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Environmental dependence of the correlations between stoichiometric and fatty acid-based indicators of phytoplankton nutritional quality

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Abstract

Marine phytoplankton is simultaneously affected by multiple environmental drivers. To-date integrative assessments of multiple combined effects are rare on the relationship between elemental stoichiometry and biochemicals in marine phytoplankton. We investigated responses of stoichiometric (N:C and P:C ratios) and fatty acid-based (polyunsaturated fatty acid, PUFA) indicators of nutritional quality to three N:P supply ratios (10:1, 24:1, and 63:1 mol mol^{-1}), three temperatures (12, 18, and 24°C) and two pCO_2 levels (560 and 2400 μatm) in the marine phytoplankters *Rhodomonas* sp. and *Phaeodactylum tricornutum*. Overall, warming and nutrient deficiency showed dramatic effects, but increased pCO_2 had modest effects on the two indicators of nutritional quality. Specifically, warming showed strong positive effects on N:C and P:C ratios in *Rhodomonas* sp. but negative effects on PUFAs in both species. The low N- and low P-media led to low contents of both nutrients but high contents of PUFAs in the biomass of *Rhodomonas* sp., while the response of *P. tricornutum* was more complex: N:C ratios were lowest at the intermediate N:P supply but P:C ratios responded negatively to P deficiency and positively to N deficiency. Large variations in the two indicators of nutritional quality can be attributed to species-specific physiological optima and interactions between the three manipulated variables. Our results suggest that stoichiometric and FA-based indicators of nutritional quality may change differentially in response to warming and nutrient deficiency in marine phytoplankton, highlighting the relevance of simultaneous considerations of the two indicators of nutritional quality, when assessing food web dynamics under future ocean scenarios.

Ecological stoichiometry provides useful insights into the functioning of ecosystems based on the balance of multiple chemical substances in ecological interactions and processes (Sterner and Elser, 2002). Stoichiometric analysis has long been applied to chemical elements (Redfield, 1958; Hessen, 1997; Sterner and Elser, 2002; Galbraith and Martiny, 2015), and it may also be applied to certain biochemicals according to the extended stoichiometric hypothesis developed by Anderson and Pond (2000). Similar to elements like N and P, certain biochemicals, e.g., ω3- and ω6-polyunsaturated fatty acids (PUFAs) are essential for animals, and thus they have been used as indicators of nutritional quality of food (Hessen, 2008; Müller-Navarra, 2008). A concept incorporating elemental stoichiometry and essential fatty acids (FAs) was been developed in studies of herbivorous zooplankton nutrition in limnology (Gulati and Demott, 1997; Boersma et al., 2001; Ravet and Brett, 2006). However, systematic studies are still lacking on the relations between elements and FAs in marine phytoplankton and their importance for zooplankton (Lampert, 2009). As evidence for climate change on marine biota continues to accumulate (IPCC, 2014), insight into the responses of elemental ratios and biochemicals in marine phytoplankton to changing environments is needed to predict the planktonic food web dynamics in the oceans.

Multiple environmental factors will be concurrently altered by climate change in the oceans (Boyd et al., 2015). As one of the most critical problems of climate change, rising atmospheric CO_2 will increase partial CO_2 pressure (pCO_2) in the oceans [851–1370 μatm by 2100 and 1371–2900 μatm by 2150 (RCP8.5 scenario of the IPCC report 2014)] (IPCC 2014). Parallel to the change of pCO_2, rising temperature and enhanced nutrient deficiency due to shallower mixing depths will also impact much of the surface ocean (Doney et al., 2012; Moore et al., 2013). For example,

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future warming will be superimposed on the strong present-day seasonal and inter-annual temperature variability of the source regions of the study strains: −3–21°C during 1990–1999 in the North Sea (Boersma et al., 2016) and ~0–21°C during 1990–2014 in the Baltic Sea (http://www.helcom.fi/baltic-sea-trends/environment-fact-sheets/; last accessed date: 12.05.2016), with an increased annual mean sea surface temperature projected to reach 29.8°C in 2100 across the North Atlantic (0–60° N) (Lewandowska et al., 2014). Surface inorganic N and P are the most limiting nutrients for primary production in many areas of the oceans (Moore et al., 2013). While a high anthropogenic inputs such as the high N:P atmospheric deposition of ~370 mol mol^{-1} drives toward a global scenario of an increase in the N:P ratio in the oceans (Peñuelas et al., 2012), a low N:P ratio occurs in many regions such as the N:P ratio of ~6 mol mol^{-1} in the center of the South Pacific Gyre (Bonnet et al., 2008; Moore et al., 2013). Although significant effort has been made to test the effect of single environmental factor on elemental ratio and FA composition of phytoplankton (Hutchins et al., 2009; Toseland et al., 2013; Bi et al., 2014), it is still a major challenge to understand the combined effects of multiple environmental drivers (MEDs) on chemical composition of phytoplankton (Domis et al., 2014; Verspagen et al., 2014; Cross et al., 2015).

Increasing attention is devoted to the interplay between MEDs on phytoplankton under the projected climate change scenarios (Xu et al., 2014a; Boyd et al., 2015; Flynn et al., 2015). To date, studies have focused on two concurrent variables (Rhee and Gotham, 1981; Staehr and Sand-Jensen, 2006; Sommer et al., 2015), while a few have examined three-way (Feng et al., 2008; Shi et al., 2015) or multi-way interactive effects of environmental factors (Xu et al., 2014b; Brennan and Collins, 2015). In most studies above, certain traits of phytoplankton have been intensively investigated, e.g., growth, photosynthesis, respiration, cell size and/or elemental content. However, less work has been conducted to simultaneously test stoichiometric and FA-based indicators of nutritional quality, and their relationship in response to the interactions of MEDs across different taxonomic groups.

In the present study, we focus on taxonomic comparisons of phytoplankton C:N:P stoichiometric and PUFA responses to N:P supply ratios, temperatures and pCO_2, as well as the relationship between the two properties of nutritional quality. We chose two marine phytoplankters as representatives of common groups, the diatom *Phaeodactylum tricornutum* and the cryptophyte *Rhodomonas* sp. The ecological relevance of both phytoplankton groups are paramount, with diatoms generating most of the organic matter that serves as food in the seas (Armbrust, 2009), and cryptophytes being amongst the most common, and possibly among the most productive, flagellates in most aquatic environments (Sommer, 1987; Klaveness, 1989; Ikavalko, 1998). *P. tricornutum* and *Rhodomonas* sp. have long been used as model species in different studies such as evolutionary history of diatom genomes (Bowler et al., 2008), or unique photosynthetic antenna proteins of cryptophytes (Collini et al., 2010), and planktonic trophic dynamics (Jónasdóttir, 1994; Malzahn et al., 2007; Arndt and Sommer, 2014). Thus, studies on model algal species would be the basis to derive general findings for other algae and consumers. In this study, we addressed the following questions: (i) How does the combination of N:P supply ratio changes, temperature rise and increasing pCO_2 affect stoichiometric (N:C and P:C biomass ratios) and FA-based (PUFA contents) indicators of nutritional quality? (ii) How do the two indicators of nutritional quality correlate with each other? (iii) Does the correlation between the two indicators of nutritional quality vary with the changing culture conditions?

