Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions

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Several observations and model calculations suggest that chemically mediated interactions can structure planktonic food webs. However, only recently have improvements in chemical methods, coupled with ecological assays, led to the characterization of chemical cues that affect the behaviour and/or physiology of planktonic organisms. We are currently beginning to elucidate if or how chemical signals can directly affect the interactions between species and even shape complex community structures in aquatic systems. Here, we highlight recent research on the nature and action of chemical signals in the pelagic marine and freshwater environments, with an emphasis on kairomones and defence metabolites.

Introduction

The open water appears to be a homogenous environment, yet gradual changes in nutrients, light, temperature, mechanical cues (e.g. currents and turbulence) and chemical signals provide directional information to its inhabitants. The physical properties of water render it an ideal medium for the evolution of chemical signal interactions. Its relatively high viscosity results in signal gradients that persist longer than chemical gradients in a turbulent atmosphere. Thus, an interaction between two individuals could be mediated by infochemicals that convey information by evoking a behavioural or physiological response in the receiver [1]. Known signals include dissolved gases (Box 1), functionalized hydrocarbons (Figure 1), peptides [2] and proteins [3].

Here, we explore two topics that illustrate current methodological and conceptual challenges in plankton chemical ecology. First, we highlight difficulties in characterizing signals, using kairomone-mediated interactions as examples. Second, we discuss phytoplankton defence strategies to illustrate the challenges encountered when investigating community effects mediated by a complex interplay of toxins, nutrients and infochemicals. Related

fields involving, for example, intraspecific chemical communication or allelopathic interactions, have been reviewed elsewhere [2,4]. We do not distinguish between freshwater and marine environments, despite the fact that research in these disciplines is traditionally separated. The limitations, challenges and concepts in these two domains are comparable and possible synergisms in uncovering aspects of chemical defence and signalling should not be affected by historical barriers.

Recent progress has led to the identification of a comparably small set of chemical signals and defence metabolites that are studied intensively to elucidate species interactions. However, we are still far from a comprehensive understanding of chemical signalling in aquatic communities, and linking this signalling with selection processes that have resulted in the rich diversity of phenotypes that we observe today is the ultimate goal of current research.

Kairomones

Kairomones are chemical signals in the information transfer between species that impart a benefit to the receiving organism while not being beneficial for the producer. For example, kairomones can be used to locate food or to detect predators. In many cases, there are disadvantages for the emitter. Consequently, kairomone release might be reduced but it cannot be fully suppressed either because the emission of signals creates a larger total benefit in another information context or because emission is unavoidable, as is the case for excretory products. This coevolutionary scenario suggests that emitters might be selected to either minimize their signal release or modify signal chemical structure and, conversely, receivers might be selected to increase their sensitivity to existing signals or evolve recognition of new cues.

DMS

Little information is currently available about the relevant signals in the kairomone-assisted location of food. Whereas the role of dissolved organic molecules in the induction of chemotaxis in planktonic bacteria [5] and

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Box 1. The role of dimethyl sulfide in plankton ecology

