

# Effects of diet nutritional quality on the growth and grazing of *Noctiluca scintillans*

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**ABSTRACT:** *Noctiluca scintillans* is a cosmopolitan red tide-forming heterotrophic dinoflagellate which can feed on a variety of algae. In this study, we examined the effects of diet nutritional quality on its ingestion and reproduction. Functional and numerical response experiments were conducted using 3 types of algae: a diatom (*Thalassiosira weissflogii*), a chlorophyte (*Platymonas helgolandica*) and a dinoflagellate (*Prorocentrum dentatum*) that were grown under nitrogen- (N-) and phosphorus- (P-) replete, N-depleted and P-depleted conditions. Ingestion and growth rates of *N. scintillans* were fitted using Type II and modified Type II models, respectively. *N. scintillans* generally exhibited higher maximum ingestion rate under nutrient-deficient conditions than when fed on N- and P-sufficient prey, presumably in order to maximize its nutrient pool and meet growth requirements. All phytoplankton cultures, except P-deficient *T. weissflogii*, supported the growth of *N. scintillans*. However, nutrient deficiency, especially P-deficient prey, yielded lower growth rates of *N. scintillans* than their nutrient-sufficient counterparts. No optimum curve was obtained for P-deficient *T. weissflogii*, which may become toxic under P limitation. Based on the hyperbolic regression models simulated for *N. scintillans* growth rate using different resources' nutritional contents as variables, P limitation appears to be the major constraint affecting *N. scintillans* reproduction and survival under nutrient deficiency. Polyunsaturated fatty acids, e.g.  $\alpha$ -linolenic acid (18:3 $\omega$ 3, ALA) and eicosapentanoic acid (20:5 $\omega$ 3, EPA), are also important in determining food quality for *N. scintillans* based on their high correlation with *N. scintillans* growth rate.

**KEY WORDS:** *Noctiluca scintillans* · Diet nutritional quality · Elemental composition · PUFAs · Functional response · Numerical response

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## INTRODUCTION

Resource limitation can have profound effects on predator–prey interactions (Brett & Goldman 1997, Glibert et al. 2013). Alteration of the mineral composition of phytoplankton cells due to resource limitation can alter their nutritional value to consumers, and may create an indirect bottom-up effect (Sterner & Elser 2002, Cebrian et al. 2009, Felpeto & Hairston 2013). Typically, phytoplankton grown in low-nutrient (phosphorus [P] and/or nitrogen [N]) environments become relatively poor-quality food (high C:P or C:N) for consumers (Sterner & Elser 2002,

Glibert et al. 2013). P limitation is known to reduce algal quality for grazers with high somatic growth rates (more rRNA) or P-containing structural components, such as skeleton and bones (Sterner & Elser 2002). N limitation also can reduce the egg production and growth efficiency of some copepods (Checkley 1980, Kjørboe 1989, Jones et al. 2002).

There are, however, other nutritional factors affecting the quality of algae as a food source for grazers, such as essential amino acids, fatty acids, etc. (Kleppel et al. 1998, Anderson & Pond 2000, Guisande et al. 2002, Anderson et al. 2004). Polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid

(20:6 $\omega$ 3, DHA), eicosapentanoic acid (20:5 $\omega$ 3, EPA) and  $\alpha$ -linolenic acid (18:3 $\omega$ 3, ALA) etc., are one of the crucial classes of fatty acids. They are critical structural components and precursors of signaling molecules, which are involved in many diverse biological and biochemical processes, and important to maintain physiological functions in consumers (Ackman et al. 1980, Sargent et al. 1987, Caramujo et al. 2008, Lund et al. 2008). In aquatic ecosystems, PUFAs are primarily produced by planktonic algae and are also affected by nutrient availability (Sargent et al. 1987, 2002). Researchers have conducted many tests on the role of PUFA in determining phytoplankton food quality for grazers (Müller-Navarra et al. 2000). Most research on this subject has used mesozooplankton to investigate the effect of PUFA composition of phytoplankton prey on their growth, survival, egg production and hatching success (Gulati & Demott 1997, Breteler et al. 2005, Chen et al. 2012), but little is known about the importance of PUFA for heterotrophic dinoflagellates (Lund et al. 2008). It is still challenging to define what a poor-quality diet is, and to determine which biochemical compounds are essential for heterotrophic dinoflagellates because of their weak homeostasis and complicated n-3 PUFAs upgrading process (Lund et al. 2008, 2009, Meunier et al. 2012, Calbet et al. 2013).

Food quality and quantity are expected to have significant effects on feeding and population growth of consumers. These effects can be evaluated in terms of functional and numerical responses. Functional responses are used to define the specific food intake rate (i.e. per zooplankton biomass, per unit of time) as a function of ambient food density (Holling 1959). Generally, they are classified into 3 main types as Type I (linear response), Type II (concave upward response) and Type III (sigmoid response) (Holling 1959). Type IV and V response curves, in which consumer intake rate peaks and then declines, also effectively define the functional response of consumers to food containing resource-driven toxicity (Crawley 1992). Numerical responses per se associate with functional responses and describe the relationship between food availability and population growth rate or abundance of a consumer (only growth rate was considered in this study) (Holling 1959, Kot 2001). Type II and modified Type II (i.e. has a growth threshold) numerical responses are most commonly used in experimental and modeling studies (Kot 2001).

*Noctiluca scintillans* is a prominent 'red tide' organism in many temperate and subtropical neritic waters (Elbrächter & Qi 1998, Harrison et al. 2011). Even when in non-bloom condition it still may constitute a

significant fraction of zooplankton biomass in many coastal regions (Nakamura 1998a, Tada et al. 2004, Chen et al. 2011, Harrison et al. 2011). This voracious grazer can feed on an extensive variety of food items including bacteria, phytoplankton, microzooplankton, copepod eggs, fish eggs, protozoans and even detritus, with phytoplankton considered as the main food item in the field (Kirchner et al. 1996, Elbrächter & Qi 1998). *N. scintillans* populations thus exert substantial grazing pressure on the phytoplankton community and may influence algal biomass, production and community composition (Kiørboe & Titelman 1998, Nakamura 1998a, Umani et al. 2004). On the other hand, quality and quantity of prey (e.g. phytoplankton) in turn are likely to affect their population dynamics. Many studies addressing the effects of diet and nutritional quality on the marine food web have been done with copepods (e.g. *Acartia tonsa*) and protozoans (e.g. *Oxyrrhis marina* and *Gyrodinium dominans*) (Kleppel et al. 1998, Chu et al. 2008, Mayor et al. 2011), but little is known about *N. scintillans*, despite its importance as a grazer on these autotrophs.