Methods

Study organisms and culture conditions

The cryptophyte *Rhodomonas* sp. and the bacillariophyte *P. tricornutum* (SAG, 1090-1b) were cultivated at a salinity of 37 psu in temperature-controlled rooms. The light intensity was constant at 100 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ at a light:dark cycle of 16:8 h. The culture medium was prepared with sterile filtered (0.2 μm pore size, Sartobran® P 300; Sartorius, Goettingen, Germany) North Sea water and enrichment nutrient solutions (macronutrients and micronutrients) based on the modified Provasoli's culture medium (Provasoli, 1963; Ismar et al., 2008). Macronutrients were added as sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4), and dissolved background concentrations were negligible. For the diatom culture, also sodium silicate pentahydrate ($Na_2SiO_3 \cdot 5H_2O$) was added at a concentration of 88 μmol $\cdot$ L^{-1}. Initial pCO_2 of the culture medium was manipulated by bubbling with the target pCO_2. Each culture was kept in a sealed cell culture flask with 920 mL culture volume. All cultures were shaken manually twice per day at a set time. Three replicates were set up for each treatment.

Experimental setup

First, batch culture experiments were performed for each algal species under three N:P supply ratios, three temperatures and two pCO_2 levels. N:P supply ratios were manipulated as 10:1 mol mol^{-1} (35.2 μmol $\cdot$ L^{-1} N and 3.6 μmol $\cdot$ L^{-1} P), 24:1 (88 μmol $\cdot$ L^{-1} N and 3.6 μmol $\cdot$ L^{-1} P) and 63:1 mol mol^{-1} (88 μmol $\cdot$ L^{-1} N and 1.4 μmol $\cdot$ L^{-1} P). Temperatures were set to 12, 18 and 24°C, and target values of the two pCO_2 levels were 560 and 2400 μatm. The chosen levels of N:P supply ratios, temperature and pCO_2 cover the ranges of typical changes of the three factors in natural conditions and they are also in agreement with future ocean projections (Peñuelas et al., 2012; Moore et al., 2013; IPCC, 2014; Lewandowska et al., 2014; Boersma et al., 2016). A temperature range of 6°C was set according to the ocean general circulation model under the IPCC SRES A1F1 scenario

(Lewandowska et al., 2014). The range of temperature also covers the optimum growth temperatures for both algal species (Hammer et al., 2002; Bojko et al., 2013). The observed maximal growth rate (μ_{max}) was estimated from cell number changes during the exponential growth phase (Bi et al., 2012).

Once batch cultures reached the early stationary phase, semi-continuous cultures were started with the specific growth rate (μ, day^{-1}) of 20% of μ_{max} for each treatment. The equivalent daily renewal rate (D, day^{-1}) can be estimated by $D = 1 - e^{-\mu \cdot t}$, where t is renewal interval (day) (here $t = 1$day). The incubation water was exchanged with fresh filtered seawater preacclimated to the desired pCO_2 level and CO_2-enriched water. Renewal of the cultures was carried out at the same hour every day. The steady state in semi-continuous cultures was assessed based on the net growth rate (r). When r was zero (at steady state), μ was equivalent to D.

Sample analysis

Algal cell density and pH were measured daily. For pH measurements the electrode was calibrated daily using standard pH buffers (pH 4 and pH 7; WTW, Weilheim, Germany). At steady state, sampling was carried out during the same hour as the daily renewal of the cultures to avoid the effect of diel variations (Lacour et al., 2012) and subsequent variability in the data. For each treatment replicate, one sample was taken for analysis (the size of samples = 3 in each treatment). All cultures were sampled for the following parameters: cell density, dissolved inorganic carbon (DIC), total alkalinity (TA), pH, particulate organic carbon, nitrogen and phosphorus (POC, PON and POP), and FAs.

DIC sampling and measurement were conducted according to Hansen et al. (2013). DIC samples were taken with a peristaltic pump into 10-mL glass vials. The filtration was conducted using a single-use syringe filter (0.2 μm, Minisart RC25; Sartorius, Goettingen, Germany) which was connected to the intake tube of the pump. Vials were immediately sealed after filling. Subsequent analysis was carried out with a gas chromatographic system (8610C; SRI-Instruments, CA). TA samples were filtered through GF/F filters (Whatman GmbH, Dassel, Germany) and analyzed with the Tirino plus 848 (Metrohm, Filderstadt, Germany). The remaining carbonate parameter pCO_2 was calculated using CO2 SYS (Pierrot et al., 2006) and the constants supplied by Hansson (1973) and Mehrbach et al. (1973) that were refitted by Dickson and Millero (1987) and the KSO_4 dissociation constant from Dickson (1990) (Supporting Information Table S1).

For elemental and FA analysis, algal cells were harvested by filtration on pre-combusted and hydrochloric acid-treated GF/F filters (Whatman GmbH, Dassel, Germany). After filtration, samples for elemental analysis were immediately dried and stored in a desiccator, and samples for FA analysis were frozen at −80°C. The determination of POC and PON was carried out after Sharp (1974) by gas chromatography in an organic elemental analyzer (Thermo Flash 2000; Thermo Fisher Scientific, Schwerte, Germany). POP was analyzed colorimetrically by converting organic phosphorus compounds to orthophosphate (Hansen and Koroleff, 1999). FAs were measured as fatty acid methyl esters (FAMEs) using a gas chromatograph (Trace GC-Ultra; Thermo Fisher Scientific, Schwerte, Germany) according to the procedure described in detail in Arndt and Sommer (2014). The FAME 19:0 was added as internal standard and 21:0 added as esterification control. The extracted FAs were dissolved with n-hexane to a final volume of 100 μL. Sample aliquots (1 μL) were given into the GC by splitless injection with hydrogen as the carrier gas. Individual FAs were integrated using Chromcard software (Thermo Fisher Scientific, Schwerte, Germany) and identified with reference to commercially available standards, Supelco 37 component FAME mixture and Supelco Menhaden fish oil.

