Stoichiometric regulation of phytoplankton toxins

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Abstract

Ecological Stoichiometry theory predicts that the production, elemental structure, and cellular content of biomolecules should depend upon the relative availability of resources and the elemental composition of their producer organism. We review the extent to which carbon- and nitrogen-rich phytoplankton toxins are regulated by nutrient limitation and cellular stoichiometry. Consistent with theory, we show that nitrogen limitation causes a reduction in the cellular quota of nitrogen-rich toxins, while phosphorus limitation causes an increase in the most nitrogen-rich paralytic shellfish poisoning toxin. In addition, we show that the cellular content of nitrogen-rich toxins increases with increasing cellular N:P ratios. Also consistent with theory, limitation by either nitrogen or phosphorus promotes the cellular quota, or toxicity, of the investigated carbon-rich toxins. These observed relationships may assist in predicting and managing toxin-producing phytoplankton blooms. Such a stoichiometric regulation of toxins is likely not restricted to phytoplankton, and may well apply to carbon- and nitrogen-rich secondary metabolites produced by bacteria, fungi, and plants.

Key words: Ecological Stoichiometry, harmful cyanobacteria, Harmful Algal Blooms, natural toxins, nutrient limitation, secondary metabolites.

Introduction

It is well-established that the elemental composition of both prokaryotic and eukaryotic cells strongly depends upon the supplies of chemical elements, such as the key nutrients nitrogen (N) and phosphorus (P) (Sterner & Elser 2002; Klausmeier *et al.* 2004). Primary producers use light energy to convert CO_2 and nutrients into an enormous variety of organic compounds, including fatty acids, amino acids, and nucleic acids. These three key groups of biomolecules differ significantly in their elemental composition, notably in their relative carbon (C), N, and P contents. Similarly, there are major differences in the C:N:P stoichiometry of secondary cellular metabolites, which are known to serve as signaling molecules as well as deterrents against pathogens and herbivores (Keller & Surette 2006; Iason *et al.* 2013). Some of the secondary metabolites produced by planktonic aquatic primary producers (unicellular and colonial cyanobacteria and eukaryotic algae, collectively termed "phytoplankton" hereafter) are toxic to invertebrates, fish, birds, and mammals. Blooms of such toxic phytoplankton are of increasing global concern because their appearance and proliferation are driven by high anthropogenic loadings of N and P to surface waters (eutrophication) (Anderson *et al.* 2002; Smith 2003). Thus, there is a strong need to gain a better understanding of how the absolute and relative supplies of key nutrients affect the production of toxic compounds by bloom-forming phytoplankton.

Toxins produced by phytoplankton are stoichiometrically diverse, ranging from N-rich alkaloids to C-based polyketides (Table 1). Previous studies involving particular phytoplankton taxa have demonstrated that the production and cellular contents (internal cell "quota") of C- and N-rich toxins respond strongly to the relative availability of nutrients (Granéli *et al.* 1998; Sivonen & Jones 1999; Granéli & Flynn 2006; Van de Waal *et al.* 2009). Here, we present the first quantitative analysis of the regulation of phytoplankton toxins by nutrient limitation. We

analyzed >500 published studies and compiled a total of 180 datasets, covering 31 phytoplankton species representing 19 different genera (Fig. S1; Table S1). Using these data, we analyzed the toxin cell quotas or toxicities of phytoplankton cells grown under either N- or P-limitation, and we compared these responses with cells grown in nutrient-sufficient controls. Based on stoichiometric theory (Sterner & Elser 2002) and current understanding of phytoplankton physiology, we predicted that production of C-rich toxic compounds would be elevated under both N- and P-limitation because, under such conditions, cellular C is by definition in excess and can accumulate in secondary pools. Following similar reasoning, we also predicted that production of N-rich toxins would be inhibited under N-limitation (because N is shunted into intracellular pools to support active growth), but amplified under P-limitation (because N is in excess and accumulates in secondary intracellular pools).