In the present study, we examined the functional and numerical responses of *N. scintillans* fed food resources of different qualities (in terms of C, N, P and fatty acid contents) and quantities, and investigate the critical constraints affecting its growth and reproduction. Preliminary studies have showed that *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum*, which have similar sizes (10–11  $\mu$ m) but quite contrasting fatty acids profiles (Chen et al. 2012, Xu et al. 2012), can support the growth of *N. scintillans* (Wu et al. 1994, Kiørboe & Titelman 1998). The diatom *T. weissflogii* and dinoflagellate *P. dentatum* represented the 2 main food sources for *N. scintillans* in the field (Elbrächter & Qi 1998, Umani et al. 2004, Liu & Wong 2006). *P. helgolandica* can support fast growth of *N. scintillans* in laboratory conditions even though this alga is seldom found in nature where *N. scintillans* occur or bloom (Wu et al. 1994). We used these 3 algal species grown in 3 different nutrient regimes (nutrient-replete, N-depleted and P-depleted) as the food sources of *N. scintillans*. These treatments were intended to create a wide range of algal biochemical and mineral compositions.

## MATERIALS AND METHODS

### Experimental organisms and culture conditions

*Noctiluca scintillans* were collected with a 120  $\mu$ m plankton net from the pier of Port Shelter in eastern

Hong Kong in October 2011. *N. scintillans* cells (several hundred individuals) were then isolated using a plastic dropper and cultured in 1 to 2 l glass beakers with 0.2  $\mu\text{m}$ -filtered (Maxi Capsulate Filter, Pall) autoclaved seawater collected from the same area. *N. scintillans* was then kept in a temperature-controlled chamber at  $23 \pm 1^\circ\text{C}$  with light intensity of  $50 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  in a 14:10 h light:dark cycle. This culture was used throughout the study. *N. scintillans* cultures were subsequently fed every 3 d with a mixture of *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* at a concentration  $>1 \mu\text{g C ml}^{-1}$ . In the preliminary study we found that continuous shaking and rotation caused deleterious effects on *N. scintillans* growth. Hence, cultures were only gently agitated manually twice a day to keep the prey items homogeneously distributed. *N. scintillans* cells were collected by reverse filtration with 123  $\mu\text{m}$  mesh, transferred to fresh-filtered autoclaved seawater every week and kept in the same conditions as described above.

Cultures of *T. weissflogii* and *P. dentatum* used in this study were obtained from the algae collection at the coastal marine laboratory, Hong Kong University of Science and Technology. *P. helgolandica* (strain M1-3, which originates from Yantian, Shenzhen) was obtained from Jinan University, China. These phytoplankton cells were first grown as batch cultures in f/2 (Si) medium (Guillard & Ryther 1962) under the same laboratory condition as the *N. scintillans* culture. The culture media was prepared with 0.2  $\mu\text{m}$ -filtered autoclaved natural seawater (salinity 35 psu) collected from Port Shelter. When the cultures reached late exponential phase, phytoplankton cells were inoculated into fresh growth media with 3 nutrient regimes (nutrient-replete [f/2], nitrogen-depleted [-N] and phosphorus-depleted [-P]) and were grown as batch cultures. Nutrient-replete cultures were grown in f/2 media with silicon (Si) for *T. weissflogii* cultures and without Si for *P. helgolandica* and *P. dentatum*. -N and -P cultures were prepared in the same manner but without adding  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  respectively while keeping the concentrations of other nutrients as in the f/2 medium. Growth and grazing experiments were started once the cultures reached exponential phase (culture period of 6 to 8 d), which allowed us to harvest enough prey cells for the experiment and also to obtain N- and P-deficient phytoplankton cells. All the cultures were sampled for cellular C, N, P and fatty acid analyses, cell counting and estimation of cell volumes before the feeding and growth experiments were started.

### Functional and numerical responses of *N. scintillans* to diets with different nutritional qualities

The experiment consisted of 2 parts: a functional response and a numerical response. These 2 experiments were designed to study the feeding and growth responses of *N. scintillans* to food with different qualities as a function of prey concentration. To avoid any potential effects of food carryover, *N. scintillans* used in this study were starved for 24 h prior to the experiment to empty their food vacuoles. Each experiment consisted of 9 treatments representing 3 algal prey species with 3 nutritional statuses.

**Functional response experiment.** In order to study the feeding responses of *N. scintillans* to diets with different nutritional qualities, starved *N. scintillans* cells were incubated with 9 different food items (NP-sufficient, N-deficient and P-deficient cultures of *T. weissflogii*, *P. helgolandica* and *P. dentatum*). For each food type, 6 to 8 different food suspensions with concentrations ranging from 300 to 25 000 cells  $\text{ml}^{-1}$  were prepared by diluting the stock cultures with appropriate amounts of filtered autoclaved seawater. Approx. 300 starved *N. scintillans* cells were inoculated into 50 ml food suspensions of each concentration in 125 ml polycarbonate bottles in triplicate. Two bottles containing prey items without *N. scintillans* were used as controls. All cultures were incubated under dim light, but keeping the other conditions the same as described above, for 6 h. At the end of the incubation, subsamples of 1 ml were taken from each bottle to estimate the final prey densities. Changes in cell densities of *N. scintillans* during the incubation period were neglected for the calculation of clearance rate ( $F$ ) since the incubation period was 0.25 d only (Nakamura 1998b).

**Numerical response experiment.** The same phytoplankton species and nutrient regimes as in the feeding experiment were used as prey in the growth experiment. Food suspensions of 6 to 7 different prey concentrations ranging from 1000 to 40 000 cells  $\text{ml}^{-1}$  were prepared by diluting stock cultures with f/20 (for NP-sufficient cultures) or f/20 without  $\text{NO}_3^-$  (for N-deficient cultures) or f/20 without  $\text{PO}_4^{3-}$  (for P-deficient cultures) media. Approx. 100 starved *N. scintillans* cells were inoculated into 100 ml of food suspension of each concentration in triplicate. Cultures of *N. scintillans* without prey (100 ml filtered, autoclaved seawater in triplicate) served as controls. All cultures were incubated under the same conditions as those in the feeding experiment for 3 d. Cultures were gently agitated manually 2 to 3 times a day to avoid cell aggregation and settlement. To esti-

mate the final prey and grazer densities, subsamples of 1 ml and duplicate subsamples of 30 to 40 ml respectively were taken from each bottle at the end of the incubation.

### Estimation of cell densities and biovolumes

All subsamples were preserved in acidic Lugol's solution (final concentration 2%). To determine the prey cell densities, aliquots of 100 to 250  $\mu$ l of the preserved samples were transferred to 96-well plates (Falcon) and manually counted at  $\times 100$  or  $\times 200$  magnification using an inverted microscope (Olympus CK30). Samples taken from dense cultures were diluted with filtered seawater before counting. Duplicate counts were taken from each sample.

Subsamples of *N. scintillans* cultures were settled in petri dishes and counted at  $\times 10$  magnification using a dissecting microscope (Leica MZ6).

Biovolumes of phytoplankton cells were measured using a Z2 particle counter (Beckman Coulter), while the diameter of *N. scintillans* cells (approx. 100 cells) were measured using an inverted microscope without adding any fixative.