Statistics

Generalized linear mixed models (GLMMs) were used to investigate the factors determining phytoplankton stoichiometric and FA composition. GLMMs combine the properties of two statistical frameworks that are widely used in ecology and evolution, linear mixed models and generalized linear models (Bolker et al., 2009). They provide a more flexible approach for analyzing non-normal data such as count or proportion compared to classical statistical procedures (Bolker et al., 2009) and have been increasingly applied in ecology (Ye et al., 2013; Jamil et al., 2014). In this study, C:N:P stoichiometric ratios (as mol mol^{-1}) and cellular contents (as ng $cell^{-1}$ for C and N, and pg $cell^{-1}$ for P), and FA contents (as μg mg^{-1} C^{-1}) were considered as response variables, with N:P supply ratio, temperature and pCO_2 as fixed effects. Target distributions were tested and link functions were consequently chosen. For all response variables, models containing first order effects of the three factors, and second and third order interactions of all factors were tested. Model selection with the Akaike Information Criterion corrected (AICc) was used to determine the model that best predicted targets, with a lower AICc value representing a better fit of the model. Following Bolker et al. (2009), changes of 10 U or more in AICc values were considered as a reasonable improvement in the fitting of GLMMs. In case AICc values were comparable (<10 U difference), the simpler model was thus chosen, unless there were significant second or third order interactions detected. Differences in AICc values for all responses of cellular C:N:P contents and ratios were <10 between different models, with an exception of N:C biomass ratios in *P. tricornutum*, which showed around 11 points less of AICc in the model containing second order interactions than that only containing the first order effect (Supporting Information Table S2). Differences in AICc values for TFAs, SFAs and MUFAs in *Rhodomonas* sp., and PUFAs and EPA in

P. tricornutum were less than 10 between different models; however, those for PUFAs, ALA, EPA and DHA in *Rhodomonas* sp. and TFAs, SFAs and MUFAs in *P. tricornutum* were up to 50 points lower in the model containing only first order effects than in those also containing second and third order effects (Supporting Information Table S2).

μ_{max} values did not differ substantially between different N:P supply ratios in *Rhodomonas* sp. and *P. tricornutum* (Bi et al., 2012). Thus, the effects of temperature and pCO_2 on μ_{max} were tested for each algal species using two factorial ANOVA. Dependent variables were checked for normality using the Shapiro–Wilk test and transformed (square) if required.

Linear regression analyses were used to test the relationship between N (and P) cell quota (Q_N and Q_P, as $\mu g\ mg^{-1}\ C^{-1}$) and the contents of each FA group (TFAs, SFAs, MUFAs, and PUFAs) under N (and P) deficiency (N:P supply ratios = 10:1 and 63:1). The same analysis was done for the relationship between cellular FA and POC contents.

GLMMs, ANOVA and linear regressions were conducted in SPSS 19.0 (IBM Corporation, NY). Significance level was set to $p < 0.05$ in all statistical tests.

Results

The observed maximal growth rate (μ_{max})

For both algal species, μ_{max} did not significantly differ between different temperature or pCO_2 treatments. However, the values of μ_{max} in *Rhodomonas* sp. showed a trend to increase with increasing temperature over the entire range of N:P supply ratio and pCO_2 (0.51 ± 0.06 to $0.66 \pm 0.03\ day^{-1}$), while those in *P. tricornutum* (0.84 ± 0.02 to $0.87 \pm 0.07\ day^{-1}$) showed no detectable pattern.

N:C and P:C biomass ratios

The results of GLMMs showed that N:C biomass ratios responded significantly to temperature changes in both algal species (Table 1). Species-specific responses were also observed, with a significant effect of N:P supply ratio and the interaction between temperature and N:P supply ratio on N:C biomass ratios in *Rhodomonas* sp., and a significant effect of pCO_2 and the interaction between temperature and pCO_2 on N:C biomass ratios in *P. tricornutum* (Table 1). Specifically, N:C biomass ratios in *Rhodomonas* sp. showed a positive response to increasing temperature under N deficiency (N:P supply ratio = 10:1) and the balanced nutrient condition (N:P supply ratio = 24:1), but a negative response to increasing temperature under P deficiency (N:P supply ratio = 63:1) (Table 2; Supporting Information Fig. S2a). In contrast, N:C biomass ratios in *P. tricornutum* showed a trend to decrease with increasing temperature under the low pCO_2 condition, but a trend to increase under the high pCO_2 condition (Table 2; Supporting Information Fig. S3b).

Similar to N:C biomass ratios, P:C biomass ratios also showed significant responses to temperature changes in both algal species according to the GLMMs (Table 1). Species-specific responses were found, with the significant interaction between temperature and pCO_2 on P:C biomass ratios in *Rhodomonas* sp., and a significant effect of N:P supply ratio and the interaction between temperature and N:P supply ratio on P:C biomass ratios in *P. tricornutum* (Table 1). Specifically, P:C biomass ratios in *Rhodomonas* sp. were higher at higher temperatures, but this positive response became weaker as pCO_2 increased (Table 2; Supporting Information Fig. S3c). Despite the non-significant response to N:P supply ratio, P:C biomass ratios in *Rhodomonas* sp. was around two times higher under N-deficient and balanced nutrient conditions than those under P deficiency (Table 2; Supporting Information Fig. S2b). In contrast to the responses to temperature rise in *Rhodomonas* sp., P:C biomass ratios in *P. tricornutum* were higher at the lowest temperature under N-deficient and balanced nutrient conditions (Table 2; Supporting Information Fig. S2b). Moreover, P:C biomass ratios in *P. tricornutum* decreased significantly with increasing N:P supply ratio, being around two to three times higher under N deficiency than under P deficiency.

Polyunsaturated fatty acids

GLMM results showed that the contents of PUFAs responded significantly to N:P supply ratio in both algal species, and PUFAs in *P. tricornutum* also responded significantly to the interaction between temperature and N:P supply ratio (Table 1). The contents of PUFAs in *Rhodomonas* sp. were markedly higher under N and P deficiency; however, PUFAs in *P. tricornutum* were slightly higher under P deficiency and lower temperatures (Table 2; Supporting Information Fig. S4d). Moreover, responses of PUFAs to increasing temperature in *Rhodomonas* sp. shifted from positive, negative to unimodal as N:P supply ratio increased, although the effects of temperature were not statistically significant.

