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The craving for phosphorus in heterotrophic dinoflagellates and its potential implications for biogeochemical cycles

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Abstract

By altering the nutritional quality of primary producers, nutrient availability indirectly influences herbivores' population dynamics. In turn, the resulting relationship between diet, growth, and wastes has consequences for nutrient cycling at the ecosystem level. We studied the link between dinoflagellates nutritional requirements and feeding behavior, and its influence on nutrient cycling. We show that long-term shifts in dissolved PO_4 concentration in the North Sea are closely linked to biomass trends of heterotrophic dinoflagellates and support this observation with experimental data indicating particularly high phosphorus requirements in dinoflagellates. At the seasonal scale, we observe a negative correlation between natural dinoflagellate abundances and the concentration of dissolved P, and we estimate that, in spring, up to 30% of dissolved P can end up in microzooplankton biomass. Our study highlights that accounting for organismal metabolic requirement provides significant insight in interpreting and predicting nutrient cycles at the ecosystem level.

Food quality is a major driver of zooplankton population dynamics (Gulati and Demott 1997; Danielsdottir et al. 2007). Because phytoplankton nutritional value strongly varies with the abiotic environment (Sterner and Elser 2002; Persson et al. 2010), herbivore zooplankton often feed on prey not matching their metabolic requirements. While autotrophs' carbon (C), nitrogen (N), and phosphorus (P) contents are influenced by the relative accessibility of light, carbon dioxide, and dissolved nutrients and as a result are highly variable (Sterner and Elser 2002), the elemental composition of heterotrophic plankton is less plastic (Meunier et al. 2012*a*, 2014). Differences between prey C : N : P stoichiometry and predator metabolic requirements thus cause a mismatch which may have consequences for zooplankton population dynamics as it directly influences feeding rates and growth (Hantzsche and Boersma 2010; Jeong et al. 2010; Zhang et al. 2017).

Since the 1970s, a succession of conventions and directives (e.g., OSPAR, Water Framework Directive) has been adopted in Europe to reduce N and P inputs to areas affected or likely to be affected by eutrophication. The consequences of these measures can be seen in the North Sea where dissolved P concentrations increased until 1978 and decreased afterwards (Sarker and Wiltshire 2017). The overall phytoplankton carrying capacity of the North Sea was shown to be related to the shift in dissolved P concentration (Sarker and Wiltshire 2017). Interestingly, there is also a parallel trend in time between the concentration of dissolved PO_4 and the biomass of heterotrophic dinoflagellates in the same area (Fig. 1). This observation indicates that, on a long time scale, there is a positive correlation between the nutrient concentration of the water (here measured as dissolved PO_4) and the density of heterotrophic dinoflagellates. Fast growth is a universal trait in heterotrophic dinoflagellates (Landry and Calbet 2004; Sherr and Sherr 2007), also in those found in the North Sea (Mieruch et al. 2010), which, according to the Growth Rate Hypothesis, suggests that these organisms

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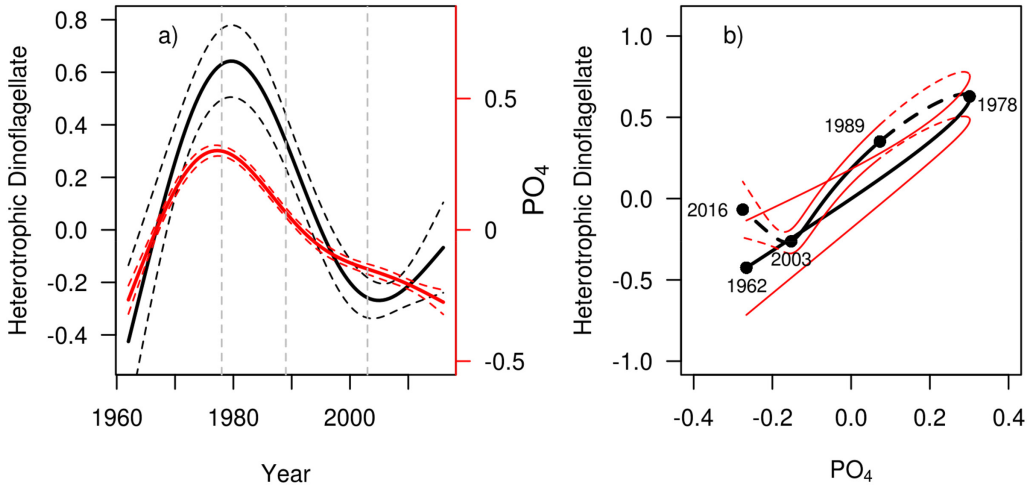

Fig. 1. Long-term trend analysis of the Helgoland Roads dataset (*see* Supporting Information Material): (**a**) Long term trends of PO_4 (red) and heterotrophic dinoflagellates (black) extracted by GAM analysis, dashed lines indicate the standard error of the trend, vertical lines show the years 1978,1989, and 2003. (**b**) Relationship between PO_4 and heterotrophic dinoflagellate long term trends including their confidence intervals (red). Periods with completely overlapping confidence intervals trends are indicated by dashed lines.

should have high P requirements. This hypothesis stipulates that fast-growing organisms have high P-requirements to synthesize P-rich ribosomal RNA (Sterner 1995; Elser et al. 1996; Sterner and Elser 2002). Thus, the link between P-availability and the biomass of heterotrophic dinoflagellates may result from the physiology of these organisms.