Possibly the best-studied marine volatile is the biogenic trace gas DMS [(CH$_3$)$_2$S], which is produced during an enzymatic reaction from the algal metabolite dimethylsulphoniopropionate (DMSP) [(CH$_3$)$_2$S$^+$(CH$_2$)$_2$COO$^-$]. Although it has multifunctional properties in algae [68], the ecological reasons for its cleavage and the associated DMS-production are still unknown [69]. DMS has attracted much research interest not only because of its effect on cloud formation and climate processes [70], but also because its release has been linked to an activated defence of algae against herbivores [38] and viruses [71]; it also provides prey-related chemical cues to grazers [7]. This single metabolite thereby directly affects trophic interactions in multiple ways (Figure I) [72].
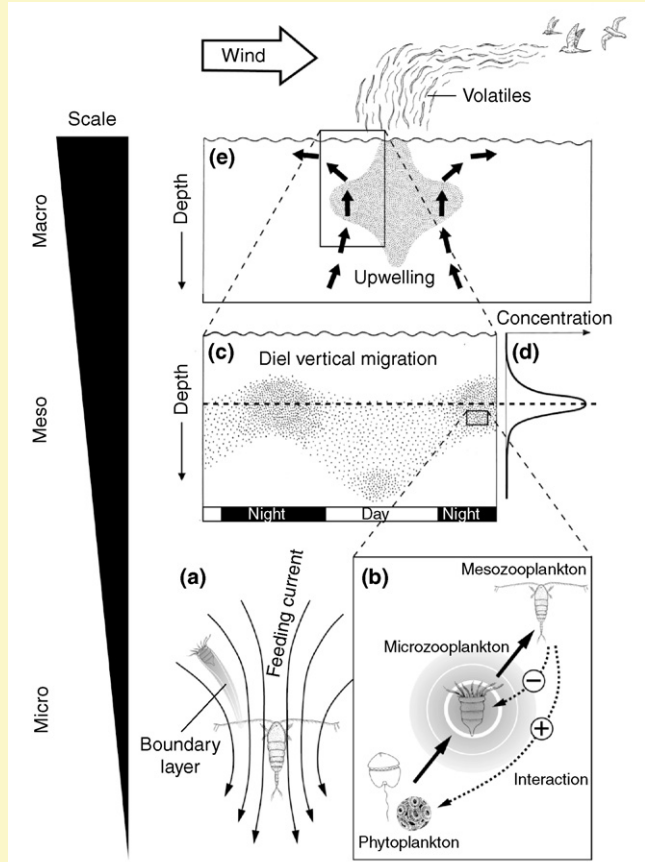
Figure I. The function of DMS and other volatile infochemicals in aquatic trophic interactions from the microscopic to global scale. (a) A suspension-feeding copepod (mesozooplankton) produces a feeding current that deforms the boundary layer at the surface of microzooplankton. This layer is enriched with infochemicals such as DMS and enables the copepod to detect the prey and to react with a feeding response. (b) Infochemicals in microbial tritrophic interactions. Representatives of the three trophic levels include dinoflagellate and coccolithophorid phytoplankton, tintinnid microzooplankton, and a mesozooplankton copepod, respectively. The interaction between phyto- and microzooplankton produces DMS and is represented by the circular shaded area around the microzooplankton. Such infochemicals are exploited by higher-order mesozooplankton when searching for microzooplankton prey. Dotted lines indicate the mode of the trophic interaction with negative effects on the microzooplankton and indirect positive effects for the phytoplankton. (c,d) The accumulation of infochemicals from microzooplankton prey (d) provides chemical cues to migrating mesozooplankton [individual dots in (c)]. (e) Local upwelling (e.g. along a frontal zone) generates biological activity (dotted area) that increases the sea–air flux of DMS and other volatile infochemicals. The resulting chemical gradient serves as a directional cue to seabirds. Reprinted, with permission, from Ref. [72].

the chemoattraction of protozoan grazers [6] has been demonstrated, the chemical nature of the involved signals is mainly unknown (Box 2, Box 3). Recently, however, a signal that triggers search behaviour in common pelagic
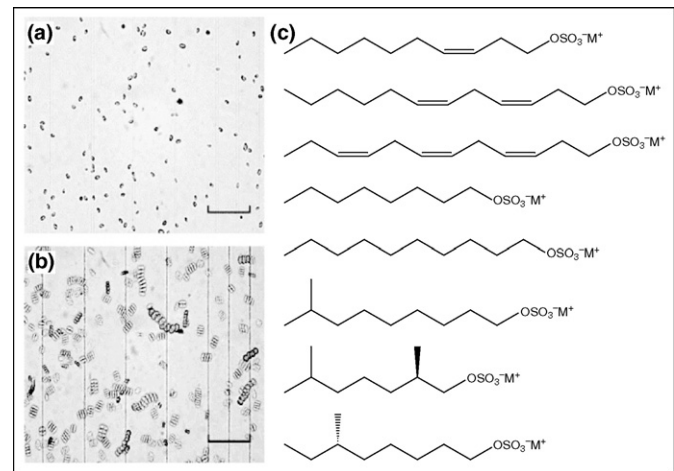
Figure 1. Structure elucidation of kairomones inducing morphological responses in green algae. The unicellular green alga *Scenedesmus subspicatus* forms colonies if grown in the presence of the waterflea *Daphnia* or in the presence of water in which this herbivore was kept. The same response is observed when purified metabolites from *Daphnia* or synthetic compounds are administered. Microscopic pictures of *Scenedesmus* controls (a) and then ten days after the addition of 1 ng ml^{-1} 8-methylnonyl sulphate (b) (scale bar = 50 μm). (c) Chemical structures of the active kairomones from *Daphnia*. Reprinted with permission from Ref. [21].

herbivores was characterized. Plumes of dimethylsulfide (DMS), which are released upon cell lysis by certain phytoplankton species (Box 1), trigger a tail-flapping response in the copepod *Temora longicornis*. This behavioural change results in altered flow patterns and probably assists copepods to find food [7]. The identification of the involved signal is important for general considerations about food finding by copepods. It is likely that pathogens or grazers are involved in the cell disintegration that triggers DMS release. This process could promote selective grazing on infected cells, attract herbivores to patches with high food concentration or assist predation on actively feeding herbivores. Such a mechanism would bias the food uptake in hitherto unpredicted ways.