Because of their high abundance and nutritional values, ALA (α-linolenic acid; 18:3n-3), EPA (eicosapentaenoic acid; 20:5n-3) and DHA (docosahexaenoic acid; 22:6n-3) in *Rhodomonas* sp. and EPA in *P. tricornutum* were considered as the most important single PUFAs. Significant responses were observed for ALA and EPA to N:P supply ratio in *Rhodomonas* sp., and DHA to temperature in *Rhodomonas* sp., but not for EPA in *P. tricornutum* (Table 1; Supporting Information Table S3). The contents of ALA in *Rhodomonas* sp. were markedly higher under N and P deficiency. While ALA, EPA, and DHA in *Rhodomonas* sp. showed a clear trend to decrease with increasing temperature in most cases, the contents of EPA increased with increasing temperature under N deficiency (Supporting Information Fig. S5).

Correlations between Q_N (and Q_P) and polyunsaturated fatty acids under N (and P) deficiency

Significant positive correlations were observed between Q_N and PUFAs under N deficiency for both algal species (Fig. 1). However, there was no significant correlation between Q_P

Table 1. Overview of the significant results of the best-fit GLMMs testing for the effects of temperature, N:P supply ratio and pCO_2 on N:C and P:C biomass ratios, the contents of polyunsaturated fatty acids, and the contents of important single polyunsaturated fatty acids in *Rhodomonas* sp. and *Phaeodactylum tricornutum*.

Response variable	Factor	Coefficient ± SE	*t*	*p*	*n*
Rhodomonas sp.					
N:C biomass ratio (mol mol^{-1})	Intercept	−2.924 ± 0.254	−11.497	**<0.001**	54
	T	0.058 ± 0.013	4.380	**<0.001**	
	pCO_2	<0.001 ± <0.001	−0.494	0.624	
	N:P	0.016 ± 0.005	3.042	**0.004**	
	T×pCO_2	<0.001 ± <0.001	−0.537	0.594	
	T×N:P	−0.001 ± <0.001	−4.014	**<0.001**	
	pCO_2×N:P	<0.001 ± <0.001	1.478	0.146	
P:C biomass ratio (mmol mol^{-1})	Intercept	0.441 ± 0.295	1.496	0.141	54
	T	0.083 ± 0.015	5.402	**<0.001**	
	pCO_2	<0.001 ± <0.001	1.466	0.149	
	N:P	−0.007 ± 0.006	−1.143	0.259	
	T×pCO_2	<0.001 ± <0.001	−2.111	**0.040**	
	T×N:P	<0.001 ± <0.001	−1.299	0.200	
	pCO_2×N:P	<0.001 ± <0.001	−0.351	0.727	
PUFAs (μg mg^{-1} C^{-1})	Intercept	153.82 ± 24.031	6.401	**<0.001**	51
	T	−1.514 ± 1.126	−1.344	0.185	
	pCO_2	<0.001 ± 0.006	−0.066	0.948	
	N:P	0.785 ± 0.247	3.182	**0.003**	
ALA (μg mg^{-1} C^{-1})	Intercept	3.644 ± 0.268	13.574	**<0.001**	51
	T	−0.011 ± 0.013	−0.875	0.368	
	pCO_2	<0.001 ± <0.001	0.246	0.807	
	N:P	0.006 ± 0.003	2.192	**0.033**	
EPA (μg mg^{-1} C^{-1})	Intercept	36.584 ± 4.355	8.401	**<0.001**	51
	T	−0.255 ± 0.204	−1.247	0.218	
	pCO_2	−0.001 ± 0.001	−0.837	0.407	
	N:P	−0.117 ± 0.045	−2.610	**0.012**	
DHA (μg mg^{-1} C^{-1})	Intercept	17.056 ± 1.312	12.999	**<0.001**	51
	T	−0.334 ± 0.061	−5.427	**<0.001**	
	pCO_2	<0.001 ± <0.001	−0.780	0.439	
	N:P	0.009 ± 0.013	0.657	0.514	
Phaeodactylum tricornutum					
N:C biomass ratio (mol mol^{-1})	Intercept	−2.265 ± 0.090	−25.289	**<0.001**	53
	T	−0.018 ± 0.005	−3.738	**0.001**	
	pCO_2	<0.001 ± <0.001	−5.665	**<0.001**	
	N:P	0.001 ± 0.002	0.860	0.394	
	T×pCO_2	<0.001 ± <0.001	4.543	**<0.001**	
	T×N:P	<0.001 ± <0.001	1.492	0.142	
	pCO_2×N:P	<0.001 ± <0.001	0.445	0.659	
P:C biomass ratio (mmol mol^{-1})	Intercept	1.992 ± 0.212	9.412	**<0.001**	53
	T	−0.028 ± 0.011	−2.459	**0.018**	
	pCO_2	<0.001 ± <0.001	−0.356	0.724	
	N:P	−0.025 ± 0.004	−6.076	**<0.001**	
	T×pCO_2	<0.001 ± <0.001	0.422	0.675	
	T×N:P	0.001 ± <0.001	2.812	**0.007**	
	pCO_2×N:P	<0.001 ± <0.001	−0.578	0.566	

TABLE 1. Continued

Response variable	Factor	Coefficient ± SE	*t*	*p*	*n*
PUFAs (μg mg^{-1} C^{-1})	Intercept	4.420 ± 0.100	44.408	**<0.001**	51
	T	−0.008 ± 0.005	−1.421	0.162	
	pCO_2	<0.001 ± <0.001	0.707	0.483	
	N:P	0.007 ± 0.002	3.330	**0.002**	
	T×pCO_2	<0.001 ± <0.001	−0.694	0.492	
	T×N:P	<0.001 ± <0.001	−2.986	**0.005**	
	pCO_2×N:P	<0.001 ± <0.001	0.625	0.536	

Significant *p* values are shown in bold; *n* is the number of observations. T: temperature; N:P: N:P supply ratio; PUFAs: polyunsaturated fatty acids; ALA: α-linolenic acid (18:3n-3); EPA: eicosapentaenoic acid (20:5n-3); DHA: docosahexaenoic acid (22:6n-3).

and PUFAs under P deficiency. The correlations between Q_N and PUFAs were species-specific and temperature-dependent. In *Rhodomonas* sp., Q_N correlated positively with PUFAs over the entire range of temperature (Fig. 1a). In contrast, Q_N in *P. tricornutum* showed no significant correlation with PUFAs over the entire range of temperature, with a significant positive correlation observed when excluding data from the lowest temperature (Fig. 1b).

Responses of both PON/PUFAs and POP/PUFAs ratios to temperature and N:P supply ratio differed between the two species, with those in *Rhodomonas* sp. being more responsive than in *P. tricornutum* (Fig. 2). In *Rhodomonas* sp., PON/PUFAs and POP/PUFAs were generally higher under the balanced nutrient condition and the highest temperature (Fig. 2a,b). However, PON/PUFAs in *P. tricornutum* showed no clear difference between different N:P supply ratio or temperature treatments (Fig. 2c), and POP/PUFAs showed a trend to decrease with increasing N:P supply ratio (Fig. 2d).