Material and Methods

Data was obtained by ISI Web of Science searches with the terms: ("phytoplankton" or "cyanobacteri*" or "dinoflagellate*") and "*toxin*" and ("nutrient*" or "nitr*" or "phosph*") (Fig. S1; Tables S1 and S2). Estimates of toxin quota or toxicity of phytoplankton grown under low N and/or low P were recovered from the stationary growth phase of batch culture experiments, and from steady state conditions of continuous cultures. Toxin quotas or toxicities of cells grown under nutrient-sufficient conditions, or at maximum growth rates during the exponential phase, served as controls. Studies reporting earlier published data were not included. We made a distinction between N-rich and C-rich toxins based on elemental composition. Toxins with a molar C:N ratio <6.6 (i.e. less than the Redfield ratio) were considered "N-rich", whereas toxins with a higher molar C:N ratio were considered "C-rich" (Table 1). For each study we took

toxin quotas or toxicities and calculated the average response ratio (RR) by normalizing toxin quotas or toxicities from the low N and low P conditions to the control. Furthermore, we derived cellular N:P ratios expressed as molar ratios.

Toxins were found to be represented by varying numbers of studies (Fig. S1). Some toxins were produced by multiple species, and these toxins were represented by different numbers of species (Fig. S1). To correct for biases that may result from such unbalanced and nested data structure, we worked with weighted means and variances. First, we calculated the average response and variance for each toxin that was represented by at least three or more independent studies (i.e. $n>3$). In case a toxin was produced by multiple species, and one species was represented by more than two studies, we first calculated the mean of this species and then calculated the average response of the toxin across species. In case multiple species were represented by multiple studies, the weighted average response of a toxin across species could be calculated according to (Borenstein *et al.* 2009):

$$M_{RR,W} = \frac{\sum_{i=1}^{n} M_{RR} W_i}{\sum_{i=1}^{n} W_i} \quad (1)$$

with weight W_i being the reciprocal of the variance $V_{tox,\,sp}$, i.e. the variance among studies for each species *i*, calculated as:

$$V_{tox,sp} = \frac{1}{n-1} \sum_{i=1}^{n} (RR_i - M_{RR})^2 \quad (2)$$

Similarly, the overall weighted average response of toxins across the means of all N- or C-rich toxins was calculated. We assessed the statistical significance of differences between the calculated RRs and their respective controls with a one sample t-test comparing the weighted mean RR with 1, which represents the absence of a response ($\alpha<0.05$).

Results

The assembled studies yielded 100 datasets with N-rich toxins (49 with N-limitation and 51 with P-limitation) and 80 datasets with C-rich toxins (37 with N-limitation and 43 with P-limitation; Fig. S1). N-rich toxins predominantly consisted of Paralytic Shellfish Poisoning (PSP) toxins produced by marine dinoflagellates (n=57), followed by microcystins (MCs) produced by freshwater cyanobacteria (n=34). Data on C-rich toxins comprised 12 compounds, groups of compounds, or toxicities associated to C-rich toxins. The C-rich toxins were almost exclusively found in marine ecosystems and consisted of various toxins produced by dinoflagellates (including Diarrhetic Shellfish Poisoning (DSP) toxins, n=18, karlotoxins (KMTX), n=11, and Neurotoxic Shellfish Poisoning (NSP) toxins, n=10), diatoms (Amnesic Shellfish Poisoning (ASP) toxins, n=9), and prymnesiophytes (hemolytic activity (HA), n=12). Various toxins were only represented by a limited number of studies (n<3) and were therefore excluded from our analyses. For most of the toxins, experiments with N-limitation and P-limitation tended to be equally well-represented (Fig. S1).

The impact of nutrient limitation on toxin quota or toxicity varied greatly and depended strongly on the stoichiometric nature of the toxins. Consistent with theoretical predictions, our analysis revealed that N-rich toxins generally decreased upon N-limitation while C-rich toxins generally increased upon limitation by either N or P (Figs. 1 and 2). More specifically, cellular quota of both N-rich PSP toxins and MCs decreased significantly upon N-limitation, irrespective of the producing taxonomic group. Upon P-limitation, cellular quota of dinoflagellate PSP toxins increased by 32%, while MCs decreased by 75% (Fig. 1; Table 2). Relative changes in C-rich toxins were generally larger than the N-rich toxins, particularly under P-limitation, although variances within toxins were also higher. Significant increases in cellular toxin quota ranged

from 99% in NSP toxins under N-limitation to 215% in KMTX under P-limitation (Fig. 2; Table 2).