Inducible responses

Many studies have shown prey responses to predator kairomones that trigger inducible defences in behavioural, morphological and physiological traits. The phenotypic plasticity of morphological traits has been particularly conspicuous, such as spine induction in rotifers [8,9], ciliates [10] and cladocerans [11,12] including *Daphnia* [13,14]. Adaptive changes of life-history parameters, such as shifts in resource allocation between somatic growth and reproduction [15], or the induction of behavioural traits, such as vertical migration [16,17] are less obvious.

Daphnia and rotifers themselves release chemical cues that induce their food source, unicellular green algae, to form grazing-resistant colonies (Figure 1) [18]. When predators are absent, the algae grow as isolated cells, but in the presence of chemical signals from predators, the algae undergo a morphological change and form colonies, which are more protected from grazing, owing to a size-mismatch between the predator and its prey (Figure 1) [18]. This initial observation prompted intense research towards the elucidation of the structure of the kairomone in *Daphnia* [19]. Recently, Yasumoto *et al.* [20,21] reported a major

breakthrough with a bioassay-guided characterization of kairomones from a 10-kg sample of *Daphnia*. After several chromatographic steps, eight aliphatic sulfates that are active at low concentrations ($<1 \, \text{ng ml}^{-1}$) were isolated and fully characterized (Figure 1) [20,21]. Interestingly, the green alga *Scenedesmus* reacts to all extracted aliphatic sulfates, although saturated compounds are the most effective. The amphiphilic nature of the kairomones appears to be more important for activity than are specific processes based on ligand–receptor interactions. The finding that the active substances are structurally

similar to commercially used anion surfactants has major implications for ecotoxicological testing, given that even sublethal concentrations of commercial detergents that are commonly detected in freshwater [22] might trigger significant ecological effects [23].

Pelagic chemical communication has been observed mainly in microplankton, but recent results indicate that signals might also have an important role in nanoplankton. The pelagic bacterium *Flectobacillus* sp. shows high variation in cell size and reacts to chemical cues of its predators. In the presence of the bacterivorous flagellate *Ochromonas* sp., the bacteria produces long filamentous cells, which are harder to ingest [24]. This response was induced by infochemicals as the predators were kept in dialysis bags that prevented direct contact but enabled chemical cues to disperse.

Associations with non-predator sources

A higher level of complexity is demonstrated by the fact that the production of several kairomones is not only related to the predators themselves, but also to other biotic sources associated with the predators or with the predation process. For example, bacteria might be involved in the production of factors released from freshwater and marine fish that induce vertical migration in *Daphnia* or brine shrimp, respectively [25,26]. Because multiple players that might be linked by symbiotic interactions are required to maintain an active signalling network, it is possible that relatively small modifications in bacterial communities can result in major changes of trophic cascades.

Release by conspecifics

Infochemicals can also be released by conspecifics. Such alarm cues from prey are now known to be involved in responses that were initially attributed to fish-borne kairomones [27]. For example, *Daphnia* exhibit a stronger reaction to kairomones from fish that had been fed conspecifics compared with cues from fish that were fed earthworms [28]. Furthermore, kairomones released by feeding predators more strongly induced morphological defences against invertebrate predators in *Daphnia* than did signals of the crushed conspecifics alone [29]. It is likely that these alarm cues are not specifically evolved pheromones, but instead are substances contained in the body of *Daphnia* that are released or activated when they are caught. Because such alarm cues from *Daphnia* alone would only provide general information about predation risk rather than about a specific predator, they would not be reliable enough to reduce tradeoffs that are associated with defences [30]. *Daphnia* fall prey to visually hunting vertebrates, such as fish, which select larger individuals, and to gape-limited invertebrate predators including *Chaoborus* larvae, which select smaller ones. Both types of predator induce life-history adaptations in opposite directions. Some *Daphnia* might shift their resource allocation in response to fish cues into reproduction and in response to invertebrate cues into somatic growth [15]. For these adequate defences in multi-predator environments, *Daphnia* require additional cues, besides alarm signals, that are specific to the predator [31].

The many organisms that respond to kairomones [32] support the idea of infochemically driven plankton

communities, but we currently have only limited knowledge of the chemical cues involved. Other than the exceptions described above, attempts to identify kairomones of pelagic organisms have encountered major difficulties (Box 2) and, thus, only resulted in partial characterizations (e.g. Ref. [33]). However, knowledge of the structure of the cues would enable researchers to quantify and evaluate responses, to utilize biomanipulations and to assess the effect of potential cross talk in the complex pelagic environment. Moreover, knowledge of the nature of kairomones is a prerequisite for the elucidation of the physiological and/or ecological relevance of their emission. Only then will it become possible to address fully why evolution has resulted in the sometimes disadvantageous kairomone release.