Discussion

Our results show complex and in many cases interactive influences of temperature, N:P supply ratio and pCO_2 on stoichiometric and FA-based nutritional quality in marine phytoplankton (Fig. 3 for a systematic summary). Overall, warming and nutrient deficiency showed dramatic effects on the nutritional quality of the two algal species, while increased pCO_2 had more modest effects, with significant interactive effects observed between temperature and N:P supply ratio (or pCO_2). The relative importance of warming and nutrient deficiency is in principle consistent with a postulated ranking of environmental factors for five major phytoplankton groups, which showed temperature, photosynthetic available radiation and nutrients (N and P) as the most important factors for phytoplankton abundance (Boyd et al. 2010). Specifically, we could observe differential shifts between positive and negative correlations between stoichiometric and FA-based indicators of nutritional quality with temperature. Our results thus highlight that one type of nutritional quality indicator alone, either elemental or biochemical can only incompletely reflect phytoplankton quality for higher trophic levels in marine food webs.

Phytoplankton stoichiometric responses

Temperature showed the most consistent significant contribution to the variation in elemental nutritional quality of the two algal species in our study (Table 1), showing up to 83% changes in C:N:P stoichiometry in response to warming (Fig. 3). These significant impacts of temperature on algal stoichiometry in this study are in agreement with recent results of field research, which demonstrate temperature as the primary factor explaining variation in algal N:P and C:P ratios on a global scale (Yvon-Durocher et al., 2015). Although the global patterns of C:N:P ratios in ocean plankton communities exhibit a strong latitudinal trend, i.e. higher N:P and C:P ratios in warmer environments (Martiny et al., 2013; Yvon-Durocher et al., 2015), taxonomic differences in responses of phytoplankton to warming were observed in previous work (Thompson et al., 1992; Taucher et al., 2015) and the present study (Fig. 3). Several mechanisms are proposed to illustrate stoichiometric responses to temperature. The temperature-dependent physiology hypothesis predicts that organisms in warm environments require fewer P-rich ribosomes, relative to N-rich proteins, to sustain growth and maintenance (Thompson et al., 1992; Woods et al., 2003; Yvon-Durocher et al., 2015). In line with this hypothesis, we observed an overall decreased P:C biomass ratios and increased N:C biomass ratios with increasing temperature in *P. tricornutum*. In contrast, the growth rate hypothesis (GRH) suggests that low C:P and N:P biomass ratios in rapidly growing organisms reflect increased allocation to P-rich ribosomal RNA, as rapid protein synthesis by ribosomes is required to support fast growth (Elser et al., 2000). This positive linkage between P content and growth rate can explain the overall increased N:C and P:C biomass ratios and higher growth rates at higher temperatures in *Rhodomonas* sp. The inconsistent predictions between the temperature-dependent physiology hypothesis and the GRH might be explained by calculations in a recent review (Cross et al., 2015), which suggest that the increased P content associated with rapid growth in warm conditions may be

Table 2. N:C and P:C biomass ratios and polyunsaturated fatty acid contents (mean ± SD) under three N:P supply ratios, three temperature scenarios and two pCO_2 levels in *Rhodomonas* sp. and *Phaeodactylum tricornutum*.

Treatment	N:C biomass ratio (mol mol^{-1})	P:C biomass ratio (mmol mol^{-1})	PUFAs (μg mg^{-1} C^{-1})
Rhodomonas sp.			
N deficiency			
LCO_2, 12°C	0.079 ± 0.001	3.007 ± 0.192	148.2 ± 2.141
LCO_2, 18°C	0.115 ± 0.009	5.671 ± 0.273	174.6 ± 6.402
LCO_2, 24°C	0.145 ± 0.003	6.630 ± 1.747	165.4 ± 9.035
HCO_2, 12°C	0.072 ± 0.001	3.302 ± 0.363	146.9 ± 1.037
HCO_2, 18°C	0.107 ± 0.004	4.825 ± 0.409	181.8 ± 14.17
HCO_2, 24°C	0.126 ± 0.002	5.889 ± 0.241	172.9 ± 10.97
Balanced nutrient condition			
LCO_2, 12°C	0.158 ± 0.005	3.680 ± 0.132	127.8 ± 6.511
LCO_2, 18°C	0.171 ± 0.002	6.333 ± 0.275	125.1 ± 9.947
LCO_2, 24°C	0.210 ± 0.024	8.765 ± 1.121	66.43 ± 11.03
HCO_2, 12°C	0.133 ± 0.006	4.586 ± 1.266	122.7 ± 12.37
HCO_2, 18°C	0.138 ± 0.003	3.480 ± 1.266	112.4 ± 13.91
HCO_2, 24°C	0.176 ± 0.006	7.048 ± 0.326	79.06 ± 5.651
P deficiency			
LCO_2, 12°C	0.122 ± 0.002	2.146 ± 0.342	190.8 ± 6.952
LCO_2, 18°C	0.117 ± 0.003	2.292 ± 0.250	228.4 ± 27.39
LCO_2, 24°C	0.120 ± 0.010	3.820 ± 0.267	154.2 ± 14.45
HCO_2, 12°C	0.143 ± 0.004	1.989 ± 0.207	173.4 ± 6.105
HCO_2, 18°C	0.117 ± 0.001	2.164 ± 0.176	195.8 ± 6.843
HCO_2, 24°C	0.116 ± 0.003	2.801 ± 0.141	178.4 ± 0.226
Phaeodactylum tricornutum			
N deficiency			
LCO_2, 12°C	0.081 ± 0.005	5.344 ± 0.145	84.39 ± 9.188
LCO_2, 18°C	0.085 ± 0.002	3.679 ± 1.170	71.98 ± 4.441
LCO_2, 24°C	0.067 ± 0.002	3.889 ± 0.115	67.31 ± 2.032
HCO_2, 12°C	0.064 ± 0.001	4.738 ± 0.112	82.37 ± 2.328
HCO_2, 18°C	0.078 ± 0.002	4.094 ± 0.307	71.64 ± 1.861
HCO_2, 24°C	0.072 ± 0.001	4.116 ± 0.103	68.84 ± 2.613
Balanced nutrient condition			
LCO_2, 12°C	0.080 ± 0.002	2.873 ± 0.163	77.68 ± 14.67
LCO_2, 18°C	0.078 ± 0.001	2.411 ± 0.102	77.19 ± 1.568
LCO_2, 24°C	0.072 ± 0.003	2.817 ± 0.628	66.72 ± 11.25
HCO_2, 12°C	0.065 ± 0.001	2.843 ± 0.384	83.09 ± 8.866
HCO_2, 18°C	0.064 ± 0.001	2.241 ± 0.020	76.63 ± 1.868
HCO_2, 24°C	0.071 ± 0.007	2.429 ± 0.244	71.96 ± 3.437
P deficiency			
LCO_2, 12°C	0.093 ± 0.001	1.691 ± 0.119	89.62 ± 8.571
LCO_2, 18°C	0.098 ± 0.001	1.807 ± 0.023	82.00 ± 1.215
LCO_2, 24°C	0.091 ± 0.001	1.912 ± 0.134	67.86 ± 0.763
HCO_2, 12°C	0.083 ± 0.001	1.680 ± 0.041	103.3 ± 2.057
HCO_2, 18°C	0.082 ± 0.001	1.505 ± 0.067	81.40 ± 1.115
HCO_2, 24°C	0.094 ± 0.001	1.929 ± 0.249	70.96 ± 10.98

