

# Phytoplankton defence mechanisms: traits and trade-offs

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## ABSTRACT

In aquatic ecosystems, unicellular algae form the basis of the food webs. Theoretical and experimental studies have demonstrated that one of the mechanisms that maintain high diversity of phytoplankton is through predation and the consequent evolution of defence mechanisms. Proposed defence mechanisms in phytoplankton are diverse and include physiological (e.g. toxicity, bioluminescence), morphological (e.g. silica shell, colony formation), and behavioural (e.g. escape response) traits. However, the function of many of the proposed defence mechanisms remains elusive, and the costs and benefits (trade-offs) are often unquantified or undocumented. Here, we provide an overview of suggested phytoplankton defensive traits and review their experimental support. Wherever possible we quantify the trade-offs from experimental evidence and theoretical considerations. In many instances, experimental evidence suggests that defences are costless. However, we argue that (i) some costs materialize only under natural conditions, for example, sinking losses, or dependency on the availability of specific nutrients, and (ii) other costs become evident only under resource-deficient conditions where a rivalry for limiting resources between growth and defence occurs. Based on these findings, we suggest two strategies for quantifying the costs of defence mechanisms in phytoplankton: (i) for the evaluation of defence costs that are realized under natural conditions, a mechanistic understanding of the hypothesized component processes is required; and (ii) the magnitude of the costs (i.e. growth reduction) must be assessed under conditions of resource limitation.

**Key words:** grazing protection, morphological defences, physiological defences, behavioural defences, benefits, costs, phytoplankton community, resource availability.

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## I. INTRODUCTION

The oceans cover more than two-thirds of the Earth's surface. In these ecosystems, the unicellular phytoplankton form the foundation of the food webs. All autotrophic phytoplankton utilize the same resources, namely, light and a few species of inorganic nutrients. Simple competition experiments and models have shown that at steady state, the number of coexisting species cannot exceed the number of limiting resources unless further mechanisms are involved (Hardin, 1960; Tilman, 1981; Sommer, 1985). In reality, phytoplankton communities are much more diverse than expected based on the competitive exclusion principle, a problem known as 'the paradox of the plankton' (Hutchinson, 1961). However, phytoplankton communities are seldom if ever at steady state due to spatiotemporal variation at all scales, thus often preventing competitive exclusion. An additional and commonly suggested explanation of higher than expected diversity of phytoplankton is the combination of predation and defence mechanisms (Darwin, 1859; Paine, 1966; Levin, 1970; Smetacek, 2001; Leibold *et al.*, 2017). Indeed, experimental manipulations of natural phytoplankton communities have demonstrated that the removal of grazers leads to a significant reduction in phytoplankton biodiversity (Leibold *et al.*, 2017).

This concept is, for example, expressed in the 'killing the winner' principle (Winter *et al.*, 2010). According to this principle, two species, a competition specialist and a defence specialist, may coexist on one limiting resource if the selective loss of the competition specialist due to predation prevents this population from seizing the limiting resource, and thus the resource becomes available to the defence strategist as well (Thingstad *et al.*, 2005; Winter *et al.*, 2010). The promotion of diversity in phytoplankton through grazing pressure and the consequent employment of defence mechanisms requires trade-offs, that is, the advantage of a defence mechanism must come at some cost (Thingstad *et al.*, 2014; Våge *et al.*, 2014). If there was no cost to grazer resistance, all species would evolve towards a state of equal defence, and community diversity would not be promoted by grazing.

Defence mechanisms (or at least proposed defence mechanisms) in phytoplankton against grazers are diverse and include morphological, physiological, and behavioural defences. A defence device may be constitutive (always present), or induced (only expressed or intensified in response to the presence of grazers or pathogens). In phytoplankton, there are examples of both types of defence mechanisms. For example, a diatom shell that may reduce grazing mortality (Hamm *et al.*, 2003) is always present (constitutive defence), but a thicker shell may be induced in the presence of zooplankton grazers (induced defence) (Pondaven *et al.*, 2007).

A considerable amount of research has been devoted to describing defence mechanisms in phytoplankton. Yet the associated trade-offs have largely been left unquantified. Thus, it remains unclear how much a particular defence mechanism reduces grazing mortality or increases pathogen

resistance, or what the costs of particular defence mechanisms are. The situation is further complicated by the fact that some defences may be effective against some grazers, but not against others. Models of phytoplankton communities therefore have to assume defence trade-offs to maintain species diversity or use other ways to circumvent this issue (e.g. assume constant immigration at a low rate in order to prevent the community from being reduced to one species over time) (Norberg *et al.*, 2001; Terseleer *et al.*, 2014; Acevedo-Trejos *et al.*, 2015), and only a few models are built on known and quantified defence trade-offs (Yoshida *et al.*, 2003; Merico, Bruggeman & Wirtz, 2009). Given the likely importance of defences and associated defence trade-offs in promoting and sustaining the diversity of phytoplankton in the ocean, the limited qualitative and quantitative understanding of defences in phytoplankton is either unexpected, or reflects difficulties in finding ways to fill this gap in knowledge.

Previous reviews of defence mechanisms in phytoplankton (Tillmann, 2004; Van Donk, Ianora & Vos, 2011) have focused on either morphological or physiological defences, on a few defensive traits from either of these groups, or on proposed defence mechanisms within a specific group of phytoplankton, or even within a single species. Examples of the latter are reviews of the inducibility of colony formation in the green alga *Scenedesmus* spp. as a defence mechanism (Van Donk, Lüring & Lampert, 1999), or the defensive role of calcification in coccolithophores (Monteiro *et al.*, 2016). Further, de Bernardi & Giussani (1990) evaluated how colony formation and toxicity affect the suitability of green algae as food for herbivores, Nejstgaard *et al.* (2007) discussed the role of coloniality and life stage for grazing mortality in *Phaeocystis* sp., and Turner (2014) explored to what extent the chemical compounds produced by harmful algal blooms (HABs) serve as grazing deterrents or toxins to mesozooplankton. Additional aspects of chemical defences in non-HAB phytoplankton were discussed by Wolfe (2000), Pohnert (2005), Pohnert, Steinke & Tollrian (2007), and Ianora, Miralto & Romano (2012).

While previous reviews have thus considered either specific taxa or specific anti-predator devices with an emphasis on the defence aspect, we here examine trade-offs of defence mechanisms across phytoplankton groups. In other words: what are the demonstrated and suspected benefits and costs of various defence mechanisms, and to what extent can benefits and costs be quantified? The currencies of benefits and costs are reductions in mortality rates and growth rates (as defence costs detract from growth), respectively, and the resulting fitness of an individual is the difference between the two. Wherever possible, we aim at assessing or quantifying these. While viruses may be an important source of mortality in phytoplankton, accounting for up to one-quarter of the primary production (see Pasulka, Samo & Landry, 2015 and references therein; Fuhrman, 1999), there is hardly anything known about phytoplankton resistance towards these threats [some examples are provided in Jacobsen, Bratbak & Heldal (1996), Huheihel *et al.* (2002), Brussaard

*et al.* (2005), Evans *et al.* (2006) and Frada *et al.* (2008)]. Therefore, we predominantly focus on the resistance of phytoplankton to zooplankton grazing, as grazing mortality is also the main mortality agent in phytoplankton (approximately two-thirds of primary production) (Calbet & Landry, 2004). We distinguish between morphological, physiological, and behavioural defence mechanisms. Our main focus is on defences induced by biotic factors (presence of grazers or their cues), as these allow for an assessment or even quantification of the trade-offs. We demonstrate that among the many suggested anti-predator devices in phytoplankton, hard evidence for trade-offs – or even benefits – is scarce. We argue that this is a serious gap in our understanding of phytoplankton communities, and suggest possible ways to fill this gap.

## II. PHYTOPLANKTON DEFENCE MECHANISMS

### (1) Morphological defences

Numerous studies investigating phytoplankton–zooplankton interactions have shown or suggested that the feeding success of herbivorous zooplankton depends greatly on the properties of the phytoplankton prey species. Morphological traits such as cell size (Yoshida, Hairston & Ellner, 2004; Long *et al.*, 2007; Friedrichs *et al.*, 2013), shape (Hessen & Van Donk, 1993), life-history stage (Dutz & Koski, 2006; Kolb & Strom, 2013), structure of cell wall (Hamm *et al.*, 2003; Pondaven *et al.*, 2007; Harvey, Bidle & Johnson, 2015; Liu *et al.*, 2016), and hardness of cell (DeMott, 1995) have all been suggested to affect grazing rates of herbivorous zooplankton. Evidence of trade-offs, where the intensity of a morphological defence mechanism has been induced by the presence of grazers or otherwise manipulated experimentally, is listed in Appendix A1.

#### (a) Cell size, colony formation, and chain formation

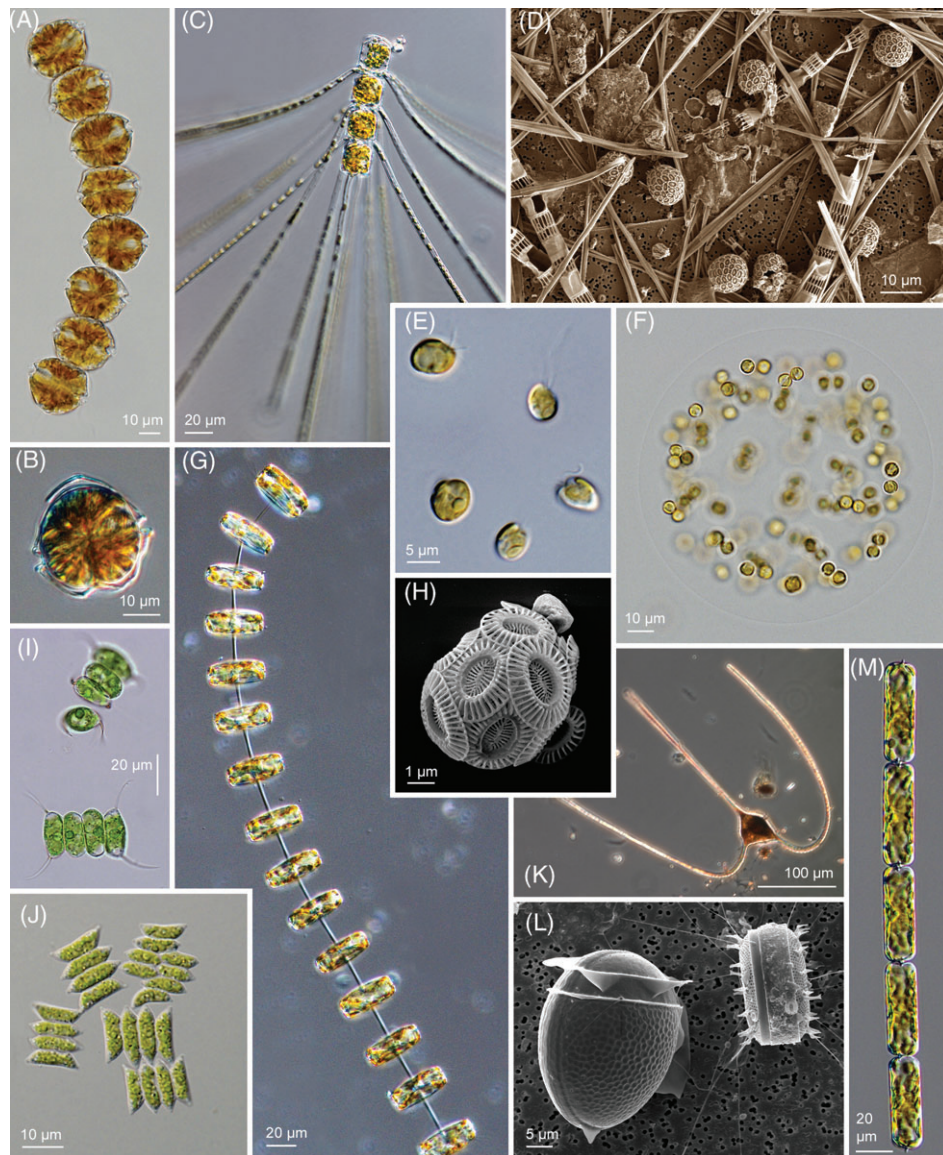
Cell size *per se* offers partial protection against grazing; large phytoplankton cells generally experience lower grazing mortality than smaller ones (Smetacek, Assmy & Henjes, 2004). The reasoning behind this is that small cells are grazed upon by small grazers, whereas large cells are consumed by larger grazers, and the total biomass of grazers declines with increasing grazer size (Kiørboe, 2011). Consequently, the mortality rate of organisms in pelagic ecosystems declines with size (McGurk, 1987), and this applies within phytoplankton communities (Latasa *et al.*, 1997; Landry *et al.*, 2000; Strom, Macri & Olson, 2007). The inherent trade-off is that the diffusion-limited specific nutrient uptake (Kiørboe, 1993) as well as the average chlorophyll-specific light absorption coefficient (i.e. the package effect) (Raven & Kubler, 2002) decline with size, and so does the growth rate in approximately the same manner, although there are large size-independent variations among phytoplankton taxa (Marañón, 2015). This fundamental trade-off may partly explain the size diversity of phytoplankton in the ocean.

