able to acclimate to filtrates when exposed at low concentrations, which implies that as blooms form, cells associated with the bloom become resistant to the toxins they are emitting.[24] Autotoxicity, therefore, may play a role in algal bloom dynamics. The autotoxic compounds were not identified.

Chemically-mediated switches from asexual to sexual modes of reproduction (a process called mixis) have recently been examined in rotifer populations.[25] Although populations of rotifers belonging to the class *Monogononta* are primarily composed of asexually-reproducing females, under stressful conditions such as crowding or food depletion a proportion of females within the population undergo mixis, resulting in the production of sexually-reproducing males and females that produce hardy resting eggs.[26] Mixis is induced when rotifer populations reach a threshold, analogous to quorum sensing in cooperative bacteria.[13] Within the *Brachionus manjavacas* (ex *B. plicatilis*) species complex, the mixis induction signal is relatively conserved:[25] mixis was similarly induced by conditioned media from multiple *B. plicatilis* strains, suggesting little divergence in genes encoding the signal over the past 10 million years. Snell *et al.*[27] examined the identity of the responsible signal using rotifer-conditioned media coupled with mixis induction assays. They proposed that the signal molecule binds to a receptor in the mother, which triggers her oocytes to become mictic. The incidence of mixis was reduced by the addition of a protease and protected and by protease inhibitors, indicating that the signaling molecule is protein based. Active HPLC fractions that promoted mixis contained a 39 kDa molecule, the N-terminus of which was 100% similar to a steroidogenesis-inducing protein from human ovarian follicular fluid, indicative of the genetic conservation of reproductive hormones. A protein can act as an effective mixis-inducing signal because it allows for high target specificity, low detection limits, and quick signal breakdown.[27]

Mate selection by male *Brachionus manjavacas* rotifers is also chemically-mediated, and appears linked to female age.[28] Males were previously shown to select mates based upon contact with a glycoprotein on females' corona.[29] In a no-choice assay, male rotifers copulated with young (3 hour old) females significantly more often than with very recently hatched (0.2–1 hour old) or older (6–72 hour old) females.[28] Males couldn't discern virgins from non-virgins, nor could they distinguish between amictic and mictic females. Since younger females have a higher probability of being virgins, selectively mating with younger females whose eggs have not already been fertilized may maximize male reproductive success.[28]

Although the identities of most copepod pheromones remain a mystery, the effects of diffusible female copepod pheromones on male mate-tracking behavior continue to be a focus of signaling studies. Male copepods have been proposed to use both mechanical flow patterns and chemical stimuli such as pheromones to track, capture and identify females. In Y-maze studies, males of the parasitic sea louse *Caligus rogercresseyi* tracked to maze legs containing either juvenile or adult females of the same species over legs that only contained seawater.[30] The species-specificity of copepod pheromones has also been addressed using 3D video recordings of copepod behavior.[31] Males of three copepod species (*Centrophagous typicus*, *Centrophagous hamatus*, and *Temora longicornis*) were exposed to females and their tracking behavior was analyzed in a series of no-choice experiments.[31] Males displayed non-specific capture behavior, pursuing and capturing heterospecifics and conspecifics at comparable rates. However, post-capture, males became more selective, and released the majority of heterospecific females prior to mating. It appears that dissolved, pre-contact pheromones lack information regarding species identity of the target female. Contact cues such as surface glycoproteins, or mechanical cues such as genital fitting, may act as more reliable, species-specific signals for copepods.[31] As heterospecific encounter rates can be as high as 2000 encounters $m^{-3}$ $d^{-1}$, mating attempts with heterospecifics are likely a common and energetically-costly aspect of copepod reproductive behavior.[31]

An unusual tracking behavior has been documented in the estuarine copepod, *Oithona davisae*.[32] Whereas mates of many copepod species track rapidly up a pheromone trail, reaching a target female in a matter of seconds, *O. davisae* males spiral around the trail, taking over 30 seconds to capture a female. Male spiraling behavior may be a response to the erratic feeding behavior of conspecific females, characterized by passively sinking, then jumping upwards up to 1 mm every 2 to 5 seconds.[32] This jumping behavior may create gaps in the pheromone trail if the cue cannot diffuse quickly enough to fill in the gaps between jumps, in response to which males compensate by spiraling around the general area of the pheromone. This tracking strategy seems fitting for oceanic dwellers where chances of mate encounters are low, but since *O. davisae* is often found at high densities in semi-enclosed estuaries and inlets, this costly behavior may be a remnant of an oceanic ancestor. *O. davisae* tracking behavior also makes males conspicuous to predators,

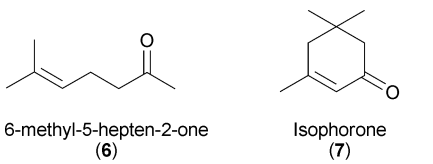
potentially leading to increased male predation and creating female-biased sex ratios.[32]

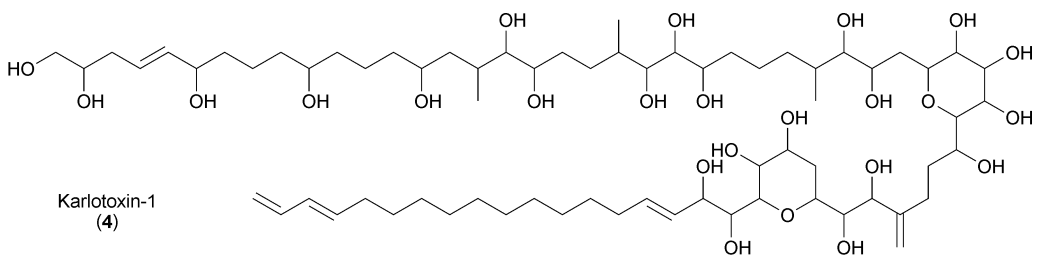
## 3 Host–parasite interactions

Toxin-producing dinoflagellates may incur costs due to their susceptibility to infection by parasites.[33] Blooms of the dinoflagellate *Karlodinium veneficum* (ex *K. micrum*) around Chesapeake Bay, USA can be ichthyotoxic and are also hosts to the parasitic dinoflagellate *Amoebophrya* sp. In co-culturing experiments using multiple *K. veneficum* host strains, there was a significant positive correlation between host karlotoxin concentration and susceptibility to *Amoebophrya* sp. infection.[33] Even though infection by *Amoebophrya* sp. led to decreased intracellular and extracellular toxin concentrations compared to uninfected controls, it is unlikely that the parasite catabolizes *K. veneficum* toxins. It is more likely that infection led to host lysis and subsequent bacterial degradation of toxins or that infection by *Amoebophrya* sp. inhibited toxin production.[33] Heightened susceptibility to infection could be due to these strains having larger cell sizes or higher cell densities, which would create increased surface area for parasitic attack.[33] *Amoebophrya* sp. may also successfully parasitize *K. veneficum* by having a cell membrane sterol composition similar to its host, which lowers the susceptibility of *Amoebophrya* sp. to the lytic effects of karlotoxins[34] (for a more detailed look at karlotoxin–sterol interactions, see section 5.1). Upon host death, toxin release from cells was rapid, implying that the use of parasitic dinoflagellates to mitigate bloom toxicity would not be an effective control strategy.[33] Recently, the molecular structures of karlotoxin-1 (**4**) and karlotoxin-2 (**5**) have been determined, although their absolute and relative stereochemistries remain unassigned.[35]

Compared to freshwater systems, there has been a relative dearth of studies that examine chemically-mediated tracking towards potential hosts by fish parasites. A few marine studies have examined host-tracking mechanisms in copepod sea lice.[30,36] The sea louse *Caligus rogercresseyi*, a known parasite of salmonids, tracked to water conditioned with Atlantic salmon (*Salmo salar*) exudates over either seawater controls or exudates of the copepod predator non-host fish, *Hypsoblennius sordidus*, in a Y-maze study.[30] Sea lice also tracked to exudates of rainbow trout, but not to coho salmon exudates, even though both of these species are known hosts for *C. rogercresseyi*.[30] Unfortunately, no analysis of specific compounds was conducted.

In a more chemically-focused study, Bailey *et al.*[36] assessed chemical cues that the sea louse *Lepeophtheirus salmonis* uses to track towards its host, Atlantic salmon (*S. salar*). Using Y-maze behavioral studies, *L. salmonis* larvae tracked towards salmon-conditioned water, lipophilic extracts of salmon-conditioned water, and two purified compounds identified from salmon solid-phase extraction eluates (6-methyl-5-hepten-2-one (**6**) and isophorone (**7**)). Responses to **7** were dose-dependent with maximal responses between 0.01–0.1 mg $ml^{-1}$.[36] When added to salmon-conditioned water, 2-aminoacetophenone (**8**) and 4-methylquinazoline (**9**), which were identified in non-host (*Scophthalmus maximus*) conditioned seawater extracts, prevented positive tracking to salmon-conditioned water by juvenile copepods. In a related study, *L. salmonis* responded to water conditioned with cubed pieces of *S. salar* flesh with stimulation of antennule neurons followed by movement in the legs and antennules.[37] Neurons were most consistently stimulated by fractions of salmon-conditioned water containing water-soluble compounds 1–10 kDa in size.[37] It appears that parasitic copepods can accurately recognize appropriate salmonid hosts using specific reliable chemical cues, and that these kairomones vary substantially in molecular structure.

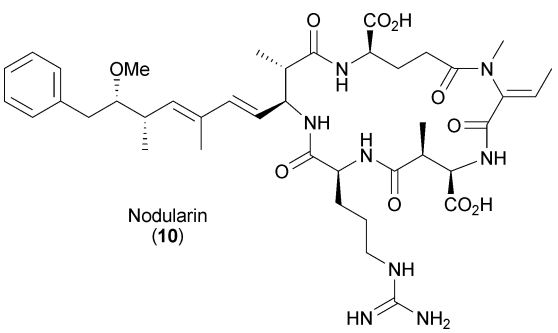


## 4 Allelopathy

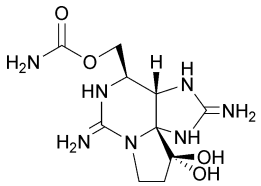
Most recent studies on the use of inhibitory compounds in competition, referred to as allelopathy, have focused on interactions among phytoplankton.[38,39] Many allelopathic microalgae are also known to produce potent toxins which can have detrimental effects on vertebrates but are rarely responsible for competitive outcomes between phytoplankton. A common theme is that yet-unidentified, non-neurotoxic metabolites account for the allelopathic effects observed within phytoplankton communities. In many cases, allelopathic compounds have been neither isolated in pure form nor have their structures been elucidated. However, their presence is indicated by the growth-inhibitory nature of phytoplankton filtrates or extracellular extracts.