N deficiency, N:P = 10:1 mol mol^{-1}; Balanced nutrient condition, 24:1 mol mol^{-1}; P deficiency, 63:1 mol mol^{-1}. LCO_2, 560 μatm; HCO_2, 2400 μatm. PUFAs: polyunsaturated fatty acids.

masked by larger decreases in P content due to other factors such as patterns of nutrient condition and biodiversity (Woods et al., 2003).

Indeed, we observed significant interactions between temperature and N:P supply ratio (or pCO_2), showing that N:P supply ratio or pCO_2 can change the extent or even reverse the effect of temperature on N:C and P:C biomass ratios in our two study taxa (Supporting Information Figs. S2 and S3). Our results show that the effect of warming on stoichiometric responses was more pronounced under lower N:P supply ratios, or under the low pCO_2 condition in *Rhodomonas* sp., and under lower N:P supply ratios in *P. tricornutum*. Because of the interactions between temperature and nutrients, the influence of increasing global temperature on phytoplankton growth showed seasonal changes in natural phytoplankton communities in lakes (Frederiksborg Slotssø, Denmark) (Staehr and Sand-Jensen, 2006). Our study suggests that nutrient availability and pCO_2 can alter the effect of warming on elemental quality of phytoplankton, which furthers our understanding of MEDs influences on phytoplankton stoichiometry.

Furthermore, stoichiometric responses to temperature, N:P supply ratio and pCO_2 differed between the two species in our study (Fig. 3). For example, we observed up to 83% enhancement of N:C biomass ratios in response to warming in *Rhodomonas* sp., but a much less pronounced change ($\leq$2%) in *P. tricornutum*. Our results are consistent with the findings by Thompson et al. (1992) and Montagnes and Franklin (2001), which showed stronger changes in stoichiometry in the cryptophyte *Rhodomonas salina* but moderate responses in *P. tricornutum*. These different responses may be attributed to different adaptations to temperature (Montagnes and Franklin, 2001), nutrients (Bi et al., 2012), and variable mechanisms regulating C acquisition (Giordano et al., 2005; Wu et al., 2014) between the two species. The expression of the CO_2 concentrating mechanisms (CCMs) has been well characterized in several algal groups such as diatoms (Hopkinson et al., 2011; Raven et al., 2012), while less is known about its mechanism in cryptophytes. Also, environmental conditions can modulate the CCM activity (Beardall and Giordano, 2002; Raven et al., 2012), but limited data are currently available on the interactions of multiple factors. Therefore, additional research is needed to fully assess the mechanisms and mathematical dynamics of the interplay between temperature and nutrients (or pCO_2) on phytoplankton stoichiometry.

Phytoplankton polyunsaturated fatty acid responses

N and P deficiency had the most significant contribution to the variation of PUFA contents in the two algal species in this work (Table 1), showing up to 76% changes in PUFAs in response to nutrient deficiency (Fig. 3). Although light intensity and temperature are probably the most commonly observed factors affecting FA composition of photosynthetic

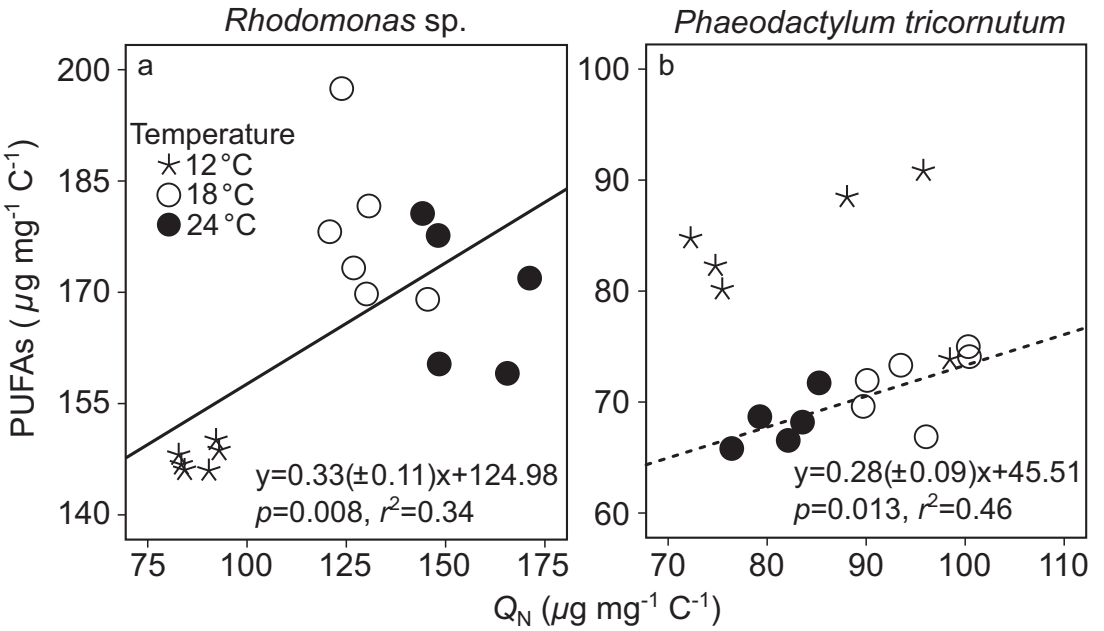

Fig. 1. Linear regressions between N cell quota (Q_N) and polyunsaturated fatty acids (PUFAs) under N deficiency for (**a**) *Rhodomonas* sp. and (**b**) *Phaeodactylum tricornutum*. Solid lines: regressions over the entire range of temperature; broken lines: regressions at higher temperatures (18 and 24°C).