Some species of phytoplankton have an inflated cell volume due to the presence of a vacuole, most notably diatoms (Menden-Deuer & Lessard, 2000) or the dinoflagellate *Noctiluca lucens* (Kiørboe, 2013), and the consequent larger cell size implies lower grazing mortality as discussed above. The trade-off in these special cases is less clear, since the inflated cell size also allows the phytoplankton cell to increase its resource acquisition rate, and hence growth, a strategy known as the ‘Winnie-the-Pooh’ strategy (Thingstad *et al.*, 2005). We consider the case of diatoms in further detail below (see Section II.1b). We are unaware of examples of grazer-induced changes in prey cell size.

Variations in size can be achieved through formation of colonies, which may be induced by the presence of grazers (Appendix A1). Colonies consist of morphologically identical clonal cells, and possess a range of different anatomies comprising one-dimensional chains (e.g. *Alexandrium* spp., many diatoms; Fig. 1A, C, G, M), two-dimensional plates (e.g. *Pediastrum* spp., *Scenedesmus* spp.; Fig. 1I, J), and three-dimensional structures (e.g. *Phaeocystis* spp.; Fig. 1F). A morphological change from solitary cells to colonies can thus cause difficulties for herbivores with a narrow range of prey-size preferences. The freshwater green algae of the genus *Scenedesmus* respond to infochemicals of various herbivores by forming large four- to eight-celled colonies (Fig. 1I, J). This phenotypic plasticity benefits the algae because a high proportion of colonies in the algal population leads to reduced clearance rates and/or reduced population growth rates of the grazers (Hessen & Van Donk, 1993; Lüring & Van Donk, 1996; Lüring, De Lange & Van Donk, 1997). Likewise, the marine prymnesiophyte *Phaeocystis* sp. responds to grazer-mediated infochemicals by changing its colony size by several orders of magnitude (Jakobsen & Tang, 2002). While microzooplankton-associated infochemicals enhance colony formation in *Phaeocystis* sp. (Jakobsen & Tang, 2002; Long *et al.*, 2007), mesozooplankton-associated infochemicals suppress colony enlargement (Long *et al.*, 2007; Lundgren & Granéli, 2010). These two responses are adaptive since copepod grazing pressure on *Phaeocystis* sp. single cells is lower than on colonies, and the growth rate and/or clearance rate of microzooplankton is lower on *Phaeocystis* sp. colonies than on solitary cells (Fig. 1E, F) (Long *et al.*, 2007). A similar response is found in the chain-forming diatom *Skeletonema* sp., and in the dinoflagellate *Alexandrium* sp. in the presence of mesozooplankton, resulting in impaired chain formation in both species, and consequently reduced mortality due to grazing (Fig. 1A, B) (Selander *et al.*, 2011; Bergkvist *et al.*, 2012; Bjærke *et al.*, 2015). Suppression of chain length in diatoms, however, is not a suitable mechanism to avoid grazing by microzooplankton, since they prefer to graze on solitary cells and short chains (Bergkvist *et al.*, 2012; Bjærke *et al.*, 2015).

As opposed to the obvious benefits of such morphological changes, the costs to organism fitness are still mainly speculative (Appendix A1). Impaired chain formation in the dinoflagellate *Alexandrium* sp. due to waterborne cues from mesozooplankton results in the occurrence of solitary cells





**Fig. 1.** Mosaic of morphological traits in phytoplankton. (A) One-dimensional chain of the dinoflagellate *Alexandrium catenella*. (B) Solitary cell of the dinoflagellate *Alexandrium catenella*. (C) One-dimensional chain of the diatom *Chaetoceros convolutes* with hollow spines containing chloroplasts. (D) Scanning electron-microscopy (SEM) image of phytoplankton natural assemblage from the North Sea (Bergen, Norway), including *Skeletonema* sp., *Pseudo-nitzschia* sp., and various coccolithophorids. (E) Motile solitary cells of the prymnesiophyte *Phaeocystis globosa*. (F) Three-dimensional non-motile colonial structure of the prymnesiophyte *Phaeocystis globosa*. (G) One-dimensional chain of the diatom *Thalassiosira rotula*. (H) SEM image of the cosmopolitan coccolithophore *Emiliana huxleyi*, whose cell wall plates (liths) are made of calcium carbonate. (I) Spiny solitary cells (top) and a one-dimensional chain (bottom) of the green alga *Scenedesmus quadricauda*. (J) One-dimensional chains (four cells) and a two-dimensional plate (eight cells) of the green alga *Scenedesmus obliquus*. (K) A large cell of the dinoflagellate *Ceratium trichoceros*. (L) SEM image of the dinoflagellate *Phalacroma* sp. with a cellulosic cell wall (left), and the diatom *Thalassiosira* sp., whose cell wall (frustule) is made of silica (right). (M) One-dimensional chain of the diatom *Dactyliosolen fragilissimus* with short and straight marginal spines. Figure credits: A, B, E, F, I, J, Gert Hansen, University of Copenhagen; C, G, M, Tara Ivanochko, University of British Columbia; D, H, K, L, Helge Abildhauge Thomsen, Technical University of Denmark.

and/or short chains (Fig. 1B) with lower swimming velocities than their long-chain counterparts (Fig. 1A) (Selander *et al.*, 2011). Long chains allow higher swimming velocities that enable the cells to perform diel vertical migrations to harvest nutrients at depth during the night, and light at the surface during the day. The ability of solitary cells or short chains to

perform such migrations is reduced, which may consequently lead to a cost in terms of decreased growth rates (Selander *et al.*, 2011).

Another cost related to large body size is sinking, with large particles sinking faster than small ones of the same density. Thus, large colonial *Scenedesmus* spp. experience up

to four times higher sinking rates relative to their unicellular counterparts, which can subsequently increase the risk of sinking out of the euphotic zone (Lürling & Van Donk, 2000). The enhancement in sinking velocities due to colony formation is partly countered by lower cell densities of colonial cells relative to single cells, facilitated by a higher content of fatty acids in colonial *Scenedesmus* spp. cells than in unicells (12–21% lipids in colonial cells *versus* 6–7% in unicells; Lürling, De Lange & Van Donk, 1997). With unchanged cell densities, Stokes' law would predict an eightfold increase in settling rates due to coloniality. The energetic costs of the synthesis of lipids from carbohydrates can be roughly estimated (Table 1). Note that the energetic costs of the synthesis are relatively low compared to the energetic value of the deposited lipids themselves. The combined energetic costs of lipid synthesis and the energetic value of the deposited lipids are approximately 15% higher in colonial relative to single cells (Table 1). Under light-limiting conditions, the growth of colonies would therefore be approximately 15% slower than that of single cells, which can be considered a cost of defensive colony formation.

Next to the higher investments in lipids, colony formation in *Scenedesmus* spp. leads to a reduction of chlorophyll-specific light absorption coefficients by 17–67% compared to unicells (Lürling, 1999), which may additionally decrease the growth rate. Yet, none of these presumed costs of colony formation in *Scenedesmus* spp. have led to measurable decreases in growth rates or efficiencies of photosystem II in experiments. Such differences would, however, become evident only under light-limiting conditions (such as often experienced by field populations), and the light intensities used in these studies may have been sufficient to overcome the energetic costs of colony formation ( $100\text{--}125\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$ ) (Lürling, 1999, 2003; Lürling & Van Donk, 2000).

Finally, colony formation may lead to a lower nutrient uptake rate per cell when nutrients are limiting, due to competition among colonial cells. Uptake rates are limited by the rate at which diffusion and advection can transport nutrients to the cell or colony surface. In the absence of ambient fluid motion, diffusive transport of nutrients to a spherical colony increases with the radius of the colony (Kjørboe, 2008), while the number of cells increases with the colonial radius squared (or a little less; Ploug, Stolte & Jørgensen, 1999), so the flux of nutrients per cell scales approximately inversely with colony size. However, this effect is partly countered by the effect of ambient fluid motion that increases with colony size, leading to an overall reduction in growth rates of colonial *Phaeocystis* sp. cells by a factor of 2–3 relative to single cells at limiting nutrient concentrations and typical levels of colony size and ambient turbulence (Ploug, Stolte & Jørgensen, 1999). The authors suggested that this would be the price for reduced grazing mortality of colonial cells. At nutrient-replete conditions, however, there would be no difference in nutrient uptake rates between solitary and colonial cells.

As noted, the costs of colony formation would mainly be evident if nutrients and/or light are limited. Therefore,

a probable reason why most of the studies summarized in Appendix A1 failed to detect costs of induced defences, in the form of reduced growth or otherwise, may be resource availability. Resource-replete environments can support energy-demanding growth and reproduction, and at the same time maintain defence, as there is no conflict between resource allocation to growth or defence. By contrast, when the organisms are exposed to resource-deficient conditions, metabolic rivalry for limiting resources between growth and defence may occur (Yoshida, Hairston & Ellner, 2004; Wang *et al.*, 2015; Zhu *et al.*, 2016). Thus, the costs of defence should be assessed at resource-limiting conditions in future work.

#### (b) Shape, extrusomes, and cell wall structure

Morphological variations such as long spines, ejectisomes, or reinforced cell walls can be beneficial to organisms as defence mechanisms. Spines may act as a grazing deterrent, which has been suggested for diatoms (e.g. *Corethron* sp., *Chaetoceros* sp.; Fig. 1C) (Crawford, Hinz & Honeywill, 1998) and green algae (e.g. *Scenedesmus* sp.; Fig. 1I) (Hessen & Van Donk, 1993). Discharging of ejectisomes in cryptophytes (e.g. *Cryptomonas* sp., *Chroomonas* sp.) may have a similar function (Rhiel, Ammermann & Hillebrand, 2016), although there is no direct evidence of reduced grazing pressure due to these structures. Similarly, the ecological role of the calcite armour in coccolithophores and silica shells in diatoms has long been proposed to act as a defence against predation [see Hamm *et al.* (2003), Monteiro *et al.* (2016) and references therein; for other proposed roles of calcite armour and silica frustules see Monteiro *et al.*, 2016 and references therein; Flynn, Clark & Wheeler, 2016; De Tommasi, Gielis & Rogato, 2017] (Fig. 1D, H, L). Only recently has experimental evidence supporting the defensive role of the calcite armour been provided: the clearance rate or growth rate of some heterotrophic protists is reduced by 29–99% and 35–73%, respectively, when fed calcified strains of *Emiliania* sp. relative to non-calcified strains (Appendix A1) (Harvey *et al.*, 2015).

The costs associated with calcification can be grouped into energetic, nutrient, and sinking costs (Monteiro *et al.*, 2016). Energetic costs include transport [calcification substrates ( $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$ ) into, and end product ( $\text{H}^+$ ) from the cell], metabolic (production of organic materials), mechanical (secretion of liths), and structural (construction and maintenance of structural components) costs, which are together estimated to be between one-fifth and one-third of the total photosynthetic budget, and are similar for small (e.g. *Emiliania huxleyi*;  $4\text{--}9\ \mu\text{m}$ ) and large coccolithophore species (e.g. *Calcidiscus pelagicus*;  $12\text{--}20\ \mu\text{m}$ ) (Raven & Crawford, 2012; Monteiro *et al.*, 2016). Genetically identical naked and coccolith-bearing individuals can be obtained by altering environmental conditions, and their growth rates subsequently quantified under identical conditions. As a result, naked cells of an oceanic *E. huxleyi* isolate are found to grow up to 15% faster than their larger coccolith-bearing counterparts (Lecourt, Muggli & Harrison, 1996), but conversely, naked cells of a coastal *E. huxleyi* isolate grow up to 15% slower than the coccolith-forming



Table 1. Cost of lipid formation in colonial and single cells of *Scenedesmus acutus* estimated from composition of the cells, the metabolic cost of lipid biosynthesis, and the energetic value of cell constituents

	<i>Scenedesmus acutus</i> unicells	<i>Scenedesmus acutus</i> colonial cells
Carbon content (pg C cell <sup>-1</sup> ) <sup>a</sup>	10	10
Lipid content (5%) <sup>b</sup>	6.5	21
Carbohydrates and protein content (5%)	93.5	79
Fatty acid content (mol FA cell <sup>-1</sup> ) <sup>c</sup>	$2.5 \times 10^{-15}$	$8.1 \times 10^{-15}$
Energetic value of cells (kJ cell <sup>-1</sup> ) <sup>d</sup>	$3.61 \times 10^{-10}$	$4.22 \times 10^{-10}$
Biochemical cost of lipid synthesis (kJ cell <sup>-1</sup> ) <sup>e</sup>	$4.35 \times 10^{-12}$	$1.41 \times 10^{-11}$
Total cost of lipid synthesis (kJ cell <sup>-1</sup> )	$3.65 \times 10^{-10}$	$4.36 \times 10^{-10}$

<sup>a</sup>Derived from a cell volume of  $74 \mu\text{m}^3$  (Lürling, De Lange & Van Donk, 1997) and a carbon to volume relationship for chlorophytes (Menden-Deuer & Lessard, 2000).

<sup>b</sup>Lürling, De Lange & Van Donk (1997).

<sup>c</sup>Derived from a total fatty acid (FA) content of 6.5 and 21% of dry mass for unicells and colonies, respectively (Lürling, De Lange & Van Donk, 1997), and assuming an average FA molecular weight of  $266 \text{ g mol}^{-1}$ . The average number of cells in a colony is 5 (Lürling, De Lange & Van Donk, 1997).

<sup>d</sup>Assuming that the caloric value of lipids is  $37.7 \text{ kJ g}^{-1}$ , and of carbohydrates and proteins is  $16.7 \text{ kJ g}^{-1}$ ; cell mass is 20 pg, assuming that the carbon content of cells is 50% of dry mass.