### Analyses of elemental and fatty acid compositions

Samples for analyses of cellular C, N and P were taken by filtering 15 to 25 ml of phytoplankton cultures and ~3000 starved *N. scintillans* cells (cells were starved for 24 h as described above) onto pre-combusted (450°C, 4 to 5 h) GF/C glass-fiber filters. Prior to analyses the filters were dried at 60°C for 24 h and stored in a desiccator. Cellular C and N were analyzed with a CHN elemental analyzer (Perkin-Elmer) and cellular P was analyzed as orthophosphate after acidic oxidative hydrolysis with 1% HCl (Grasshoff et al. 1999).

To analyze the composition of fatty acids of prey and *N. scintillans*, 50 ml of dense phytoplankton cultures and ~6000 starved *N. scintillans* cells were filtered onto pre-combusted GF/F filters respectively, and frozen at -80°C until analysis. Samples were saponified and extracted using a mixture of  $\text{CHCl}_3$ :methanol:water (8:4:3, v:v:v) as described in Folch et al. (1957). Extracted lipids were analyzed by gas chromatography according to Kattner & Fricke (1986). Fatty acids were determined by comparing retention times with mixed standards including: 10:0, 11:0, 12:0, 13:0, 14:0, 14:1, 15:0, 15:1, 16:0, 16:1 $\omega$ 7, 16:3 $\omega$ 4, 17:0, 17:1, 18:0, 18:1 $\omega$ 9, 18:2 $\omega$ 6, 18:3 $\omega$ 3,

18:4 $\omega$ 3, 19:0, 20:0, 20:2 $\omega$ 6, 20:3 $\omega$ 6, 20:4 $\omega$ 6, 23:0, 20:5 $\omega$ 3, 22:0, 22:1 $\omega$ 9, 22:2, 22:6 $\omega$ 3, 24:0 and 24:1. The fatty acid 19:0 was added prior to sample analysis as an internal standard.

### Calculation of rates

In the feeding experiment, clearance rates of *N. scintillans* ( $F$ , ml  $\text{Noc}^{-1} \text{d}^{-1}$ ) were calculated using a modified equation from Frost (1972) (see also Heinbokel 1978, Harris et al. 2000):

$$F = \ln(C'_t/C_t) \times V/(t \times n) \quad (1)$$

Where  $C'_t$  and  $C_t$  (cells  $\text{ml}^{-1}$ ) are the prey concentrations at the end of the incubation in control and experimental bottles, respectively;  $V$  is the volume of the culture (ml),  $t$  (d) is the incubation period and  $n$  is the number of *N. scintillans* cells (Noc) used.

Ingestion rates of *N. scintillans* ( $I$ , cells  $\text{Noc}^{-1} \text{d}^{-1}$ ) were calculated from clearance rates and average prey concentration ( $C$ ) of each treatment according to Frost (1972):

$$I = F \times C \quad (2)$$

Average prey concentrations during incubations were calculated as:

$$C = (C_t - C_0)/\ln(C_t/C_0) \quad (3)$$

where  $C_0$  and  $C_t$  are prey concentrations at the start and end of the incubations, respectively. The results were fitted to Type II response equation using the software SigmaPlot v.11.0 (Systat Software):

$$I = (I_{\max} \times C)/(K_S + C) \quad (4)$$

where  $I$  is the ingestion rate (cells  $\text{Noc}^{-1} \text{d}^{-1}$ ),  $I_{\max}$  is the maximum ingestion rate,  $C$  is the average prey concentration,  $K_S$  is a half-saturation constant. Ingestion rates were calculated both on a cell and biomass basis.

In the growth experiment growth rates of *N. scintillans* ( $\mu$ ,  $\text{d}^{-1}$ ) were calculated as:

$$\mu = \ln(n_t/n_0)/t \quad (5)$$

where  $n_0$  and  $n_t$  are *N. scintillans* cell densities (cells  $\text{ml}^{-1}$ ) in the bottle at the beginning and end of time interval  $t$  (d). Growth rates ( $\mu$ ) of *N. scintillans* as a function of average prey concentration was fitted to a classic Type II response equation modified with an extra parameter to account for the food abundance at which growth rate was 0:

$$\mu = [\mu_{\max} \times (C - S)]/[K_m + (C - S)] \quad (6)$$

where  $\mu_{\max}$  is the maximum growth rate of *N. scintillans*,  $K_m$  is the 'prey concentration sustaining  $1/2 \mu_{\max}$ ' (Jeong et al. 2004),  $C$  is the average prey concentration and  $S$  is the threshold prey concentration ( $\text{ng C ml}^{-1}$ ) for maintaining positive growth of *N. scintillans*.

### Data analysis

Statistical analyses were performed with SPSS (IBM SPSS Statistics v.19) and R software v. 3.0.2 (R Development Core Team 2013). The data were checked for normality with the Shapiro-Wilk test and for homogeneity of variances with the Levene's test. The differences of N, P and fatty acids contents of phytoplankton cells between different treatments were analyzed using 1-way ANOVA and Tukey's post-hoc test. To compare various candidate models for *N. scintillans* growth,  $R^2$  was used as a selection method; the higher the  $R^2$  the better the model fit. All the model tests were performed using the nlme package in R.

## RESULTS

### Elemental and fatty acid profiles of algal diets

Phytoplankton cell volumes in nutrient-depleted cultures were generally higher than those in nutrient-replete cultures (Table 1). NP-sufficient *Prorocentrum dentatum* contained higher amounts of cellular C, N and P than those of NP-sufficient *Platymonas helgolandica* and *Thalassiosira weissflogii*, even though they are similar in cell sizes (Table 1). Manipulation of nutrients yielded distinctively different elemental compositions within each algal group

(Table 1, Fig. 1). Phytoplankton cells grown in N- and P-depleted conditions contained significantly lower amounts of cellular N and P respectively than their nutrient-replete counterparts (Table 1).

*T. weissflogii*, *P. helgolandica* and *P. dentatum* presented quite contrasting fatty acid profiles (Fig. 2), particularly for PUFA components, containing eicosapentanoic acid (20:5 $\omega$ 3, EPA),  $\alpha$ -linolenic acid (18:3 $\omega$ 3, ALA) or docosahexaenoic acid (20:6 $\omega$ 3, DHA), respectively, as the major fatty acid. N and/or P deficiency did not show much influence on the fatty acid profiles of these phytoplankton species. Fatty acid profiles were much more variable between than within algal taxonomic classes (Fig. 2, Table S1 in the Supplement at [www.int-res.com/articles/suppl/m527p073\\_supp.pdf](http://www.int-res.com/articles/suppl/m527p073_supp.pdf)). NP-sufficient *T. weissflogii* was rich in EPA (20%) and contained small proportions of DHA (1.65%) and ALA (1.95%), while NP-sufficient *P. dentatum* was rich in DHA (18.37%) and contained only 1.33% EPA and 0.91% ALA. NP-sufficient *P. helgolandica* contained a high proportion of ALA (26%), but lacked DHA. ALA and EPA were the 2 major PUFAs present in all phytoplankton cells used in our study. Portions of the sum of ALA and EPA ( $\Sigma\text{ALA}+\text{EPA}$ , % of total fatty acids) in N- and P-deficient cultures were lower than those in NP-sufficient cultures (Fig. 3); the differences were, however, not statistically significant. No clear trends in total fatty acid content ( $\Sigma\text{FA}$ ,  $\text{pg cell}^{-1}$ ) and  $\Sigma\text{ALA}+\text{EPA}$  content ( $\text{pg cell}^{-1}$ ) were found in relation to N and/or P deficiency in phytoplankton species (Fig. 3).