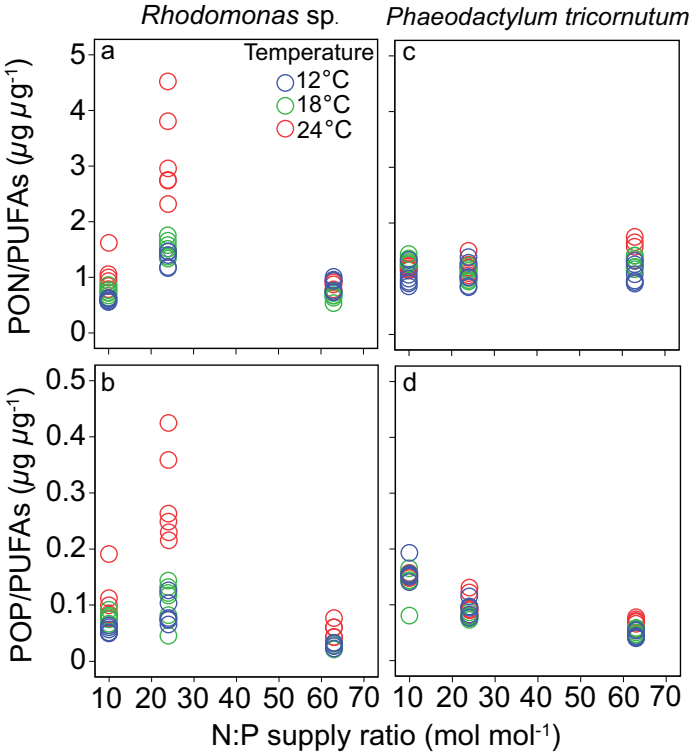

Fig. 2. The ratios of particulate organic nitrogen (and phosphorus) vs. polyunsaturated fatty acids (PON/PUFAs and POP/PUFAs) as functions of N:P supply ratio under different temperatures for (**a** and **b**) *Rhodomonas* sp. and (**c** and **d**) *Phaeodactylum tricornutum*.

tissues or organisms, nutrient availability has also shown a significant impact on FA composition of algae (Guschina and Harwood, 2006). For example, the importance of nutrient availability on FA composition was observed in mesocosm experiments conducted with natural phytoplankton communities of marine, brackish and freshwater systems (Brepohl, 2005). Our results provided additional evidence for the importance of N:P supply ratio on PUFAs in two marine phytoplankters (representing two algal groups). Furthermore, the overall responses of PUFAs to nutrient deficiency showed no consistent pattern between the two species, with more than 58% enhancement in *Rhodomonas* sp. but less than 9% changes in *P. tricornutum* (Fig. 3). Interspecific differences in the responses of PUFAs to nutrient deficiency may be attributed to the association of PUFAs with different lipid types such as triacylglycerols, phospholipids and phospholipids substitutions (Bi et al., 2014). Moreover, nutrient levels seem to influence the slope of the correlations between cellular POC and FAs in *Rhodomonas* sp., showing higher slopes under the balanced nutrient condition (Supporting Information Fig. S6 a–d), suggesting that a larger part of C may be fixed in non-FA molecules, e.g., carbohydrates, under nutrient replete conditions.

Warming showed an overall negative effect on PUFAs in both algal species in our study, resulting in <20% changes in PUFAs (Fig. 3). This negative effect of warming on PUFAs was also found in tropical *Rhodomonas* sp. and *P. tricornutum* (Jiang and Gao, 2004) and another diatom *Odontella aurita*

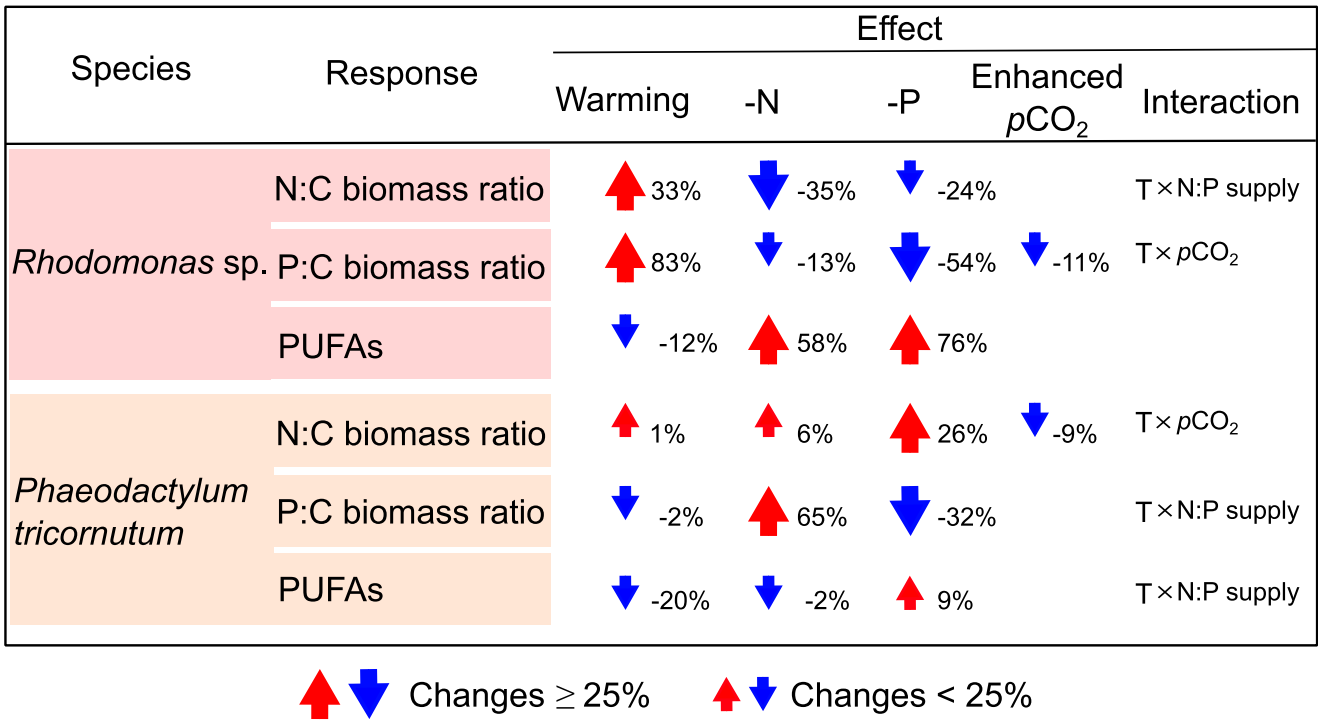

Fig. 3. The changes in stoichiometric (N:C and P:C biomass ratios) and fatty acid-based (polyunsaturated fatty acids, PUFAs) indicators of nutritional quality in response to warming, N and P deficiency (-N and -P), and enriched pCO_2 in *Rhodomonas* sp. and *Phaeodactylum tricornutum* based on the data in Table 1 and Table 2. Here is shown not only significant and substantial effects on the two indicators of nutritional quality, but also moderate and non-significant first order effects, some of these include significant two-factorial interactions. Significant interactions between temperature (T) and N:P supply ratio (or pCO_2) are presented based on GLMM results in Table 1, and corresponding response patterns are shown in Supporting Information Fig. S2–S4. Red arrows indicate a mean percent increase, and blue arrows indicate a mean percent decrease in a given response.