<sup>e</sup>Assuming that 57 mol of ATP is needed for the synthesis of 1 mol FA palmitate (C16) (Berg, Tymoczko & Stryer, 2002), and that 1 mol of ATP provides  $30.5 \text{ kJ mol}^{-1}$  of energy.

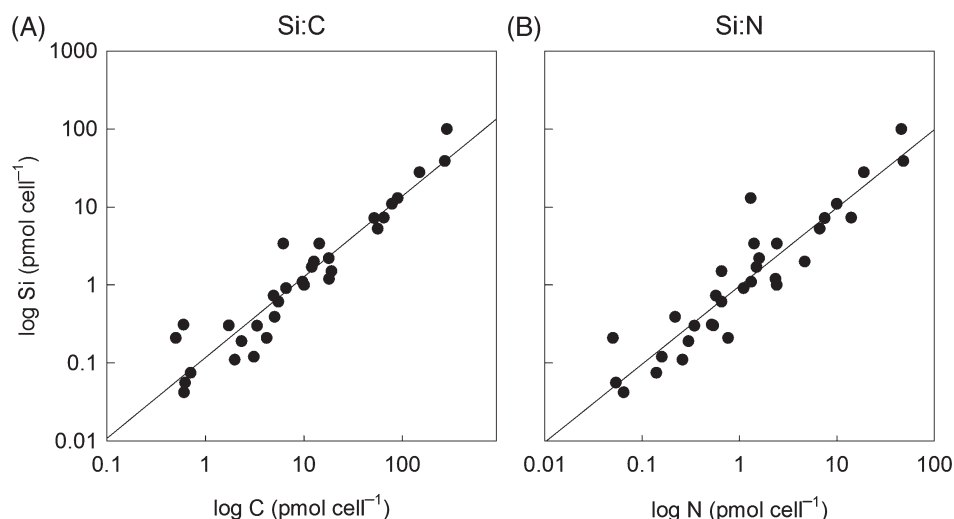
cells (Paasche & Klaveness, 1970). Therefore, the estimated energetic costs of calcification are not unequivocally evident in observed growth rates of coccolithophores. Regarding nutrient costs, formation of the calcite armour seems to be a rather inexpensive process, since the demands for nitrogen and phosphorus are very low, and calcification generally requires only inorganic carbon and calcium (Lavoie, Raven & Levasseur, 2016; Monteiro *et al.*, 2016). Finally, despite the fact that calcification largely enhances sinking velocities of calcifying cells (Lavoie, Raven & Levasseur, 2016), the impact of calcification on loss rates *via* sinking out of the mixed layer may be negligible for small coccolithophore species (e.g. *Emiliania huxleyi*), because the vertical eddy diffusivities observed in the mixed layer are always sufficient to compensate for sinking rates and, hence, to keep the cells suspended (Monteiro *et al.*, 2016). Sinking costs become significant only for larger calcifying species (e.g. *Calcidiscus* sp.) in regions with low turbulent mixing (Monteiro *et al.*, 2016). Thus, overall, the trade-offs of calcification are not immediately evident.

Similarly, diatoms possess silicified armour (Fig. 1D, L) that may have evolved as a mechanical protection against predation. The level of silicification varies greatly among

diatom species, which led Assmy *et al.* (2013) to propose two different strategies in diatoms: defence specialists with thick shells and slow growth *versus* competition specialists with thin shells and rapid growth. A representative of the defence strategy, *Fragilariopsis kerguelensis*, possesses an exceptionally strong silica wall (Hamm *et al.*, 2003) that has been suggested to reduce losses to mesozooplankton grazing by reducing the proportion of cracked and crushed cells, which could potentially enable viable gut passage (Jansen & Bathmann, 2007; Kruse *et al.*, 2009; Assmy *et al.*, 2013). Competition-specialized diatoms, on the other hand, have thinner shells but also have the ability to proliferate rapidly during favourable conditions. On account of the fact that thinner frustules are crushed at much lower forces than the thick ones (e.g. for *Thalassiosira punctigera* and *F. kerguelensis*:  $\sim 200$  and  $\sim 750 \mu\text{N}$ , respectively), thin-shelled diatoms may therefore experience higher grazing mortality than those with thick-shells (Hamm *et al.*, 2003). In line with this, it has been speculated that mesozooplankton (Hamm *et al.*, 2003) and benthic foraminifera (see Austin, Austin & Paterson, 2005 and references therein) are likely to have evolved specialized tools to break open the frustules. Finally, this high variation in silica content among diatoms (Brzezinski, 1985; Conley, Kilham & Theriot, 1989) (Fig. 2) could be related to their life strategy, and may represent a trade-off between growth and grazing losses.

While it appears intuitive that a diatom shell may provide mechanical protection against grazing (as cells stay enclosed in tightly sealed silica boxes at all times and open them only during sexual reproduction), and that thick-shelled diatoms should be better protected than thin-shelled ones, direct evidence remains very limited. Recently, a single experiment demonstrated that fast-growing, thin-shelled *Thalassiosira weissflogii* experience 2–3 times higher grazing pressure from copepods than slow-growing thick-shelled cells of the same species (Liu *et al.*, 2016). Likewise, there is only weak experimental evidence that diatoms can survive gut passage better than non-silicified phytoplankton (Fowler & Fisher, 1983). Finally, there is some indication that diatoms exposed to chemical cues released by herbivores accumulate more silicon per cell (Pondaven *et al.*, 2007), thus providing indirect evidence that silicification provides grazer resistance. However, the lack or scarcity of hard evidence is striking in light of the common assumption that diatom shells provide protection against predation. Indeed the shell may have evolved for entirely different purposes, for example, to allow diatoms to have a large vacuole and, as a consequence, inflated size that on its own may reduce grazing mortality (see Section II.1a). There is a strong need for experiments that test this hypothesis.

The anticipated benefits of a thick shell must be traded off against the costs. Silicon deposition in diatoms depends on their growth rate, thus suggesting a cost to silicification; since many diatoms do not store sufficient quantities of silicon for new valve formation, they must harvest most of the required amounts immediately before ( $G_2$  interphase) and during cell division (Brzezinski, 1985). Thus, slow growth



**Fig. 2.** Silica-to-carbon (A) and silica-to-nitrogen (B) content in marine diatoms. Regression lines:  $\log \text{Si} = \log 0.1175 + 1.0356 \log \text{C}$  ( $r^2 = 0.899$ ;  $P < 0.05$ ; mean Si:C = 0.24);  $\log \text{Si} = \log 0.9758 + 1.0019 \log \text{N}$  ( $r^2 = 0.862$ ;  $P < 0.05$ ; mean Si:N = 1.42). Note that the axes are plotted on a logarithmic scale. Data derived from Parsons, Stephens & Strickland (1961), Eppley, Holmes & Paasche (1967), Harrison *et al.* (1977), and Brzezinski (1985).

under non-limiting silicon conditions leads to an extended period available for silicon uptake, resulting in heavier silicification of cell walls in defence specialists; the opposite will be true for high growth rates in competition specialists (Martin-Jézéquel, Hildebrand & Brzezinski, 2000). This also implies that external abiotic forces involved in regulating growth rates, such as availability of nutrients (nitrogen, phosphorus) (Claquin *et al.*, 2002; De La Rocha *et al.*, 2010), light (Taylor, 1985; Liu *et al.*, 2016), temperature (Furnas, 1978; Redalje & Laws, 1983), or trace metals (iron, zinc) (Hutchins & Bruland, 1998; Takeda, 1998; De La Rocha *et al.*, 2000), will indirectly control the process of silicification.

Other costs include biochemical costs directly related to the uptake and deposition of silicon, the costs associated with the dependency of diatoms on dissolved silica in the environment, and the costs related to the potential losses due to elevated sinking rates of heavy, silicified cells. Silicification seems to be a rather cheap process since it requires only 1 mol ATP per 1 mol Si (Raven, 1983), meaning that a diatom cell with a Si:C molar ratio of 0.24 (Fig. 2) would invest less than 2% of its total energy into silicification (a more-extended analysis with a similar conclusion is provided by Lavoie, Raven & Levasseur, 2016) (Table 2). In fact, silicification is much cheaper than the deposition of polysaccharides (e.g. cellulose), a common cell wall component in phytoplankton: the combustion of 0.1 g of glucose would enable the precipitation of more than 1 g of Si in the cell walls, but only 0.09 g of polysaccharides (Raven, 1983). The use of dissolved silica instead of polysaccharides thus represents an energetic advantage rather than a cost for diatoms.

The growth of diatoms is dependent on the availability of dissolved silica in the environment (Brzezinski, Olson & Chisholm, 1990; Raven & Waite, 2004), which represents another potential cost that cannot be directly estimated from

Table 2. Estimating the energetic cost of silica formation in diatoms from cell composition and cost of biosynthesis

Carbon content (pg C cell <sup>-1</sup> ) <sup>a</sup>	450
Silica content (pg Si cell <sup>-1</sup> ) <sup>a</sup>	200
Energetic value of cells (kJ cell <sup>-1</sup> ) <sup>b</sup>	$1.52 \times 10^{-8}$
Biochemical cost of silicification (kJ cell <sup>-1</sup> ) <sup>c</sup>	$2.23 \times 10^{-10}$

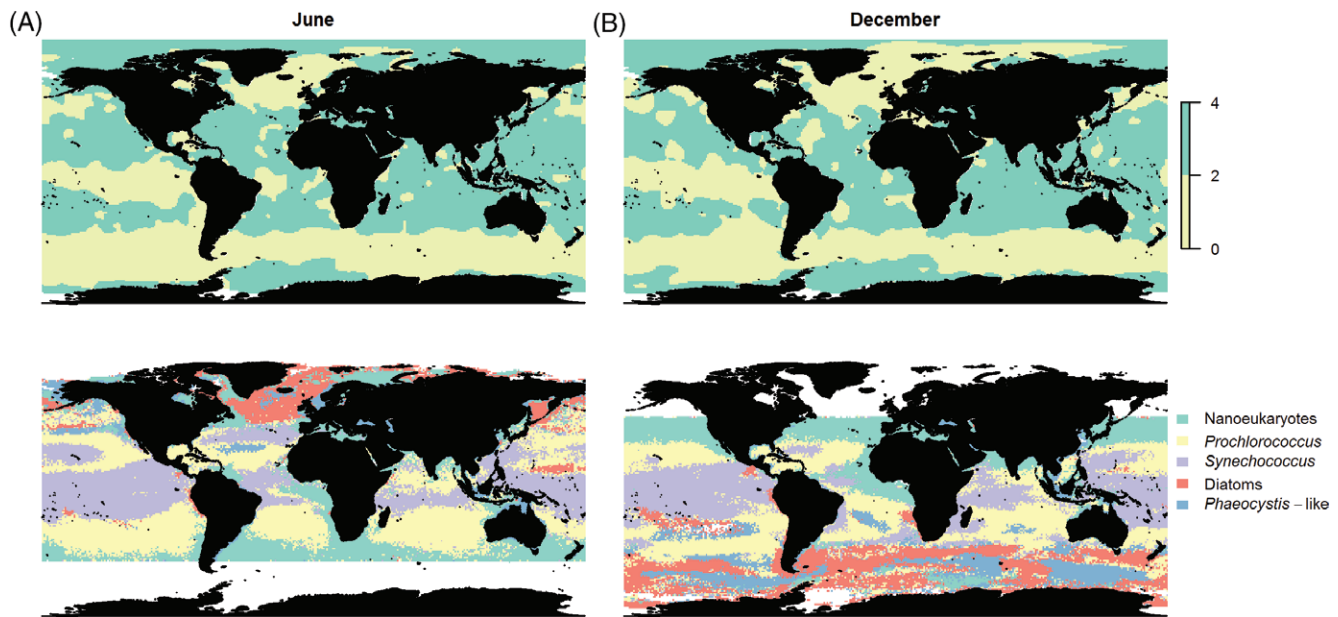
<sup>a</sup>Derived from a carbon to volume relationship for diatoms from Menden-Deuer & Lessard (2000), assuming a spherical shape with a diameter of 25  $\mu\text{m}$ , and a Si:C molar ratio of 0.24 (Fig. 2).

<sup>b</sup>Assuming that the carbon content of cells is 50% of dry mass, and is distributed between carbohydrates and proteins only (no lipids) with a caloric value of 16.7 kJ g<sup>-1</sup>.

<sup>c</sup>Assuming 1 mol ATP per 1 mol Si (Raven, 1983), and that 1 mol of ATP provides 30.5 kJ mol<sup>-1</sup> of energy.

laboratory experiments. Diatoms exhibit strong seasonal cycles with large-scale blooms in spring and summer that typically occur at high latitudes and in eutrophic regions (Fig. 3) (Alvain *et al.*, 2005). Those areas are usually characterized by low Si:N ratios (Fig. 3). If we assume that the average ratio of Si:N in diatoms is 1.4 (Fig. 2), we can expect that in regions with lower ambient Si:N ratios, silica will be exhausted before other macronutrients (Taylor *et al.*, 1993; Nelson *et al.*, 2001). Depletion of dissolved silica thus ends the diatom bloom, putting them at a competitive disadvantage compared to the succeeding phytoplankton groups whose populations may increase as long as other macronutrients are available (Taylor *et al.*, 1993).