*Noctiluca scintillans* contained  $24.74 \pm 1.56 \text{ ng C cell}^{-1}$ ,  $6.86 \pm 0.31 \text{ ng N cell}^{-1}$ ,  $0.31 \pm 0.04 \text{ ng P cell}^{-1}$  and  $8.76 \pm 2.36 \text{ ng } \Sigma\text{FA cell}^{-1}$ . The atomic ratio of C:N was lower ( $4.20 \pm 0.14$ ), but N:P ( $49 \pm 6.76$ ) was much higher than the Redfield ratios (C:N = 6.625:1, N:P =

Table 1. Cell size (equivalent spherical diameters [ESD],  $\mu\text{m}$ ), cellular carbon, nitrogen, phosphorus and fatty acid contents ( $\Sigma\text{FA}$ ) of *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* grown under nutrient-replete, N-depleted (-N) and P-depleted (-P) conditions. All values are mean  $\pm$  SD. Superscript letters indicate significant differences between nutrient treatments (Tukey,  $p < 0.05$ )

Algae	Treatment	ESD ( $\mu\text{m}$ )	Carbon ( $\text{pg cell}^{-1}$ )	Nitrogen ( $\text{pg cell}^{-1}$ )	Phosphorus ( $\text{pg cell}^{-1}$ )	$\Sigma\text{FA}$ ( $\text{pg cell}^{-1}$ )
<i>T. weissflogii</i>	f/2	$11.19 \pm 0.12$	$78.19 \pm 2.33$	$15.77 \pm 0.48^a$	$2.3 \pm 0.09^a$	$15.69 \pm 1.93$
	-N	$14.10 \pm 0.10$	$63.38 \pm 2.9$	$8.87 \pm 0.31^b$	$1.62 \pm 0.2^b$	$24.16 \pm 0.90$
	-P	$11.03 \pm 0.03$	$84.65 \pm 2.31$	$12.71 \pm 0.34^a$	$0.19 \pm 0.02^c$	$19.54 \pm 0.24$
<i>P. helgolandica</i>	f/2	$10.11 \pm 0.02$	$99.51 \pm 0.64$	$18.44 \pm 0.15^a$	$2.41 \pm 0.27^a$	$23.57 \pm 2.77$
	-N	$10.56 \pm 0.07$	$113.64 \pm 1.19$	$7.64 \pm 0.1^b$	$2.83 \pm 0.16^a$	$13.9 \pm 0.03$
	-P	$11.43 \pm 0.07$	$191.2 \pm 8.32$	$16.53 \pm 0.88^c$	$0.16 \pm 0.02^b$	$20.66 \pm 0.96$
<i>P. dentatum</i>	f/2	$11.01 \pm 0.04$	$164.11 \pm 2.73$	$34.89 \pm 0.59^a$	$4.61 \pm 0.49^a$	$31.2 \pm 7.04$
	-N	$11.77 \pm 0.06$	$345.69 \pm 8.61$	$25.32 \pm 0.73^b$	$4.89 \pm 0.27^a$	$97.48 \pm 1.20$
	-P	$14.10 \pm 0.10$	$674.53 \pm 18.93$	$91.34 \pm 3.32^c$	$1.28 \pm 0.04^b$	$216.24 \pm 15.26$

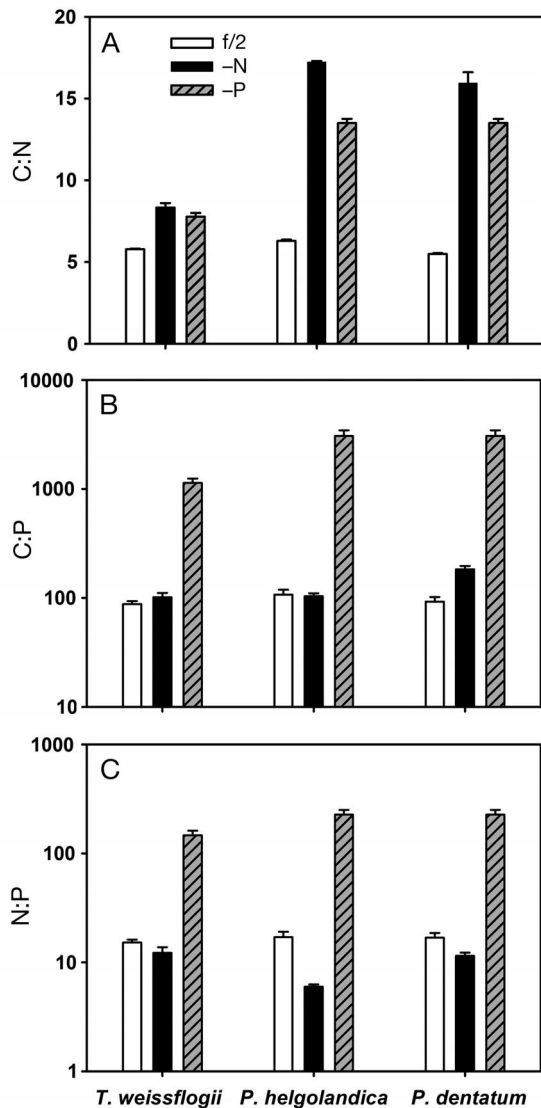


Fig. 1. Atomic ratios (mean  $\pm$  SD;  $n = 3$ ) of cellular carbon, nitrogen and phosphorus (C:N, C:P and N:P ratios) of *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* grown under nutrient-replete (f/2), N-depleted (-N) and P-depleted (-P) conditions

16:1). Saturated fatty acids 14:0, 16:0, and 18:0 accounted for about 58% of total fatty acid contents. The most abundant PUFAs in *N. scintillans* were the n-3 PUFAs (35%), of which DHA, EPA and ALA accounted for 28%, 6% and 1.4%, respectively.