Under balanced, non-limiting growth conditions, molar phytoplankton N:P ratios typically converge upon the so-called 'Redfield ratio' of N:P=16 (Sterner & Elser 2002). Indeed, in the control treatments of the experiments we assembled, cellular N:P ratios resembled the Redfield ratio, whereas cellular N:P stoichiometry was almost always lower than Redfield under N-limitation and higher than Redfield under P-limitation (Fig. 3). N-rich toxins showed a positive linear relationship with cellular N:P ratios. The response ratios of the N-rich PSP toxins and MCs decreased below 1 at N:P ratios below the Redfield ratio, but increased beyond 1 when N:P ratios exceeded the Redfield ratio (Fig. 3a). In contrast, the C-rich DSP toxins and HA increased at N:P ratios both below and above the Redfield ratio (Fig. 3b).

Discussion

Our results demonstrate that the synthesis of N-rich and C-rich toxins is regulated by the relative availabilities of N and P (Figs. 1 and 2). Upon N-limitation, cellular quota of the N-rich PSP toxins and microcystins decreased, while upon P-limitation, cellular quota of the most N-rich PSP toxins increased. Cellular quota of microcystin, however, showed a strong decrease, and nodularin did not change upon P-limitation (Fig. 1). These contrasting responses to P-limitation may reflect the fact that, unlike eukaryotic algae, cyanobacteria can shunt excess N into the storage compound cyanophycin (Allen 1984), a process that likely prevents N from accumulating primarily as microcystin. C-rich toxins generally increased upon limitation by both N and P (Fig. 2). Most of the C-rich toxins were produced by eukaryotic algae and are polyketides, whose synthesis is tightly coupled with cellular C acquisition (Staunton &

Weissman 2001). Thus, under conditions of nutrient limitation, the relative excess of energy and newly-synthesized organic C cannot be used for cell growth and instead appears to be shunted into C-rich molecules such as lipids and polyketides, including those that have toxic effects. We also observed a general increase of N-rich toxins with cellular N:P ratio (Fig. 3a), while C-rich toxins showed an initial decrease with N:P ratios until reaching the Redfield ratio, after which their content increased (Fig. 4b). These findings are consistent with the 'V-shaped' response to N:P ratios that has been reported previously for hemolytic activity associated with C-rich compounds (Granéli & Flynn 2006; Granéli *et al.* 2012).

While most of the selected datasets reported cellular toxin quotas, a few (~6%) assessed toxicities relative to a standard toxin analogue, for instance as saxitoxin equivalents (Table S1). Changes in these relative toxicities are generally associated with changes in cellular toxin quota, yet may also relate to a shift in the toxin composition (Cembella 1998; Sivonen & Jones 1999). Because the few selected studies reporting relative toxicities generally showed none, or only minor shifts in toxin composition, we considered the observed changes in relative toxicities to mainly reflect changes in the N-rich toxin quota. All reported hemolytic activities of prymnesiophytes were assessed by bioassays and have been ascribed to various C-rich compounds, such as glycolipids and the polyether prymnesin (Edvardsen & Imai 2006; Granéli *et al.* 2012). Although hemolytic activity showed a high average response to nutrient limitation, the variance was also very high (Table 2), which may reflect a higher inherent variability of bioassay data relative to direct chemical measurements of the cellular quota of a specific compound. Nevertheless, our analysis reveals that the response of hemolytic activity by prymnesiophytes towards both N- and P-limitation is consistent with the general response of C-rich toxins, and therefore presumably reflects changes in cellular quotas of a C-rich compound.

Toxins produced by various harmful phytoplankton taxonomic groups have been shown to exhibit a broad range of potential functions, including grazer deterrence, prey capture, and resource uptake (see also Granéli & Turner 2006; Glibert & Burkholder 2011). The question remains whether our results reflect a possible role of toxins in acquiring N and P, or if the toxin quota merely responds to the relative availability of N and C within the cells. Production of N-rich toxins generally decreased with N-limitation, suggesting a cost rather than a benefit under N-limitation. Cellular contents of N-rich cyanobacterial toxins strongly decreased or remained unaltered upon P-limitation, whereas the quota of N-rich dinoflagellate PSP toxins increased. C-rich toxins in dinoflagellates and diatoms, as well as hemolytic activity caused by prymnesiophytes, also increased with P-limitation, and the dinoflagellate karlotoxin and NSP toxins increased significantly with N-limitation. These findings suggest that toxin synthesis may be related to resource acquisition, specifically C-rich toxins for acquiring P. Indeed, C-rich toxins produced by prymnesiophytes seem to be involved in prey capture (Skovgaard *et al.* 2003; Tillmann 2003). Furthermore, nutrient limitation may stimulate feeding by mixotrophic dinoflagellates and prymnesiophytes (Legrand *et al.* 2001; Smalley *et al.* 2003), though it is not clear whether this is related to their toxicity (Stoecker *et al.* 2006). Thus, whether toxins stimulated by nutrient limitation are indeed involved in resource acquisition and prey capture, or whether they merely respond to a relative excess of non-limiting resources required for their synthesis, needs to be further elucidated.