Heterotrophic dinoflagellates often make up more than 50% of microzooplankton biomass in temperate coastal areas and typically occur in high abundance following phytoplankton blooms (Sherr and Sherr 2007; Löder et al. 2011*a*). This microzooplankton group plays crucial roles in marine coastal systems. They influence primary producer biomass, the recycling of nutrients, and the transfer of nutrients and energy to higher trophic levels (Landry et al. 1993; Irigoien et al. 2005; Löder et al. 2011*b*; Aberle et al. 2013). Despite of their ecological significance, the parameters driving the feeding behaviors and population dynamics of heterotrophic dinoflagellates remain very ill-explored (Sherr and Sherr 2007; Schmoker et al. 2013; Zhang et al. 2017).

Given the importance of food quality for growth, heterotrophic dinoflagellates have adopted a variety of feeding behaviors in response to low prey quality to minimize the metabolic costs of ingesting nutrient-poor prey. For instance, they can choose their food based on prey parameters such as size and taxonomical identity (for review *see* Sherr and Sherr 2007). Phytoplankton nutritional value also directly influences ingestion rates. In feeding experiments with the heterotrophic dinoflagellate *Oxyrrhis marina*, Meunier et al. (2012*b*) showed a positive selection for P-rich algal cells and they also observed that *O. marina* compensates for low nutritional quality of one food item through compensatory feeding on a second food item which contains the limiting element. Alternatively, post-ingestion mechanisms, such as selective transfer efficiencies of ingested elements and excretion of excess elements, might also be effective mechanisms to balance unbalanced food (Anderson et al. 2005; Frost et al. 2005; Zhang et al. 2017). The grazing activity of heterotrophic dinoflagellates can thus affect their environment not only through selective feeding, i.e., by removing targeted elements, but also by the selective retention of scarce elements and the excretion of abundant ones (Vanni 2002; Meunier et al. 2016). As such, heterotrophic dinoflagellates may, due to their fast growth and high biomass especially in spring and summer, influence the recycling of nutrients on a seasonal scale.

We hypothesize that the long-term changes in nutrient availability, and especially P, that occurred in the North Sea altered phytoplankton nutritional value and in turn influenced heterotrophic dinoflagellates' dynamics. We propose that the long-term fluctuations in P availability in the North Sea have been driving changes in heterotrophic dinoflagellates' biomass since these organisms have high P requirements. Here, we test the hypothesis that heterotrophic dinoflagellates have high metabolic P demands and we evaluate the potential consequences for the cycling of P in coastal systems. Assessing elemental metabolic demands requires information on the consumer's growth and feeding responses to different prey qualities. Hence, in this paper we studied the trophodynamic responses of the ubiquitous heterotrophic dinoflagellate *Gyrodinium dominans* to P-rich and P-poor phytoplankton prey. Because microzooplankton can substantially influence nutrient cycling (Landry and Calbet 2004; Calbet 2008), we evaluate whether heterotrophic dinoflagellate abundances in the field are also correlated to dissolved PO_4 concentrations at the shorter seasonal scale. We used data from the Helgoland Roads Time Series (North Sea,

Germany), which contains daily measurements since 1962 (Wiltshire et al. 2010) and therefore provides a unique tool to study the relationship between heterotrophic dinoflagellate densities and dissolved P concentration. We hypothesize that heterotrophic dinoflagellates selectively retain P in their biomass by minimizing P excretion. Thus, on a seasonal scale, heterotrophic dinoflagellate abundance should negatively correlate with the concentration of dissolved P, indicating their key role in the P cycle.

Material and methods

In order to verify whether P availability can drive the long-term dynamics of heterotrophic dinoflagellates' populations, we need to understand the extent to which these organisms are influenced by and rely on P. Hence, we fed *G. dominans* with P-rich and P-poor phytoplankton prey and measured its growth rate and functional response. This heterotrophic dinoflagellate species is a well suited model organism as it is found in pelagic open waters and can be abundant in the North Sea (Löder et al. 2011*a*). Consumer dynamics are influenced by nutrient availability but they can also on a shorter time scale, through selective nutrient retention and excretion, influence nutrient concentrations in the environment. To assess whether seasonal fluctuations in heterotrophic dinoflagellate biomass can influence dissolved PO_4 concentrations, we first studied the feeding behavior of *G. dominans* and its ability to selectively retain and excrete specific nutrients. We tested whether this consumer actively chooses prey of higher nutritional quality and adjusts the amounts of P and N ingested and excreted. Because little is known about selective feeding, we also studied whether prey elemental P content itself, or the content in biochemical compounds is the driving force for selection. Then, we evaluated whether seasonal heterotrophic dinoflagellates' population dynamics influence dissolved PO_4 concentrations in the field.