There have been many studies on the allelopathic effects of freshwater cyanobacteria, but studies involving marine cyanobacteria are somewhat rarer. Nodularin (**10**) is a potent toxin produced by the brackish cyanobacterium *Nodularia spumigena* that promotes liver tumor formation,[40] and can also bioaccumulate in birds,[41] zooplankton,[42] and fish.[43,44] The allelopathic effects of toxic *N. spumigena* and non-toxic *Aphanizomenon flos-aquae*, both from the Baltic Sea, were compared using cell-free filtrates of each cyanobacterium.[45] Interestingly, non-toxic *A. flos-aquae* was inhibitory towards the cryptophyte *Rhodomonas* sp., reducing competitor cell numbers by 29% and cellular chlorophyll-a content by 34%.[45] In contrast, toxic *N. spumigena* only reduced competitor cell numbers by 14% and cellular chlorophyll-a content by 12%. Pure **10** added to *Rhodomonas* sp. cultures did not cause a significant change in any *Rhodomonas* growth parameters, suggesting that metabolites other than **10** are responsible for the mild allelopathic effects observed for *N. spumigena*.[45] Alternatively, some of its competitive dominance may be caused by *N. spumigena* having a higher pH tolerance compared to competitor species.[46] In co-culturing experiments, pH in cultures increased during growth, which could explain eventual *N. spumigena* competitive success.[46]



Nodularin (**10**)

Dinoflagellates belonging to the genus *Alexandrium* continue to be studied extensively, largely because they produce saxitoxin (**11**) and related compounds that cause paralytic shellfish poisoning in humans and occasionally form large-scale harmful algal blooms.[47] Although **11** is not allelopathic, *Alexandrium* spp. and their exudates are.[39] Planktonic organisms including chlorophytes, cryptophytes, diatoms, dinoflagellates, and ciliates were each exposed to filtrates from three diverse strains of *A. ostenfeldii* originating from New Zealand, Canada, and Denmark.[39] Responses to filtrates included cell lysis, cell elongation, cyst formation, reduced motility, and temporary immobilization with effects depending on the strain of *A. ostenfeldii* to which competitors were exposed.[39] Although the allelopathic compounds employed by *A. ostenfeldii* remain unidentified, it appears that outer cell membranes of competitor cells are a frequent target.[39] The allelopathic effects were inversely correlated with target cell density, which may be due to a saturation effect dependent on the density of absorbing particles.[39] These taxonomically-broad allelopathic effects may help *A. ostenfeldii* form small patches within the water column where they are locally abundant.[39]



STX (**11**)

The allelopathic effects of *Alexandrium* spp. are not limited to *A. ostenfeldii*, and do not appear linked to bacteria associated with the dinoflagellate. In a study by Tillmann *et al.*[48] cultures of six species (*A. tamarense*, *A. ostenfeldii*, *A. lustanicum*, *A. minutum*, *A. catenella*, and *A. taylori*) treated with broad-spectrum antibiotics to remove associated bacteria caused lysis of several autotrophic and heterotrophic plankton species. Filtrates of *Alexandrium* spp. treated with antibiotics were also lytic towards the cryptophyte *Rhodomonas salina*.[48] The extent of cell lysis was variable depending on the target species and *Alexandrium* species involved. None of the *Alexandrium* spp. showed any statistical difference in allelopathic potency whether treated with broad-spectrum antibiotics or not, suggesting that extracellular bacteria are unlikely to be involved in production of allelopathic compounds.[48] Although antibiotics removed a majority (up to >99%) of associated bacteria, it is possible that intracellular bacteria may be involved in *Alexandrium* spp. allelopathy.[48] Nevertheless, it appears that *Alexandrium* spp. allelopathy is common within the genus, and may play a role in bloom maintenance.

Adolf *et al.*[49] investigated how allelopathy may be a useful strategy to mixotrophic dinoflagellates, which can photosynthesize *and* consume other cells. Karlotoxin-1 (**4**) and karlotoxin-2 (**5**) from the mixotroph *Karlodinium veneficum* were isolated[50] and their structures recently elucidated.[35] Partially purified karlotoxins suppressed growth rates of several raphidophytes, dinoflagellates, and the cryptophyte *Storeatula major*, although for some species, the waterborne concentrations of karlotoxins required to suppress growth (>500 ng/ml) would rarely be found around natural blooms.[49] These compounds may play an additional role in predator–prey interactions (see section 5.3), and their mechanism of action may be linked to the sterol composition of competitors and grazers (see section 5.1).[51]

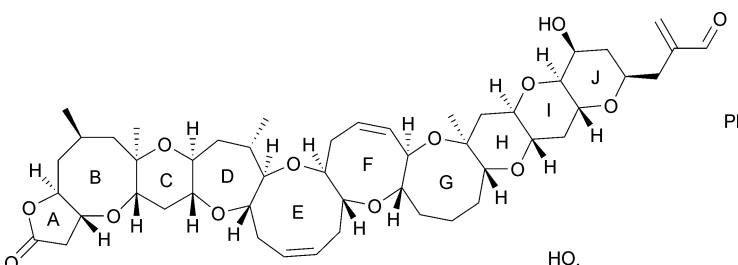
The toxic haptophyte *Prymnesium parvum* is a bloom-forming alga that is allelopathic, capable of immobilizing and lysing competitor cells, and can feed on a range of organisms from bacteria to other phytoplankton.[6] Uronen *et al.*[52] examined the effects of *P. parvum* filtrates on *Rhodomonas salina* and associated bacterial communities. When *R. salina* was exposed to either cultured *P. parvum* or cell-free *P. parvum* filtrates which

contained associated bacteria as well as *P. parvum* exudates, *R. salina* cells were rapidly damaged or lysed, resulting in dissolved organic carbon release within 30 minutes of exposure.[52] Bacterial biomass increased significantly when *R. salina* was exposed to either of the aforementioned treatments, suggesting that bacteria can take advantage of this new source of carbon.[52] For the mixotroph *P. parvum*, there are potential positive direct and indirect effects that arise from the use of allelopathic compounds. Directly, competitor species are removed from the water column, and indirectly, the increase in bacterial biomass creates a potential additional food source for *P. parvum*.[52] Alternatively, *P. parvum* may directly utilize dissolved organic carbon and nitrogen released during competitor cell lysis, based upon recent stable isotope studies demonstrating that *P. parvum* is capable of utilizing organic carbon and nitrogen from sewage effluent as substrates for growth.[53]

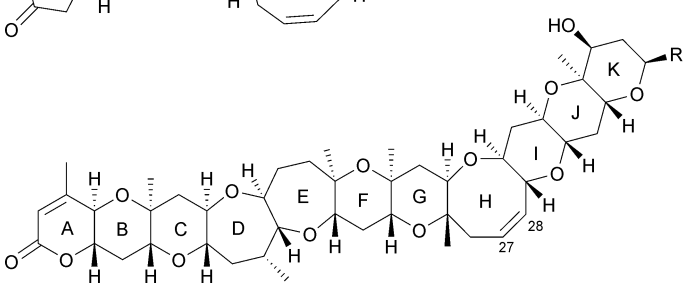
The dinoflagellate *Karenia brevis* blooms frequently in the Gulf of Mexico, producing neurotoxic brevetoxins (**12–16**) that can lead to massive fish kills[54] and sea mammal mortality.[55,56] These compounds do not appear linked to the allelopathic success of *K. brevis*,[38,57] although the allelopathic mechanisms of *K. brevis* exudates have recently been investigated. Prince *et al.*[38] found that extracellular extracts from natural bloom samples of *K. brevis* inhibited the growth of four (*Amphora* sp., *Asterionellopsis glacialis*, *Prorocentrum minimum*, and *Skeletonema costatum*) out of five competitor species tested. Cell membranes appeared to be a target of *K. brevis* allelopathy: three competitors (*Akashiwo sanguinea*, *A. glacialis*, and *P. minimum*) developed cell membrane damage when exposed to extracellular extracts from *K. brevis* cultures.[38] All five competitors suffered inhibited photosystem II activity, used as a measure of photosynthetic efficiency. Photosystem II was inhibited by 68% in *S. costatum*, but it is unclear whether photosystem II was a target for allelochemicals or whether cellular stress led to decreased efficiency.[38] Other hypothesized allelopathic mechanisms, namely disruption of competitor esterase activity or production of iron-sequestering siderophores, did not appear to be mechanisms of *K. brevis* allelopathy.[38] Allelopathic potency was variable between culture extracts, possibly due to small differences in growth stage, culture pH, or nutrient limitation.

Some *Karenia brevis* competitors appear to employ strategies to undermine the allelopathic effects of *K. brevis*, which could account for variability from year to year in allelopathic potency of bloom samples.[38] The diatom *Skeletonema costatum* is one competitor species that may possess such a strategy.[58] Extracellular extracts of *K. brevis* bloom samples that were co-cultured with *S. costatum* were significantly less allelopathic than extracts from *K. brevis* blooms not exposed to live *S. costatum*. Undermining of allelopathy could be due to *S. costatum* interrupting the biosynthesis or exudation of allelochemicals, metabolizing allelochemicals, or producing compounds that counteract *K. brevis* allelochemicals.[58] Since it is a superior exploitation competitor, *S. costatum* may also prevent *K. brevis* from acquiring the resources necessary to produce allelochemicals. In laboratory co-culturing experiments, only two of ten phytoplankton species, the diatoms *S. costatum* and *A. glacialis*, reduced *K. brevis* allelopathic potency, suggesting that the ability to undermine *K. brevis* allelopathy is relatively rare within the Gulf of Mexico phytoplankton community.[58] It is also possible that *S. costatum* produces allelochemicals of its own as a competitive strategy. Yamasaki *et al.*[59] found that filtrates of bacteria-free cultures of *S. costatum* decreased the growth of its competitors *Heterosigma akashiwo* and *Chaetoceros muelleri*. The resistance of competitors to allelopathic species is likely to be a profitable focus of future research.

Polyunsaturated aldehydes (PUAs) are implicated in allelopathic interactions in Norwegian waters. Two of the dominant phytoplankton in these waters are the haptophyte *Phaeocystis pouchetii* and the diatom *Skeletonema costatum*, both of which can release (2*E*,4*E*)-decadienal (**1**).[16] Three diatom species (*S. costatum*, *Chaetoceros socialis*, and *Thalassiosira antarctica*) cultured from Austnesfjorden, Norway, were grown with commercially-purchased **1**, and suffered decreased growth in a concentration-dependant manner.[16] However, in field samples, higher *P. pouchetii* densities correlated with higher diatom diversity. Since both *P. pouchetti* and *S. costatum* were frequently the two most common phytoplankton species in field samples and both can produce **1**, the authors speculated that these species may dominate the community by being somewhat resistant to the effects of **1** at natural bloom concentrations.[16]



PbTx-1 (**12**)



PbTx-2 (**13**) R = $CH_2C(=CH_2)CHO$
PbTx-3 (**14**) R = $CH_2C(=CH_2)CH_2OH$
PbTx-6 (**15**) R = H-ring epoxide (27*S*,28*R*) of **13**
PbTx-9 (**16**) R = $CH_2CH(CH_3)CH_2OH$

In another study, three commercially-purchased PUAs (**1–3**) caused a concentration-dependent decrease in the growth rates of six taxonomically diverse phytoplankton species (*Skeletonema marinoi*, *Dunaliella tertiolecta*, *Isochrysis galbana*, *Amphidinium carterae*, *Tetraselmis suecica* and *Micromonas pusilla*), although the six species did not respond identically to all three compounds.[60] These compounds also disrupted target cell membranes, and degraded cellular chlorophyll. The PUA with the longest alkyl chain (**1**) stunted cellular growth rates more than the other two compounds. Interestingly, one of the target species, the diatom *S. marinoi*, itself produces **2** and **3**,[61] and was affected less by these compounds than other target species, suggesting that this diatom is partially resistant to compounds it produces.[60] Often, phytoplankton species that were more susceptible to PUAs were smaller in size and had less-developed cell walls and high lipid content.[60] Diatom cell physiological state can also influence the production of and response to PUAs.[62] Total PUA production was maximized in the diatom *Skeletonema marinoi* when cells were nutrient-limited and in stationary phase. PUA concentrations increased more than three-fold from exponential growth phase to stationary phase if cells were damaged.[62]

## 5 Predator–prey interactions

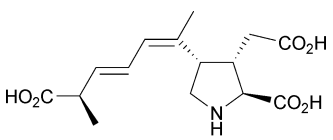
### 5.1 Constitutive defenses

Some secondary metabolites produced by phytoplankton act as constitutive anti-grazer defenses, being produced more or less constantly. While many studies have focused on demonstrating the direct physiological effects of phytoplankton toxicity on consumer species, indirect effects including altering consumer behavior have also been investigated. Behavioral changes such as decreased feeding rates can translate into reduced reproductive success. Exposure to the toxic phytoplankton *Prymnesium parvum* can cause inactivity in the copepods *Eurytemora affinis* and *Acartia bifflosa*, without the copepods actually consuming *P. parvum*, resulting in reduced copepod reproductive success.[63] Sopanen *et al.*[64] found that cell-free filtrates of *P. parvum* also negatively impacted copepod survivability, suggesting that the anti-grazer impacts of *P. parvum* on *E. affinis* are chemically-based. Mixed diets containing *P. parvum* and non-toxic *Rhodomonas salina* reduced copepod feeding activity, but the diets were not as detrimental to copepod health as were *P. parvum* filtrates.[64] Although only demonstrated in lab-based studies, impacts of toxic phytoplankton species on copepod grazer behavior may have large implications for copepod population dynamics and reproductive ecology in the field.