#### Functional responses of *N. scintillans* to diets with different nutritional qualities

Over the series of food concentrations studied, ingestion rates of *T. weissflogii*, *P. helgolandica* and *P. dentatum* with different nutrient status can be de-

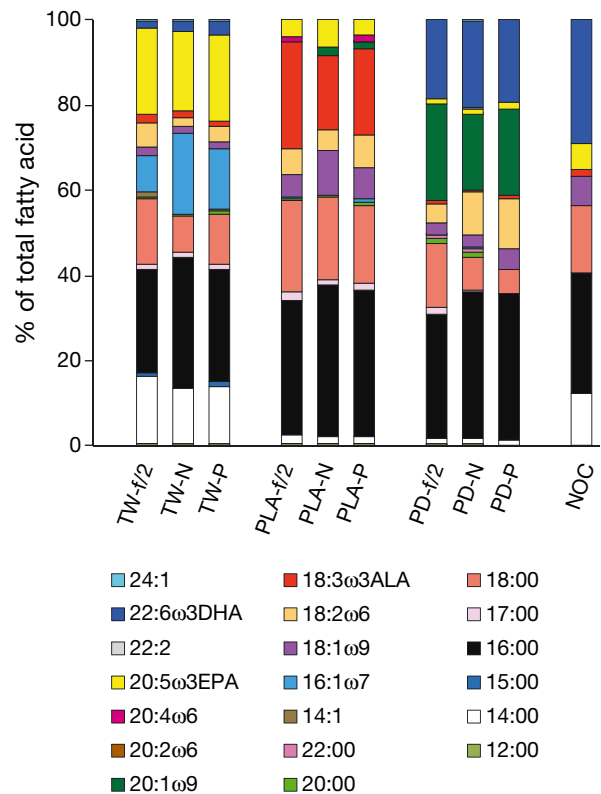


Fig. 2. Distribution of fatty acids in *Noctiluca scintillans* (NOC) and its 3 prey algae *Thalassiosira weissflogii* (TW), *Platymonas helgolandica* (PLA) and *Prorocentrum dentatum* (PD) grown under nutrient-replete (f/2), N-depleted (-N) and P-depleted (-P) conditions

scribed by Type II functional curves (Eq. 4, Fig. 4). Generally,  $I_{\max}$  was higher on nutrient-deficient cells, especially P-deficient cells, than on their NP-sufficient counterparts both on a carbon and cell biomass basis. However,  $I_{\max}$  of *N. scintillans* fed P-deficient *T. weissflogii* was only 195 ng C Noc<sup>-1</sup> d<sup>-1</sup> (2036 cells Noc<sup>-1</sup> d<sup>-1</sup>, Table 2). This rate was much lower than those observed for *N. scintillans* fed NP-sufficient and N-deficient *T. weissflogii*, which were 293 and 357 ng C Noc<sup>-1</sup> d<sup>-1</sup>, respectively. Based on carbon biomass, *N. scintillans* ingestion reached the saturation level at higher prey concentrations for nutrient-deficient compared to NP-sufficient prey.

#### Numerical response of *N. scintillans* to diets with different nutritional qualities

All phytoplankton cultures, except P-deficient *T. weissflogii*, supported the growth of *N. scintillans*. Numerical response curves describing the *N. scintillans* growth rates in relation to prey concentrations were

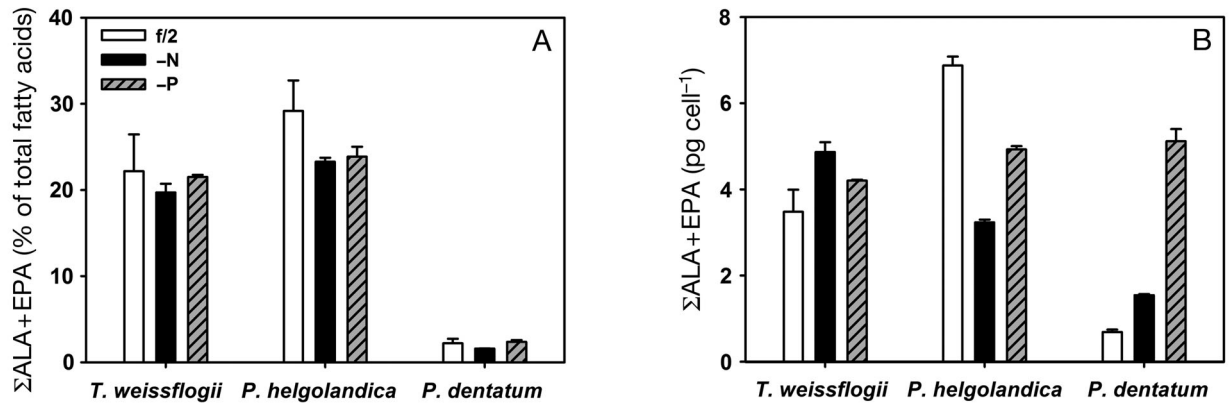


Fig. 3.  $\Sigma$ ALA+EPA (A) distribution and (B) absolute content of *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* grown under nutrient-replete (f/2), N-depleted (-N) and P-depleted (-P) conditions