Growth rate

To assess the growth response of heterotrophic dinoflagellates to different prey qualities, we fed *G. dominans* ad libitum every 24 h with P-rich (nP prey) and P-poor *Rhodomonas salina* (Np prey). For the different limitations, *R. salina* cultures were grown 3 d in F/2 medium (Guillard and Ryther 1962) without P (treatment "Np") and without N addition (treatment "nP") in 1-L bottles under high light (185 μmol m^{-2} s^{-1}) at 18°C at a 12 : 12 light : dark cycle (for details *see* Meunier et al. 2016). The heterotrophic dinoflagellates were taken from a stock culture regularly fed *R. salina* grown in F/2 medium (Guillard and Ryther 1962). All dinoflagellate cultures were kept in the dark. To measure cellular C, N, and P contents of algae and dinoflagellates, the density of each culture was determined using a CASY particle counter (SCHÄRFE SYSTEMS, Reutlingen, Germany) and 200 μg C were filtered on precombusted GF-F filters. Cellular C and N contents were measured with a Vario Micro Cube elemental analyser (Elementar, Hanau, Germany) while P was analyzed as orthophosphate after acidic oxidative hydrolysis with 5% H_2SO_4 (Grasshoff et al. 1999). The culture treatments led to significant differences in elemental contents of phytoplankton and heterotrophic dinoflagellate cells (one-way ANOVA, Table 1). We hypothesized that, to fulfil their high P requirements, heterotrophic dinoflagellates might be able to directly use dissolved P. Hence, we also tested whether *G. dominans* is able to directly use dissolved P to grow. We incubated *G. dominans* from our stock culture without prey in nutrient free artificial seawater but with 7.68 nmol P mL^{-1}. We also created a control without P addition. All incubations were done in 500 mL bottles using four replicates and were started with 5000 dinoflagellates mL^{-1}. After 3 d the density of each incubation vessel was determined using a CASY particle counter (SCHÄRFE SYSTEMS, Reutlingen, Germany) and used to calculate growth rates with the following equation

$$\mu = \left(\frac{\ln {}^{C_t}/_{C_0}}{t} \right)$$

where μ is the growth rate (d^{-1}), C_0 and C_t are the heterotrophic dinoflagellate concentrations at the beginning and at the end of the experiment (cells mL^{-1}), and t the incubation time (day). These growth rates were finally analyzed with a one-way ANOVA using prey type as predictor followed by a Tukey-HSD posthoc test.

Table 1. Nutrient contents and ratios of algae and dinoflagellates used for the experiments. C, N, and P cell contents (mean of four replicates ± SD) of P-rich (nP), P-limited (Np), and P-pulsed (Np) cultures. Different letters (A,B) indicate significant differences between treatments.

		nP	Np	Np-pulsed
R. salina	C (pg $cell^{-1}$)	72.3 (3.7)^{A}	58.9 (6.1)^{B}	66.5 (9.4)^{AB}
	N (pg $cell^{-1}$)	5.8 (0.5)^{A}	10.7 (0.98)^{B}	12.1 (1.3)^{B}
	P (pg $cell^{-1}$)	0.80 (0.14)^{A}	0.20 (0.03)^{B}	0.74 (0.25)^{A}
	C : N (molar)	14.7 (1.2)^{A}	6.4 (0.1)^{B}	6.4 (0.4)^{B}
	C : P (molar)	238 (38)^{A}	752 (132)^{B}	253 (85)^{A}
	N : P (molar)	16.2 (1.8)^{A}	117 (19)^{B}	39.8 (14.8)^{C}
G. dominans	C (pg $cell^{-1}$)	450 (108)^{A}	388 (32)^{A}	
	N (pg $cell^{-1}$)	74.2 (8.2)^{A}	73.3 (5.8)^{A}	
	P (pg $cell^{-1}$)	6.7 (0.7)^{A}	4.5 (1.1)^{B}	
	C : N (molar)	7.1 (0.8)^{A}	6.2 (0.4)^{A}	
	C : P (molar)	173 (24)^{A}	238 (66)^{A}	
	N : P (molar)	24.4 (3.5)^{A}	39.1 (2.5)^{B}	

Functional response

To measure the functional response of the heterotrophic dinoflagellate *G. dominans*, we incubated 2000 dinoflagellates mL^{-1} of the nP and Np *G. dominans* cultures (see above) in 100 mL plastic beakers with nP and Np prey, respectively, in

eight concentrations between 1 and 100 phytoplankton cells per dinoflagellate. We also created control bottles without predators for each prey concentration. The experimental bottles were incubated at 18°C in the dark for 2 h. At the end of the experiment, the density of remaining prey was determined with a CASY particle counter. These counts together with the prey C content measurements were used to calculate ingestion rates as the amount of C ingested per *G. dominans* and hour (Frost 1972; Tarran 1991). These ingestion rates were then analyzed by linear logarithmic regression.

Selective feeding

We tested whether heterotrophic dinoflagellates selectively ingest P-rich prey. To do so, a mixture of 50% nP and 50% Np prey (same prey used for the growth rate assessment) was prepared in 100-mL plastic beakers (six replicates) and diluted with artificial seawater, which contained no nutrients to a predator : prey ratio of 1 : 25. To test whether selective feeding behavior is influenced by feeding history, we used the *G. dominans* cultures fed with either nP or Np prey for the growth rate assessment (see above) and offered the prey mix to each precondition type. These incubations were conducted after Meunier et al. (2012*b*) and ran for 2 h.

Whether prey elemental P content itself, or the content in biochemical compounds is the driving force for selection was assessed in a second, parallel, selectivity experiment. We used the same experimental setup described above but exposed the Np prey to a P pulse (Np-pulsed prey) immediately before being fed to the heterotrophic dinoflagellates. Starving algae are known to soak up nutrients very fast when new nutrients are supplied and store them for later use (Boersma 2000). Schoo et al. (2014) confirmed that a single P pulse only influences the nutrient stoichiometry of P-limited *R. salina*, while the biochemical composition (i.e., the fatty acid content), which takes longer to change, remains identical to that of the P-limited algae. Hence, the Np culture received a pulse of 38.4 μmol P L^{-1} and the prey could take up this nutrient for 30 min before being fed to the heterotrophic dinoflagellates for the selectivity experiment. The elemental content of the Np-pulsed prey was measured 30 min after the P pulse as well as at the end of the experiment following the sampling procedure described above. We observed that the pulse treatment cancelled the difference in P content with the nP prey (Table 1). There was no difference in P content of the Np-pulsed prey before and after the selectivity experiment.