Herbivores whose ancestors were exposed to chemically-defended prey may respond differently to chemical defenses than grazers that have a limited shared history with the prey species. Florida estuarine rotifers (*Brachionus ibericus*) were willing to feed on the Florida red tide dinoflagellate *Karenia brevis* in a mixed diet, whereas rotifers from a Russian inland sea, *B. manjavacas* (ex *B. plicatilus*), refused *K. brevis* in an identical mixed diet.[65] However, Russian *B. manjavacas* fed on mixtures containing *K. brevis* four days into the experiment, indicating an eventual acclimation to the *K. brevis* feeding deterrent. Brevetoxins (PbTx-2 (**13**), -3 (**14**) and -9 (**16**)) were not responsible for the observed effects, but the deterrence has a chemical basis, since lipophilic fractions from *K. brevis* cells were deterrent when coated onto dried yeast particles at natural concentrations. However, the deterrent compound(s) were found to be labile, and thus remain unidentified.

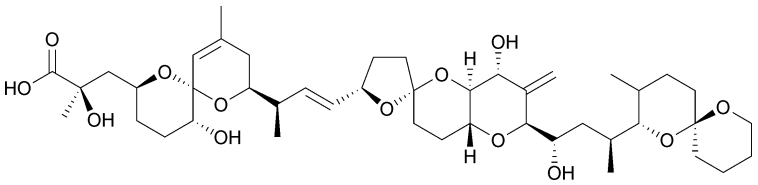
A recent study with the copepod *Acartia tonsa* demonstrated that negative effects of *K. brevis* on copepod egg production and survivability were not due to a chemical deterrent, but were likely caused by the nutritional inadequacy of *K. brevis* as a food source.[66,67] Copepods attempted to compensate for the nutritional inadequacy of *K. brevis*: the highest ingestion rates were observed for copepods fed solely *K. brevis*, but these copepods suffered low survivorship and low egg production rates. Egg production rates were not significantly different between starved copepods and those fed *K. brevis*, indicating that *K. brevis* is not chemically-defended from copepod grazers, but that *K. brevis* is a nutritionally inadequate food source.[66] Speekmann *et al.*[67] also concluded that *K. brevis* is a low quality food item for *A. tonsa.* When *A. tonsa* was fed sole or mixed diets containing *K. brevis* and the non-toxic dinoflagellate *Peridinium foliaceum*, egg production rates were significantly higher for copepods that were fed *P. foliaceum* versus copepods fed solely *K. brevis*.[67] These results, coupled with those of Kubanek *et al.*,[65] suggest that *K. brevis* is not chemically-defended against all important grazers, but is still a poor food for zooplankton.

The potential role of domoic acid (**17**) as an anti-grazer defense produced by diatoms of the genus *Pseudo-nitzschia* has recently been examined. Bargu *et al.*[68] found that krill exposed to abnormally high concentrations of dissolved **17** fed significantly less on a non-toxic food source than krill unexposed to **17**. However, other studies have suggested that *Pseudo-nitzschia* spp. are not chemically-defended against copepods. Olson *et al.*[69] found that copepod grazing impacts on field populations of *Pseudo-nitzschia* sp. were negligible but this lack of grazing was not attributed to **17**. In a similar field-based study, Olson *et al.*[70] found no correlation between low grazing rates and particulate or dissolved **17** concentrations in field samples. Due to the lack of recent experimental studies directly testing the impact of **17** on plankton consumers using ecologically-relevant concentrations and exposure methods, little can be concluded about the putative anti-predatory role of this toxin.




Domoic acid (**17**)

Zooplankton grazing on toxic phytoplankton may be affected by prey cell concentrations. Grazing on okadaic acid-producing *Dinophysis* spp. by the copepods *Temora longicornis* and *Centrophages typicus* only occurred when cell densities of *Dinophysis* spp. were low or when other food items were present.[71] No field-collected copepods positively selected for *Dinophysis* spp. at any cell density, although copepod feeding rates on *Dinophysis* spp. increased when offered a mixture of phytoplankton.[71] Chemical defenses of this genus may prevent copepods from controlling blooms by grazing, except in situations where *Dinophysis* spp. is



Okadaic acid (**18**)

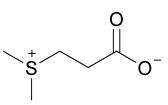
present at low concentrations. However, in natural systems the spatial distributions of especially toxic cells may vary,[72] which should be considered when interpreting results from feeding studies. Regardless of its uncertain capacity to serve as an anti-grazer defense, low abundance of okadaic acid (**18**) were also detected in copepods after feeding on *Dinophysis* spp.,[71] suggesting that copepods can act as vectors to transfer **18** to higher trophic levels.

Phytoplankton may also employ chemical defenses for protection against microzooplankton grazers. Lab grazing rates on a non-toxic strain of *Karlodinium veneficum* or the non-toxic cyptophyte *Storeatula major* were double that of grazing on toxic *K. veneficum* strain. The addition of partially purified, waterborne karlotoxins (*e.g.*, **4–5**) from *K. veneficum* reduced grazing pressure by the heterotrophic dinoflagellate *Oxyrrhis marina* on non-toxic *K. veneficum* and *S. major*.[73] However, since 90% of karlotoxins are cell-bound,[73] the effects of waterborne karlotoxins may not adequately simulate the route of exposure of the toxin to grazers. *O. marina* consumed less non-toxic *K. veneficum* cells when part of a mixed diet containing toxic *K. veneficum* than when offered non-toxic monocultures, suggesting that *K. veneficum* toxins affect *O. marina* feeding behavior. However, this does not exclude the possibility that other, uncharacterized compounds are involved in deterrence. Toxins produced by *K. veneficum* also appear to be allelopathic (see section 4) and may aid *K. veneficum* in prey capture (see section 5.3).[49] In a related study, feeding by the copepod *Acartia tonsa* was inversely related to the proportion of toxic *K. veneficum* cells in mixed diets.[74] Copepod mortality was not influenced by the consumption of toxic cells, suggesting that *K. veneficum* defenses may deter *A. tonsa* from feeding without killing the copepod.[74]

Susceptibility of *Karlodinium veneficum* grazers to karlotoxins may be related to the sterol composition of grazer cell membranes, with grazers whose membranes are rich in ergosterol and cholesterol hypothesized to be more susceptible to toxicity.[51] Fish erythrocytes incubated with dissolved ergosterol or cholesterol were less susceptible to hemolysis by partially purified karlotoxins compared with erythrocytes incubated with gymnodinosterol, suggesting that cholesterol and ergosterol are target molecules for karlotoxin activity.[51] Interestingly, gymnodinosterol was found to be the major steroid component of *K. veneficum* cell membranes, which may account for the resistance of *K. veneficum* to its own toxins. The high cholesterol composition of *Oxyrrhis marina* cell membranes may make this grazer vulnerable to cell lysis when exposed to karlotoxins.[51] However, the mechanism by which karlotoxins interact with cholesterol or ergosterol is not known.

Lewitus *et al.*[75] studied how the toxicity of the mixotrophic dinoflagellate *Pfiesteria piscicida* affects trophic interactions. Toxic, moderately toxic, and non-toxic strains of *P. piscicida* were incubated with *Rhodomonas* sp., to determine the impact of *Pfiesteria* strain toxicity on prey consumption. Toxic *P. piscicida* grazed on *Rhodomonas* sp. significantly less than did either the moderately toxic or non-toxic strains, suggesting that more toxic strains are less mixotrophic.[75] Sequestration of prey chloroplasts was observed for the less toxic strains.[75] When the three strains of *P. piscicida* were exposed to the ciliate predators *Euplotes woodruffi* and *E. vannus*, the toxic strains were consumed significantly less than the other two strains, leading to the hypothesis that toxin production may be act as an anti-grazer defense.[75] However, toxins from *Pfiesteria* spp. have still not been fully characterized, despite more than a decade of effort by several groups. Moeller *et al.*[76] reported multiple metal-containing organic toxins from *P. piscicida*, but their complete molecular structures could not be determined due to instability.

Previous studies have shown that dimethylsulfoniopropionate (DMSP; **19**) is an effective defense against microzooplankton grazers, although it does not appear to function as a toxin but rather as a signal to grazers.[77,78] Adding **19** to natural Gulf of Alaska and Puget Sound protist assemblages decreased feeding rates by 28–75% in lab experiments.[78] However, **19** reduced feeding rates in only four of 17 field microcosm experiments. These opposing effects were attributed to community microzooplankton acclimating to **19** in microcosm studies due to the longer duration of these studies compared to lab experiments or to grazing inhibition masked by stimulatory effects of **19** on community members not present in the lab-based study.[78] DMSP (**19**) appears to have multiple roles as a signaling molecule that can stimulate and inhibit grazing in a concentration-dependent manner, similar to the response of microzooplankton to amino acids.[79] In a related study, addition of valine, cysteine, proline, or serine to cultures of the tintinnid *Favella* sp. reduced feeding rates by 80% relative to controls.[79] Amino acids may make reliable signaling molecules for several reasons: long term exposure to amino acids had no impact on ciliate growth or mortality; low concentrations of amino acids were needed to induce a response; and the observed effects were reversible once the signal was removed. Amino acids with smaller side chains were also more inhibitory than those with longer side chains, suggesting some chemical specificity of the behavioral response.[79]



DMSP (**19**)

### 5.2 Activated defenses

Diatoms are known to produce a variety of chemical defenses that are activated after cellular damage. Ultimately, multiple pathways are used to create compounds from the oxidation of

membrane lipids, some of which appear function as anti-predatory defenses.[80] These compounds include oxypilins, fatty acid hydroperoxides, and polyunsaturated aldehydes (PUAs), some of which act as teratogens interfering with copepod reproduction and development.[17] Precursor molecules to these anti-predatory compounds are stored by many diatoms as polyunsaturated fatty acids that are enzymatically converted following cell damage.[80] Egg production and hatching success of the co-occurring copepods *Acartia clausi*, *Calanus helgolandicus* and *Temora longicornis* were negatively affected after feeding on field-collected *Cerataulina pelagica*, although PUAs were not detected in any samples.[81] However, when surface seawater samples were re-analyzed for fatty acid derivatives, hydroxyl and keto derivatives of the PUA precursor molecules eicosapentoic acid and docosahexaenoic acid were detected, providing evidence of oxylipins other than PUAs in diatom-dominated field samples.[81] Low fecundity was also reported for all three copepod species, although egg viability was high. *In situ* fecal pellet production was low, indicating that copepods ate less during a bloom which may account for low copepod fecundity.[81] The compounds present during a *C. pelagica* bloom were only partially characterized, and so it is possible that there were multiple active anti-grazer compounds present in this study.