Finally, as defined by Stokes' law, sinking rates of organisms increase with size and density. Silicon frustules increase the overall density of diatoms, which consequently elevates their sinking rates. The sinking velocities of actively growing single-celled diatoms measured in the laboratory



**Fig. 3.** Global distributions of ambient Si:N ratios (mol mol<sup>-1</sup>, A), and global distributions of the main phytoplankton groups (B). Top panels show monthly mean maps of Si:N ratios for June and December. Values <2 mol mol<sup>-1</sup> (yellow) represent areas where dissolved silica becomes depleted before inorganic nitrogen; and values >2 mol mol<sup>-1</sup> (green) represent areas where inorganic nitrogen is a growth-limiting nutrient. Data derived from the World Ocean Atlas 2013 (Boyer *et al.*, 2013). Bottom panels show monthly mean distributions of the prevailing phytoplankton groups in June and December: nanoeukaryotes (green), *Prochlorococcus* spp. (yellow), *Synechococcus* spp. (purple), diatoms (red), and *Phaeocystis*-like organisms (blue). White regions indicate areas with no data. Based on the PHYSAT method of Alvain *et al.* (2005).

range from 0.02 m day<sup>-1</sup> for small diatoms to 2 m day<sup>-1</sup> for large ones (Fig. 4). If we assume that the depth of the euphotic zone is 50 m, then the specific sinking-loss rates of small and large diatoms are about  $4 \times 10^{-4}$  and  $4 \times 10^{-2}$  day<sup>-1</sup>, respectively; loss rates through sinking out of the euphotic zone therefore are small relative to typical reproductive rates of diatoms ( $\sim 1$  day<sup>-1</sup>). Massive sinking losses of diatoms, as reported at the end of blooms, are facilitated by the cells coagulating into large, rapidly sinking aggregates, and may be considered part of diatom life history rather than a cost of silicification (Smetacek, 1985).

### (c) Life history stage

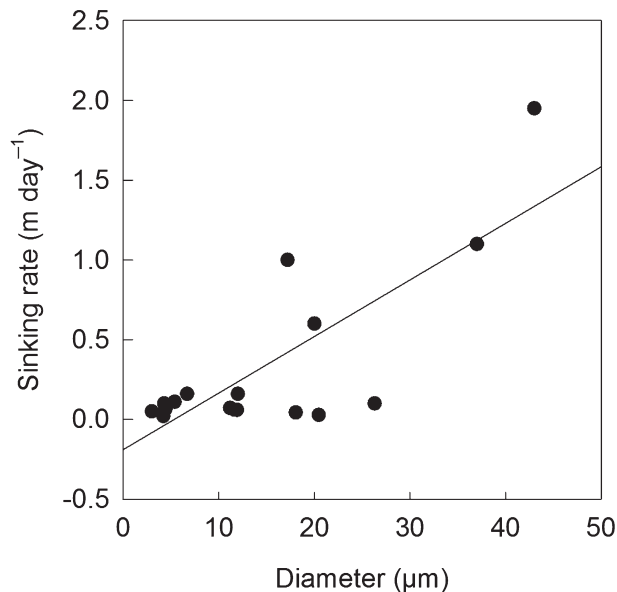
Solitary cells of haptophytes, including *Emiliania huxleyi* and *Phaeocystis globosa*, have a haploid–diploid life cycle with at least two morphologically different phases (see Appendix A1) (Dutz & Koski, 2006; Kolb & Strom, 2013). In *E. huxleyi*, the diploid cells are non-motile and produce calcite liths, whereas haploid cells have two flagella and organic liths. A defence mechanism of some sort is induced in the latter motile type in the presence of microzooplankton under nutrient-sufficient conditions, which leads to a rapid and persistent reduction in grazer ingestion rates (Appendix A1) (Kolb & Strom, 2013). Since the defence mechanism is unknown, any costs to the organism's fitness remain unquantified. The heteromorphic life cycle of *Phaeocystis* spp. involves a transition between free-living solitary cells (Fig. 1E), which are highly vulnerable to microzooplankton grazing but are not grazed upon

efficiently by mesozooplankton, and mucilaginous colonies (see Section II.1a; Fig. 1F) (Dutz & Koski, 2006). Solitary cells of *P. globosa* occur in nature in at least three different types differing in size, ploidy, and morphology; yet, their position and function in the life cycle are poorly known (Dutz & Koski, 2006). Similarly to the above-reported decrease in microzooplankton ingestion on the motile haploid *E. huxleyi*, the motile haploid cells of *P. globosa* reduce vulnerability to mesozooplankton grazing (Appendix A1); however, the mechanisms as well as the cost of defence still need to be addressed (Dutz & Koski, 2006).

### (2) Physiological defences

Several physiological traits in phytoplankton, such as toxin production and the production of other chemical substances, bioluminescence, and altered nutritional value of algae, are proposed to represent physiological defence mechanisms. The production of defensive chemical compounds can be activated by a variety of cues, by grazing, or by motion. Defensive chemical compounds have been found to reduce grazer feeding activity, or cause grazer reproductive failure, and even death (Fulton & Paerl, 1987; Dutz, 1998; Colin & Dam, 2003; Selander *et al.*, 2006; Bergkvist, Selander & Pavia, 2008; Wohlrab, Iversen & John, 2010). Many of these defensive traits are constitutive, but their production may increase in the presence of grazers (Selander *et al.*, 2006; Harðardóttir *et al.*, 2015). Interestingly, nutrient-stressed environmental conditions, as found in the aftermath of algal





**Fig. 4.** Sinking rate (SR) of diatoms ( $\text{m d}^{-1}$ ) versus cell diameter ( $D$ ;  $\mu\text{m}$ ). Data were obtained from Smayda (1970) and Waite *et al.* (1997), and are based on laboratory measurements of sinking rates of ten actively growing diatom species (including several strains) ranging in size from 3 to 40  $\mu\text{m}$  [ $\text{SR} = 0.0355D - 0.1901$  ( $r^2 = 0.63$ ;  $P < 0.05$ )].

blooms, lead to nutrient-deficient phytoplankton that can either pass through the grazer's gut intact, or result in reduced grazer ingestion rates (Van Donk & Hessen, 1993). In line with this, the low-food-value of slow growing algae can be an efficient defence strategy in nutrient-depleted environments (Van Donk & Hessen, 1993; Yoshida *et al.*, 2003; Yoshida, Hairston & Ellner, 2004). Certain physiological changes (e.g. production of chemical compounds) can also appear under adverse abiotic or biotic environmental conditions, thus suggesting other metabolic roles [e.g. offensive (see Driscoll, Hackett & Ferrière, 2016, and references therein) or allelopathic (Bar-Yosef *et al.*, 2010)] besides defensive. Evidence for physiological defensive traits is summarized in Appendix A2. A detailed description of chemical compounds produced by phytoplankton is beyond the scope of this review.

(a) Cue-activated increased production of constitutive chemicals

The evolutionary coexistence of prey and predator may impact the levels of toxins produced by phytoplankton, meaning that the induced responses of prey coinciding in evolutionary time and space with predators might be different from responses to predators with greater temporal and spatial distance (Bergkvist, Selander & Pavia, 2008). Moreover, the shared history may also determine the effects that defensive chemicals have on the herbivores (Fulton & Paerl, 1987; DeMott, Zhang & Carmichael, 1991; Colin & Dam, 2003; Bergkvist, Selander & Pavia, 2008).

Three major types of grazer responses have been observed:

(i) no response to the chemicals; (ii) low feeding rates on toxic

algae when offered as a sole diet, or selection against toxic algae when offered a mixed diet; and (iii) high feeding rates at first (sole or mixed diets), followed by reduced ingestion, and/or reduced metabolic rate, and/or increased mortality of the grazers. The first type of response, where a herbivore can graze on toxic algae without obvious adverse effects is often interpreted to be a result of evolutionary history between the prey and predator populations (Fulton & Paerl, 1987; Teegarden & Cembella, 1996; Colin & Dam, 2003; Wohlrab, Iversen & John, 2010). However, it may simply be due to the fact that the definition of 'toxic algae' refers to their toxicity to human consumers and not zooplankton.

The second type of response is commonly referred to as deterred feeding. This type of response enables the herbivore to avoid a potentially dangerous diet prior to ingesting harmful amounts, meaning that the grazer has developed the ability to recognize and avoid toxic prey (Fulton & Paerl, 1987; DeMott, Zhang & Carmichael, 1991; Teegarden & Cembella, 1996; Colin & Dam, 2002; Selander *et al.*, 2006) either *via* sensing the prey (Schultz & Kiørboe, 2009) or *via* capturing, tasting, and then rejecting the prey (Gill & Harris, 1987; Sykes & Huntley, 1987; Xu *et al.*, 2017). Finally, for the third type of response, the ingestion of toxic algae is required to affect the grazer, often followed by physical incapacitation and hence reduced ability to feed afterwards, or reduced growth with subsequent, long-term reduced grazing (Ives, 1987; DeMott, Zhang & Carmichael, 1991; Colin & Dam, 2003; Sopanen *et al.*, 2011). Even though the last two types of response both result in overall reduced grazing pressure on phytoplankton, and subsequently reduced growth or increased mortality of the grazers, they may have very different implications to the prey. The prey organisms that are avoided in the first place, or captured and instantly rejected by the grazers, will survive and consequently gain a competitive advantage. It is straightforward to comprehend how this strategy may have evolved. On the contrary, it is difficult to understand the benefits of the last type of response, as this requires elimination of some cells to elicit a response, and hence, could only evolve if the prey population mainly consists of closely related cells ('kin selection'). Moreover, reduced grazing due to toxic effects on the grazer is equally beneficial to competing algal species, and thus does not provide the producer of the toxin any competitive advantage. This still remains an unresolved issue, and is very different from physiological defences in terrestrial plants: a tree can afford to lose a few toxic leaves to kill or repel its consumers, but a single-celled phytoplankton cannot.

The levels of paralytic shellfish toxins (PSTs) produced by a number of dinoflagellate genera, and microcystins produced by a few genera of cyanobacteria, increase with direct or indirect exposure to grazers (Selander *et al.*, 2006; Jang, Jung & Takamura, 2007). From the studies summarized in Appendix A2, it is clear that in some – but far from all – cases, increased toxin production is advantageous to the phytoplankton, and that the observed responses of the

grazers to toxic dinoflagellates or cyanobacteria are not only diverse but also depend on the particular combination of prey and predator as well as on the strains involved (also see Turner, 2014 and references therein).

The costs of PST production are much more difficult to assess than the benefits. A few attempts to quantify these costs through changes in growth have yielded similar results: induced cells (elevated PST production) grow as fast as uninduced cells (Selander *et al.*, 2006; Bergkvist, Selander & Pavia, 2008), and toxic and non-toxic strains of the same species grow at the same rate (John *et al.*, 2015). This apparent lack of cost may, however, depend on nutrient conditions. Dinoflagellates generally produce PSTs in N:P balanced (N:P = 16), and/or high-N (N:P > 16) environments, but not under low-N (N:P < 16) conditions (Béchemin *et al.*, 1999; Maestrini *et al.*, 2000; Guisande *et al.*, 2002; John & Flynn, 2002; Selander, Cervin & Pavia, 2008; Lim *et al.*, 2010). Similarly, waterborne cues from the mesozooplankton *Acartia* sp. have been found to increase the production of PSTs in the dinoflagellate *Alexandrium minutum* in N-high, but not in N-low environments, suggesting that toxin production is dependent on nitrogen availability (Selander, Cervin & Pavia, 2008). Moreover, PSTs are synthesized in the G<sub>1</sub> phase of the cell cycle, whose duration can be prolonged by external factors such as temperature or phosphate limitation (Taroncher-Oldenburg, Kulis & Anderson, 1999). Thus, increased duration of the cell cycle due to phosphate limitation may result in cellular accumulation of PSTs, but as soon as P-replete conditions are restored high growth rates are regained and subsequently the PST quota per cell decreases (Lippemeier *et al.*, 2003). Since PST is an alkaloid rich in nitrogen (C:N = 1.4; average C:N ratio in dinoflagellates is 4.9) (Menden-Deuer & Lessard, 2000), it is likely that nitrogen rather than carbon or phosphorus will limit PST production (e.g. Flynn *et al.*, 1994; Lippemeier *et al.*, 2003; Selander, Cervin & Pavia, 2008). Overall, this means that the costs of defence may be unmeasurably low when resources are plentiful, but could be more significant in N-depleted environments, although this still remains to be demonstrated. To date, no attempts have been made to measure the cost of elevated production of cyanobacterial toxins.

Some diatoms of the genus *Pseudo-nitzschia* can produce domoic acid (DA), and cellular DA content increases with exposure to copepod grazers (Harðardóttir *et al.*, 2015). The proposed role of DA as a chemical defence mechanism in this genus is still under debate, since there is little evidence of a correlation between grazing rates and DA content (Olson *et al.*, 2008; Harðardóttir *et al.*, 2015). Despite its inducibility and potential effects on grazers, no association exists between elevated production of DA and a cost to its production in terms of growth rates (Harðardóttir *et al.*, 2015). However, as DA is an amino acid and the synthesis of amino acids requires nitrogen, it may be that dependence on nitrogen availability represents a potential cost of DA production that would become evident only under N-limiting conditions.

#### (b) Grazing-activated production of chemicals

Some chemical substances in phytoplankton are grazing-activated (wound-activated or post-ingestion-activated). Here, phytoplankton cells must be injured, which often means eliminated, before the chemicals actually affect the herbivores. Therefore, such grazing-activated chemical compounds will be beneficial to the algae only if at least part of the population is comprised of clonal or closely related cells ('kin selection'). However, this strategy will also be beneficial to their competitors (e.g. Flynn & Irigoien, 2009). The production of compounds, such as apo-fucoxanthinoids in diatoms and dimethylsulphoniopropionate (DMSP) in haptophytes increases when the cells become stressed due to environmental limitations (Shaw, Andersen & Harrison, 1997; Fredrickson & Strom, 2009), suggesting that these chemical substances may have other metabolic functions besides defence.