Chemical defence of unicellular algae

In contrast to the direct observation of morphological or behavioural responses, the demonstration of an ecologically relevant chemical defence of phytoplankton is currently a major challenge. Thus, it has not yet been fully resolved whether our limited knowledge about plankton chemical defence simply reflects the fact that it has only a minor role in this environment or whether systematic problems in the elucidation of the involved chemicals and their ecological roles limit scientific progress. Some general considerations might lead to support for the first explanation. Energy flow and biomass partitioning differ between sessile and pelagic organisms [34]. In contrast to terrestrial plants and benthic algae, planktonic primary producers have no requirement for massive supportive tissue and rapidly complete their life cycle. Accordingly, >99% of the global biomass is found in the terrestrial environment; however, turnover in plankton is so high, that still almost half of primary production occurs in the sea [34]. It can be argued that planktonic primary producers support more consumption and are regulated by top-down forces to a greater degree than are sessile plants. Accordingly, variable selective forces could drive differences in defence allocation patterns in pelagic versus terrestrial or benthic habitats, and chemical or mechanical defence would not be widespread in the pelagic environment [34]. However, this general consideration is contradicted by numerous observations and models that demonstrate a role of mechanical defence, toxins or other chemical defence metabolites in the modulation of pelagic trophic interactions (e.g. Refs [35–37]). Here, we group the different defence strategies of phytoplankton against herbivores into three functional categories.

Release of toxins and feeding repellents: costly but effective?

The prerequisite for defence by released toxins or repellents is that predators demonstrate selective feeding [38–40]. In such cases, grazers must utilize chemical cues before ingestion, which suggests a role for surface-bound chemicals and chemical exudates that accumulate in the diffusion-limited boundary layer surrounding the phytoplankton cells [41]. These chemicals create the 'active space', a zone that is influenced by diffusion and turbulence, resulting in a concentration gradient that provides directional information

about their source [42]. However, if grazers cannot use specific chemical cues in selective feeding, or when alternative prey is unavailable, phytoplankton that invested in defence mechanisms might still be ingested.

The requirement of both an active space around the phytoplankton cells and a behavioural response of the herbivore in the complex environment make the direct action of chemicals difficult to demonstrate in laboratory experiments. In this context, strains of phytoplankton species that produce different quantities or types of defence metabolite are a useful tool, although the risk of misinterpretation owing to feeding on an unbalanced monospecies diet cannot be ignored [43,44]. Methods in which toxins can be delivered using otherwise neutral food pellets would enable a direct comparative approach in laboratory studies and would provide direct insight into their defensive value [45]. Despite significant efforts, no universal solution to this problem has been found, although successful single examples have been reported using microencapsulation techniques, liposomes or phytoplankton that have been impregnated with potentially noxious chemicals [46–48]. Other approaches use bioassays where mixtures of phytoplankton species with different toxic potential are administered and the (selective) clearance rate and/or the performance of the herbivore is monitored (Box 4).

Box 4. Can the effects of phytoplankton toxins be separated from overlaying influences of food quality?

More than 200 phytoplankton species are known to be toxic or harmful to a range of organisms, inducing mass mortality of fish and harming mammals [79]. However, the relevance of these toxins for defence against the appropriate predators of the zooplankton is often unclear. It is now accepted that there is no general relationship between the production of phytoplankton toxins and implicated consequences for planktonic grazers; case studies are needed that incorporate selected algae and herbivores.

Karenia brevis is a pelagic bloom-forming dinoflagellate that can produce neurotoxic brevetoxins that cause mass mortalities of clams, crabs and fish; however, as yet, its mode of action on sympatric zooplankton grazers is poorly understood (see Ref. [80] for recent references). Prince *et al.* [80] aimed to clarify the interaction between *K. brevis* and a major co-occurring predator using a mixed diet approach. When offered diets with varying proportions of *K. brevis* and a non-toxic cryptomonad, survivorship of the herbivorous copepod *Acartia tonsa* decreased with increasing *K. brevis* content. However, extracellular extracts of *K. brevis* did not affect the copepods, which rules out a direct toxic function of the brevetoxins. If offered the mixed diets, copepods slightly preferred the non-toxic food source. However, the benefit for the toxin-producing *K. brevis* could be negated by a nearly fivefold increase in food uptake when copepods were raised on *K. brevis*-rich diets. This result was attributed to poor food quality of *K. brevis*, which is compensated for by an increased food uptake.

The situation becomes more complex if the reproductive success of the copepods is also considered. Predator reproductive success depends on egg production rate, egg hatching and naupliar survival. Thus, toxins that affect these processes provide a strategy for an indirect chemical defence. In the experiments with *A. tonsa*, egg production rates decreased with increasing proportion of *K. brevis* in the food, but the egg-hatching success remained constantly high [80]. The decreased egg production could not be correlated to toxicity, making inadequate food quality of this alga the probable case for its adverse effects. This example demonstrates that, even with a simplified scenario of three species, it is a major task to separate unambiguously the impact of toxicity and food quality.