At the end of the 2 h incubation, we fixed the samples with formalin (formaldehyde 20% buffered with hexamine) and stored them cool and dark. In order to count and discriminate nP and Np prey cells, 2.973 mL of each sample were settled in sedimentation chambers (HYDROBIOS) for 24 h, and counted under a Zeiss Axiovert 135 inverted microscope using epifluorescence (Meunier et al. 2012*b*). This method is based on color differences between Np (red) and nP (green) *R. salina* caused by a lack of phycoerythrin in N-limited algae. Prey selectivity, α, was calculated according to Chesson (1978, 1983). Significance of the selectivity was tested against $\alpha = 0.5$ (Student's *t*-test), using the different replicates of the selection experiment.

Nutritional geometry

In a separate experiment, we fed a gradient of prey with different N : P ratios to *G. dominans* and measured how the N : P ratio of the heterotrophic dinoflagellates is influenced by this prey quality gradient (Supporting Information Fig. S1). Sampling and analyses for N and P were performed following the same procedure described above. This experiment allowed us to identify an inverse-sigmoidal response which is characteristic of regulators (Supporting Information Fig. S1), i.e., organisms with a certain degree of stoichiometric homeostasis (Meunier et al. 2014). Since the middle plateau of this inverse-sigmoidal curve corresponds to the N : P ratio which heterotrophic dinoflagellates aim at maintaining, it can be interpreted as the amount of N and P *G. dominans* need to obtain from their diet (assuming identical uptake and metabolism). To test whether these requirements are fulfilled, we calculated the N and P ingested by both preconditioned dinoflagellates fed on mixes of 50% nP and 50% Np prey (selective feeding experiment, see previous paragraph). To do so, we determined *G. dominans* ingestion rates for each prey quality and multiplied these with the N and P content of each prey type. We tested differences in N and P content between different diets (diet of nP dinoflagellates, diet of Np dinoflagellates, and plateau N : P) with one-way ANOVAs. We used a graphical representation employed in nutritional geometry (Raubenheimer et al. 2009) to identify whether heterotrophic dinoflagellates adjust their feeding behavior to fulfil their nutrient requirements. In this chart, the straight lines represent the N and P that consumers can obtain from individual food types and the area between these lines illustrates the nutrients consumers can obtain based on the proportion and quantity of each food type in their diet.

Excretion

In order to illustrate how changes in food quality alter excretion N : P (i.e., the ratio of elements released to the environment), we used a simple mass balance model to estimate excretion N : P ratios. We multiplied *G. dominans* maximum ingestion rate (calculated from the selective feeding experiment) by the N and P content of nP and Np prey, then subtracted the N and P content of *G. dominans* (i.e., N and P excreted), and finally calculated the N : P ratios of these estimated N and P excretion. These results were analyzed using a one-way ANOVA with excreted N : P as the dependent variable and food precondition as factor.

Seasonal trend analysis

To evaluate whether seasonal fluctuations of heterotrophic dinoflagellate abundances in the field are correlated to dissolved PO_4 concentrations, we conducted a statistical analysis of the Helgoland Roads dataset (German Bight, North Sea). For this analysis only heterotrophic dinoflagellates were considered, mixotrophs were not included in the dataset. Time resolution of this dataset ranges from 3 to 5 data points per week, depending on the time period. The relationship between heterotrophic dinoflagellate abundances and dissolved PO_4 concentrations were calculated as:

$$[PO_4] = e^{a} \times \text{Heterotrophic dinoflagellates}^{b}$$

using a generalized linear model (GLM) following the Gamma distribution. All analyses were carried out in the R software (v3.3.2) and the mgcv package (v1.8-17) (Wood 2011; R Core Team 2017).

Results

Growth and feeding response to prey P content

We assessed the growth response of heterotrophic dinoflagellates to different prey qualities (Fig. 2a). We observed the highest growth rate for *G. dominans* fed with P-rich prey ($\mu = 0.80$ d^{-1}) while P-poor prey significantly reduced the heterotrophic dinoflagellates' growth rate ($\mu = 0.47$ d^{-1}, one-way ANOVA, Tukey-HSD posthoc test $p < 0.01$). We also observed no significant difference in growth rate between the control and the treatment in which *G. dominans* was only supplied with dissolved P (one-way ANOVA, Tukey-HSD posthoc test $p = 0.56$). The growth rates in the control and the phosphate treatment were however slightly higher than zero, indicating that the heterotrophic dinoflagellates could still use the prey present in their food vacuoles to grow or rely on heterotrophic bacteria present in the filtered-sea water. The functional responses of the heterotrophic dinoflagellate *G. dominans* were different when fed with nP and Np prey (Fig. 2b). For all prey concentrations, ingestion rates on P-rich prey were higher than on P-poor prey (paired *t*-test, $p < 0.05$). The maximum C ingestion rate was also higher for nP prey (Vmax = 1.33 ngC d^{-1}) than for Np prey (Vmax = 1.18 ngC d^{-1}). Interestingly, the ratios of those maximum ingestion rates relative to the body carbon content of *G. dominans* fed on nP prey and Np prey are very similar. Thus, the carbon-specific maximum ingestion rates on both prey may not be different. However, as the growth rates on both prey were clearly different, the nutritional values of both prey must be different.