Even though there is evidence that apo-fucoxanthinoids produced by a few diatom species reduce grazer feeding activity (Appendix A2) (Shaw, Andersen & Harrison, 1997), it remains unclear whether this observed reduction is due to low feeding rates on harmful prey (the component acts as a deterrent) or physical incapacitation of the grazers. The costs of apo-fucoxanthinoid production to the diatoms are unknown.

When algal cells are subjected to either physical or chemical stresses that cause cell lysis, the algal DMSP becomes exposed to the enzyme DMSP lyase that triggers the production of dimethyl sulphide (DMS) and acrylate (Wolfe, Steinke & Kirst, 1997). However, activity of DMSP lyase is strain-specific; strains with high-activity DMSP lyase exhibit 33% slower growth than low-activity DMSP lyase strains (Wolfe & Steinke, 1996), possibly indicating very high costs of producing and storing DMSP-cleaving enzymes. High-activity DMSP lyase, however, appears beneficial to the algae, as reduced grazing rates of herbivore microzooplankton have been observed in a sole diet (Wolfe & Steinke, 1996; Wolfe, Steinke & Kirst, 1997; Strom *et al.*, 2003). Moreover, grazers avoid or reject the toxic prey in a mixed diet, although the presence of high-activity DMSP lyase strains does not impair feeding on preferred prey (Wolfe, Steinke & Kirst, 1997; Strom *et al.*, 2003), thus indicating that DMSP and its products are potential grazing deterrents (Appendix A2). By contrast, the chemical signature of DMSP and related compounds can act as a cue that attracts foragers, as observed in the dinoflagellate *Oxyrrhis marina* (Seymour *et al.*, 2010). Also, these compounds may induce what has been interpreted as search behaviour in the copepod *Temora longicornis* [but not in *Calanus helgolandicus* (Breckels *et al.*, 2013)], which may then lead to increased foraging of this copepod on herbivorous microzooplankton (Steinke, Stefels & Stamhuis, 2006). Steinke, Stefels & Stamhuis (2006) speculate that these chemical compounds may mediate tritrophic interactions among DMSP-producing phytoplankton, microzooplankton, and mesozooplankton. Local elevations in DMSP/DMS concentrations can also alert higher-order predators, such as planktivorous reef fish (DeBose, Lema &

Nevitt, 2008) or seabirds (Nevitt, Veit & Kareiva, 1995) to large aggregations of zooplankton, therefore indirectly reducing mortality of DMSP-producing phytoplankton. Overall, the potential benefit of DMSP as a defence mechanism appears to be complex, since it may reduce grazing mortality (directly or indirectly), but at the same time attract other grazers.

Another group of wound-activated chemical compounds comprises volatile oxidation products of the membrane lipids, as found in some bloom-forming diatom species (Wichard *et al.*, 2005; Ianora, Miralto & Romano, 2012). Chemical compounds from this group have low acute toxicity effects on adult planktonic grazers, but depress the viability of grazer gametes (D'Ippolito *et al.*, 2002; Pohnert *et al.*, 2002) and offspring (Miralto *et al.*, 1999; D'Ippolito *et al.*, 2002; Pohnert *et al.*, 2002; Ianora *et al.*, 2004; Fontana *et al.*, 2007) (Appendix A2). However, it has been argued that this so-called 'insidious' effect (i.e. killing the offspring of the grazer) cannot be evolutionarily sustained as a defence mechanism, as it is not beneficial to the producers over the time scales from ingestion to a potential reduction in grazing, and because competing species are equally benefited (Flynn & Irigoien, 2009). Nonetheless, since metabolically valuable fatty acids are direct precursors for the production of volatile products, the diatoms avoid the expense of producing alternative secondary metabolites (Pohnert *et al.*, 2002). However, producing and storing the enzymes responsible for the lipid enzymatic cascade may represent a potential cost, which must still be quantified.

#### (c) Exopolymer substances

Another type of chemical substances produced by phytoplankton is extracellular exopolymer material. These exopolymers can reduce feeding rates of grazers either by indirectly serving as an alternative food source, hence reducing ingestion of the cells (Passow & Alldredge, 1999), or directly by depressing grazer feeding (Dutz, Breteler & Kramer, 2005). Increased production of transparent exopolymer particles (TEPs) in *Phaeocystis globosa* is directly linked with bloom progression. Thus, elevated TEP concentrations in the surroundings could result from predation and disruption of colonies (Dutz, Breteler & Kramer, 2005) (Appendix A2), or simply from changed nutrient status of the cells as the bloom develops (i.e. an overflow mechanism). Similarly, the presence of a mucus layer of exopolymer secretions (EPS) outside the cell wall of the 'Texas brown tide'-forming pelagophyte *Aureoumbra lagunensis* reduces herbivore grazing rates, and consequently their growth rates (Liu & Buskey, 2000) (Appendix A2). EPS production increases with age of the *Aureoumbra* sp. population as well as under hypersaline conditions (see Liu & Buskey, 2000). Production costs of TEP and EPS are unquantified, but may be minimal if the synthesis of exopolymer substances is part of an overflow mechanism.

#### (d) Bioluminescence

A minority of marine dinoflagellates are bioluminescent in nature (Buskey & Swift, 1983). Luminescence can be induced by grazer-associated infochemicals (Lindström *et al.*, 2017), and the emission capacity of dinoflagellates is determined by environmental conditions such as nutrient levels, light, temperature, and salinity (see White, 1979 and references therein). Under favourable conditions, bioluminescence leads to decreased feeding efficiency on dinoflagellates either directly (Esaías & Curl, 1972; White, 1979; Buskey, Mills & Swift, 1983; Buskey & Swift, 1983) or indirectly (Mensing & Case, 1992; Fleisher & Case, 1995) (Appendix A2). A direct reduction in feeding efficiency is mediated by disrupted grazer swimming behaviour in response to flashing dinoflagellates (Buskey, Mills & Swift, 1983; Buskey & Swift, 1983). Indirect reductions may come about through the hypothesized 'burglar alarm' effect: flashes of light attract secondary predators that reduce grazer numbers, which could then lead to reduced mortality of the light-emitting dinoflagellate (Mensing & Case, 1992; Fleisher & Case, 1995). The potential cost is in the synthesis of scintillons, organelles responsible for the emission of light in dinoflagellates. Scintillons comprise three components needed for light emission: the substrate luciferin, a luciferin-binding protein, and the enzyme luciferase, with each of the two proteins being active at a different pH (Schultz *et al.*, 2005). The reaction that leads to the emission of light is thus pH dependent (Hastings, 1983; Schultz *et al.*, 2005), and may only require energy (in terms of ATP) for opening voltage-gated channels in the scintillon membrane (Schultz *et al.*, 2005).

#### (e) Low-food-value algae

Nutrient-limiting environmental conditions, as found following phytoplankton blooms, lead to nutrient-deficient (low-food-value) algae that can either pass through the grazer's gut intact to a greater extent than nutrient-sufficient cells (Van Donk & Hessen, 1993), result in reduced ingestion rates of grazers (Cowles, Olson & Chisholm, 1988; Van Donk & Hessen, 1993), and/or cause low growth rates of grazers (Yoshida *et al.*, 2003; Yoshida, Hairston & Ellner, 2004) (Appendix A2). However, it remains unknown whether such nutrient-deficient cells can be detected (and rejected) by herbivores before ingestion. Low-food-value algae are thus less-edible prey, which either represents an efficient strategy for grazing resistance in nutrient-depleted environments, or simply is an unavoidable effect of the low nutritional value of nutrient-starved cells. However, for *Chlorella* sp., the growth rate of a population under nutrient-deficient conditions and in the presence of grazers ('defended algae') was 28% below that of a population grown under the same nutrient deficiency but in the absence of grazers ('undefended algae') (Yoshida, Hairston & Ellner, 2004), indicating that this strategy has an associated cost.



### (3) Behavioural defences

Resting stages and motility state may function as grazer-avoidance adaptations. It has been suggested that reduced excystment of resting cysts in the presence of high grazer abundance (Hansson, 1996, 2000; Rengefors, Karlsson & Hansson, 1998) and induced production of temporary cysts in the presence of parasites (Toth *et al.*, 2004) represent such adaptations. Motility itself can be viewed as a grazer-avoidance adaptation in phytoplankton since motile organisms can avoid grazers, for instance by increased swimming velocities away from grazers, or by escape jumps when entrained in a grazer feeding current (Jakobsen, 2001; Harvey & Menden-Deuer, 2012). Evidence of behavioural defences is summarized in Appendix A3.

#### (a) Resting stages

A common trait among phytoplankton is the production of dormant resting stages, such as cysts in dinoflagellates or spores in diatoms, which have been regarded as a strategy for survival during periods of adverse environmental conditions (see Rengefors, Karlsson & Hansson, 1998 and references therein). Moreover, some phytoplankton can respond to unfavourable abiotic as well as biotic environmental conditions by forming temporary cysts that, unlike dormant cysts, can hatch within a few hours (see Lundgren & Granéli, 2011, and references therein; Toth *et al.*, 2004). Regardless of what factors induce the formation of cysts or spores, empirical evidence indicates that they can function as a defence mechanism against parasite infection (Toth *et al.*, 2004) as well as predation (Montresor, Nuzzo & Mazzocchi, 2003; Kuwata & Tsuda, 2005; Lundgren & Granéli, 2011). The presence of parasites in the environment induces life-history shifts in the dinoflagellate *Alexandrium ostenfeldii*, which rapidly forms temporary cysts that are more resistant to infection than vegetative cells (Toth *et al.*, 2004). The function of cysts as a defence mechanism against grazing can be seen as: (i) a predator-avoidance strategy, where germination rates decrease or are completely suppressed when grazing zooplankton are present (Hansson, 1996, 2000; Rengefors, Karlsson & Hansson, 1998); and (ii) a morphological defence strategy, since cysts are relatively inedible to mesozooplankton (reduced ingestion rates by >75%) owing to their morphological properties (Montresor, Nuzzo & Mazzocchi, 2003; Lundgren & Granéli, 2011). The trade-offs are fairly straightforward: encysted cells experience much lower grazing mortality and parasitic attacks, but pay a cost in the form of lost opportunities for resource acquisition and proliferation.

#### (b) Motility

Several species from various phytoplankton groups are motile. Motile organisms can regulate their movement behaviour based on chemical or hydromechanical cues from grazers, for example, by directed swimming away from regions of high herbivore concentrations (Harvey &

Menden-Deuer, 2012), or by escape jumps when entrained in zooplankton feeding currents (Jakobsen, 2001). In addition, motile prey when captured by microzooplankton may escape by jumping or swimming away (Jakobsen, Everett & Strom, 2006; Nielsen & Kiørboe, 2015). However, changes in swimming behaviour are only effective against certain grazing strategies, for example, when the grazer generates a fluid signal strong enough for the prey to detect it. Grazer-induced changes in phytoplankton movement behaviour generally lead to reduced grazer ingestion rates due to decreased encounter rates, and thus increased survivorship of the prey (Buskey, 1997; Jakobsen, 2001; Tillmann & Reckermann, 2002; Jakobsen, Everett & Strom, 2006; Selander *et al.*, 2011; Harvey & Menden-Deuer, 2012). Such changes in behaviour may provide a clear benefit in terms of predator avoidance, but again involve certain costs. Firstly, swimming itself requires energy, although it is usually considered relatively inexpensive for microbes (Berg, 1993). Powerful escape jumps, however, are energetically costly, but such events are typically rare and brief, and so represent only a minute fraction of the organism's energy budget (Jiang, 2011). Secondly and probably more importantly, are the costs associated with the production and maintenance of a propulsion apparatus (one or a few flagella, or many cilia); these are difficult to quantify, but may also be low (Johnson *et al.*, 2009). Finally, the most significant cost to motility is an elevated predation risk for motile phytoplankton compared to non-motile, because motility itself can lead to increased encounters with predators. In addition, rheotactic predators (e.g. copepods) may detect fluid disturbances generated by swimming cells that can consequently lead to elevated encounter rates with the predator (Kiørboe *et al.*, 2014). While motility will have other functions, such as foraging in mixotrophs, or locating nutrient patches in autotrophs, there will be an obvious trade-off between escaping and hiding strategies.

### III. CONCLUSIONS

(1) At steady state, the number of coexisting species in phytoplankton communities cannot exceed the number of limiting resources unless additional mechanisms are involved. One such mechanism is predation, which may increase the diversity of phytoplankton communities by facilitating the evolution of defence mechanisms, provided that the employment of defences has an associated cost. While empirical and theoretical studies have documented the importance of predation in maintaining and enhancing biodiversity, the function of many proposed defence mechanisms still remains elusive, and any trade-offs often remain unquantified or unknown.

(2) Many suggested defensive traits are inducible, that is, their intensity increases in the presence of grazers or grazer cues. This suggests that these proposed traits indeed have a defensive function, whether or not the mechanism is understood. It also suggests the presence of a cost to such

defences: if there was no cost, why would phytoplankton only use these defences when needed?