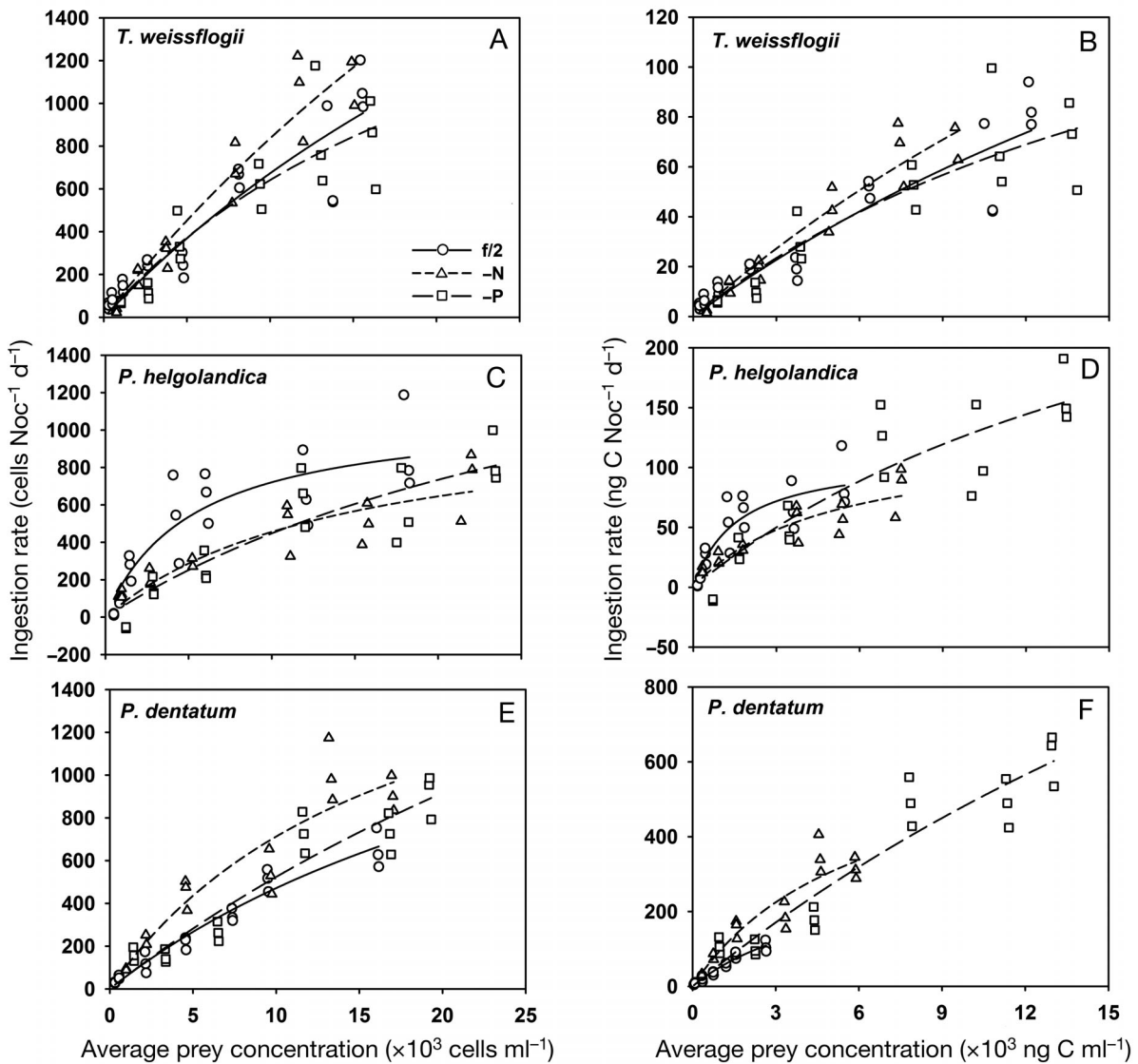


Fig. 4. Ingestion rates of *Noctiluca scintillans* as a function of average prey concentrations of (A,B) *Thalassiosira weissflogii*, (C,D) *Platymonas helgolandica* and (E,F) *Prorocentrum dentatum* grown under nutrient-replete (f/2), N-depleted (-N) and P-depleted (-P) conditions, both on cell and carbon biomass basis. Note the different x- and y-axes scales in (B), (D), and (F)

Table 2. Summary of estimations of maximum ingestion rate ( $I_{\max}$ ) and half-saturation ( $K_S$ ) values from Type II models ( $R^2$ ) in the functional response experiments on *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* grown under nutrient replete (f/2), N-depleted (-N) and P-depleted (-P) conditions. All treatments on all algae were significant at  $p < 0.0001$

Algae	Treatment	$I_{\max}$ (ng C $\text{Noc}^{-1} \text{d}^{-1}$ )	$K_S$ (ng C $\text{ml}^{-1}$ )	$R^2$
<i>T. weissflogii</i>	f/2	293	3580	0.86
	-N	357	3636	0.93
	-P	195	2196	0.81
<i>P. helgolandica</i>	f/2	110	525	0.80
	-N	126	1623	0.79
	-P	389	6785	0.82
<i>P. dentatum</i>	f/2	331	5383	0.95
	-N	676	6036	0.87
	-P	2469	40392	0.89

Table 3. Summary of estimations of asymptotic growth ( $\mu_{\max}$ ), growth threshold ( $S$ ), and half-saturation constant ( $K_m$ ) from the modified Type II model ( $R^2$ ) in the numerical response experiments on *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* grown under nutrient replete (f/2), N-depleted (-N) and P-depleted (-P) conditions. Dashes mean unsuccessful model fitting. All treatments for all algae were significant at  $p < 0.0001$  (except *T. weissflogii* -P; no model fitted)

Algae	Treatment	$\mu_{\max}$ ( $\text{d}^{-1}$ )	$S$ (ng C $\text{ml}^{-1}$ )	$K_m$ (ng C $\text{ml}^{-1}$ )	$R^2$
<i>T. weissflogii</i>	f/2	0.54	51	74	0.85
	-N	0.39	201	532	0.94
	-P	-	-	-	-
<i>P. helgolandica</i>	f/2	0.71	22	348	0.92
	-N	0.64	138	598	0.97
	-P	0.36	326	488	0.83
<i>P. dentatum</i>	f/2	0.35	145	707	0.89
	-N	0.49	830	7941	0.94
	-P	0.22	2591	6187	0.92

markedly distinct within and between algal prey classes (Table 3, Fig. 5). *N. scintillans* growth rates followed a modified Type II numerical response (Eq. 6) except when they were fed with P-deficient *T. weissflogii*. No optimum curve was fitted for P-deficient *T. weissflogii*, but a Type IV-like numerical response was observed, in which *N. scintillans* rose to a growth rate of  $0.08 \text{ d}^{-1}$  and declined thereafter. When fed NP-sufficient algae, high asymptotic growth ( $\mu_{\max}$ ), low half-saturation constant ( $K_m$ ) and low growth threshold ( $S$ ) for *N. scintillans* were obtained, while the opposite was observed on N- and P-deficient algal prey. Of the

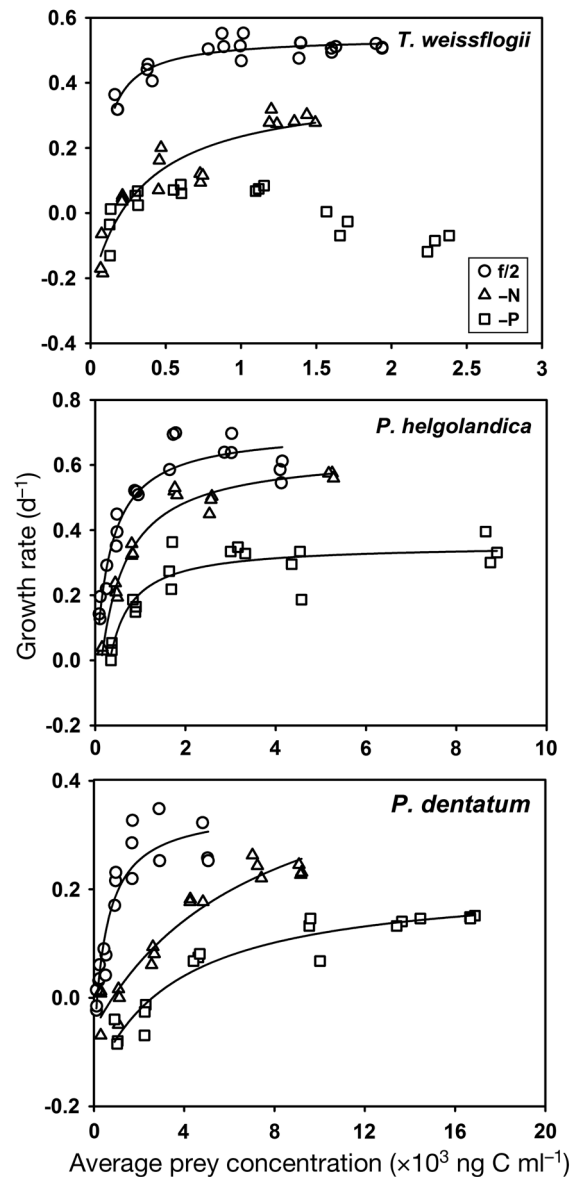


Fig. 5. Numerical responses of *Noctiluca scintillans* as a function of average prey concentrations of *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* grown under nutrient-replete (f/2), N-depleted (-N) and P-depleted (-P) conditions. Note the different x- and y-axes scales

3 phytoplankton species studied, *P. helgolandica* was the optimum prey for *N. scintillans* as it yielded the highest  $\mu_{\max}$  in all nutrient statuses. Although NP-sufficient *T. weissflogii* was also able to support rapid growth of *N. scintillans*, resource limitation, especially P limitation, made it the lowest-quality food item among the choices in this study.