We observed that *G. dominans* feeds selectively when offered a mix of nP and Np prey (Fig. 3a). Irrespective of the different pre-conditioning, these heterotrophic dinoflagellates selected positively for P-rich prey (Fig. 3a). The two types of pre-conditioned *G. dominans* had similar selectivity index $\alpha = 0.59$ and 0.58 (significantly different from $\alpha = 0.5$) for nP and Np preconditions, respectively. However, when offered a mix of nP and Np-pulsed prey, the heterotrophic dinoflagellates did not show any sign of selective feeding (Fig. 3b). Both pre-conditioned *G. dominans* had the same selectivity index $\alpha = 0.51$ (not significantly different from $\alpha = 0.5$).

While the amount of N ingested varied significantly between pre-condition types and was significantly different from the N target (one-way ANOVA $p < 0.05$), we observed no significant difference between the P target and the P ingested by *G. dominans* (two one way ANOVA $p = 0.54$; Fig. 4a). This indicates that heterotrophic dinoflagellates tightly regulate the amount of P they ingest while their N ingestion is more flexible. To illustrate how different prey types may affect nutrient recycling, we estimated the excretion N : P ratios for *G. dominans* feeding on different food qualities (Fig. 4b). Excretion N : P ratios were significantly higher for heterotrophic dinoflagellates feeding on P-poor algae than for those feeding on P-rich algae (one-way ANOVA, $p < 0.05$).

Seasonal trends of heterotrophic dinoflagellate abundances and P cycle

We detected a clear correlation between PO_4 concentrations and heterotrophic dinoflagellate biomass. Seasonal trends of PO_4 and heterotrophic dinoflagellate extracted via generalized additive models (GAMs, *see* Supporting Information) show an opposite pattern (Fig. 5a). Increasing heterotrophic dinoflagellate biomass (from ca. week 10) coincides with decreasing PO_4 concentrations. The highest heterotrophic dinoflagellate biomass occurs with a certain lag, due to the normal time it takes for them to grow, from the PO_4 concentrations minima. Similarly, a GLM of weekly averaged PO_4 concentration vs. heterotrophic dinoflagellate biomass showed a clear opposite relationship, PO_4 concentration decreases as heterotrophic dinoflagellate biomass increases (Fig. 5b). This model was statistically significant at the 5% level and follows the formula:

$$[PO_4] = e^{1.1521} \times \text{Hetetrotrophic dinoflagellates}^{-0.1199}$$

In order to ensure that the seasonal trends observed are influenced by heterotrophic dinoflagellates, we calculated the biovolume contribution of heterotrophic dinoflagellates to the total biovolume of dinoflagellate and phytoplankton. We observed that between weeks 10 and 23 heterotrophic dinoflagellates can comprise up to 15% of this biovolume (Supporting Information Fig. S2).

Discussion

Although it is believed that microzooplankton play a major role in nutrient recycling (Johannes 1964; Irigoien et al. 2005), their importance has rarely been evaluated at the ecosystem level. Here, we show that heterotrophic dinoflagellates are potentially able to sequester a considerable