PUAs may also undermine the nutritional quality of diatoms as food items for copepods. The enzymes that convert fatty acids into PUAs can reduce the nutritional quality of diatom exudates, which in turn may prevent efficient assimilation of diatom fatty acids into copepod tissue.[82] Wichard *et al.*[82] linked cellular fatty acid depletion with the formation of PUAs in disrupted diatom cells. Diatom enzymes remained active in the foregut of the copepod *C. helgolandicus*, indicating that the nutritional quality of diatoms may continue to decline as copepods consume them. Diatom diets supplemented with fatty acids increased the amount of PUAs produced by creating more substrate for the diatom enzymes to convert to PUAs. Because egg hatching success of the copepod *Temora longicornis* was still high despite the increase in PUAs, these compounds may not be directly toxic to this copepod, but may instead reduce the nutritional quality of diatom prey.[82]

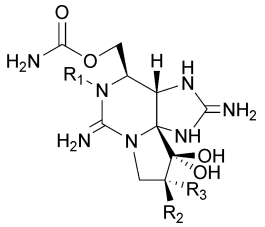
Despite a number of studies concluding that PUAs are responsible for reduced copepod reproductive success, several other studies have rejected this hypothesis. Poulet *et al.*[83] observed significant decreases in copepod egg production rates within two to three days after incubating *Calanus helgolandicus* females with mixed, natural assemblages of diatoms. This trend was reversible with a change in diet, and no correlation between the presence of diatoms known to produce PUAs and egg production rates was found.[83] The negative effects on copepod reproduction were attributed to nutritional deficiencies or other unidentified anti-grazer compounds produced by the diatoms, although PUAs were not directly measured.[83] In a companion study, Wichard *et al.*[84] found no correlation between field measurements of PUAs and copepod reproductive parameters, including egg production rates, hatching success, and abnormal larvae development.

Li *et al.*[85] tested the effects of single and mixed diatom diets (*Phaeodactylum tricorinutum* and/or *Skeletonema costatum*) on the egg production, hatching success, and naupliar development of the copepod *Acartia bifilosa*. Females survived at higher rates on mixed diets than on monocultures when food concentrations were kept constant, although higher ingestion rates were observed for controls containing either the flagellate *Platymonas subordiformis* or the green alga *Nannochloropsis oculata*, which adds to the evidence that diatoms may not be high quality food for copepods.[85] There was no effect on hatching success of copepod eggs incubated with diatom exudates or filtered seawater controls (a less-than-ideal experimental design given that copepod eggs would not normally be exposed to high concentrations of diatom compounds this way); however, the diet of maternal copepods strongly affected the hatching success, indicating that diatom diets negatively affect the reproductive success of copepods.[85]

The effects of several diatoms and a chryptophyte on the reproductive success of the copepod *Temora longicornis* has also been investigated.[86] Every diatom tested negatively affected copepod reproduction. Concentrations of total PUAs and polyunsaturated fatty acids, as well as the concentrations of other PUA precursor molecules and sterols, were determined for each prey species. Often, copepods had high fecundity when feeding on PUA-rich diatoms, whereas the fecundity of copepods feeding on PUA-deficient diatoms was low.[86] Interestingly, the most fertile copepods that consumed non-PUA producing diatoms also experienced the largest reduction in egg hatching success. Reductions in copepod reproductive success were not attributed to nutritional deficiencies of lipids in diatom food nor to PUAs, although the reductions could have resulted from a lack of vitamins and proteins or presence of other deterrent compounds.[86] Similarly, PUAs from pelagic diatoms appeared to have limited impacts on the benthic copepod *Tisbe holothuriae*, with no observed effects of PUAs on the reproductive success and larval survival.[87] The importance of PUAs as anti-grazing compounds and their impacts on copepod reproduction clearly cannot be generalized and remain an active area of investigation. Although several studies suggest that PUAs are not involved in diatom–copepod interactions, several of these do not directly measure PUAs. The physiological state[62] of the diatom or unknown feeding deterrents that are also derived from the lipid peroxidation pathway may cause some of the detrimental effects on copepods.[80,81]

### 5.3 Induced defenses

Induction of chemical defenses in the presence of grazers has been observed for a few toxic phytoplankton species. Concentrations of cellular gonyautoxins (GTX 1–4; **20–23**) significantly increased in the dinoflagellate *Alexandrium minutum* when co-cultured with the copepod *Acartia tonsa*, compared to *A. minutum* not exposed to copepods.[88] Concentrations of **20–23** increased more in dinoflagellate cells exposed to higher densities of copepods, and to actively-feeding rather than starved copepods.[88] In choice feeding assays, induced *A. minutum* cells were consumed less than non-induced *A. minutum* cells using non-toxic *Prorocentrum micans* as a control food. Because the alternative prey (*P. micans*) was consumed at equal rates when mixed with either induced or non-induced *A. minutum*, it is likely that the copepods rejected induced *A. minutum* and instead consumed the non-toxic control plankton, as opposed to being incapacitated by toxic cells.



GTX-1 (**20**) $R_1$ = OH, $R_2$ = H, $R_3$ = $OSO_3^-$
GTX-2 (**21**) $R_1$ = $R_2$ = H, $R_3$ = $OSO_3^-$
GTX-3 (**22**) $R_1$ = $R_3$ = H, $R_2$ = $OSO_3^-$
GTX-4 (**23**) $R_1$ = OH, $R_2$ = $OSO_3^-$, $R_3$ = H

The induction of *A. minutum* chemical defenses appears to vary based upon exposure to species-specific consumer cues.[89] Two strains of *A. minutum* (strains no. 83 and CNR A5) were exposed to waterborne cues from the copepods *Acartia clausi*, *Centropages typicus*, and *Pseudocalanus* sp. When *C. typicus* adults were caged away from phytoplankton cells to expose *A. minutum* to predator cues without the risk of consumption, GTX concentrations increased five-fold in strain no. 83 compared to no-copepod controls, whereas for strain CNR A5 GTX concentrations increased twenty-fold.[89] When exposed to *A. clausi* exudates, only one *A. minutum* strain displayed increased GTX concentrations, and neither strain responded with increased GTXs when exposed to waterborne cues of *Pseudocalanus* sp.[89] Bergkvist *et al.*[89] offered several hypotheses for the variable induction of GTX production in *A. minutum* when exposed to different grazers. First, *Pseudocalanus* sp. may pose less of a threat to *A. minutum* than do other copepod species, since it is a filter-feeder that cannot efficiently capture large particles like *A. minutum*; thus, *A. minutum* would not strongly benefit from inducing chemical defenses against *Pseudocalanus* sp.[89] It is likely that the induction of anti-grazer defenses evolved due to grazing pressure from a copepod that was capable of feeding on *A. minutum*.[89] The history of exposure to specific consumers could play a role in the induction of toxins as well. *A. minutum*, *C. typicus*, and *A. tonsa* are adapted to warmer waters, whereas *Pseudocalanus* sp. is more adapted to cold waters and rarely encounters *A. minutum* in the field.[89] Since *Pseudocalanus* sp. fed on *A. minutum* but did not induce toxin production, it is likely that the chemical cues received by *A. minutum* came from copepod grazers, and were not alarm cues that were released from the destruction of *A. minutum* cells.[89]

Selander *et al.*[90] investigated induced GTX production in the same *A. minutum* strains as Bergkvist *et al.*[89] under nutrient-limiting conditions. Both predator presence and high nitrate concentrations led to increased cellular GTX content. These results support the Carbon-Nutrient Balance Hypothesis,[91] because paralytic shellfish toxins including **20–23** are alkaloids with a low C:N ratio of ~1.4, which should favor production of nitrogen-rich metabolites when surplus nitrogen is available. The results also support the Optimal Defense Model,[92] since defenses increase when needed, *i.e.*, in the presence of grazers rather than being wasted in the absence of danger.[90] The authors cautioned that direct demonstrations of paralytic shellfish toxins functioning as anti-grazer defenses have not been demonstrated to date, and the above studies only provide correlative evidence of their function as anti-grazer defenses.

The effects of other *A. minutum* toxins on copepod feeding behavior and reproductive success have also been investigated by Barreiro *et al.*[93] When a toxic and a non-toxic strain of *A. minutum* were each fed to the copepod *A. clausi*, feeding rates and total consumption of the toxic strain were significantly lower than for non-toxic *A. minutum* controls. Mortality of copepods fed mixed diets was intermediate compared to those fed either toxic or non-toxic strains. Since there was no significant difference in toxin accumulation in copepods fed toxic *versus* mixed strain treatments, it is likely that the observed intermediate mortality levels were a result of an amelioration effect of non-toxic food within the mixed diet.[93] Copepod egg production was suppressed on the toxic diet, whereas egg production rates were similar between non-toxic and mixed diets. Favorable egg production rates in mixed culture treatments may have also been due to an amelioration effect. Alternatively, the low ingestion rates measured for copepods feeding on a toxic diet may have directly led to lower egg production rates.[93] Egg hatching success was negatively affected by the toxic diet, and hatching success of the mixed and toxic treatments was not significantly different. Phytoplankton community composition may also determine consumption of toxic species: ingestion and clearance rates by *A. clausi* depended on the presence of other, non-toxic phytoplankton in the community.[94] Alternatively, Estrada *et al.*[95] found no evidence suggesting that related toxins produced by *Alexandrium catenella* function as anti-grazing defenses in a microcosm study. The authors suggested several hypotheses to explain why negative effects of *A. catenella* may have been missed: concentrations of *A. catenella* cells were lower compared to other studies; toxin concentrations may also have been too low to affect copepods in the microcosm; and the dominant toxins detected in the microcosm were the least potent of the paralytic shellfish toxin suite.[95]

Ingestion of *Alexandrium* spp. by copepods may depend on cell concentrations as well as toxin composition and concentration.[96] When three different *Alexandrium* strains of varying toxicities (high, intermediate, and non-toxic) were offered in mixed diets to four different copepod species, three copepod species did not appear to differentiate between cells of intermediate and high toxicity, but both cell types were consumed less than the non-toxic strain at low cell densities.[96] This suggests that these copepods responded to the presence or absence of a toxin, without differentiating between cellular toxin concentrations when overall phytoplankton cell densities were low.[96] Moreover, when the overall concentration of *Alexandrium* cells increased, copepods could no longer select for non-toxic prey and instead decreased their overall phytoplankton consumption. At high cell densities of *Alexandrium*, some copepods are likely to reduce overall consumption of phytoplankton rather than only reducing the consumption of toxic cells; therefore grazer biomass may be more important in *Alexandrium* bloom dynamics than the specific response of grazers to phytoplankton toxicity.[96]

The effects of microalgal toxins on grazers may also act as selective agents influencing the population genetics and evolution of grazer species.[97,98] Connell *et al.*[98] found that multiple mutations arising in soft clam *Mya arenaria* populations can confer resistance to saxitoxin (**11**) produced by *Alexandrium* spp. Four resistant genotypes for the saxitoxin-binding sodium channel were found in a survey of *M. arenaria* populations from areas

historically known to experience *Alexandrium* spp. blooms, whereas clams from non-bloom areas were typically sensitive to intoxication. An intermediate level of saxitoxin resistance was measured for certain heterozygous genotypes in *in vitro* nerve trunk assays.[98] Sensitive *versus* resistant phenotypes displayed differences in burrowing and feeding capabilities, toxin accumulation, and survivability, indicating a fitness advantage to resistant phenotypes when exposed to *Alexandrium* toxins.[99] The rate of selective pressure that toxins impose on clam populations, whether this selective pressure is variable within clam populations, and the fitness costs for saxitoxin-resistance remain unknown and topics for future study.[98]

Paralytic shellfish toxins may not account for all observed negative effects on susceptible bivalve populations. Extracts of toxic *Alexandrium tamarense* cultures did not affect the immune responses of the clams *Mya arenaria* and *Ruditapes philippinarum*, whereas non-toxic *A. tamarense* extracts negatively impacted hemocyte activity in these clams, which indicates that bioactive compounds other than paralytic shellfish toxins can cause detrimental effects on exposed bivalve populations.[100] The compound(s) responsible remain to be identified.