(3) In response to grazing, phytoplankton have evolved various morphological defensive traits. Size *per se* offers partial protection against grazing, as larger cells are subject to lower mortality than smaller cells, and this is traded off against lower growth rates (which may partly account for the size diversity of phytoplankton communities). Variations in size can also be achieved through colony formation (or suppression of colony formation) in response to grazing pressure. However, such size modifications are only beneficial against certain grazers (e.g. mesozooplankton) and can simultaneously promote grazing by other zooplankton (e.g. microzooplankton). Associated costs include impaired chain formation in motile phytoplankton that perform diel vertical migrations (e.g. *Alexandrium* spp.) leading to reduced ability to complete such migrations due to insufficient swimming velocities, and increased size-associated sinking speeds in large non-motile organisms. To mitigate the latter problem, colonial cells often contain more lipids than unicells (e.g. *Scenedesmus* spp.), which may be interpreted as an additional buoyancy mechanism to reduce sinking. Synthesis of lipids, however, will have a cost. Additionally, increases in size can lead to a reduction of chlorophyll-specific light absorption coefficients as well as nutrient uptake under nutrient-limiting conditions due to competition among colonial cells. These costs will depend strongly on environmental conditions, but can be quantified.

(4) The presence of calcite armour in coccolithophores and silicified cell walls in diatoms may reduce grazing mortality. Surprisingly, experimental evidence supporting mechanical protection against grazing by the shell remains limited. Even though calcification and silicification seem to be rather inexpensive processes in terms of energy expenditure, the dependency of coccolithophores and diatoms on ambient concentrations of inorganic calcium and silicon represents an obvious cost that can only be evaluated in the context of the prevailing environmental conditions. The impact of calcification and silicification on loss rates *via* sinking out of the euphotic zone is another cost that appears to be small relative to the typical growth rates of coccolithophores and diatoms.

(5) Phytoplankton produce a variety of chemical substances that have proposed defensive functions. Their effects on grazers are very variable: no response, deterred feeding, and toxic. Toxic effects cannot be considered a defensive trait since they will also benefit competitors. Therefore, cheaters should thrive, and it is difficult to envisage how such defence mechanisms could evolve as they seem to be an evolutionarily unstable strategy. Interpretation of these compounds as defensive compounds is partly inspired by comparison with defensive chemicals in terrestrial plants; however, unlike a tree or a bush that can afford to sacrifice a few leaves to deter grazers, the phytoplankton cell would die. Clearly, there is a need for theoretical exploration of how such 'defences' could evolve and function. Nonetheless, there are a few cases where convincing deterrent effects have been described, for example, when a cell is not eaten due to production of a chemical substance. Also, chemicals

with a suspected defensive function may attract secondary predators. The evolutionary coexistence of prey and predator may determine what effects those chemicals will have on the grazers.

(6) Resting stages and motility can be viewed as behavioural defence mechanisms in phytoplankton, and for these defensive traits the assessment of benefits and costs is fairly straightforward. For instance, delayed excystment due to unfavourable biotic conditions leads to lost opportunities for resource acquisition and proliferation, but at the same time reduces grazing mortality. Motile cells that are captured by herbivores can sever contact with a predator by jumping or swimming away, again reducing grazing mortality. On the other hand, fluid disturbances generated by swimming cells can be detected by rheotactic grazers, leading to elevated encounter rates and consequently increased mortality.

(7) Although many morphological, physiological, and behavioural defence mechanisms in phytoplankton are described, the associated trade-offs are often not considered. In many cases even the benefits of employing certain defences are undocumented, and often the costs remain unknown and unquantified (Appendices A1–A3). One reason for this lack of experimental evidence for the costs of defence mechanisms is that these costs are often only realized under natural conditions, for example, when vertical migration is prevented due to reduced swimming speeds; or when the employment of certain traits depends on the availability of specific nutrients. In such cases, a mechanistic understanding of the component processes (e.g. reduced swimming speed due to grazer-induced unicellularity in chain-forming phytoplankton), combined with simple fitness-optimization models may allow quantification of the costs under specific environmental conditions (e.g. depth of mixed layer). Another reason for the lack of cost estimates is that some costs may be manifest only when cells are resource limited. Resource-sufficient environments, to which experimental organisms are most often exposed, can support resource- and energy-demanding growth, reproduction, and defence, as there is no conflict in resource allocation between growth and defence. However, when an organism is exposed to nutrient-deficient conditions, a metabolic rivalry for limiting resources between growth and defence is more likely to occur. Examples of this include reduced light harvesting in colonial cells that only takes place under light-limiting conditions; or reduced growth of toxin-producing cells (N-rich toxins) that may be found only under nitrogen-limiting conditions.

(8) Based on the above findings, two strategies for quantifying the costs of defences can be suggested: (i) for defence costs that are only realized under natural conditions, the evaluation of costs (and benefits) requires a mechanistic understanding of the hypothesized component processes. (ii) The magnitude of growth reduction should be assessed under conditions of resource limitation. In order to isolate the relevant resource(s) that should be limited in such experiments (e.g. nitrogen for PST-producing cells, or light for colony formation), an understanding of the processes involved in the defence is required. Simple

fitness-optimization models can then be used to predict the optimal strategy and assess trade-offs under a range of relevant environmental conditions; a similar approach has been employed in studies on foraging or vertical migration trade-offs in zooplankton (e.g. Visser, 2007; Sainmont, Thygesen & Visser, 2013).

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## APPENDICES. MORPHOLOGICAL, PHYSIOLOGICAL AND BEHAVIOURAL DEFENSIVE TRAITS IN PHYTOPLANKTON

Appendix A1. Morphological defence mechanisms in phytoplankton, when exposed to specific grazers at given environmental conditions as well as assessments of associated benefits and costs. \*, defensive traits that were not induced by grazers, but were solely compared with prey that do not possess the defence mechanisms (e.g. naked *E. huxleyi* versus calcified *E. huxleyi*); –, no differences observed; ?, not reported, or unknown benefits of specific defensive traits, as phytoplankton were not exposed directly to grazers but only to their cues/filtrate

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Scenedesmus subspicatus</i>	<i>Daphnia magna</i>	Nutrient-replete medium	Colony formation (spine-armoured cells)	Reduced clearance rate of grazers by 30–44%	–	Hessen & Van Donk (1993)
<i>Scenedesmus acutus</i>	<i>Daphnia magna</i>	Nutrient-replete medium	Colony formation	–	–	Lampert, Rothhaupt & von Elert (1994)
<i>Scenedesmus obliquus</i>	<i>Daphnia magna</i> filtrate	Nutrient-replete medium and <i>Microcystis aeruginosa</i> competitor	–	?	–	Zhu <i>et al.</i> (2015)
<i>Scenedesmus acutus</i>	<i>Daphnia magna</i> filtrate	Nutrient-replete medium	Cell-size increase and colony formation	?	Reduced carrying capacity (cells ml <sup>-1</sup> ) by 32%	Lürling (1998)
<i>Scenedesmus acutus</i>	<i>Daphnia magna</i> filtrate	Nutrient-replete medium	Colony formation	?	Increased settling velocities by 77%	Lürling & Van Donk (2000)
<i>Scenedesmus obliquus</i>	<i>Ceriodaphnia reticulata</i> filtrate	Nutrient-replete medium	Colony formation	?	–	Lürling (2003)
	<i>Daphnia galeata</i> × <i>hyalina</i> filtrate					
	<i>Daphnia magna</i> filtrate					
	<i>Daphnia pulicaria</i> filtrate					
<i>Scenedesmus acutus</i>	<i>Daphnia cucullata</i>	Nutrient-replete medium	Colony formation	Reduced clearance rate by 35% and population growth rate of grazers by 28%	–	Lürling & Van Donk (1996)
<i>Scenedesmus acutus</i>	<i>Daphnia pulex</i>	P-insufficient medium	Colony formation	–	–	O'Donnell, Fey & Cottingham (2013)
	<i>Daphnia magna</i> filtrate	P-sufficient medium	Colony formation	?	–	
<i>Scenedesmus acutus</i>	<i>Daphnia magna</i> filtrate	Nutrient-replete medium	Colony formation	?	–	Lürling & Van Donk (1997)
	<i>Daphnia galeata</i> filtrate					
	<i>Brachionus calyciflorus</i> filtrate					
	<i>Eudiaptomus gracilis</i> filtrate					
	<i>Bosmina longirostris</i> filtrate					

Appendix A1. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Scenedesmus acutus</i>	<i>Daphnia cucullata</i>	Nutrient-replete medium	Colony formation	Reduced clearance rate by 61–96% and population growth rate of grazers by 32% Reduced clearance rate by 0–93% and population growth rate of grazers by 5%	Increased fatty acid concentration by 71% and changed composition	Lürling, De Lange & Van Donk (1997)
<i>Scenedesmus obliquus</i>	<i>Daphnia magna</i>			Reduced clearance rate by 61–96% and population growth rate of grazers by 32% Reduced clearance rate by 0–93% and population growth rate of grazers by 5%	Increased fatty acid concentration by 50% and changed composition	
<i>Scenedesmus subspicatus</i>	<i>Daphnia cucullata</i>			Reduced clearance rate by 61–96% and population growth rate of grazers by 32% Reduced clearance rate by 0–93% and population growth rate of grazers by 5%	Increased fatty acid concentration by 50% and changed composition	
	<i>Daphnia magna</i>			Reduced clearance rate by 61–96% and population growth rate of grazers by 32% Reduced clearance rate by 0–93% and population growth rate of grazers by 5%	Reduced specific light absorption coefficient by 17–67%	Lürling (1999)
<i>Phaeocystis globosa</i>	<i>Pseudodiaptomus poplesia</i> filtrate <i>Euplates</i> sp. filtrate	Nutrient-replete medium Nutrient-depleted medium Nutrient-replete medium Nutrient-depleted medium	Colony formation	?	Reduced growth rate by 30%	Wang <i>et al.</i> (2015)



Appendix A1. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
	<i>Oxyrrhis marina</i> filtrate	Nutrient-replete medium			–	
		Nutrient-depleted medium			Reduced growth rate by 18%	
<i>Phaeocystis globosa</i>	<i>Gyrodinium dominans</i> grazing on other algae – filtrate <i>Glenodinium</i> cf. <i>danicum</i> grazing on other algae – filtrate <i>Temora longicornis</i> grazing on other algae – filtrate	Nutrient-replete medium	Colony formation	?	–	Tang (2003)
<i>Phaeocystis antarctica</i>	Natural zooplankton assemblage filtrate	Nutrient-replete medium	Colony formation	?	–	Tang <i>et al.</i> (2008)
<i>Phaeocystis globosa</i>	<i>Acartia tonsa</i> (live and filtrate) <i>Euphoties</i> sp. (live and filtrate)	Nutrient-replete medium	Colony formation suppressed Colony formation	Reduced feeding rate of grazers by 75% Reduced population growth rate of grazers by 68%	– Reduced growth rate by 9%	Long <i>et al.</i> (2007)
<i>Phaeocystis globosa</i>	<i>Acartia</i> sp.	Nutrient-replete medium P-insufficient medium	Colony formation suppressed	–	–	Lundgren & Grandli (2010)
		N-insufficient medium		Increased mortality of grazers by 8–19% Increased mortality of grazers by 8–46%		
<i>Phaeocystis globosa</i>	<i>Noctiluca scintillans</i> <i>Gyrodinium dominans</i>	Nutrient-replete medium	Colony formation	–	–	Jakobsen & Tang (2002)
				Reduced population growth rate of grazers by >89%		
<i>Microcystis aeruginosa</i>	<i>Ochromonas</i> sp. filtrate	Nutrient-replete medium	Colony formation	Reduced clearance rate by 83% and population growth rate of grazers by 23%	Increased settling velocities by 86%; effective quantum field of photosystem II decreased	Yang <i>et al.</i> (2009)
<i>Desmodosmus subspicatus</i>	<i>Daphnia</i> kairomone	Nutrient-replete medium and <i>Monoraphidium griffithii</i> competitor	Colony formation	?	Increased competitive exclusion rate	Yokota & Sterner (2011)

## Appendix A1. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Skeletonema marinoi</i>	<i>Acartia tonsa</i>	Nutrient–replete medium	Chain formation suppressed	Reduced clearance rate of grazers by 30%	–	Bergkvist <i>et al.</i> (2012)
	<i>Centropages hamatus</i>		Chain formation suppressed	?		
	<i>Temora longicornis</i>		Chain formation suppressed	?		
<i>Skeletonema marinoi</i>	<i>Gyrodinium dominans</i>	Nutrient–replete medium	Chain formation	?		Bjærke <i>et al.</i> (2015)
	<i>Acartia clausi</i>		Chain formation suppressed	Reduced clearance rate of grazers by 74–92%	–	
	<i>Strobilidium spiralis</i>		Chain formation	Reduced clearance rate of grazers by 100%		
<i>Cochlodinium polykrikoides</i>	<i>Acartia tonsa</i>	Nutrient replete medium	Chain formation	Reduced clearance rate of grazers by 84%	–	Jiang, Lonsdale & Gobler (2010)
<i>Alexandrium tamarense</i>	<i>Centropages typicus</i> (live and filtrate)	Nutrient–replete medium	Chain formation suppressed	Potentially reduced encounter rates with grazers due to reduced velocity by >38%	–	Sclander <i>et al.</i> (2011)
<i>Alexandrium tamarense</i>	<i>Calanus</i> sp. filtrate	Nutrient–replete medium	Chain formation suppressed	?	–	Sclander <i>et al.</i> (2012)
	<i>Centropages typicus</i> filtrate					
<i>Thalassiosira weissflogii</i>	<i>Acartia tonsa</i> filtrate	Nutrient–replete medium	Cell wall silicification	?	–	Pondaven <i>et al.</i> (2007)
<i>Thalassiosira weissflogii</i>	<i>Calanus helgolandicus</i> filtrate	Nutrient–replete medium and low irradiance	Cell wall silicification*	Reduced clearance rate by 43–68%, fecundity of grazers by 88%, and egg hatching success by 71% relative to <i>T. weissflogii</i> at high irradiance	Reduced growth rate by 67%	Liu <i>et al.</i> (2016)
	<i>Parvocalanus crassirostris</i>					
<i>Emiliania huxleyi</i>	<i>Oxyrrhis marina</i>	Nutrient–replete medium	Calcified cells*	Reduced population growth rate of grazers by 66% relative to naked cells	–	Harvey <i>et al.</i> (2015)
	<i>Gyrodinium dominans</i>			Reduced ingestion rate by 29% and population growth rate of grazers by 35% relative to naked cells		