Our findings illustrate that the extent to which phytoplankton are able to produce toxins, and thereby exploit their putative function, will partially depend on the relative availabilities of N and P in the environment. Because N and P often limit phytoplankton growth in both freshwater and marine ecosystems (Elser *et al.* 2007), the cellular production of C-rich toxins

avoids resource allocation conflicts between population growth and secondary metabolite synthesis. Production of N-rich toxins, however, does decrease with N-limitation, suggesting that N is allocated to growth rather than secondary metabolites. Growth and the synthesis of biochemicals together will determine the elemental stoichiometry of phytoplankton, which shows distinct differences between taxonomic groups. For instance, dinoflagellates exhibit higher C:N and C:P ratios as compared to prymnesiophytes and diatoms (Quigg *et al.* 2003), while cyanobacteria have generally higher optimal N:P ratios than diatoms (Hillebrand *et al.* 2013). Most of the C-rich toxins studied here were indeed produced by dinoflagellates (i.e. 10 out of 12 toxins; Fig. S1), and the reported N-rich toxins were produced by cyanobacteria (i.e. MC, PSP, NOD and CYN) or seem to have a common origin with cyanobacteria (i.e. PSP; Moustafa *et al.* 2009; Hackett *et al.* 2013). We did not observe differences in the elemental composition between taxonomic groups, yet our results demonstrate that the cellular quota of the N-rich PSP toxins and microcystins are strongly correlated with N:P stoichiometry, and this relationship is irrespective of the producing taxonomic group, both at the genus as well as at the species level (Fig. 3). To what extent the stoichiometry of a taxonomic group may influence the stoichiometry of the toxins that can be produced remains to be elucidated. Obviously, cellular elemental ratios strongly reflect the intracellular content of major biochemicals such as proteins and nucleic acids (Sterner & Elser 2002). Here, we show that they may also determine, at least partially, the stoichiometry of secondary metabolites that can be synthesized by the cell.

The strong association of phytoplankton toxins with N:P stoichiometry has important implications for interpreting and predicting the responses of phytoplankton-associated toxins to human-induced changes in the magnitude and ratios of N and P supplies to surface waters. One of the clearest and most predictable responses of both freshwater and marine ecosystems to

224 increased nutrient loading is the profound stimulation of phytoplankton biomass, often by orders
225 of magnitude (Smith 2003; Dolman *et al.* 2012; Orihel *et al.* 2012). Total toxin concentrations in
226 natural waters will therefore tend to increase with eutrophication-driven increases in total
227 phytoplankton biomass, but the magnitude of this response will be amplified if nutrient
228 enrichment also selects for dominance by toxin-producing taxa (Anderson *et al.* 2002; Heisler *et*
229 *al.* 2008; Dolman *et al.* 2012; Scott *et al.* 2013) or clones (Kardinaal *et al.* 2007; Touzet *et al.*
230 2007) ENREF_25 ENREF_12 and if nutrient limitation promotes the internal accumulation of toxic
231 molecules, as shown in our analysis. These insights also provide a means for anticipating which
232 toxins will be triggered as a function of N- or P-limitation during bloom termination. Thus, the
233 stoichiometric relationships we have uncovered may provide a practical basis for better
234 forecasting the potential occurrence of toxic compounds in the aquatic environment, possibly
235 allowing the creation of an "early warning system" for more detailed chemical analyses of
236 suspended particulate matter, both in drinking water supplies and in surface waters that support
237 fisheries and heavy recreational use.