Chemical cues from a grazer can also induce morphological defenses in phytoplankton. *Phaeocystis globosa* can change its morphology in response to different grazer cues, switching between colonies and single cells in order to defend itself from grazing.[101] To avoid predation pressure from larger copepods, *P. globosa* remains as single cells that are too small to be preferred prey. To avoid smaller grazers such as ciliates, the colony morph is advantageous, because it is too big to be consumed by these grazers. *P. globosa* colony formation was suppressed by 70–75% when exposed to chemical cues from a natural copepod dominated mesozooplankton assemblage or from the copepod *Acartia tonsa* feeding on *P. globosa*, although the average number of cells per colony did not change.[101] Conversely, cues from the grazing ciliate *Euplotes* sp. stimulated a 25% increase in colony formation in *P. globosa* compared to unexposed controls.[101] *P. globosa* can therefore change its morphology to avoid predation by chemically assessing local predation threats. The Antarctic haptophyte *Phaeocystis antarctica* is also capable of inducing morphological defenses in response to grazer cues from a natural mesozooplankton assemblage.[102] Grazer cues were less than 12 kDa in size, based upon diffusion through dialysis membrane.[102] Although specific waterborne chemical cues from ciliates and copepods appear to be responsible for the observed induced morphological changes, these compounds have not yet been identified.[102]

Chemical cues can induce both morphological and behavioral changes in zooplankton. The shell morphology of planktonic larvae of the intertidal snail *Littorina scutulata* changes in response to chemical cues from consumers (zoea larvae of *Cancer* spp.) and from snail larvae consumed by *Cancer* spp. larvae.[103] Snail larvae exposed to predator exudates had significantly rounder shells and smaller apertures than those not exposed to predator cues, which coincided with significantly higher survival rates compared to unexposed larvae.[103] This is a rare example of morphological defenses in marine zooplankton, despite vast numbers of studies on this topic in freshwater systems. Chemical cues from caged predators can also induce behavioral changes in marine zooplankton.[104] Urchin (*Strongylocentrotus droebachiensis*) larvae swam at lower average depths when a caged predator (the ctenophore *Bolinopsis infundibulum*) was introduced to the top of a water column, compared with larvae that were not exposed to a predator, suggesting that swimming depth choice may represent an escape response by urchins.[104] In contrast, oyster (*Ostrea edulis)* larvae did not significantly change swimming depth compared to controls. Behavioral changes in response to a potential chemical cue may be important in minimizing predation upon the pelagic larvae of marine benthic invertebrates.[104]

### 5.4 Prey tracking and recognition

Protozoans can detect and track towards bacterial prey using chemical cues, including cell surface carbohydrates and amino acids. Mohapatra and Fukami[105] investigated heterotrophic nanoflagellate migration into capillary tubes containing three different marine bacterial species or cellular surface extracts containing bacterial surface compounds and compared both treatments to aged seawater and 0.5 M sodium chloride controls. The highest positive chemotactic response to both surface chemistry extracts and whole cells was measured for the bacterium *Pseudomonas* sp.[105] Clearance rates by heterotrophic nanoflagellates were also measured using these bacteria as prey items, and *Pseudomonas* sp. was ingested at the highest rates.[105] Prey selection by nanoflagellates is not based solely on geometry and size, but also on the surface biochemistry of prey.

Cell surface receptors of marine planktonic protozoa, specifically lectins that allow discrimination among multiple prey types according to prey surface carbohydrates, have recently been investigated.[106] Wootton *et al.*[106] found a calcium-dependent, mannose-binding lectin from surface protein preparations of the dinoflagellate *Oxyrrhis marina*. Mannose was detected on the surface of prey (*Isochrysis galbana*) cells, indicating that *O. marina* could use mannose-binding lectin to identify *I. galbana* as a prey item.[106] After mannose-binding lectin functioning was blocked in live *O. marina*, feeding on prey cells was inhibited by 60% and the predator no longer discriminated between mannose-coated beads *versus* control beads.[106] Thus, chemoreception at cell surfaces can be used by protozoa to distinguish between different prey cells.

Zooplankton can use a suite of cues found in exudates of phytoplankton to locate prey patches. The response of the predatory dinoflagellate *Oxyrrhis marina* to planktonic thin layers has been observed using lab-generated thin layers containing either live prey (*Isochrysis galbana*) or filtrates from *I. galbana*.[107] After the introduction of prey to thin layers, more *O. marina* individuals tracked to the thin layer. Swimming speeds and turning rates of *O. marina* also increased, although these effects were less consistent when prey filtrates were added to thin layers than in the presence of live prey.[107] Since both prey filtrates and live prey caused *O. marina* to aggregate in thin layers, chemical cues appear to be used in *O. marina* prey tracking, although these kairomones remain unidentified.

Copepods can differentially respond to a variety of physical and chemical cues to gather information about their environment.[108] The copepods *Temora longicornis* and *Acartia tonsa* responded to velocity gradients and phytoplankton exudates contained within thin layers by increasing swimming speed,

turning rates, and residence times in these layers.[108] These behavioral responses may allow the copepod to effectively search for and locate food items by quickly scanning the area.[108] Thin layers may help the copepod maintain a desirable position in the water column, and help transport them to new locations based on velocity gradient responses. The interplay between multiple stimulatory cues is important to determine the behavior of zooplankton.[108]

### 5.5 Prey capture and consumption

*Karlodinium veneficum* can utilize karlotoxins (*e.g.*, **4–5**) to immobilize potential prey.[49] Since karlotoxins are at least 90% cell-associated,[73] the authors speculated that cell–cell contact is necessary to expose prey to karlotoxins and to immobilize prey cells, which could then be more easily captured and ingested by *K.* veneficum.[49] When prey cells (*Storeatula major*) were treated with 25 ng/ml mixed karlotoxins and exposed to two different *K. veneficum* strains, prey ingestion rates were significantly higher than when prey cells were not pre-treated with karlotoxins, suggesting that these compounds make prey capture easier for *K. veneficum.*[49]

Jellyfish are important members of the marine plankton, and recent work has investigated the toxicity of jellyfish venoms used for prey capture.[109] Recently, a 27.5 kDa glycoprotein (ClGp1) that is toxic to human HepG2 cells was isolated from the oral arms (mesenteric tentacles) of the blue jellyfish *Cyanea lamarckii*.[109] Up to 26.8% of this glycoprotein is composed of carbohydrate portions, and it likely includes mannose and *N*-acetylglucosamine or sialic acid side chains. It is probable that this protein represents one of many glycoproteins present in jellyfish venom.[109] Toxins can also be differentially distributed between tentacle types, based upon the ecological function of the tentacle. *C. lamarkii* mesenteric tentacle extracts were seven times more hemolytic and significantly more toxic to human HepG2 cells than fishing tentacle extracts.[110] A similar pattern was observed in extracts of mesenteric and fishing tentacles of the lion's mane jellyfish (*Cyanea capillata*).[110] Higher levels of toxicity and hemolytic activity in the mesenteric tentacles indicates that oral arms contribute more to the digestion of prey items than fishing tentacles.[110]

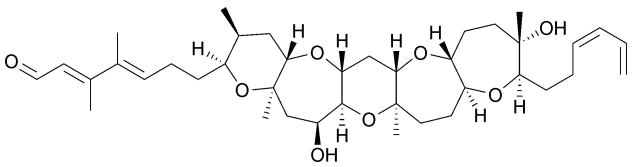
## 6 Community and ecosystem effects

Several recent studies have considered the fate of phytoplankton toxins after they are released into the environment, which may depend on the presence of certain community members. Hagstrom *et al.*[111] found that while diatom-associated bacteria may have promoted production or cellular release of domoic acid (**17**) from the diatom *Pseudo-nitzschia multiseries*, these bacteria did not alter decomposition rates of the toxin after it was released from *P. multiseries* cells.[111] The presence of mussel pseudo-feces and bottom sediment samples did increase the rate of toxin degradation, which may be caused by sediment bacteria and/or enzymes present in these samples degrading or utilizing **17** as substrate.[111]

In addition to the multiple effects discussed in earlier sections, polyunsaturated aldehydes (PUAs) can also affect marine bacterial community composition.[18] Three commercially purchased PUAs (**1–3**) known to be produced by diatoms including *Skeletonema marinoi* were added at multiple concentrations (13–150 μM) to cultures of 33 marine bacteria. Depending on PUA concentration and/or target species identity, PUAs had either had a positive, negative, or neutral effect on bacterial growth.[18] To assess chemical specificity, bacteria were exposed to two compounds: ethanal was used to mimic a PUA aldehydic group, and decanoic acid was used to mimic the carbon chain of **1**. Not surprisingly, neither compound had an effect on bacterial growth, suggesting that these functional groups may not act independently on bacteria.[18] Resistant bacteria may use detoxifying enzymes, such as NADPH dehydrogenase, to cope with PUA exposure, and are also expected to out-compete susceptible bacteria during *S. marinoi* blooms.[18]

Myers *et al.*[112] found that brevetoxins produced by the red tide dinoflagellate *Karenia brevis* are either adsorbed or metabolized by other members of the phytoplankton community. When extracellular extracts of *K. brevis* were added to cultures of *Skeletonema costatum*, concentrations of brevetoxin B (PbTx-2; **13**) significantly decreased after 24 hours compared to *S. costatum*-free controls, and no additional brevetoxin-like derivatives, metabolites, or other degradation products were detected in samples when analyzed by LC-MS and ELISA.[112] The ability of competitors to remove **13** from the water column was not limited to *S. costatum*: diatoms, cryptophytes, and dinoflagellates were all capable of removing approximately 80% of dissolved **13** within 24 hours.[112] Metabolism or adsorption of **13** is likely related to its Michael-accepting α,β-unsaturated aldehyde-containing side chain, since PbTx-3 (**14**) whose side chain is reduced compared with **13** was not affected, but PbTx-1 (**12**) with a similar side chain also decreased in concentration from exposure to phytoplankton. PbTx-2 (**13**) is probably removed by adsorption to competitor cells or by complexation with competitor biomolecules through the formation of covalent bonds or strong non-covalent interactions.[112] It is also possible that competitor phytoplankton species can metabolize **13**, producing novel degradation products or other metabolites.

While Myers *et al.*[112] found that bacteria-free strains of *S. costatum* were capable of removing waterborne **13**, suggesting that bacteria are not necessary for toxin degradation, some algicidal bacteria can lead to the release of brevetoxins into the water column.[113] The fate of **13–14** released into the environment was monitored by measuring brevetoxin concentration and overall toxicity after cell lysis by algicidal bacteria.[113] High pH, and not degradation by bacteria, likely caused hydrolysis of **13–14** into less toxic open A-ring derivatives.[113] In field samples, brevetoxins can also be modified when released into the environment following cell lysis.[114] Waterborne **13** can be reduced to **14**, which can remain in the water column even after *K. brevis* cell counts have diminished below detectable levels.[114] Brevenal (**24**), a recently-discovered brevetoxin antagonist to sodium channels,[115] was also detected in field samples.[114] Field concentrations of brevetoxins were variable, although the most common compounds were **12–14**. PbTx-2 (**13**) was the most common intracellular brevetoxin present in natural bloom samples, while the most common waterborne and aerosol-associated toxin was **14**. PbTx-1 (**12**) was not detected in sea spray samples, although trace amounts of **24** were detected.[114]



Brevenal (**24**)

Brevetoxin accumulation in shellfish tissues after exposure to *K. brevis* blooms was examined by Pierce and Henry.[114] The oyster *Crassostrea virginica* and the clam *Merceneria mercenaria-campenchenesis* contained detectable quantities of brevetoxin metabolites, although no "parent" brevetoxins were detected in shellfish tissues. The metabolites that were formed by shellfish were still toxic in mouse assays.[114] Clams appeared to accumulate toxins at slower rates than oysters, but they also retained their toxicity for a longer period of time compared to oysters.[114] Sedimentation of *K. brevis* cells can concentrate brevetoxins (*e.g.*, **12–16**), exposing benthic deposit feeders to the toxins in addition to filter feeders such as clams and mussels.[116] This action shifts toxins into a different but similarly important trophic pathway.