Unfortunately, in most experiments demonstrating the role of chemical defense, no distinction has been made between the delivery of toxins or repellents within a food particle or their release in the active space. In addition, overlaying effects of food quality, morphology and culture artefacts are rarely considered, even if these factors are crucial for the correct interpretation of feeding behaviour. The task becomes increasingly challenging if situations of similar complexity to those in the field are addressed. Here, multiple species coexist that each might influence the abundance, behaviour and chemical properties of others [49]. Recent progress in the genetic transformation of microalgae [50] and in the use of transgenic microalgae in ecological studies [51] might enable investigations of the effect of mutants with altered metabolic profiles on the performance of herbivores. Ideally, these should be implemented in comparatively large-scale enclosure (mesocosm) studies to obtain a direct insight into the ecological role of metabolites under near natural conditions.

Induced defence of microalgae: a flexible strategy?
An efficient way to minimize the cost of defence is the use of induced defence strategies where defensive metabolites are only produced during high predation risk. Such processes are common in higher plants [52] and have also been identified in seaweeds [53]. However, it was only recently demonstrated that comparable mechanisms also exist in the pelagic environment. Waterborne cues from feeding copepods can induce the production of paralytic shellfish toxin in a dinoflagellate that forms harmful algal blooms [49]. The elevated amount of toxins in the induced algal cells correlate with an increased resistance to copepod grazing, suggesting the direct role of induction in the chemical defence of the dinoflagellate. An induced increase in toxin production has also been observed for other phytoplankton species, such as *Microcystis aeruginosa* [54], and it will be interesting to study whether such processes are common strategies of phytoplankton to reduce the cost of defence.

Community defence of plankton: does it exist?
The effects of activated defence mechanisms that result in toxin production after cell disruption (Box 1) [38,55], or constitutive chemical defences with toxins stored intracellularly, are a matter of debate [56]. Defence based on such mechanisms implies that the toxin-producing algal cell must be consumed. Thus, it would not directly benefit from developing defences that require part of its metabolic energy and so selection should act against these strategies. Furthermore, effects of defence metabolites can be indirect or delayed, for example when they reduce the reproductive capacities of predators [48]. Nevertheless, these mechanisms could be effective at the population level. Group selection, which would be a prerequisite for the evolution of such defense strategies, is considered to evolve only under specific narrow conditions (e.g. involving kin selection) and is poorly explored, especially in unicellular organisms [57,58]. For a long time, it was assumed that phytoplankton species reproduce asexually during bloom formation and, thus, that a chemical defence could increase the survivorship of the clonal population without protecting the single cell. Accordingly, the evolution of a common defence mechanism of bloom-forming cells would be possible even if other clones of the same species or other co-existing species would also benefit [41]. Here, a major paradigm-shift is arising based on several recent genetic investigations including microsatellite studies that address the relatedness within algal blooms. It appears that this diversity can be surprisingly high [59–61]. For example, on average, 94% of cells in each sample obtained during blooms of the diatom *Ditylum brightwellii* were genetically distinct [62]. These findings question whether such a high degree of intraspecific genetic diversity translates into variability in ecological function. To answer if and how the observed activated and constitutive defence mechanisms benefit surviving clonal cells, model calculations, extensive genetic and chemical profiling, as well as enclosure experiments, are required.

For evolutionary considerations, the metabolic costs involved in chemical defences should also be considered. It is striking that the activated defences of phytoplankton that have been discovered so far depend on primary metabolic processes [41,63]. Thus, they do not require the specific production of cost-intensive secondary metabolites, as is the case for the microalgal toxins discussed previously. For example, the precursor for activated dimethylsulfide production is also involved in the osmoregulation of intact cells (Box 1); the rapid wound-activated release of polyunsaturated aldehydes, which results in a decrease of reproductive success of feeding herbivores, also depends on processes that are closely associated with primary metabolism [48,64]. This defence relies on storage lipids, which are transformed by lipase and lipoxygenase enzymes after wounding or ingestion. It is likely that these lipids, as well as the enzymes involved, are also required for the regulation of cellular processes in intact cells [63]. In these two cases, the cost of the defence might therefore be negligible and the evolution of such defences could thus be driven by the need for processes involved in primary metabolism rather than by the need for feeding pressure reduction, which might be a beneficial side effect.

Conclusions
New chemical methodologies in combination with advanced ecological experiments facilitate studies of the role of chemical signals and defence metabolites in aquatic environments. The search for active compounds in plankton chemical ecology is by no means only of interest to a small community of chemists. Once the structures of signals are known, it opens new avenues for directed ecological manipulations and for the monitoring of interspecific interactions in highly complex communities. These investigations will pave the way for the development of new theories and models on how plankton biodiversity evolved.