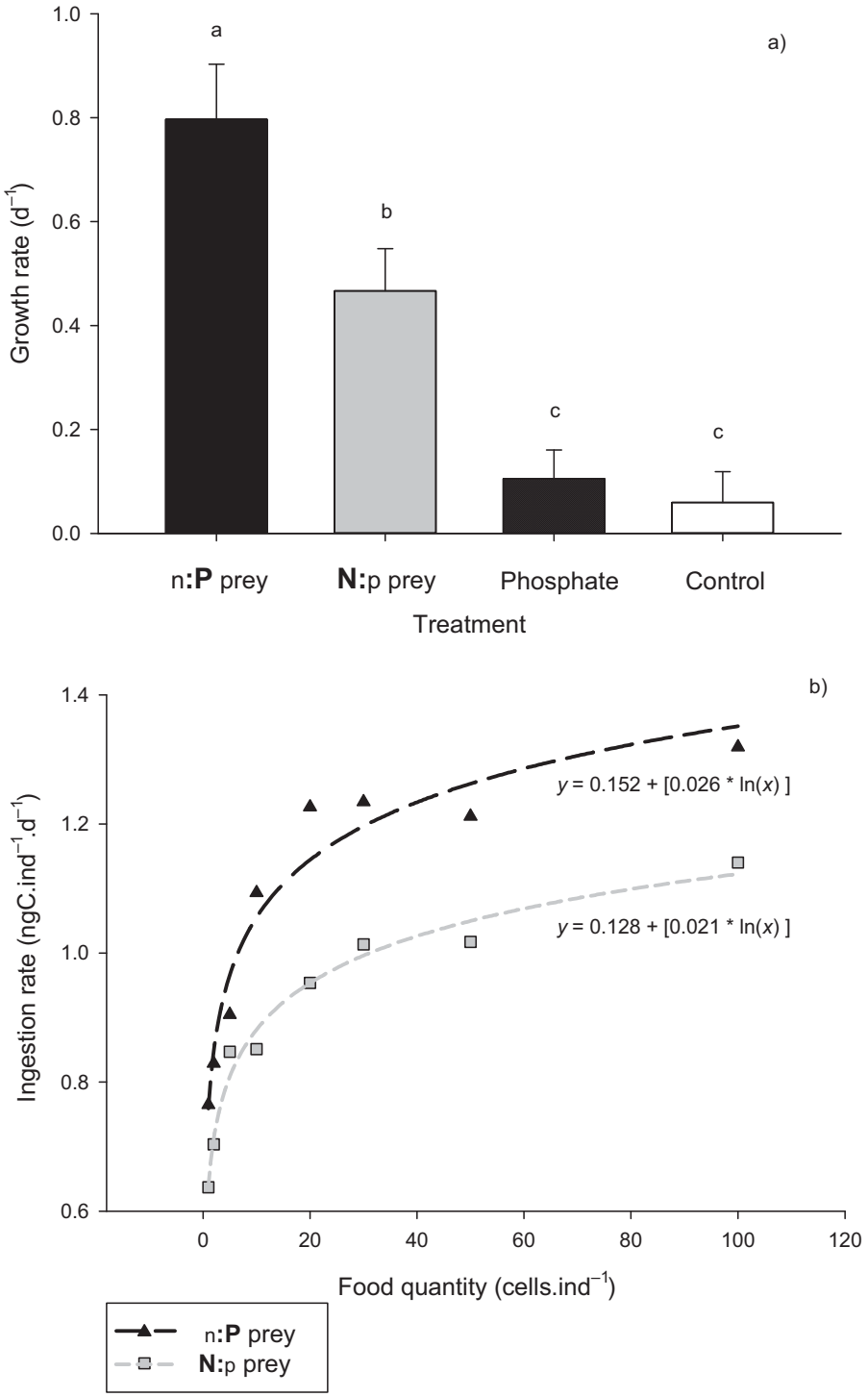

Fig. 2. Growth rates (**a**) of *G. dominans* fed with different food sources. Different bar colors indicate different prey treatments: black indicate P-rich prey, gray indicates P-poor prey, black white-dotted indicates dissolved P, and white is the control. Data indicate means and SD of four replicates. Different letters indicate significant differences between treatments. Ingestion rates (**b**) of *G. dominans* fed with P-rich (black triangles) and P-poor prey (gray squares). The dotted lines are non-linear logarithmic regressions.

part of the available P within a system. The negative relationship between dissolved P concentration and dinoflagellate biomass is particularly strong in spring when increasing heterotrophic dinoflagellate biomass coincides with decreasing dissolved P concentrations. We attribute this relationship to a trophic cascade between dissolved P, phytoplankton,

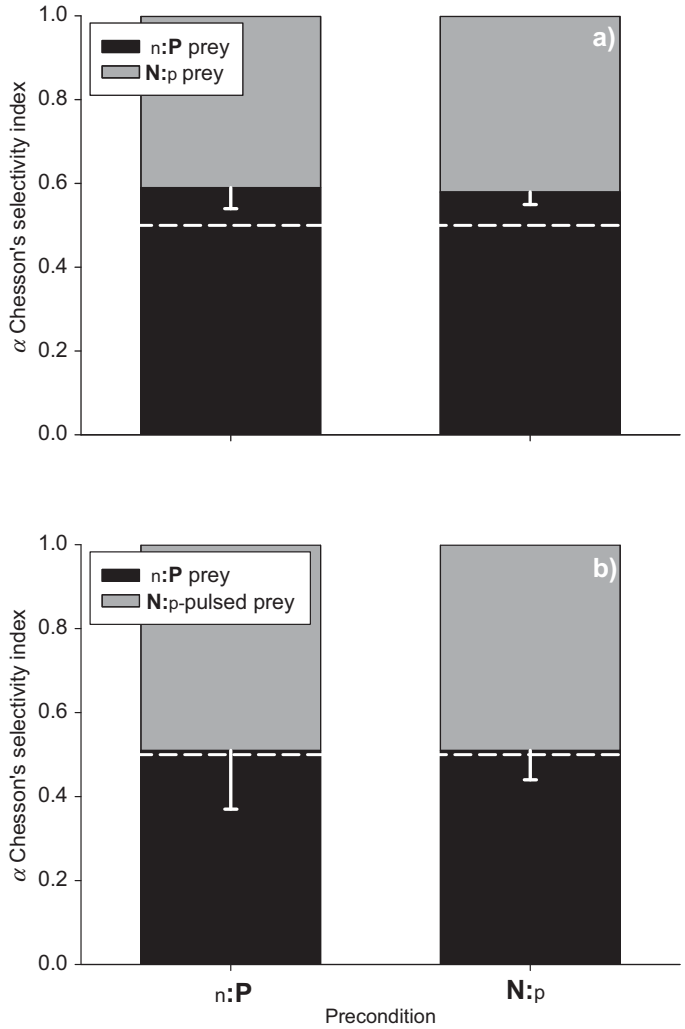

Fig. 3. Selective feeding of *G. dominans*. Selectivity between P-rich (black bars) and P-poor prey (gray bars) (**a**) and between P-rich (black bars) and P-pulsed prey (gray bars) (**b**) of *G. dominans* preconditioned for 3 d on P-rich and P-poor prey. Selectivity was calculated as α Chesson's selectivity index (mean ± SD). Each value represents the mean of six replicates.