Since shellfish can remain toxic well after an algal bloom has dissipated, the effects of exposure to chronic, sub-lethal doses of accumulated compounds on omnivorous fish and sea mammals are important to study, as their prey can act as vectors for brevetoxin transfer.[117] Planktivorous striped mullet (*Mugil cephalus*) were exposed to either lysed *Karenia brevis* or to live, whole *K. brevis* cells. Fish exposed to lysed suspensions did not accumulate any toxins into their tissues, but they died within 80 minutes of exposure. In treatments where fish were exposed to whole *K. brevis* cells, they accumulated toxins in muscle and viscera tissues, presumably by consumption.[117] In a second experiment, omnivorous pinfish (*Lagodon rhomboides*) and Atlantic croakers (*Micropogonias undulatus*) were fed hard clams (*Mercenaria* sp.) that were contaminated with brevetoxins. After 14 days of exposure to toxic clams, both pinfish and croakers contained high brevetoxin concentrations in muscles and viscera, without displaying any adverse effects. Toxin profiles of fish were often identical to those of the shellfish or plankton to which they were exposed, usually consisting of PbTx-3 (**14**), metabolites cys-PbTx-2, and OxCys-PbTx-2 for the pinfish and croakers, whereas the mullet contained PbTx-2 (**13**), **14**, PbTx-6 (**15**), and PbTx-9 (**16**).[117] Curiously, brevenal (**24**) was measured in *K. brevis* cells, but not in fish tissue. A variety of functionally- and taxonomically-distinct fish were caught and analyzed during a non-bloom period, and 70% of fish caught contained brevetoxins.[117] These experiments clearly indicate that the mode of delivery and route of exposure to brevetoxins is crucial in determining whether or not brevetoxins are ichthyotoxic or are passed through marine food webs, and that these toxins can remain in animal tissue for at least weeks or months.[117]

Although the accumulation of brevetoxins has been well described among shellfish, studies addressing other physiological consequences of sub-lethal exposure are somewhat rarer. Keppler *et al.*[118] investigated the sub-lethal effects of brevetoxins using biomarkers, including lysosomal destabilization assays, in the oyster *Crassostrea virginica* after exposure to both pure **14** and to a natural bloom consisting of the raphidophytes *Chattonella subsalsa* and *Fibrocapsa japonica*, both of which have been previously reported to produce brevetoxins, particularly **14**.[119,120] Exposure to live bloom samples caused a significant increase in cellular damage to oyster tissue after 96 hour exposure, as did exposure to dissolved **14** at 1 and 10 nM.[118] High liposomal destabilization may translate into reduced reproductive fitness of oysters,[121] indicating that bloom exposure may be costly to oysters.[118]

Recruitment success of bivalves from the plankton to the benthos may be prevented by phytoplankton toxins. When fed different densities of the brown tide alga *Aureococcus anophagefferens* in a diet mixed with the nutritious haptophyte *Isochrysis galbana*, growth of larvae of the clam (*Mercenaria mercenaria*) was inhibited by the presence of *A. anophagefferens* in a density-dependent manner.[122] Increased mortality and delayed development were also observed in larvae exposed to *A. anophagefferens*, and exposed larvae had reduced clearance rates. These effects appear to be linked to unidentified metabolites associated with *A. anophagefferens* cells, since filtrates of *A. anophagefferens* cultures induced a small decrease in larval growth rates.[122] *A. anophagefferens* may therefore inhibit the recruitment of clam larvae to the benthos.

Harmful algal blooms can act as potential refuges for fish attempting to avoid visual predators, although the refuges themselves may incur costs to the fish. In flume studies, three-spined stickleback fish (*Gasterosteus aculeatus*) significantly tracked to maze arms containing the toxic cyanobacterium *Nodularia spumigena* over arms containing water conditioned with a predator, the European perch (*Perca fluviatilis*).[123] Stickleback did not differentiate between filtered seawater and either cyanobacteria or predator cues in control runs. Stickleback appeared to integrate multiple cues in order to pick suitable refuges. In this case, the very turbid *N. spumigena* bloom may hide the fish from visual predators, while also exposing sticklebacks to a higher toxicity risk.[123] Juvenile stickleback fed mixed diets including *N. spumigena* suffered fitness costs, including decreased growth and feeding rates, while accumulating nodularin (**10**) in their tissues.[124] Field-collected sticklebacks that were subsequently fed a non-toxic diet were able to partially detoxify their tissues after two weeks.[124] This suggests that sticklebacks must make tradeoffs between long-term survival and short-term exposure to cyanobacterial toxins.

Sub-lethal exposures to domoic acid (**17**) may impact gene expression in asymptomatic fish.[125] Acute exposure to injected **17** changed global gene expression in zebrafish at concentrations lower than those causing observable behavioral changes.[125] Down-regulation of genes involved in immune functioning, RNA processing, ion transport, and signal transduction were observed after exposure to **17** at concentrations well below the $EC_{50}$ for zebrafish, demonstrating that low levels of toxins maintain the potential for neurological impact. Exposure to low concentrations of **17** most often caused down-regulation in 10 of 11 functional gene groups, whereas exposure to higher doses (approximately twice the $EC_{50}$) caused up-regulation of eight of 11 functional groups. Genes that were most affected by **17** were found in transcription factor and signal transduction functional groups.[125]

Domoic acid (**17**) has also been found to accumulate in fish which may serve as vectors to other trophic levels. After force-feeding coho salmon (*Oncorhynchus kisutch*) an aqueous solution

containing **17** at ecologically-relevant concentrations, salmon accumulated **17** in their tissues without causing observable behavioral impacts and **17** was still present at 25% of initial concentrations after 96 hours.[126] Concentrations of **17** were highest in the kidneys, which may be the primary route of toxin excretion for these fish.[126] In contrast, **17** caused obvious detrimental effects including inhibition of swimming when injected into fish tissue, demonstrating that coho salmon are neurologically susceptible to **17**, but not when exposed to the toxin orally. In symptomatic fish after intracoelomic injection, the highest concentrations of **17** were found in brain tissue, in contrast to orally-exposed fish, for whom **17** concentrations were lowest in brain tissue.[126]

Accumulation of **17** in other commercially valuable species has also been demonstrated.[127] Approximately 30% of male squid (*Loligo opalescens*) sampled during a toxic *Pseudo-nitzschia* sp. bloom in Monterey Bay, California, USA, had **17** in their stomachs and viscera. Toxic male squid also tended to have *Pseudo-nitzschia australis* frustules in their stomachs, although this may have been a result of the squid consuming krill, a common grazer of *P. australis*. Squid enter the bay to spawn, and females don't typically feed during spawning, which could explain why females did not accumulate **17**.[127]

Toxins belonging to various structural classes may accumulate in fish tissues when multiple toxic algal species are present during mixed blooms.[128] Domoic acid (**17**), saxitoxin (**11**), and saxitoxin-related compounds (**20–23**) were detected in the muscle and viscera of sardines and anchovies caught in Monterey Bay, California, USA, when both *Pseudo-nitzschia* sp. and *Alexandrium* sp. were present in field samples.[128]

Some filter-feeding species accumulate more okadaic acid (**18**) than others when exposed to the dinoflagellate *Dinophysis acuminata*.[129] During an intense bloom, all organisms studied, including mussels, clams, ascidians, and polychaete worms contained measurable amounts of **18**, with the mussels *Mytilus galloprovincialis* and *Modiolus barbatus* consistently exhibiting the highest concentrations. Mussels that were suspended in the water column accumulated significantly less **18** than mussels collected from the same sampling site but from benthic substrates, which suggests exposure to toxins not only from the overlying water column, but from toxins accumulated in pseudo-feces and other settling organic material as well. This sheds light into how toxins can be transferred from the water column to underlying sediments.[129] Variable toxin accumulation in different species could also result from different consumption rates of toxic algae, as well as from different levels of exposure in slightly different habitats. This study demonstrated the need for increased studies on species-specific responses to various microalgal toxins that consider ecologically-realistic methods of toxin exposure.[129]

While the roles of copepods and shellfish as vectors for phytoplankton toxins are well documented, cladoceran zooplankters, namely *Molina mongolica*, can potentially act as vectors for the trophic transfer of paralytic shellfish toxins from the dinoflagellate *Alexandrium tamarense*.[130] *M. mongolica* that ate *A. tamarense* displayed similar toxin profiles to its food, and ingestion rates of *A. tamarense* by *M. mongolica* did not correlate with prey toxicity. When *M. mongolica* reared on *A. tamarense* were fed to fish (*Sciaenops oscellatus*) larvae, paralytic shellfish toxins were detected in fish, although no fish mortality was observed. Paralytic shellfish toxins can be transferred through trophic levels *via* a cladoceran vector and potentially be metabolized into other derivatives through this process.[130] In contrast, the tintinnid *Favella taraikaensis* retained less than 2% of ingested paralytic shellfish toxins when cultured with *A. tamarense*.[131] *F. taraikaensis* growth rates were high when feeding on *A. tamarense*, suggesting that it is a high quality food item for this tintinnid. The total (intra- plus extra-cellular) toxin content in treatments were not significantly different from controls, indicating that there is little to no metabolism of paralytic shellfish toxins by the tintinnid, which may simply excrete the compounds intact.[131]

## 7 Conclusions

The ecological roles of natural products from pelagic organisms are becoming increasingly appreciated. Specifically, allelopathic interactions and predator–prey dynamics have been strong foci of marine plankton chemical ecology research in recent years. Exciting examples of host–parasite interactions among marine planktonic organisms have been documented in the past three years, which complement the larger pool of these types of studies in freshwater plankton systems. In contrast, the importance of natural products in mutualistic interactions as well as intraspecific communication represents a relatively unexplored avenue for future research. The influence of bacteria on phytoplankton natural product biosynthesis, induction, release, metabolism, and degradation is also under-represented in the literature.

A continued lack of fully-characterized molecular structures, particularly in allelopathy and pheromone studies, remains a hindrance to appreciating the importance of natural products in pelagic communities. Without having specific compounds identified and available in pure form for manipulative experiments and for use as analytical standards, it is difficult to study patterns of production and distribution, mechanisms of action, and the costs and benefits associated with secondary metabolism. However, due to their low natural concentrations, typically high water-solubility, dispersal in large volumes of seawater, and the small size of most planktonic organisms, it is not surprising that these chemical cues are not nearly as tractable as those of benthic marine or terrestrial macroorganisms.

Recent advances in genetics and metabolomics as well as improvements in the sensitivity of analytical instrumentation will aid the discovery of natural products from marine planktonic organisms. Future discoveries of novel natural products will allow researchers to directly test hypotheses about the ecological functions of these compounds in rigorously-designed, ecologically-relevant experiments. Planktonic secondary metabolites can influence the ecology and evolution of organisms at multiple trophic levels within the marine plankton, and their effects can also trickle into other systems. Although chemical ecology involving terrestrial and benthic marine habitats are better-developed fields of study, natural products are clearly crucial in pelagic systems on multiple ecological scales, and therefore chemical ecology of the marine plankton is an increasingly fruitful area for research.