One major challenge will include the transfer of knowledge and concepts derived from laboratory experiments to natural communities. The ultimate goal is to determine and model the large-scale effects and global significance of chemical defence in plankton communities dominated by top-down control. Knowledge about the chemical structures of the signals will help, for example, to evaluate the distribution of certain signals in the

environment, their species specificity, the metabolic costs involved in their production, and the potential interference of other organisms by chemical mimicry or communication disruption. Moreover, synthetically produced infochemicals and analogues will enable us to perform large-scale manipulations, experiments that have proven useful for the evaluation of the impact of nutrient limitation on the bloom formation of phytoplankton [65].

Given that most of the substances of interest occur at low concentrations but are nevertheless remarkably effective, this research is also of importance for ecotoxicological considerations. In this area of study, toxicity assays are used to determine the impact of anthropogenic chemicals on the environment. Because such chemicals can interfere with plankton chemical communication at sublethal concentrations [66,67], there might be a large group of hitherto unrecognized compounds that could disrupt aquatic community structure and food-webs in both lakes and oceans.

Current evidence highlights the major roles that chemical communication and chemical defence have in the pelagic environment; however, substantial methodological and conceptual progress is required if we are to understand the implications for plankton ecosystem functioning. With newly available methods, such as high throughput metabolic profiling techniques in analytical chemistry and genetic techniques, the stage is set for making fundamental progress towards a better understanding of the chemical language of plankton.

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References

1 Dicke, M. and Sabelis, M. (1988) Infochemical terminology: based on cost–benefit analysis rather than origin of compounds? *Funct. Ecol.* 2, 131–139

2 Rittschof, D. and Cohen, J.H. (2004) Crustacean peptide and peptide like pheromones and kairomones. *Peptides* 25, 1503–1516

3 Hallmann, A. *et al.* (1998) The highly efficient sex-inducing pheromone system of *Volvox*. *Trends Microbiol.* 6, 185–189

4 Gross, E.M. (2003) Allelopathy of aquatic autotrophs. *Crit. Rev. Plant Sci.* 22, 313–339

5 Fenchel, T. (2002) Microbial behavior in a heterogeneous world. *Science* 296, 1068–1071

6 Tillmann, U. (2004) Interactions between planktonic microalgae and protozoan grazers. *J. Eukaryot. Microbiol.* 51, 156–168

7 Steinke, M. *et al.* (2006) Dimethyl sulfide triggers search behavior in copepods. *Limnol. Oceanogr.* 51, 1925–1930

8 Gilbert, J.J. (1966) Rotifer ecology and embryological induction. *Science* 151, 1234–1237

9 Gilbert, J.J. (1999) Kairomone-induced morphological defenses in rotifers. In *The Ecology and Evolution of Inducible Defenses* (Tollrian, R. and Harvell, C.D., eds), pp. 127–141, Princeton University Press

10 Kuhlmann, H.W. *et al.* (1999) Predator induced defenses in ciliated protozoa. In *The Ecology and Evolution of Inducible Defenses* (Tollrian, R. and Harvell, C.D., eds), pp. 142–159, Princeton University Press

11 Krueger, D.A. and Dodson, S.I. (1981) Embryological induction and predation ecology in *Daphnia pulex*. *Limnol. Oceanogr.* 26, 219–223

12 Grant, J.W.G. and Bayly, I.A.E. (1981) Predator induction of crests in morphs of the *Daphnia carinata* King complex. *Limnol. Oceanogr.* 26, 201–218

13 Tollrian, R. and Dodson, S.I. (1999) Inducible defenses in Cladocera. In *The Ecology and Evolution of Inducible Defenses* (Tollrian, R. and Harvell, C.D., eds), pp. 177–202, Princeton University Press

14 Lass, S. and Spaak, P. (2003) Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia* 491, 221–236

15 Stibor, H. (1992) Predator induced life-history shifts in a freshwater Cladoceran. *Oecologia* 92, 162–165

16 Loose, C.J. *et al.* (1993) Chemically induced diel vertical migration in *Daphnia* – a new bioassay for kairomones exuded by fish. *Arch. Hydrobiol.* 126, 329–337

17 Dodson, S.I. (1988) The ecological role of chemical stimuli for the zooplankton: predator avoidance behavior in *Daphnia*. *Limnol. Oceanogr.* 33, 1431–1439

18 Hessen, D.O. and Van Donk, E. (1993) Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. *Arch. Hydrobiol.* 127, 129–140

19 van Holthoon, F.L. *et al.* (2003) Colony formation in *Scenedesmus*: a literature overview and further steps towards the chemical characterisation of the *Daphnia* kairomone. *Hydrobiologia* 491, 241–254

20 Yasumoto, K. *et al.* (2006) Isolation and absolute configuration determination of aliphatic sulfates as the *Daphnia* kairomones inducing morphological defense of a phytoplankton. *Chem. Pharm. Bull.* 54, 271–274