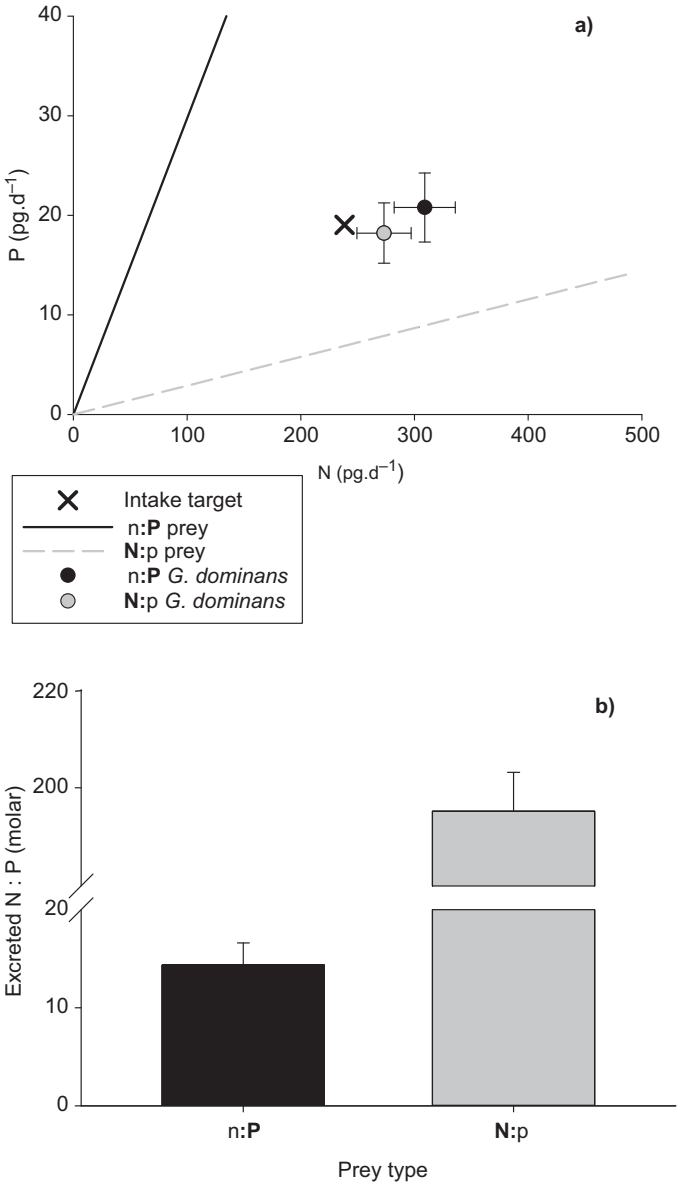

Fig. 4. (**a**) N and P required for growth vs. N and P obtained from the diet. Optimal N and P content of *G. dominans* (cross), N and P obtained from feeding for 1 d on a mixed diet of P-rich (continuous black line) and P-poor (dotted gray line) prey for *G. dominans* preconditioned 3 d on P-rich (black circle) and P-poor (gray circle) prey (mean ± SD). Each value represents the mean of six replicates. (**b**) *G. dominans* N : P excretion. N : P excretion calculated with a mass balance model of *G. dominans* feeding on P-rich (black bar) and P-poor (gray bar) prey (mean ± SD). Each value represents the mean of six replicates.

and heterotrophic dinoflagellate as well as to the fact that these consumers selectively retain P in their biomass. Interestingly, Meunier et al. (2016) analyzed the same dataset as in the present study and observed a similar link between dissolved P concentration and the abundance of nauplii. We used our data as well as that of Meunier et al. (2016), to estimate the percentage of dissolved P ending up in total microzooplankton biomass in spring. Using the C : P ratios of nauplii and heterotrophic dinoflagellates measured in the laboratory, we converted the nauplii and heterotrophic dinoflagellates C biomass measured in the field in spring into P biomass and compared it to winter dissolved PO_4 concentrations. We estimate that up to 16% of dissolved P can end up in heterotrophic dinoflagellate biomass and in total up to 30% in microzooplankton (heterotrophic dinoflagellate + nauplii) biomass. This is remarkable since the seasonal decrease in dissolved P during spring is traditionally solely attributed to the phytoplankton spring bloom (Benitez-Nelson 2000). Hence, the role heterotrophic dinoflagellates, and microzooplankton in general, play in biogeochemical cycles might be more important than generally believed.

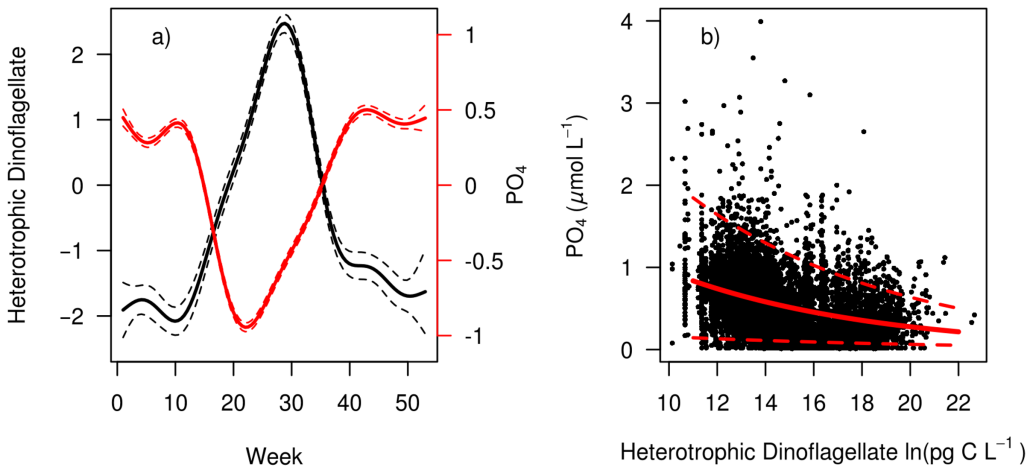

Fig. 5. Seasonal trend analysis of the Helgoland Roads dataset. (**a**) Seasonal trend extracted by GAM analysis for PO_4 (black) and heterotrophic dinoflagellates (red), dashed line represents standard error of the trend; (**b**) modelled relationship for the whole dataset between PO_4 and heterotrophic dinoflagellates, dashed line represents the confidence interval.