238 Our analysis demonstrates that the production of stoichiometrically distinct toxins can be
239 explained, at least partially, by the availabilities of key limiting nutrients in the environment and
240 within the cell. However, we note that the production of toxins may also be affected by other
241 environmental factors. In particular, C-rich toxins may be sensitive to changes in CO_2 and light
242 availability, as these resources provide phytoplankton with C and energy. Indeed, enhanced CO_2
243 availabilities have been shown to increase the production of C-rich toxins such as domoic acid
244 (Sun *et al.* 2011) and karlotoxin (Fu *et al.* 2010), particularly under P-limited conditions.
245 However, there seems no general response of C-rich toxins to increasing light intensities. More
246 specifically, increasing light intensities were shown to cause an increase (Bates 1998; Cusack *et*

al. 2002) or a decrease (Auro & Cochlan 2013) in the production of domoic acid, while the sensitivity of hemolytic activity (Baker *et al.* 2007; Granéli *et al.* 2012) and DSP toxins (Nielsen *et al.* 2013) to light intensity seems to be marginal or even absent. Enhanced light intensities were shown to cause an increase in the production of the N-rich PSP toxins (Parkhill & Cembella 1999; Etheridge & Roesler 2005) and microcystins (Wiedner *et al.* 2003; Tonk *et al.* 2005) at limiting light levels, but caused a decrease at saturating light levels. Generally, the response of N- and C-rich toxins to light availability seems less pronounced as compared to nutrient limitation, while enhanced CO_2 availabilities may further promote the production of C-rich toxins under nutrient limiting conditions.

Our results provide a broad picture of how the synthesis of toxins in response to nutrient limitation might be modified by internal allocation processes that shunt excess C and nutrients not to toxins but towards storage compounds, as in the case of N in cyanobacteria. In higher plants, nutrient limitation may result in a surplus of fixed C as well, which can also be allocated either to storage compounds or to C-rich secondary metabolites (Koricheva *et al.* 1998). Likewise, the availability of C and nutrients has been shown to affect synthesis of C- or N-rich secondary metabolites in bacteria and fungi (Sanchez & Demain 2002; Cornforth & Foster 2013). Even though secondary metabolites are highly diverse in function among producers, the synthesis of these molecules generally has a flexible component that at least partially depends on the interplay between their own elemental composition and the relative availability of key resources. Thus, toxin production can be expected to behave in broadly predictable ways across a wide variety of biota.

Ongoing anthropogenic loading of nutrients will lead to deterioration of water quality not only by promoting biomass accumulation of phytoplankton, but by affecting both the quantities

and the biochemical composition of the toxins they can produce, especially when nutrient loading leads to strong stoichiometric imbalances of N and P. For instance, nutrient inputs from nonpoint sources such as atmospheric N deposition or release of sediment-bound P (Carpenter *et al.* 1998; Elser *et al.* 2009) may shift phytoplankton from N- to P-limitation, or vice versa, and thereby alter their toxicity. Thus, application of stoichiometric theory, together with knowledge of the phytoplankton species and the stoichiometric composition of the toxins they produce, may help us predict whether nutrient loading will cause an increase, decrease, or no change in overall toxicity in natural waters (Anderson *et al.* 2002; Heisler *et al.* 2008), with important potential consequences for the various services provided by freshwater and marine ecosystems.

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Supporting Information

The following Supporting Information is available for this article:

Figure S1. Overview of investigated datasets grouped according to toxin stoichiometry, type of experimental nutrient limitation, habitat of origin, identity of toxin or toxin group, and taxonomic group (species or genus). Numbers between parentheses indicate for each category the number of datasets that were available for analysis.

Table S1. Toxin quota and toxicities of various phytoplankton species grown under low N, low P and nutrient-sufficient conditions.

Table S2. C:N:P stoichiometry of various phytoplankton species grown under low N, low P and nutrient-sufficient conditions.

Table 1. Overview of toxins grouped according to their C:N ratio.

Group	Toxins		Molecular formula	C:N ratio*
N-rich	PSP	Paralytic Shellfish Poisoning toxins (incl. saxitoxin, STX)	$C_{10}H_{19}N_7O_4$	1.5
	MC	Microcystin (incl. microcystin-LR; microcystin-RR)	$C_{49}H_{74}N_{10}O_{12}$ (MC-LR); $C_{49}H_{75}N_{13}O_{12}$ (MC-RR)	4.3
	CYN	Cylindrospermopsin	$C_{15}H_{21}N_5O_7S$	3.0
	NOD	Nodularin	$C_{41}H_{60}N_8O_{10}$	5.1
C-rich	ANTX	Anatoxin a	$C_{10}H_{15}NO$	10
	ASP	Amnesic Shellfish Poisoning toxin (incl. domoic acid, DA)	$C_{15}H_{21}NO_6$	15
	GYM	Gymnodimine	$C_{32}H_{45}NO_4$	32
	SPX	Spirolide	$C_{42}H_{61}NO_7$	42
	PLTX	Palytoxin	$C_{129}H_{223}N_3O_{54}$	43
	OVTX	Ovatoxin (incl. OVTX-a)	$C_{129}H_{223}N_3O_{52}$	43
	DSP	Diarrheic Shellfish Poisoning toxins (incl. okadaic acid, OA; dinophysis toxins, DTX-1, DTX-2)	$C_{44}H_{68}O_{13}$ (OA, DTX-2); $C_{45}H_{70}O_{13}$ (DTX-1);	-
	NSP	Neurotoxic Shellfish Poisoning toxin (incl. brevetoxin, PbTx)	$C_{49}H_{70}O_{13}$	-
	KMTX	Karlotoxins (incl. KMTX-1-1, KMTX-2)	$C_{67}H_{120}O_{24}$ (KMTX-1-1); $C_{67}H_{121}ClO_{24}$ (KMTX-2)	-
	CTX	Ciguatoxin	$C_{61}H_{88}O_{19}$	-
	MTX	Maitotoxin	$C_{164}H_{256}O_{68}S_2Na_2$	-
	HA	Hemolytic activity based on bioassays		