Negative impacts of nutrient-deficient food on *N. scintillans* maintenance and growth was evident even at low food concentrations. Of the parameters (C, N, P



and various fatty acid contents) tested in the hyperbolic regression models for NP-sufficient prey,  $\Sigma$ ALA+EPA content of the diets showed the strongest positive correlation ( $R^2 = 0.89$ , AIC = -155.35,  $n = 63$ ,  $p < 0.0001$ ) with *N. scintillans* growth rate (Table 4). In order to evaluate which parameter could best describe the growth of *N. scintillans*, hyperbolic regression models based on the growth rates obtained with each phytoplankton species individually and in combination were simulated using C, N, P and various fatty acid contents as variables (Tables S2 to S5 in the Supplement at [www.int-res.com/articles/suppl/m527p073\\_supp.pdf](http://www.int-res.com/articles/suppl/m527p073_supp.pdf)). Of all these variables, P content of the diets was the best indicator to explain the growth of *N. scintillans* (Table 5, Tables S2 to S5 in the Supplement). Furthermore, model fits were better for each algal group individually than in combination.

## DISCUSSION

In the present study, we found that differences in both elemental and fatty acid compositions of the diets resulted in markedly different effects on the feeding and growth of *Noctiluca scintillans*. N and/or P deficiency significantly reduced the growth of *N. scintillans*, showing that these phytoplankton represent prey of poor quality for *N. scintillans*.

*N. scintillans* increased its feeding rates on N- or P-deficient food, presumably to maximize the utilization of available resources and/or to resist starvation or nutrient limitation (Meunier et al. 2012, Calbet et al. 2013). However, feeding on N- and/or P-deficient prey resulted in a lower growth rate than when on a NP-sufficient diet. This is not surprising because the increased feeding effort on suboptimal food requires higher energy cost, which in turn could decrease the reproduction rate of consumers. Nevertheless, we found the same trend of growth rate variations at low prey concentrations, even if ingestion rates were similar between different treatments irrespective of prey nutritional status. This implies that the cost of maintenance, in terms of not just C but also other nutrients (e.g. N, P and fatty acids etc.), is considerable. In fact, the correlation coefficients between *N. scintillans* growth rates and different nutrient constituents (C, N, P and different fatty acid constituents) of prey showed marked differences (Tables 4 & 5), which indicate that *N. scintillans* probably has large differences in the requirement of various nutrients. This may be due to the different importance of these nutrient constituents in the physiological aspects of metabolism and reproduction.

Table 4. *Noctiluca scintillans* growth rate regression models (hyperbolic) result for NP-sufficient *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* using C, N, P and various fatty acid contents ( $\text{ng ml}^{-1}$ ) as variables. AIC: Akaike's information criterion. The best-fit model with the highest  $R^2$  is shown in **bold**. All models were significant at  $p < 0.0001$

Independent variable	Formula	$R^2$	AIC
C	$\mu = \frac{0.52 \times (x - 59.27)}{227.17 + (x - 59.27)}$	0.42	-52.30
N	$\mu = \frac{0.51 \times (x - 11.09)}{43.16 + (x - 11.09)}$	0.39	-49.42
P	$\mu = \frac{0.51 \times (x - 1.52)}{6.03 + (x - 1.52)}$	0.40	-51.03
<b><math>\Sigma</math>ALA+EPA</b>	<b><math>\mu = \frac{0.65 \times (x + 0.36)}{15.6 + (x + 0.36)}</math></b>	<b>0.89</b>	<b>-155.35</b>
$\Sigma$ PUFA	$\mu = \frac{0.55 \times (x - 3.44)}{19.4 + (x - 3.44)}$	0.52	-65.04
$\Sigma$ FAs	$\mu = \frac{0.53 \times (x - 12.78)}{51.68 + (x - 12.78)}$	0.45	-56.30

Table 5. *Noctiluca scintillans* growth rate regression models (hyperbolic) result for treatments of *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* individually and/or in combination in terms of P content ( $\text{ng P ml}^{-1}$ ). 'Total' indicates the combination of 9 phytoplankton food sources in the model; AIC: Akaike's information criterion. All models were significant at  $p < 0.0001$

Independent variable	Formula	$R^2$	AIC
<i>T. weissflogii</i>	$\mu = \frac{0.74 \times (x - 1.46)}{25.17 + (x - 1.46)}$	0.67	-61.65
<i>P. helgolandica</i>	$\mu = \frac{0.69 \times (x - 3.06)}{14.43 + (x - 3.06)}$	0.74	-95.80
<i>P. dentatum</i>	$\mu = \frac{0.33 \times (x - 5.49)}{28.52 + (x - 5.49)}$	0.80	-168.66
Total	$\mu = \frac{0.50 \times (x - 0.60)}{16.93 + (x - 0.60)}$	0.40	-129.30

*N. scintillans*, which contain high cellular contents of N (low C:N and high N:P ratios), showed neither higher requirements of N nor grew better on diets with higher N:P. We found that growth of *N. scintillans* was more dependent on the P rather than the N content of the diet. Heterotrophic dinoflagellates are

usually not strictly homeostatic, and therefore assimilated nutrient fluxes cannot be budgeted directly by comparing the elemental composition of consumers versus resources (Grover & Chrzanowski 2006, Hantzsche & Boersma 2010). As indicated by the apparent reduction in *N. scintillans* growth on P-deficient prey and high correlation coefficient between *N. scintillans* growth rates and bulk P content of diets, its strong P demand is apparent. This agrees with both the facts that mineral limitation of prey affects zooplankton proliferation, and also the growth rate hypothesis: P limitation exhibits stronger negative effects than N limitation (Elser et al. 2000). Construction of large cell membranes, luciferase and nucleic acids essentially require P (Dikarev 1982, Baldwin 1996). Therefore, P limitation might have disturbed the metabolic regulation, and thus inhibited the cell division of *N. scintillans*. However, the correlation coefficient resulting from *N. scintillans* growth rates and P content is much lower in the combination of 9 food sources (3 algal species  $\times$  3 nutrient statuses) than in each algal group individually. Taxonomy-affiliated nutrient components (e.g. fatty acids) beyond macronutrients C, N, and P also influence food nutritional quality for this grazer.