21 Yasumoto, K. *et al.* (2005) Aliphatic sulfates released from *Daphnia* induce morphological defense of phytoplankton: isolation and synthesis of kairomones. *Tetrahedron Lett.* 46, 4765–4767

22 Lürling, M. (2006) Effects of a surfactant (FFD-6) on *Scenedesmus* morphology and growth under different nutrient conditions. *Chemosphere* 62, 1351–1358

23 Lürling, M. and Beekman, W. (2002) Extractable substances (anionic surfactants) from membrane filters induce morphological changes in the green alga *Scenedesmus obliquus* (Chlorophyceae). *Environ. Toxicol. Chem.* 21, 1213–1218

24 Corno, G. and Jürgens, K. (2006) Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. *Appl. Environ. Microbiol.* 72, 78–86

25 Ringelberg, J. and Van Gool, E. (1998) Do bacteria, not fish, produce 'fish kairomone'? *J. Plankton Res.* 20, 1847–1852

26 Forward, R.B. and Rittschof, D. (1999) Brine shrimp larval photoresponses involved in diel vertical migration: activation by fish mucus and modified amino sugars. *Limnol. Oceanogr.* 44, 1904–1916

27 Pijanowska, J. (1997) Alarm signals in *Daphnia*? *Oecologia* 112, 12–16

28 Stabell, O.B. *et al.* (2003) Inducible defences in *Daphnia* depend on latent alarm signals from conspecific prey activated in predators. *Chem. Senses* 28, 141–153

29 Laforsch, C. *et al.* (2006) Inducible defenses: the relevance of chemical alarm cues in *Daphnia*. *Limnol. Oceanogr.* 51, 1466–1472

30 Gabriel, W. *et al.* (2005) Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. *Am. Nat.* 166, 339–353

31 Laforsch, C. and Tollrian, R. (2004) Inducible defenses in multipredator environments: cyclomorphosis in *Daphnia cucullata*. *Ecology* 85, 2302–2311

32 Miner, B.G. *et al.* (2005) Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* 20, 685–692

33 von Elert, E. and Pohnert, G. (2000) Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. *Oikos* 88, 119–128

34 Shurin, J.B. *et al.* (2006) All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proc. R. Soc. B* 273, 1–9

35 Mitra, A. and Flynn, K.J. (2005) Predator-prey interactions: is 'ecological stoichiometry' sufficient when good food goes bad? *J. Plankton Res.* 27, 393–399

36 Irigoien, X. *et al.* (2004) Global biodiversity patterns of marine phytoplankton and zooplankton. *Nature* 429, 863–867

37 Hamm, C.E. *et al.* (2003) Architecture and material properties of diatom shells provide effective mechanical protection. *Nature* 421, 841–843

38 Wolfe, G.V. *et al.* (1997) Grazing-activated chemical defence in a unicellular marine alga. *Nature* 387, 894–897

39 Strom, S. *et al.* (2003) Chemical defense in the microplankton I: Feeding and growth rates of heterotrophic protists on the DMS-producing phytoplankter *Emiliania huxleyi*. *Limnol. Oceanogr.* 48, 217–229

40 Teegarden, G.J. *et al.* (2001) Zooplankton feeding behavior and particle selection in natural plankton assemblages containing toxic *Alexandrium spp*. *Mar. Ecol. Prog. Ser.* 218, 213–226

41 Wolfe, G.V. (2000) The chemical defense ecology of marine unicellular plankton: Constraints, mechanisms, and impacts. *Biol. Bull.* 198, 225–244

42 Wilson, E.O. (1970) *Chemical Ecology,* Academic Press

43 Pohnert, G. *et al.* (2002) Are volatile unsaturated aldehydes from diatoms the main line of chemical defence against copepods? *Mar. Ecol. Prog. Ser.* 245, 33–45

44 Jones, R.H. and Flynn, K.J. (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science* 307, 1457–1459

45 Hay, M.E. (1996) Marine chemical ecology: What's known and what's next? *J. Exp. Mar. Biol. Ecol.* 200, 103–134

46 von Elert, E. (2002) Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. *Limnol. Oceanogr.* 47, 1764–1773

47 Kreeger, D.A. *et al.* (1996) Use of dual-labeled microcapsules to discern the physiological fates of assimilated carbohydrate, protein carbon, and protein nitrogen in suspension-feeding organisms. *Limnol. Oceanogr.* 41, 208–215

48 Ianora, A. *et al.* (2004) Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. *Nature* 429, 403–407

49 Selander, E. *et al.* (2006) Copepods induce paralytic shellfish toxin production in marine dinoflagellates. *Proc. R. Soc. B* 273, 1673–1680

50 Poulsen, N. and Kröger, N. (2005) A new molecular tool for transgenic diatoms – control of mRNA and protein biosynthesis by an inducible promoter-terminator cassette. *FEBS J.* 272, 3413–3423