Appendix A1. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
	<i>Protoperidinium</i> sp.			Reduced ingestion rate by >99% and population growth rate of grazers by 73% relative to naked cells		
<i>Emiliana huxleyi</i> haploid stage (non-calcified)	<i>Strombidinopsis acuminatum</i>	Nutrient-replete medium	(unknown defence mechanism)	Reduced ingestion rate of grazers on grazer-exposed haploid prey by 25–43% compared to naïve haploid prey	–	Kolb & Strom (2013)
			Cell type?*	Reduced ingestion rate of grazers on haploid prey by 89% compared to diploid prey		
<i>Phaeocystis globosa</i> (solitary flagellate)	<i>Temora longicornis</i> nauplii	Nutrient-replete medium	Cell type?*	Reduced ingestion rate on solitary flagellate relative to non-flagellate cells by 18–91% and increased mortality of grazers by 19–88%; poor development of grazers	–	Dutz & Koski (2006)



Appendix A2. Physiological defence mechanisms in phytoplankton, when exposed to specific grazers at given environmental conditions as well as assessments of associated benefits and costs. –, no differences observed; ?, not reported, not examined, or unknown benefits of specific defensive traits, as phytoplankton were not exposed directly to grazers but only to their cues/filtrate. A-FX, apo-fucoxanthinoid; DA, domoic acid; DMSP, dimethylsulphoniopropionate; EPS, exopolymer substance; HEPEs, hydroxyeicosapentaenoic acids; HepETEs, hydroxyepoxyeicosatetraenoic acids; PST, paralytic shellfish toxin; PUAs, polyunsaturated aldehydes; TEP, transparent exopolymer particle

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Alexandrium minutum</i> (two strains)	<i>Acartia clausi</i> <i>Centropages typicus</i>	Nutrient-replete medium	PST	Mortality of grazers – 47%		Bergkvist, Selander & Pavia (2008)
<i>Alexandrium minutum</i>	<i>Acartia tonsa</i>	Nutrient-replete medium and <i>Prorocentrum micans</i>	PST	Reduced clearance – rates on <i>A. minutum</i> by 18% (sole diet) and 31% (mixed diet)		Selander <i>et al.</i> (2006)
<i>Alexandrium minutum</i> (two strains)	<i>Acartia tonsa</i>	Nutrient-replete medium, or P-insufficient medium	PST	?	–	Selander, Cervin & Pavia (2008)
<i>Alexandrium tamarense</i>	<i>Calanus helgolandicus</i>	Nutrient-replete medium	PST	Affected escape behaviour of 29% of individuals; 14% died	–	Wohlrab, Iversen & John (2010)
	<i>Acartia clausi</i>			Affected escape behaviour of 10% of individuals; 40% died		
	<i>Oithona similis</i>			Affected escape behaviour of 65% of individuals; 21% died		
<i>Gymnodinium catenatum</i>	<i>Euterpina acutifrons</i>	Nutrient replete medium	PST	Reduced ingestion – rates within 24 h by 42–72%; 50% mortality rate after 5 days	–	da Costa, Carneiro Pereira & Fernández (2012)
	<i>Acartia grani</i>			Reduced ingestion rates within 24 h by 44–58%; 50% mortality rate after 5 days		
<i>Alexandrium lusitanicum</i>	<i>Acartia clausi</i>	Nutrient-replete medium	PST	Affected fecundity, – as gross growth efficiency decreased by 57% with increased cell concentration		Dutz (1998)
<i>Alexandrium ostenfeldii</i>	<i>Eurytemora affinis</i>	Nutrient-replete medium	PST	Little to no grazing; – rapid behavioural disturbances at high cell concentration in 60–95% of individuals		Sopanen <i>et al.</i> (2011)

## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
	<i>Acartia bifilosa</i>			Little to no grazing; rapid behavioural disturbances at high cell concentration in 35–70% of individuals		
<i>Alexandrium fundyense</i>	<i>Acartia hudsonica</i> (two geographically distinct species)	Nutrient-replete medium and <i>Tetraselmis</i> sp.	PST	Clearance rates of naïve copepods on toxic prey 70–100% lower relative to non-toxic; metabolic rate decreased by 33%; no impact on native copepods	–	Colin & Dam (2003)
<i>Karenia mikimotoi</i>	<i>Pseudocalanus elongatus</i>	Nutrient-replete medium and <i>Gyrodinium instriatum</i>	PST	Clearance rates on <i>K. mikimotoi</i> in mixed diet 67–85% lower relative to <i>K. mikimotoi</i> in sole diet	–	Schultz & Kiørboe (2009)
	<i>Temora longicornis</i>			Clearance rates on <i>K. mikimotoi</i> in mixed diet 75% lower relative to <i>K. mikimotoi</i> in sole diet		
<i>Alexandrium ostenfeldii</i> (two strains)	<i>Favella ehrenbergii</i>	Nutrient-replete medium	PST	Induced backward swimming, swelling, and lysis at high cell concentration	–	Hansen, Cembella & Moestrup (1992)
<i>Alexandrium tamarense</i> (several strains)	<i>Favella ehrenbergii</i>	Nutrient-replete medium	PST	Induced backward swimming, swelling, and lysis at high cell concentration	–	Hansen (1989)
<i>Alexandrium tamarense</i>	<i>Eurytemora herdmanni</i>	Nutrient-replete medium and <i>Lingulodinium polyedrum</i>	PST	–	–	Teegarden & Cembella (1996)
	<i>Acartia tonsa</i>			No or little grazing on <i>A. tamarense</i> in mixed diet		
<i>Alexandrium fundyense</i>	<i>Eurytemora herdmanni</i>			No or little grazing on <i>A. fundyense</i> in mixed diet		
<i>Karenia brevis</i>	<i>Acartia tonsa</i> <i>Calanus pacificus</i>	Nutrient-replete medium and <i>Gyrodinium resplendens</i>	PST (?)	Clearance rates on <i>K. brevis</i> 53% lower	–	Huntley <i>et al.</i> (1986)

## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Scrippsiella acuminata</i>	<i>Calanus pacificus</i>			Clearance rates on <i>S. acuminata</i> 71% lower	Lower growth rates than 'non-rejected' (non-toxic or less toxic) species (?)	
<i>Protoceratium reticulatum</i>	<i>Calanus pacificus</i>			Clearance rates on <i>P. reticulatum</i> 69% lower; mortality increased by 80%; no egg production	Lower growth rates than 'non-rejected' (non-toxic or less toxic) species (?)	
	<i>Paracalanus parvus</i>			Clearance rates on <i>P. reticulatum</i> 36% lower		
<i>Karenia brevis</i>	<i>Calanus pacificus</i>	?	PST (?)	Erratic mouthpart movements; nervous twitching of mouthparts; accelerated heart beat	—	Sykes & Huntley (1987)
<i>Scrippsiella acuminata</i>				Occasional mouthpart twitching; animals failed to maintain full guts		
<i>Protoceratium reticulatum</i>				Animals regurgitated their gut content; failed to maintain full gut; ceased feeding		
<i>Alexandrium fundyense</i>	<i>Acartia hudsonica</i> (two geographically distinct species)	Nutrient-replete medium and non-toxic <i>Alexandrium tamarense</i>	PST	Ingestion rates of native and naïve animals on toxic <i>A. fundyense</i> in mixed diet by 67% and 84% lower, respectively	—	Senft-Batoh <i>et al.</i> (2015)
<i>Alexandrium fundyense</i>	<i>Temora longicornis</i>	Nutrient-replete medium and <i>Rhodomonas lens</i>	PST	Increased swimming velocity by 21% (increased theoretical encounter rates with secondary predators by 56%)	—	Lasley-Rasher <i>et al.</i> (2016)
Non-toxic <i>Pseudo-nitzschia pungens</i> in dissolved DA	<i>Euphausia pacifica</i>	Nutrient-replete medium	DA	Ingestion rates reduced by 43–73% with increased DA concentration	—	Bargu, Lefebvre & Silver (2006)
<i>Pseudo-nitzschia seriata</i> <i>Pseudo-nitzschia obtusa</i>	<i>Calanus</i> spp.	Nutrient-replete medium	DA	—	—	Harðardóttir <i>et al.</i> (2015)



## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Pseudo-nitzschia seriata</i>	<i>Calanus finmarchicus</i>	Nutrient-replete medium (50% Si)	DA	Ingestion rates reduced by 73% (6 versus 12 h)	–	Tammilehto <i>et al.</i> (2012)
	<i>Calanus hyperboreus</i>			Ingestion rates reduced by 58% (6 versus 12 h)		
<i>Microcystis aeruginosa</i>	<i>Diaptomus birgei</i>	?	Microcystin	Rapid mortality at high cell concentration	–	DeMott, Zhang & Carmichael (1991)
	<i>Daphnia pulex</i>			Clearance rates reduced by 90% relative to non-toxic prey		
	<i>Daphnia hyalina</i>			Clearance rates reduced by 78% relative to non-toxic prey		
	<i>Daphnia pulex</i>			Clearance rates reduced by 50% relative to non-toxic prey; rapid mortality		
<i>Microcystis aeruginosa</i>	<i>Daphnia ambigua</i>	Nutrient-replete medium and <i>Chlamydomonas reinhardtii</i>	Microcystin	Low survivorship of grazers at high cell concentration; no reproduction; low filtering rates on <i>M. aeruginosa</i> in mixed diet	–	Fulton & Paerl (1987)
	<i>Diaphanosoma brachyurum</i>			Low survivorship of grazers at high cell concentration		
	<i>Keratella mixta</i>			Low survivorship of grazers at high cell concentration		
	<i>Brachionus calyciflorus</i>			Low growth of grazers		
	<i>Diaptomus reighardi</i>			Filtering rates on <i>C. reinhardtii</i> reduced at high concentration of <i>M. aeruginosa</i> ; low filtering rates on <i>M. aeruginosa</i>		
	<i>Eurytemora affinis</i>			?		
	<i>Tropocyclops prasinus</i>			?		
	<i>Platytia patulus</i>			?		
	<i>Bosmina longirostris</i>			Low survivorship, growth, reproduction, and filtering rates of grazers		

## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
	<i>Ceriodaphnia quadrangularis</i> <i>Sida crystallina</i>			Low filtering rates on <i>M. aeruginosa</i> Filtering rates on <i>C. reinhardi</i> reduced by the presence of <i>M. aeruginosa</i> ; low filtering rates on <i>M. aeruginosa</i>		
	<i>Simocephalus serratulus</i>			Filtering rates on <i>C. reinhardi</i> reduced at high concentration of <i>M. aeruginosa</i> , but similar on both species		
<i>Microcystis aeruginosa</i>	<i>Daphnia magna</i>	Nutrient-replete medium	Microcystin	Low survivorship of – grazers		Jang, Jung & Takamura (2007)
	<i>Moina macrocopa</i>					
<i>Planktotrix agardhii</i>	<i>Daphnia magna</i>					
	<i>Moina macrocopa</i>					
<i>Microcystis aeruginosa</i>	<i>Daphnia pulex</i>	Nutrient-replete medium and <i>Scenedesmus acutus</i>	Microcystin	Reduced filtering – rates and growth rates by 17–93% and 32–100% with increased cell concentration; low survivorship of grazers (0% in 2 days)		Lampert (1981)
Toxin from <i>Trichodesmium thiebautii</i>	<i>Artemia salina</i>	–	Cyano-bacterial toxin	Increasing mortality of grazers with increasing toxin concentration (up to 100%)	–	Hawser <i>et al.</i> (1992)
	<i>Macrosetella gracilis</i>			?		
	<i>Miracia efferata</i>			?		
	<i>Clausocalanus furcatus</i>			Increasing mortality of grazers with increasing toxin concentration (up to 98%)		
	<i>Farranula gracilis</i>			Increasing mortality of grazers with increasing toxin concentration (up to 98%)		
<i>Aureoumbra lagunensis</i>	<i>Oxyrrhis marina</i>	Nutrient-replete medium	EPS	Reduced growth – rates of grazers by 16% relative to low-EPS prey		Liu & Buskey (2000)
	<i>Euplotes</i> sp.			Reduced growth rates by 77–97% relative to low-EPS prey		

## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
	<i>Aspidisca</i> sp.			Reduced growth rates and clearance rates by 35% and 62–96% relative to low-EPS prey		
<i>Thalassiosira weissflogii</i> and <i>Rhodomonas</i> sp. in TEP from <i>Phaeocystis globosa</i>	<i>Temora longicornis</i>	Nutrient-replete medium, and N-insufficient medium	TEP	Reduced ingestion rates of nauplii and females by 44–84% and 21–45% relative to control (no TEP), as well as with increased TEP concentration by 64–80 and 43–56%	–	Dutz, Breteler & Kramer (2005)
<i>Thalassiosira weissflogii</i> in TEP	<i>Euphausia pacifica</i>	Nutrient-replete medium	TEP	Ingestion rates reduced by 53–89% relative to control (no TEP)	–	Passow & Alldredge (1999)
<i>Thalassiosira pseudonana</i> in semisynthetic compounds	<i>Tigriopus californicus</i>	Nutrient-replete medium	A-FX	Reduced feeding activity and increased mortality rates with increased A-FX concentration	–	Shaw, Andersen & Harrison (1997)
<i>Chaetoceros socialis</i>	<i>Temora stylifera</i>	Nutrient-replete medium	Oxylipins (HEPEs, HepETEs)	Egg viability reduced by 50% within 2 weeks relative to control	–	Fontana <i>et al.</i> (2007)
<i>Chaetoceros affinis</i>	<i>Calanus helgolandicus</i> <i>Temora stylifera</i>			Egg viability reduced by 20–100% within 2 weeks relative to control; produced morphologically deformed nauplii		
<i>Skeletonema costatum</i>	<i>Calanus helgolandicus</i> <i>Temora stylifera</i>	?	PUAs (linear aldehydes)	Fecundity decreased by 96% within 4 days; egg viability reduced by 100% within 7 days	–	D'Ippolito <i>et al.</i> (2002)
<i>Thalassiosira rotula</i>				Egg viability reduced by 90% within 2 weeks		



## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Skeletonema costatum</i>	<i>Calanus helgolandicus</i> ?		PUAs (decadial)	Nauplii mortality 100% within 4–14 days (females and larvae fed <i>S. costatum</i> ); egg viability reduced by 90% within 9 days; 45–65% of nauplii fed <i>S. costatum</i> morphologically deformed after 6 days	–	Ianora <i>et al.</i> (2004)
<i>Skeletonema costatum</i> , <i>Pseudo-nitzschia delicatissima</i>	<i>Acartia clausi</i>  <i>Calanus helgolandicus</i>	<i>In situ</i> study	PUAs (decatrienal, decadial)	Egg viability reduced by 76–88% during bloom relative to post-bloom conditions Egg viability reduced by 88% during bloom relative to post-bloom conditions	–	Miralto <i>et al.</i> (1999)
<i>Skeletonema marionoi</i> , <i>Melosira nummuloides</i>	<i>Tisbe holothuriae</i>	Nutrient-replete medium	PUAs	?	–	Taylor <i>et al.</i> (2007)
<i>Thalassiosira rotula</i>	<i>Calanus helgolandicus</i>	Nutrient-replete medium	PUAs, hydrocarbons, (and long-chain saturated aldehydes)	Egg viability reduced by 45% relative to control	–	Pohnert <i>et al.</i> (2002)
<i>Skeletonema pseudocostatum</i>			Long-chain saturated aldehydes	Lower fecundity relative to control		
<i>Emiliania huxleyi</i> (strains with high-DMSP lyase activity)	<i>Oxyrrhis marina</i>  <i>Dunaliella tertiolecta</i>	Non-DMSP-containing prey, and low-DMSP lyase activity <i>E. huxleyi</i> strains	DMSP	High-activity strains rejected or avoided in mixed diet; grazing rates reduced by 79% in sole diet relative to low-DMSP prey High-activity strains rejected or avoided in mixed diet; grazing rates reduced by 40–98% in sole diet relative to low-DMSP prey	–	Wolfe, Steinke & Kirst (1997)

## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
Various prey in dissolved DMSP	<i>Strombidinopsis acuminatum</i>	Nutrient-replete medium	DMSP	Feeding rates reduced by 50% relative to control	—	Fredrickson & Strom (2009)
	<i>Favella</i> sp.			Feeding rates reduced by 48–75% relative to control		
	<i>Noctiluca scintillans</i>			Feeding rates reduced by 28–40% relative to control		
<i>Emiliana huxleyi</i> (strains with high-DMSP lyase activity)	<i>Coxiella</i> sp.	Nutrient-replete medium and low-DMSP lyase activity <i>E. huxleyi</i> strains	DMSP	High-activity strains rejected or avoided in mixed diet; feeding rates reduced by 11–22% in sole diet (normal growth)	—	Strom <i>et al.</i> (2003)
	<i>Strombidinopsis</i> sp.			High-activity strains rejected or avoided in mixed diet; feeding rates reduced by 27% in sole diet (no growth)		
	<i>Metacalis</i> sp.			High-activity strains rejected or avoided in mixed diet; feeding rates reduced by 18–65% in sole diet		
	<i>Amphidinium longum</i>			High-activity strains rejected or avoided in mixed diet; feeding rates reduced by 96% in sole diet (no growth)		
	<i>Oxyrrhis marina</i> <i>Gymnodinium</i> sp.			—		
<i>Emiliana huxleyi</i> (high-DMSP lyase activity strain)	<i>Oxyrrhis marina</i>	Nutrient-replete medium	DMSP	Feeding rates reduced by 58% relative to low-DMSP <i>E. huxleyi</i>	Growth rates 33% lower	Wolfe & Steinke (1996)

## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Lingulidinium polyedra</i>	<i>Calanus finmarchicus</i>	Nutrient–replete medium	Luminescence	?	–	Lindström <i>et al.</i> (2017)
<i>Alexandrium tamarense</i>	<i>Acartia tonsa</i>	Nutrient–replete medium	Luminescence	Ingestion rates reduced—by 50–75% relative to weakly luminescent prey		White (1979)
<i>Alexandrium tamarense</i>	<i>Acartia tonsa</i>	Nutrient–replete medium	Luminescence	Ingestion rates reduced—by 30% relative to prey with reduced capacity for luminescence		Esaias & Curl (1972)
<i>Gonyaulax acatenella</i>	<i>Acartia clausi</i>			Ingestion rates reduced by 36–74% relative to prey with reduced capacity for luminescence		
	<i>Acartia longiremis</i>			Ingestion rates reduced by 64% relative to prey with reduced capacity for luminescence		
<i>Alexandrium catanella</i>	<i>Calanus pacificus</i>			Ingestion rates reduced by 82% relative to prey with reduced capacity for luminescence		
<i>Pyrocystis fusiformis</i>	<i>Holmesimysis costata</i> (mysid) <i>versus</i> <i>Porichthys notatus</i> (fish)	Nutrient–replete medium	Luminescence – burglar alarm	Increased predation rate success of fish on mysid by 29% in presence of luminescent relative to non-luminescent dinoflagellates (consequently reduced grazing rates on luminescent dinoflagellates?)	–	Mensinger & Case (1992)
<i>Pyrocystis fusiformis</i>	<i>Holmesimysis sculpta</i> (mysid), <i>Palaemonetes pugio</i> (grass shrimp), <i>Gambusia affinis</i> (mosquito fish) <i>versus</i> <i>Sepia officinalis</i> (cuttlefish)	Nutrient–replete medium	Luminescence – burglar alarm	Increased number of cuttlefish strikes; increased predation rate success of cuttlefish on prey by 95% in presence of luminescent dinoflagellates relative to control (consequently reduced grazing rates on luminescent dinoflagellates?)	–	Fleisher & Case (1995)
	<i>Palaemonetes pugio</i> (grass shrimp) <i>versus</i> <i>Euprymna scolopes</i> (squid)			Increased number of squid strikes by 41–53% in presence of luminescent dinoflagellates relative to control		



## Appendix A2. Continued

Phytoplankton	Zooplankton	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Scenedesmus subspicatus</i>	<i>Daphnia pulex</i>	P-insufficient medium	Low-food-value algae	Ingestion rates on P-limited algae reduced by 69% relative to control	Growth rates 76% lower	Van Donk & Hessen (1993)
<i>Selenastrum capricornatum</i>	<i>Daphnia pulex</i>			Ingestion rates on P-limited algae reduced by 70–77% relative to control	Growth rates 68–72% lower	
	<i>Daphnia magna</i>			Ingestion rates on P-limited algae reduced by 32% relative to control	Growth rates 68% lower	
<i>Chlorella vulgaris</i>	<i>Brachionus calyciflorus</i>	N-insufficient medium	Low-food-value algae	Growth rates of grazers lower by 32% relative to control	Growth rates 28% lower	Yoshida <i>et al.</i> (2003)
<i>Chlorella vulgaris</i>	<i>Brachionus calyciflorus</i>	N-insufficient medium	Low-food-value algae	Growth rates of grazers lower by 32% relative to control	Growth rates 28% lower	Yoshida, Hairston & Ellner (2004)

Appendix A3. Behavioural defence mechanisms in phytoplankton, when exposed to specific grazers at given environmental conditions as well as assessments of associated benefits and costs. \*, defensive traits that were not induced by grazers, but were compared with prey that do not possess that defence mechanism (e.g. motile *versus* non-motile prey); –, no differences observed.

Phytoplankton	Zooplankton/ parasite	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Ceratium hirundinella</i>	Freshwater herbivores filtrate	Nutrient-replete medium and various temperatures	Reduced excystment	Excystment rate reduced by <61%	Missed opportunities for growth	Rengefors, Karlsson & Hansson (1998)
<i>Peridinium aciculiferum</i>				Excystment rate reduced by >48%		
<i>Peridinium pusillum</i> , <i>P. wisconsinense</i>	Freshwater herbivores	<i>In situ</i> study	Reduced excystment	Excystment rates reduced or suppressed entirely at high grazer abundances	Missed opportunities for growth	Hansson (1996)
<i>Gonyostomum semen</i>	<i>Daphnia magna</i>	Natural water		Excystment rate reduced by 86%		
<i>Peridinium</i> sp.	<i>Daphnia magna</i>	Natural water	Reduced excystment	Excystment rates suppressed entirely	Missed opportunities for growth	Hansson (2000)
<i>Gonyostomum semen</i>				Excystment rates reduced by >80% or suppressed entirely		

## Appendix A3. Continued.

Phytoplankton	Zooplankton/ parasite	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Scrippsiella trochoidea</i>	<i>Acartia tonsa</i>	Nutrient-replete medium	Temporary cysts	Ingestion rates and faecal pellet production reduced by 62% and 57% compared to vegetative cells; egg production reduced by 67%	Missed opportunities for growth	Lundgren & Granéli (2011)
<i>Alexandrium ostenfeldii</i>	Parasitic protozoan <i>Parvilucifera infectans</i>	Nutrient-replete medium	Temporary cysts	Decreased infection rate of parasites by 78%	Missed opportunities for growth	Toth <i>et al.</i> (2004)
<i>Scrippsiella trochoidea</i>	Various copepod species	Nutrient-replete medium	Resting cysts	Reduced ingestion rates by >78% compared to vegetative cells; survival rate of ingested cysts 76–81%	Missed opportunities for growth	Montresor, Nuzzo & Mazzocchi (2003)
<i>Scrippsiella ramonii</i>				Reduced ingestion rates by 75–83%		
<i>Heterosigma akashiwo</i>	<i>Favella</i> sp.	Nutrient-replete medium	Escape behaviour	Increased swimming velocity by 38%, and increased vertical speed towards refuge by 29% resulting in reduced encounter rates	–	Harvey & Menden-Deuer (2012)
<i>Balanion comatum</i>	<i>Temora longicornis</i> (feeding-current feeder)	Nutrient-replete medium	Escape behaviour (jumping)*	Reduced ingestion rates by 93% relative to non-jumping <i>Heterocapsa triquetra</i>	–	Jakobsen (2001)
<i>Lingulodinium polyedra</i> , <i>Prorocentrum micans</i>	<i>Protoperidinium pellucidum</i> (pallium-feeder)	Nutrient-replete medium; dinoflagellate diet <i>versus</i> diatom diet (non-motile)	Motility*	Reduced ingestion rates by 82% relative to non-motile diatom diet; 38% and 54% survivorship of dinoflagellates: of which 17% and 11% was due to escape success before, and 21% and 43% to escape success after being captured; reduced growth rates of grazers by 43% relative to non-motile diatom diet	–	Buskey (1997)
<i>Heterocapsa triquetra</i>	<i>Noctiluca scintillans</i> (interception-feeder)	Sole diet; mixed diet with diatoms	Motility*	Reduced ingestion rates by 84% in mixed diet, and 90% in sole diet relative to diatom diet; reduced growth rates of grazers by 70% relative to diatom diet	–	Kiørboe & Titelman (1998)

## Appendix A3. Continued.

Phytoplankton	Zooplankton/ parasite	Environmental characteristics	Defensive trait	Benefit	Cost	Reference
<i>Fibrocapsa japonica</i>	<i>Oblea rotunda</i> (pallium-feeder)	Nutrient-replete medium	Motility	92% survivorship: of which 55% was due to escape success before, and 37% to escape success after being captured	–	Tillmann & Reckermann (2002)
<i>Rhodomonas salina</i>	<i>Mesodinium pulex</i> (ambush-feeder)	Nutrient-replete medium	Motility	100% survivorship: of which 38% was due to avoided encounter, and 62% to escape success after being captured	–	Jakobsen, Everett & Strom (2006)
<i>Gymnodinium simplex</i>				96% survivorship due to avoided encounter		
<i>Heterocapsa rotundata</i>				75% survivorship: of which 70% was due to avoided encounter, and 5% to escape success after being captured		

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