Our results suggest that the influence heterotrophic dinoflagellates may have on the P cycle originates from their metabolic requirements. We observed that *G. dominans* grows faster and has higher ingestion rates when fed P-rich prey than P-poor prey. Since the growth of *G. dominans* is influenced by prey P content, we expected this heterotrophic dinoflagellate to adjust its feeding behavior to maximize P intake. We recorded higher ingestion rates of P-rich prey than P-poor prey and observed that *G. dominans* preferentially ingests P-rich prey and tightly regulates the amount of P it ingests. While some studies have shown selective feeding and changes in ingestion rates associated with different prey qualities (e.g., Cowles et al. 1988; Sanders and Wickham 1993; Meunier et al. 2012*b*, 2016; Zhang et al. 2017), the mechanisms involved in prey quality recognition remain however largely unknown. Nutrient limitation of phytoplankton cells can alter the biosynthesis of specific molecules such as fatty acids and proteins (e.g., Shifrin and Chisholm 1981; Harrison et al. 1990). For instance, N-limitation was shown to influence the production of mannose located at the phytoplankton cell surface (Martel 2009) which are involved in prey biorecognition processes (Wootton et al. 2007; Roberts et al. 2011). We, however, observed that *G. dominans* did not feed selectively when offered a mix of P-rich and P-pulsed prey. This indicates that this heterotrophic dinoflagellate does not use biosynthesized molecules as infochemicals but is able to identify the elemental P content of phytoplankton prey cells, or alternatively that the changes in biochemistry are far faster than has been measured for, for example, fatty acids.

The craving for P in *G. dominans* is likely linked to its fast growth and can be explained by the Growth Rate Hypothesis, which posits that fast-growing organisms have high P requirements (Sterner 1995; Elser et al. 1996; Sterner and Elser 2002). Growth rate is therefore an important trait influencing nutritional requirements and subsequently feeding behavior. Further, metabolic rates are tightly linked to size and small organisms tend to have higher maximal growth rates (Savage et al. 2004; Kempes et al. 2012). Consequently, we suggest that the results we obtained for *G. dominans* are not species specific but could be generalized to heterotrophic dinoflagellates, which are small and fast growing organisms. Our results support previous work showing that *O. marina* selectively feeds on P-rich prey (Meunier et al. 2012*b*), presents strong resistance against and resilience from P-limitation (Meunier et al. 2012*a*), and grows slower when fed with P-poor prey (Hantzsche and Boersma 2010). Those similarities in physiological response are particularly interesting since the two heterotrophic dinoflagellate species occupy very different habitats, *G. dominans* is found in pelagic systems and *O. marina* in tidal pools. Because they share similar growth and size traits, heterotrophic dinoflagellates and other microzooplankton taxa might have high P-requirements. For instance, copepod nauplii, which are important members of microzooplankton and the most abundant metazoans on earth (Turner 2004), grow faster and are richer in P than the older copepodite stages (Elser et al. 1996; Villar-Argaiz et al. 2002) and preferentially ingest P-rich prey (Meunier et al. 2016). From a succession perspective, microzooplankton are the first consumers to respond to autotrophic biomass buildup (e.g., beginning of the phytoplankton bloom). Our findings suggest that competition for P, caused by P sequestration in micrograzers, might be important at the onset of a heterotrophic dinoflagellate bloom.

Heterotrophic dinoflagellates adjust their nutrient excretion based on the nutritional status of their prey. In laboratory grazing experiments, Andersen et al. (1986) observed that when fed P-limited prey, virtually no P was excreted

throughout the entire growth cycle of the microflagellate *Physomonas imperforata*. We estimated *G. dominans* excretion N : P ratios and determined that this heterotrophic dinoflagellate should excrete higher N : P ratios with increasing prey P-limitation, therefore selectively retaining P. The excretion N : P ratios we calculated are similar to those estimated for copepod nauplii and are lower than that of copepodites (Meunier et al. 2016) indicating that microzooplankton might retain relatively more P in their tissue than other organisms with lower P metabolic requirements. These predictions are generally supported by experimental data of consumer-driven nutrient recycling (for syntheses *see* Elser and Urabe 1999; Vanni and Mcintyre 2016) showing that excretion N : P ratios are primarily a function of prey N : P ratio and secondarily a function of consumer N : P ratio, and that rates of P release by consumers are also related to prey P content. Furthermore, studies on gelatinous zooplankton show that, despite increasing body C : P ratios, P limitation does not reduce the growth of *Pleurobrachia pileus* and *Gonionemus vertens* (Malzahn et al. 2010; Schoo et al. 2010). These results indicate that, compared to other coexisting consumers such as copepodites, adult copepods, and gelatinous zooplankton, microzooplankton have particularly high demands for P. Hence, our study highlights the ecophysiological importance of nutritional requirements and indicates that the resulting relationship between diet, growth, and excretory products substantially influences the cycling of nutrients at the ecosystem level.

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Conflict of Interest

None declared.

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