## 8 Acknowledgements

We would like to thank the many researchers who provided reprints of their recent publications and copies of papers in press.

Our research on chemical ecology of the marine plankton has been funded by the National Science Foundation grants OCE-0134843 and OCE-0726689 and by the Florida Fish & Wildlife Conservation Commission. We thank Dr. Clare H. Redshaw and E. Paige Stout for suggestions that improved the manuscript.

## 9 References


1 V. J. Paul and R. Ritson-Williams, *Natural Product Reports*, 2008, **25**, 662–695.
2 A. Ianora, M. Boersma, R. Casotti, A. Fontana, J. Harder, F. Hoffmann, H. Pavia, P. Potin, S. A. Poulet and G. Toth, *Estuaries and Coasts*, 2006, **29**, 531–551.
3 G. Pohnert, M. Steinke and R. Tollrian, *Trends in Ecology & Evolution*, 2007, **22**, 198–204.
4 C. Avila, S. Taboada and L. Nunez-Pons, *Marine Ecology – an Evolutionary Perspective*, 2008, **29**, 1–71.
5 P. Fink, *Marine and Freshwater Behavoir and Physiology*, 2007, **40**, 155–168.
6 E. Granéli and H. Pavia, in *Allelopathy: a physiological process with ecological implications*, eds. M. J. Reigosa, N. Pedrol and L. González, Springer, Dodrecht, 2006, pp. 415–431.
7 E. Graneli, M. Weberg and P. S. Salomon, *Harmful Algae*, 2008, **8**, 94–102.
8 E. Graneli, *African Journal of Marine Science*, 2006, **28**, 331–336.
9 M. Karjalainen, J. Engstrom-Ost, S. Korpinen, H. Peltonen, J. P. Paakkonen, S. Ronkkonen, S. Suikkanen and M. Viitasalo, *Ambio*, 2007, **36**, 195–202.
10 V. L. Trainer, B. Hickey and S. S. Bates, in *Oceans and human health: Risks and remedies from the sea*, eds. P. J. Walsh, S. L. Smith, L. E. Fleming, H. Solo-Gabriele and W. H. Gerwick, Elsevier Science Publishers, New York, 2008, pp. 219–237.
11 A. Vardi, *Communicative & Integrative Biology*, 2008, **1**, 1–3.
12 J. C. Nejstgaard, K. W. Tang, M. Steinke, J. Dutz, M. Koski, E. Antajan and J. D. Long, *Biogeochemistry*, 2007, **83**, 147–172.
13 J. Kubanek and T. W. Snell, in *Chemical communication among bacteria*, eds. S. C. Winans and B. L. Bassler, ASM Press, Washington, D.C., 2008, pp. 453–461.
14 R. K. Zimmer and C. A. Zimmer, *Journal of Chemical Ecology*, 2008, **34**, 822–836.
15 A. Vardi, F. Formiggini, R. Casotti, A. De Martino, F. Ribalet, A. Miralto and C. Bowler, *PLoS Biology*, 2006, **4**, 411–419.
16 E. Hansen and H. C. Eilertsen, *Journal of Plankton Research*, 2007, **29**, 87–96.
17 A. Miralto, G. Barone, G. Romano, S. A. Poulet, A. Ianora, G. L. Russo, I. Buttino, G. Mazzarella, M. Laabir, M. Cabrini and M. G. Giacobbe, *Nature*, 1999, **402**, 173–176.
18 F. Ribalet, L. Intertaglia, P. Lebaron and R. Casotti, *Aquatic Toxicology*, 2008, **86**, 249–255.
19 G. Pohnert, O. Lumineau, A. Cueff, S. Adolph, C. Cordevant, M. Lange and S. Poulet, *Marine Ecology – Progress Series*, 2002, **245**, 33–45.
20 A. Vardi, K. D. Bidle, C. Kwityn, D. J. Hirsh, S. M. Thompson, J. A. Callow, P. Falkowski and C. Bowler, *Current Biology*, 2008, **18**, 895–899.
21 C. Vidoudez and G. Pohnert, *Journal of PlanktonResearch*, 2008, **30**, 1305–1313.
22 R. Casotti, S. Mazza, C. Brunet, V. Vantrepotte, A. Ianora and A. Miralto, *Journal of Phycology*, 2005, **41**, 7–20.
23 A. Vardi, D. Eisenstadt, O. Murik, I. Berman-Frank, T. Zohary, A. Levine and A. Kaplan, *Environmental Microbiology*, 2007, **9**, 360–369.
24 K. Olli and K. Trunov, *Phycologia*, 2007, **46**, 109–112.
25 C. P. Stelzer and T. W. Snell, *Limnology and Oceanography*, 2006, **51**, 125–130.
26 J. J. Gilbert, *Journal of Experimental Biology*, 1963, **40**, 625, &.
27 T. W. Snell, J. Kubanek, W. Carter, A. B. Payne, J. Kim, M. K. Hicks and C. P. Stelzer, *Marine Biology*, 2006, **149**, 763–773.
28 T. W. Snell, J. Kim, E. Zelaya and R. Resop, *Hydrobiologia*, 2007, **593**, 151–157.
29 T. W. Snell, R. Rico-Martinez, L. N. Kelly and T. E. Battle, *Marine Biology*, 1995, **123**, 347–353.
30 J. Pino-Marambio, A. J. Mordue, M. Birkett, J. Carvajal, G. Asencio, A. Mellado and A. Quiroz, *Aquaculture*, 2007, **271**, 70–76.
31 E. Goetze, *Limnology and Oceanography*, 2008, **53**, 433–445.
32 T. Kiorboe, *Limnology and Oceanography*, 2007, **52**, 1511–1522.
33 X. M. Bai, J. E. Adolf, T. Bachvaroff, A. R. Place and D. W. Coats, *Harmful Algae*, 2007, **6**, 670–678.
34 A. R. Place, H. R. Harvey, X. Bai and D. W. Coats, *African Journal of Marine Science*, 2006, **28**, 347–351.
35 R. M. Van Wagoner, J. R. Deeds, M. Satake, A. A. Ribeiro, A. R. Place and J. L. C. Wright, *Tetrahedron Letters*, 2008, **49**, 6457–6461.
36 R. J. E. Bailey, M. A. Birkett, A. Ingvarsdottir, A. J. Mordue, W. Mordue, B. O'Shea, J. A. Pickett and L. J. Wadhams, *Canadian Journal of Fisheries and Aquatic Sciences*, 2006, **63**, 448–456.
37 D. M. Fields, M. J. Weissburg and H. Browman, *Diseases of Aquatic Organisms*, 2007, **78**, 161–168.
38 E. K. Prince, T. L. Myers and J. Kubanek, *Limnology and Oceanography*, 2008, **53**, 531–541.
39 U. Tillmann, U. John and A. Cembella, *Journal of Plankton Research*, 2007, **29**, 527–543.
40 T. Ohta, E. Sueoka, N. Iida, A. Komori, M. Suganuma, R. Nishiwaki, M. Tatematsu, S. J. Kim, W. W. Carmichael and H. Fujiki, *Cancer Research*, 1994, **54**, 6402–6406.
41 V. O. Sipia, M. R. Neffling, J. S. Metcalf, S. M. K. Nybom, J. A. O. Meriluoto and G. A. Codd, *Harmful Algae*, 2008, **7**, 99–105.
42 M. Karjalainen, B. Kozlowsky-Suzuki, M. Lehtiniemi, J. Engstrom-Ost, H. Kankaanpaa and M. Viitasalo, *Marine Biology*, 2006, **148**, 683–691.
43 M. Karjalainen, J. P. Paakkonen, H. Peltonen, V. Sipia, T. Valtonen and M. Viitasalo, *Marine Biology*, 2008, **155**, 483–491.
44 V. O. Sipia, O. Sjovall, T. Valtonen, D. L. Barnaby, G. A. Codd, J. S. Metcalf, M. Kilpi, O. Mustonen and J. A. O. Meriluoto, *Environmental Toxicology and Chemistry*, 2006, **25**, 2834–2839.
45 S. Suikkanen, J. Engstrom-Ost, J. Jokela, K. Sivonen and M. Viitasalo, *Journal of Plankton Research*, 2006, **28**, 543–550.
46 M. K. Mogelhoj, P. J. Hansen, P. Henriksen and N. Lundholm, *Aquatic Microbial Ecology*, 2006, **43**, 43–54.
47 D. M. Anderson, *Limnology and Oceanography*, 1997, **42**, 1009–1022.
48 U. Tillmann, T. Alpermann, U. John and A. Cembella, *Harmful Algae*, 2008, **7**, 52–64.
49 J. E. Adolf, T. R. Bachvaroff, D. N. Krupatkina, H. Nonogaki, P. J. P. Brown, A. J. Lewitus, H. R. Harvey and A. R. Place, *African Journal of Marine Science*, 2006, **28**, 415–419.
50 T. R. Bachvaroff, J. E. Adolf, A. H. Squier, H. R. Harvey and A. R. Place, *Harmful Algae*, 2008, **7**, 473–484.
51 J. R. Deeds and A. R. Place, *African Journal of Marine Science*, 2006, **28**, 421–425.
52 P. Uronen, P. Kuuppo, C. Legrand and T. Tamminen, *Microbial Ecology*, 2007, **54**, 183–193.
53 E. Lindehoff, E. Graneli and W. Graneli, *Harmful Algae*, 2008, in press.
54 C. Legrand, K. Rengefors, G. O. Fistarol and E. Graneli, *Phycologia*, 2003, **42**, 406–419.
55 L. J. Flewelling, J. P. Naar, J. P. Abbott, D. G. Baden, N. B. Barros, G. D. Bossart, M. Y. D. Bottein, D. G. Hammond, E. M. Haubold, C. A. Heil, M. S. Henry, H. M. Jacocks, T. A. Leighfield, R. H. Pierce, T. D. Pitchford, S. A. Rommel, P. S. Scott, K. A. Steidinger, E. W. Truby, F. M. Van Dolah and J. H. Landsberg, *Nature*, 2005, **435**, 755–756.
56 S. E. Fire, L. J. Flewelling, Z. H. Wang, J. Naar, M. S. Henry, R. H. Pierce and R. S. Wells, *Marine Mammal Science*, 2008, **24**, 831–844.
57 J. Kubanek, M. K. Hicks, J. Naar and T. A. Villareal, *Limnology and Oceanography*, 2005, **50**, 883–895.
58 E. K. Prince, T. L. Myers, J. Naar and J. Kubanek, *Proceedings of the Royal Society B – Biological Sciences*, 2008, **275**, 2733–2741.
59 Y. Yamasaki, S. Nagasoe, T. Matsubara, T. Shikata, Y. Shimasaki, Y. Oshima and T. Honjo, *Marine Ecology – Progress Series*, 2007, **339**, 83–92.
60 F. Ribalet, J. A. Berges, A. Ianora and R. Casotti, *Aquatic Toxicology*, 2007, **85**, 219–227.