51 Vardi, A. *et al.* (2006) A stress surveillance system based on calcium and nitric oxide in marine diatoms. *PLoS Biol.* 4, 411–419

52 Kessler, A. and Baldwin, I.T. (2002) Plant responses to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* 53, 299–328

53 Cronin, G. and Hay, M.E. (1996) Induction of seaweed chemical defenses by amphipod grazing. *Ecology* 77, 2287–2301

54 Jang, M.H. *et al.* (2003) Toxin production of cyanobacteria is increased by exposure to zooplankton. *Freshw. Biol.* 48, 1540–1550

55 Pohnert, G. (2000) Wound-activated chemical defense in unicellular planktonic algae. *Angew. Chem. Int. Ed.* 39, 4352–4354

56 Turner, J.T. and Tester, P.A. (1997) Toxic marine phytoplankton, zooplankton grazers, and pelagic food web. *Limnol. Oceanogr.* 42, 1203–1214

57 Crespi, B.J. (2001) The evolution of social behavior in microorganisms. *Trends Ecol. Evol.* 16, 178–183

58 Griffin, A.S. *et al.* (2004) Cooperation and competition in pathogenic bacteria. *Nature* 430, 1024–1027

59 Wilson, A.E. *et al.* (2005) Genetic variation of the bloom-forming cyanobacterium *Microcystis aeruginosa* within and among lakes: implications for harmful algal blooms. *Appl. Environ. Microbiol.* 71, 6126–6133

60 Beszteri, B. *et al.* (2005) Ribosomal DNA sequence variation among sympatric strains of the *Cyclotella meneghiniana* complex (Bacillariophyceae) reveals cryptic diversity. *Protist* 156, 317–333

61 Iglesias-Rodriguez, M.D. *et al.* (2006) Intraspecific genetic diversity in the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae): the use of microsatellite analysis in marine phytoplankton population studies. *J. Phycol.* 42, 526–536

62 Rynearson, T.A. *et al.* (2006) Spring bloom development, genetic variation, and population succession in the planktonic diatom *Ditylum brightwellii*. *Limnol. Oceanogr.* 51, 1249–1261

63 Pohnert, G. (2005) Diatom/Copepod interactions in plankton: the indirect chemical defense of unicellular algae. *ChemBioChem* 6, 946–959

64 Miralto, A. *et al.* (1999) The insidious effect of diatoms on copepod reproduction. *Nature* 402, 173–176

65 Coale, K.H. *et al.* (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* 383, 495–501

66 Hanazato, T. (2001) Pesticide effects on freshwater zooplankton: an ecological perspective. *Environ. Pollut.* 112, 1–10

67 Lürling, M. *et al.* (2006) Life-history consequences for *Daphnia pulex* exposed to pharmaceutical carbamazepine. *Environ. Toxicol.* 21, 172–180

68 Stefels, J. (2000) Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *J. Sea Res.* 43, 183–197

69 Simo, R. (2001) Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. *Trends Ecol. Evol.* 16, 287–294

70 Andreae, M.O. and Crutzen, P.J. (1997) Atmospheric aerosols: biogeochemical sources and role in atmospheric chemistry. *Science* 276, 1052–1058

71 Evans, C. *et al.* (2006) Infectious titres of *Emiliania huxleyi* virus 86 are reduced by exposure to millimolar dimethyl sulfide and acrylic acid. *Limnol. Oceanogr.* 51, 2468–2471

72 Steinke, M. *et al.* (2002) Trophic interactions in the sea: an ecological role for climate relevant volatiles? *J. Phycol.* 38, 630–638

73 Wiltshire, K.H. and Lampert, W. (1999) Urea excretion by *Daphnia*: a colony-inducing factor in *Scenedesmus? Limnol. Oceanogr.* 44, 1894–1903

74 Lurling, A. and von Elert, E. (2001) Colony formation in *Scenedesmus*: No contribution of urea in induction by a lipophilic *Daphnia* exudate. *Limnol. Oceanogr.* 46, 1809–1813

75 Stich, H.B. and Lampert, W. (1981) Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature* 293, 396–398

76 Boriss, H. *et al.* (1999) Trimethylamine induces migration of waterfleas. *Nature* 398, 382–382

77 Pohnert, G. and von Elert, E. (2000) No ecological relevance of trimethylamine in fish – *Daphnia* interactions. *Limnol. Oceanogr.* 45, 1153–1156

78 Weckwerth, W. and Morgenthal, K. (2005) Metabolomics: from pattern recognition to biological interpretation. *Drug Discov. Today* 10, 1551–1558

79 Landsberg, J.H. (2002) The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10, 113–390

80 Prince, E.K. *et al.* (2006) Fitness consequences for copepods feeding on a red tide dinoflagellate: deciphering the effects of nutritional value, toxicity, and feeding behavior. *Oecologia* 147, 479–488