(Pasquet et al., 2014). Unsaturated FAs become higher to maintain membrane fluidity, which is suggested to provide a membrane of constant viscosity, i.e., homeoviscosity, at low temperatures (Sinensky, 1974; Los et al., 2013). However, positive and unimodal responses of PUFAs to warming were also found in *Rhodomonas* sp. in our study (Table 2; Supporting Information Fig. S4d) and other algal species in previous work (Renaud et al., 1995; Piepho et al., 2012; Roleda et al., 2013). For *Rhodomonas* sp. in this work, the lower PUFAs at the lowest temperature under N deficiency was due to the markedly decrease in EPA, while other major PUFA components (ALA and DHA) showed only moderate changes under the same condition (Supporting Information Fig. S5). Our results suggest that nutrient deficiency may influence temperature-dependent modification of FA unsaturation in certain algal species, e.g., the inhibition of N deficiency on elongation or desaturation of precursors to produce EPA in *Rhodomonas* sp. at the lowest temperature.

Partial CO_2 pressure showed a non-significant effect on PUFAs in either species in our study (Table 1). This negligible effect of pCO_2 on phytoplankton FA composition was also found in monocultures of other species from different phyla such as Cyanobacteria, Bacillariophyta, and Rhodophyta (Tsuzuki et al., 1990; Shi et al., 2015), and in a natural plankton community (Leu et al., 2013). Under different pCO_2 levels, not only FAs but also other biochemicals such as sterols and amino acids can be changed in phytoplankton (Gordillo et al., 1998; Riebesell et al., 2000; Bermudez et al., 2015). For example, cellular FA, protein or $\beta-1,3$-glucan contents in the diatom *Thalassiosira pseudonana* showed no significant differences between two pCO_2 levels; however, the cellular $\beta-1,3$-glucan content showed a larger variation than FAs in response to pCO_2 (Shi et al., 2015). We thus suggest that other biochemicals, such as sterols (Müller-Navarra, 2008), may be influenced by pCO_2. Further research is recommended to explore the responses of variable biochemicals to pCO_2 and other factors.

Correlations between stoichiometric and FA-based nutritional quality of phytoplankton and implications for zooplankton nutrition

The results discussed above suggest that stoichiometric and FA-based indicators of nutritional quality responded differently under different combinations of environmental factors; therefore, both need to be studied if an integrative assessment of nutritional value to higher trophic levels is required. In the present study, we focus on the potential limitation of elements and PUFAs on zooplankton nutrition

under nutrient deficiency, and thus the relationship between Q_N (and Q_P) and PUFAs was tested under N (and P) deficiency. We found that the correlations between Q_N and PUFAs under N deficiency at higher temperatures (18 and 24°C; Fig. 1) are consistent with the findings by Bi et al. (2014), which showed a positive correlation between Q_N and PUFAs in *P. tricornutum* but a negative correlation in *Rhodomonas* sp. under N deficiency at 18°C. Moreover, we observed a shift from negative to positive correlations between Q_N and PUFAs in *Rhodomonas* sp., and a lack of significant correlation between Q_N and PUFAs in *P. tricornutum* when data at the lowest temperature (12°C) were included. The correlations between N (and P) content and PUFAs in phytoplankton have been widely studied in freshwater environments (Müller-Navarra, 1995; Wacker and Von Elert, 2001; Gladyshev et al., 2007), which helped to understand the relative importance of the two properties of nutritional food quality in regulating zooplankton nutrition (Müller-Navarra, 1995; Park et al., 2002). The present study shows species-specific and temperature-dependent covariance of Q_N and PUFAs (under N deficiency) in two marine phytoplankters, providing important empirical data to understanding of the relations of elements and FAs in marine zooplankton nutrition.

Interestingly, we also found that PON/PUFAs and POP/PUFAs changed along the gradients of temperature and N:P supply ratio (Fig. 2). According to the extended stoichiometric hypothesis, the ratio of N (or P) and essential PUFAs in phytoplankton correlates negatively with the strength of limitation of N (or P) relative to essential PUFAs in zooplankton production (Anderson and Pond, 2000). Our results therefore indicate that higher PON/PUFAs and POP/PUFAs may lead to a lower probability of N (or P) limitation relative to PUFAs for zooplankton feeding *Rhodomonas* sp. at the balanced nutrient and high temperature conditions; and decreased POP/PUFAs may lead to an increasing probability of P limitation relative to PUFAs for zooplankton feeding *P. tricornutum* as N:P supply ratio increased. However, it must be noted that zooplankton production also depends on its nutritional requirements (Anderson and Pond, 2000). It is well known that the nutritional requirements of consumers change with prey nutritional status and environmental factors by regulating behavior (e.g., ingestion process) or physiological controls (e.g., metabolism) (Mitra and Flynn, 2005; Sperfeld and Wacker, 2011; Acheampong et al., 2014). Clearly, further work is required to incorporate elements and FAs of both consumers and prey in studies of marine planktonic trophic dynamics.

General conclusions

This study evaluated the variability of major element ratios (N:C and P:C) and PUFA contents in response to MEDs (N:P supply ratio, temperature and pCO_2) in two model species as representatives of common phytoplankton groups. Overall, our results scaled the relative importance of the three environmental factors, showing that warming and N (and P) deficiency had the most pronounced effects on elemental ratios and PUFAs, respectively. The effect trends of the three factors on the nutritional quality of phytoplankton are consistent with previous work, to some extent revealing the general relevance of our findings for phytoplankton. However, species-specific responses were also observed in our study, suggesting that the implications of this study should be tested with more phytoplankton species from different groups in future studies. Moreover, significant interactions between the three environmental factors suggest that the effect of temperature can be modified by nutrient conditions and pCO_2. We also observed the covariance of Q_N and PUFAs (under N deficiency) in both algal species, and the shifts of PON/PUFAs and POP/PUFAs in dependence of temperature, N:P supply ratio and algal species. This work thus provides a useful basis for simultaneous inter-comparison of elements and FAs in future studies on food web dynamics in the face of climate change.

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Conflict of Interest

None declared.

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