61 T. Wichard, S. A. Poulet, C. Halsband-Lenk, A. Albaina, R. Harris, D. Y. Liu and G. Pohnert, *Journal of Chemical Ecology*, 2005, **31**, 949–958.
62 F. Ribalet, T. Wichard, G. Pohnert, A. Ianora, A. Miralto and R. Casotti, *Phytochemistry*, 2007, **68**, 2059–2067.
63 S. Sopanen, M. Koski, P. Kuuppo, P. Uronen, C. Legrand and T. Tamminen, *Marine Ecology – Progress Series*, 2006, **327**, 223–232.
64 S. Sopanen, M. Koski, P. Uronen, P. Kuuppo, S. Lehtinen, C. Legrand and T. Tamminen, *Marine Ecology – Progress Series*, 2008, **361**, 191–202.
65 J. Kubanek, T. W. Snell and C. Pirkle, *Limnology and Oceanography*, 2007, **52**, 1026–1035.
66 E. K. Prince, L. Lettieri, K. J. McCurdy and J. Kubanek, *Oecologia*, 2006, **147**, 479–488.
67 C. L. Speekmann, C. J. Hyatt and E. J. Buskey, *Harmful Algae*, 2006, **5**, 693–704.
68 S. Bargu, K. Lefebvre and M. W. Silver, *Marine Ecology – Progress Series*, 2006, **312**, 169–175.
69 M. B. Olson, E. J. Lessard, C. H. J. Wong and M. J. Bernhardt, *Marine Ecology – Progress Series*, 2006, **326**, 207–220.
70 M. B. Olson, E. J. Lessard, W. P. Cochlan and V. L. Trainer, *Limnology and Oceanography*, 2008, **53**, 1352–1368.
71 B. Kozlowsky-Suzuki, P. Carlsson, A. Ruhl and E. Graneli, *Harmful Algae*, 2006, **5**, 57–68.
72 O. Lindahl, B. Lundve and M. Johansen, *Harmful Algae*, 2007, **6**, 218–231.
73 J. E. Adolf, D. Krupatkina, T. Bachvaroff and A. R. Place, *Harmful Algae*, 2007, **6**, 400–412.
74 R. J. Waggett, P. A. Tester and A. R. Place, *Marine Ecology – Progress Series*, 2008, **366**, 31–42.
75 A. J. Lewitus, M. S. Wetz, B. M. Willis, J. M. Burkholder, M. W. Parrow and H. B. Glasgow, *Harmful Algae*, 2006, **5**, 427–434.
76 P. D. R. Moeller, K. R. Beauchesne, K. M. Huncik, W. C. Davis, S. J. Christopher, P. Riggs-Gelasco and A. K. Gelasco, *Environmental Science & Technology*, 2007, **41**, 1166–1172.
77 S. Strom, G. Wolfe, A. Slajer, S. Lambert and J. Clough, *Limnology and Oceanography*, 2003, **48**, 230–237.
78 K. A. Fredrickson and S. L. Strom, *Journal of Plankon Research*, 2008, in press.
79 S. L. Strom, G. V. Wolfe and K. J. Bright, *Aquatic Microbial Ecology*, 2007, **47**, 107–121.
80 A. Fontana, G. d'Ippolito, A. Cutignano, G. Romano, N. Lamari, A. M. Gallucci, G. Cimino, A. Miralto and A. Ianora, *ChemBioChem*, 2007, **8**, 1810–1818.
81 A. Ianora, R. Casotti, M. Bastianini, C. Brunet, G. d'Ippolito, F. Acri, A. Fontana, A. Cutignano, J. T. Turner and A. Miralto, *Marine Ecology-an Evolutionary Perspective*, 2008, **29**, 399–410.
82 T. Wichard, A. Gerecht, M. Boersma, S. A. Poulet, K. Wiltshire and G. Pohnert, *ChemBioChem*, 2007, **8**, 1146–1153.
83 S. A. Poulet, T. Wichard, J. B. Ledoux, B. Lebreton, J. Marchetti, C. Dancie, D. Bonnet, A. Cueff, P. Morin and G. Pohnert, *Marine Ecology – Progress Series*, 2006, **308**, 129–142.
84 T. Wichard, S. A. Poulet, A. L. Boulesteix, J. B. Ledoux, B. Lebreton, J. Marchetti and G. Pohnert, *Progress in Oceanography*, 2008, **77**, 30–44.
85 J. Li, S. Sun, C. L. Li, Z. Zhang and X. M. Pu, *Journal of Experimental Marine Biology and Ecology*, 2008, **355**, 95–102.
86 J. Dutz, M. Koski and S. H. Jonasdottir, *Limnology and Oceanography*, 2008, **53**, 225–235.
87 R. L. Taylor, G. S. Caldwell, H. J. Dunstan and M. G. Bentley, *Journal of Experimental Marine Biology and Ecology*, 2007, **341**, 60–69.
88 E. Selander, P. Thor, G. Toth and H. Pavia, *Proceedings of the Royal Society B – Biological Sciences*, 2006, **273**, 1673–1680.
89 J. Bergkvist, E. Selander and H. Pavia, *Oecologia*, 2008, **156**, 147–154.
90 E. Selander, G. Cervin and H. Pavia, *Limnology and Oceanography*, 2008, **53**, 523–530.
91 J. P. Bryant, F. S. Chapin and D. R. Klein, *Oikos*, 1983, **40**, 357–368.
92 D. F. Rhoades, in *Herbivores: their interaction with secondary plant metabolites*, eds. G. A. Rosenthal and D. H. Janzen, Academic Press, New York, 1979, pp. 3–54.
93 A. Barreiro, C. Guisande, M. Frangopulos, A. Gonzalez-Fernandez, S. Munoz, D. Perez, S. Magadan, I. Maneiro, I. Riveiro and P. Iglesias, *Marine Ecology – Progress Series*, 2006, **316**, 115–125.
94 A. Barreiro, C. Guisande, I. Maneiro, A. R. Vergara, I. Riveiro and P. Iglesias, *Acta Oecologica – International Journal of Ecology*, 2007, **32**, 279–290.
95 M. Estrada, M. M. Sala, K. van Lenning, M. Alcaraz, J. Felipe and M. J. W. Veldhuis, *Journal of Experimental Marine Biology and Ecology*, 2008, **355**, 1–11.
96 G. J. Teegarden, R. G. Campbell, D. T. Anson, A. Ouellett, B. A. Westman and E. G. Durbin, *Harmful Algae*, 2008, **7**, 33–44.
97 V. M. Bricelj, L. Connell, K. Konoki, S. P. MacQuarrie, T. Scheuer, W. A. Catterall and V. L. Trainer, *Nature*, 2005, **434**, 763–767.
98 L. B. Connell, S. P. MacQuarrie, B. M. Twarog, M. Iszard and V. M. Bricelj, *Marine Biology*, 2007, **150**, 1227–1236.
99 S. P. MacQuarrie and V. M. Bricelj, *Marine Ecology – Progress Series*, 2008, **366**, 59–74.
100 S. E. Ford, V. M. Bricelj, C. Lambert and C. Paillard, *Marine Biology*, 2008, **154**, 241–253.
101 J. D. Long, G. W. Smalley, T. Barsby, J. T. Anderson and M. E. Hay, *Proceedings of the National Academy of Sciences of the United States of America*, 2007, **104**, 10512–10517.
102 K. W. Tang, W. O. Smith, D. T. Elliott and A. R. Shields, *J. Phycol.*, 2008, **44**, 1372–1378.
103 D. Vaughn, *Ecology*, 2007, **88**, 1030–1039.
104 A. Metaxas and V. Burdett-Coutts, *Journal of Experimental Marine Biology and Ecology*, 2006, **334**, 187–195.
105 B. R. Mohapatra and K. Fukami, *Basic and Applied Ecology*, 2007, **8**, 475–481.
106 E. C. Wootton, M. V. Zubkov, D. H. Jones, R. H. Jones, C. M. Martel, C. A. Thornton and E. C. Roberts, *Environmental Microbiology*, 2007, **9**, 216–222.
107 S. Menden-Deuer and D. Grunbaum, *Limnology and Oceanography*, 2006, **51**, 109–116.
108 C. B. Woodson, D. R. Webster, M. J. Weissburg and J. Yen, *Marine Ecology – Progress Series*, 2007, **330**, 163–177.
109 H. Helmholz, S. Naatz, S. Lassen and A. Prange, *Journal of Chromatography, B*, 2008, **871**, 60–66.
110 H. Helmholz, C. Ruhnau, C. Schutt and A. Prange, *Toxicon*, 2007, **50**, 53–64.
111 J. A. Hagstrom, E. Graneli, I. Maneiro, A. Barreiro, A. Petermann and C. Svensen, *Harmful Algae*, 2007, **6**, 175–188.
112 T. L. Myers, E. K. Prince, J. Naar and J. Kubanek, *Harmful Algae*, 2008, **7**, 762–771.
113 P. B. Roth, M. J. Twiner, Z. Wanga, M. Y. B. Dechraoui and G. J. Doucette, *Toxicon*, 2007, **50**, 1175–1191.
114 R. H. Pierce and M. S. Henry, *Ecotoxicology*, 2008, **17**, 623–631.
115 M. Satake, A. J. Bourdelais, R. M. Van Wagoner, D. G. Baden and J. L. C. Wright, *Organic Letters*, 2008, **10**, 3465–3468.
116 A. G. Haubois, V. M. Bricelj and J. Naar, *Marine Biology*, 2007, **151**, 2003–2012.
117 J. P. Naar, L. J. Flewelling, A. Lenzi, J. P. Abbott, A. Granholm, H. M. Jacocks, D. Gannon, M. Henry, R. Pierce, D. G. Baden, J. Wolny and J. H. Landsberg, *Toxicon*, 2007, **50**, 707–723.
118 C. J. Keppler, A. J. Lewitus, A. H. Ringwood, J. Hoguet and T. Staton, *Marine Ecology – Progress Series*, 2006, **312**, 141–147.
119 A. J. Bourdelais, C. R. Tomas, J. Naar, J. Kubanek and D. G. Baden, *Environmental Health Perspectives*, 2002, **110**, 465–470.
120 C. J. Keppler, J. Hoguet, K. Smith, A. H. Ringwood and A. J. Lewitus, *Harmful Algae*, 2005, **4**, 275–285.
121 A. H. Ringwood, J. Hoguet, C. Keppler and M. Gielazyn, *Marine Environmental Research*, 2004, **58**, 151–155.
122 V. M. Bricelj and S. P. MacQuarrie, *Marine Ecology – Progress Series*, 2007, **331**, 147–159.
123 J. Engstrom-Ost, M. Karjalainen and M. Viitasalo, *Environmental Biology of Fishes*, 2006, **76**, 109–117.
124 J. P. Paeaekkoenen, S. Ronkkonen, M. Karjalaineno and M. Viitasalo, *Journal of Fish Biology*, 2008, **72**, 485–499.
125 K. A. Lefebvre, S. C. Tilton, T. K. Bammler, R. P. Beyer, S. Srinouanprachan, P. L. Stapleton, F. M. Farin and E. P. Gallagher, *Toxicological Sciences*, 2008, in press.

126 K. A. Lefebvre, D. P. Noren, I. R. Schultz, S. M. Bogard, J. Wilson and B. T. L. Eberhart, *Aquatic Toxicology*, 2007, **81**, 266–274.
127 S. Bargu, C. L. Powell, Z. H. Wang, G. J. Doucette and M. W. Silver, *Harmful Algae*, 2008, **7**, 45–51.
128 R. Jester, K. Lefebvre, G. Langlois, V. Vigilant, K. Baugh and M. W. Silver, *Harmful Algae*, 2008, in press.
129 S. Reizopoulou, E. Strogyloudi, A. Giannakourou, K. Pagou, L. Hatzianestis, C. Pyrgaki and E. Graneli, *Harmful Algae*, 2008, **7**, 228–234.
130 T. H. Jlang, D. Z. Wang, T. Niu and Y. X. Xu, *Toxicon*, 2007, **50**, 639–645.
131 T. Kamiyama and T. Suzuki, *Marine Ecology – Progress Series*, 2006, **317**, 57–65.