*Values refer to the described toxins, or to the average of the shown analogues.

Table 2. Results of one-sample t-tests comparing relative changes in N- and C-rich toxin quotas or toxicities between a treatment and its control, with sample size (*n*), mean change, 95% confidence interval, and *P*-value at a significance level of 0.05 (indicated in bold).

N-rich					
	n	Mean	Variance	95% CI	*P*-value
N-limitation					
PSP	5*	0.65	0.009	0.46 to 0.83	**0.011**
MC	2*	0.49	0.003	0.38 to 0.60	**0.035**
All	2†	0.53	0.002	0.44 to 0.62	**0.033**
P-limitation					
PSP	5*	1.32	0.004	1.19 to 1.44	**0.004**
MC	3*	0.25	0.008	0.08 to 0.43	**0.008**
NOD	5	0.93	0.886	0.11 to 1.76	0.440
All	3†	0.96	0.003	0.86 to 1.06	0.271
C-rich					
	n	Mean	Variance	95% CI	*P*-value
N-limitation					
DSP	2*	1.69	1.95	-1.04 to 4.43	0.353
KMTX	5	2.77	0.93	1.92 to 3.61	**0.008**
NSP	5	1.99	0.15	1.65 to 2.33	**0.002**
ASP	2*	1.04	1.02	-0.37 to 2.44	0.484
HA	2*	4.41	6.47	0.88 to 7.93	0.155
All	5†	2.00	0.11	1.36 to 2.64	**0.019**
P-limitation					
DSP	2*	2.04	4.40	-0.34 to 4.41	0.241
KMTX	6	3.15	3.72	1.61 to 4.70	**0.021**
NSP	5	2.30	0.34	1.79 to 2.82	**0.004**
ASP	2*	6.25	4.87	1.93 to 10.58	0.127
HA	2*	6.48	25.69	0.74 to 12.21	0.101
ANTX	3	1.10	0.16	0.65 to 1.56	0.351
All	6†	1.66	0.10	1.04 to 2.29	**0.002**

*Analysis based on means or weighted means across species; †Analysis based on weighted means across toxins.

Figure legends

Fig. 1. Relative changes of N-rich toxins in response to nutrient limitation. (**a**) N-limitation and (**b**) P-limitation. White symbols show weighted means for individual toxins with error bars indicating SE across means of species; grey symbols show the overall weighted means of N-rich toxins with error bars indicating SE across means of toxins. The horizontal line indicates absence of response. Asterisks indicate significances $P<0.01$ (**) and $P<0.05$ (*).

Fig. 2. Relative changes of C-rich toxins in response to nutrient limitation. (**a**) N-limitation and (**b**) P-limitation. White symbols show weighted means for individual toxins with error bars indicating SE across means of species; grey symbols show the overall weighted means of C-rich toxins with error bars indicating SE across means of toxins. The horizontal line indicates absence of response. Asterisks indicate significances $P<0.01$ (**) and $P<0.05$ (*).

Fig. 3. Relative changes of N- and C-rich toxins in response to cellular N:P ratios. (**a**) N-rich toxins and (**b**) C-rich toxins. Symbols show weighted means for individual toxins with error bars indicating SE across means of species. The horizontal line indicates an absence of response, and the vertical line illustrates the Redfield N:P ratio (N:P=16). The solid grey line in (**a**) shows the linear fit to all data ($R^2=0.989$, $n=6$, $P<0.001$).