Of the hyperbolic regression models describing growth of *N. scintillans* fed NP-sufficient preys, the one based on  $\Sigma$ ALA+EPA content represented the best-fit model (Table 4). This implies that  $\Sigma$ ALA+EPA content is also a good indicator with which to assess the quality of food for *N. scintillans*. Research on the importance of fatty acids on heterotrophic dinoflagellates has mainly focused on the trophic upgrading processes, but neglected the fact that PUFAs may also serve as critical nutrient sources (Chu et al. 2008, Lund et al. 2008, Calbet et al. 2013). For example, heterotrophic dinoflagellates such as *Oxyrrhis marina*, *Gyrodinium dominans* and *N. scintillans* tend to feed on diatoms, chlorophytes and cryptophytes which contain high EPA and ALA (Kiørboe & Titelman 1998, Chu et al. 2008, Lund et al. 2008, Calbet et al. 2013, and the present study). Lund et al. (2008) found that gelatin acacia microspheres containing ALA only can still support fast growth of *O. marina* and *G. dominans*. Growth and feeding of heterotrophic dinoflagellates largely depend on systematic group affiliation of the prey. Moreover, *N. scintillans* contained a significantly higher proportion of DHA compared to both *Platymonas helgolandica* and *Thalassiosira weissflogii*, but a lower proportion of ALA and EPA compared to *P. helgolandica* and *T. weissflogii*, respectively (Fig. 2). Changing the quantity and quality of these PUFAs in heterotrophic dino-

flagellates relies on the elongation and desaturation of dietary precursors or on the production of long-chain essential FAs de novo (Barclay et al. 1994, de Swaaf et al. 2003, Bec et al. 2006, Lund et al. 2009). These processes might have energy costs. Therefore, consumption of poor-quality food, which is deficient in or lacking certain FAs, would have negative consequences on the net growth of grazers and on the use of these lipids as an energy source during starvation (Calbet et al. 2013). However, the importance of these PUFAs may be masked by large intraspecific variations in the elemental compositions of algal preys under resource limitation. Additionally, fatty acid compositions of these 3 different algal species may co-vary with some limiting biochemical constituents, such as phospholipids, sterols and other trace elements. The significance of biochemical nutrients in protozoans has not yet been well established. Given the importance of heterotrophic dinoflagellates as trophic intermediates between microbial loops and higher trophic levels, more studies need to be done to investigate mechanisms underlying these trophic transfer and nutrient upgrading processes.

Secondary metabolites, induced by resource limitation, would interact with the effects of PUFAs and P contents in determining the overall food quality of a prey. Recent studies found that nutrients stress, especially P-related stress, trigger a significantly enhanced production of polyunsaturated aldehyde in diatoms (Ribalet et al. 2007, 2009). Diatoms can be toxic or deleterious to mesozooplankton by reducing their egg production, preventing their eggs from hatching, and/or affecting larval development (Miralto et al. 1999, Ribalet et al. 2007, Barreiro et al. 2011, Carotenuto et al. 2011). This 'diatom-toxic effect' would explain why the growth of *N. scintillans* fed on P-deficient *T. weissflogii* cells was markedly reduced compared to those fed on P-deficient *P. helgolandica* and *Prorocentrum dentatum*. Furthermore, this adverse effect was found to increase in relation to prey densities. Similar phenomena have been found in nature during diatom-*N. scintillans* succession blooms. A *N. scintillans* population which occurs during a late stage of a diatom bloom can be adversely affected, i.e. having larger cell sizes or being unhealthy, compared to individuals present during an early stage of a diatom bloom (Nakamura 1998a, Dela-Cruz et al. 2003). Our results also support the idea that the ability of *N. scintillans* to feed on various types of phytoplankton species and to reproduce rapidly is beneficial for their survival by depressing the toxic effect of a single-species diatom diet.

There are substantial ecological implications for interactions between *N. scintillans* and the quantity and quality of their algal preys on trophic transfers in pelagic ecosystems. *N. scintillans* modify quantity and distribution of n-3 PUFAs, thereby increasing the n-3 PUFAs pool and altering DHA:EPA ratios of the diets available to higher trophic levels (Watanabe 1993, Sargent 1995, Chu et al. 2008). *N. scintillans*–phytoplankton bloom formation and succession in winter and spring are reported to be highly regulated by bottom-up effects (Yin 2003). For example, during winter and spring in the coastal waters in temperate and subtropical regions, *N. scintillans* peak biomass always coincides with the spring diatom blooms. This phenomenon demonstrates that *N. scintillans* is able to respond numerically to diatom blooms. However, long residence time due to hydrographical and meteorological conditions in spring sets up certain semi-enclosed bays, e.g. Port Shelter and Tolo Harbor in Hong Kong, into a batch culture mode, which in turn causes nutrients like N and P to deplete as time elapses (Yin 2003). Phytoplankton communities during this period mostly consist of P-limited diatoms, leading to nutrient limitation of *N. scintillans*. Resource limitation of primary producers would therefore act as a bottom-up force to regulate *N. scintillans* abundance. This scenario could also occur in the late phase of algal blooms containing high biomass of nutrient-deficient phytoplankton cells.

It is also noted that *N. scintillans* abundance is substantially lower, sometimes to the point of virtually absent, during summer and fall in some coastal waters (Huang & Qi 1997, Chen et al. 2011, Mikaelyan et al. 2014). Authors mainly ascribe its disappearance to high temperature (Huang & Qi 1997, Liu & Wong 2006), even though it has a wide thermal optima from 10 to 31°C (Huang & Qi 1997, Al-Azri et al. 2007, Harrison et al. 2011, Mikaelyan et al. 2014). Other factors, i.e. changes in nutritional and other environmental conditions may also contribute to its disappearance from the upper mixed layer in the summer (Mikaelyan et al. 2014). For instance, the diatom-dominated phytoplankton population in Hong Kong waters always experience P limitation during summer and fall (Yin et al. 2004, Liu & Wong 2006, Xu et al. 2009). These observations, together with our findings, indicate that negative effects of nutrient-deficient food sources, which could be detrimental to *N. scintillans*, should not simply be neglected in research into *N. scintillans* population dynamics.

In conclusion, *N. scintillans* changed their feeding rates to compensate for the low availability of limiting nutrients. Feeding on nutrient-deficient, particu-

larly P-deficient algal prey, reduced the growth of *N. scintillans*. Nutrient-sufficient prey with high  $\Sigma$ ALA+EPA content is the optimal food item for *N. scintillans*.  $\Sigma$ ALA+EPA and P contents of diets represent good indicators of food quality for *N. scintillans*, but the importance of their influence depends on the nutrient status of food items. Additionally, resource-driven toxicity might also associate with the effects of EFA and P contents in determining the overall food quality.

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