

Differential feeding and growth of *Noctiluca scintillans* on monospecific and mixed diets

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ABSTRACT: Growth and feeding experiments using monospecific and mixed diets were conducted to investigate *Noctiluca scintillans*' food preference, and the influence of prey composition on its prey consumption and growth rates. In the feeding experiments on 14 monospecific diets of autotrophic and heterotrophic plankton, growth and feeding of *N. scintillans* showed no significant relationship with the cell size or swimming motility of the prey, but it generally grew better on diatoms and chlorophytes than other plankton. On the mixed diets composed of the diatom *Thalassiosira weissflogii* and a nutritionally superior (*Platymonas helgolandica*, chlorophyte), inferior (*Prorocentrum dentatum*, autotrophic dinoflagellate) or poor (*Lepidodinium* sp., heterotrophic dinoflagellate) prey (in terms of the ability to support *N. scintillans* growth), *N. scintillans*' feeding preference seemed to depend on the prey abundance in short (6 h) incubations. However, as incubation time increased (2 d), *N. scintillans* generally preferentially consumed the superior prey in food mixtures, regardless of prey abundance. Growth of *N. scintillans* on mixed diatom–dinoflagellate diets benefited from the increase of diatoms in the diets, but rates were lower than that achieved on *T. weissflogii* alone. When fed with diatom–chlorophyte prey pairs, growth of *N. scintillans* was slightly higher on the mixtures than on the single diatom diet, but there was no significant difference among them. Therefore, the dietary choice of *N. scintillans* may be a trade-off between maximizing food and nutrient intake and minimizing the energy cost of handling different prey. Such feeding strategies of *N. scintillans* should have great ecological significance on plankton bloom formation and succession.

KEY WORDS: *Noctiluca scintillans* · Monospecific and mixed diets · Feeding preference · Growth rates · Ingestion rates · Digestion time

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INTRODUCTION

Noctiluca scintillans is a non-toxic red tide-forming heterotrophic dinoflagellate, which is ubiquitous in temperate and subtropical coastal regions (Harrison et al. 2011). It can feed on a wide variety of prey across a broad spectrum of both size and trophic status, including bacteria, detritus, phytoplankton, protozoans, copepods, eggs of copepods and fish larvae (Kirchner et al. 1996, Quevedo et al. 1999, Umani et al. 2004). As *N. scintillans* lacks the ability to swim and actively migrate like most other dinoflagellates and mesozooplankton, it has relatively limited ability to actively select food in a mixture. Early studies on

the ecology of *N. scintillans* showed no evidence of food preference (Uhlig & Sahling 1990). However, Kiørboe & Titelman (1998) clearly demonstrated a higher ingestion rate on diatoms than dinoflagellates. They suggested that variation in *N. scintillans*' growth was mainly due to its capability to capture different types of prey (motile vs. immotile). Another study using various autotrophic flagellates as prey revealed that prey size affected the suitability of the food source. Prey items with an equivalent spherical diameter (ESD) of <5 µm were not good food items, but prey with an ESD >10 µm were good prey (Nakamura 1998b). In the framework of ecological stoichiometry, some researchers also suggested that *N.*

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scintillans, which has a low cellular carbon-to-nitrogen ratio (C:N) of 2.3–4.4 (Tada et al. 2000), needs to eat more heterotrophic than autotrophic food items to meet its high nitrogen (N) requirement (i.e. protozoans or various eggs and larvae of metazoans; Kimor 1979, Quevedo et al. 1999, Umani et al. 2004). Umani et al. (2004) also found that *N. scintillans* lived mainly on heterotrophic prey during their survey, and it may require a mixed and balanced diet to achieve a high growth rate (Broglia et al. 2003, Umani et al. 2004, Jones & Flynn 2005). Several laboratory studies on *N. scintillans*' feeding preference and its interaction with other plankters have been conducted (Buskey 1995, Kjørboe & Titelman 1998, Nakamura 1998b). However, most of these studies fed *N. scintillans* a single food source, even though its natural diet is usually a mixture of prey with various sizes, shapes, species and trophic types (Huang & Qi 1997, Umani et al. 2004, Mikaelyan et al. 2014). There are very few studies that measure its growth and prey uptake using mixed diets (Buskey 1995, Kjørboe & Titelman 1998) and there are still many questions relating to the feeding preference of *N. scintillans*. For example, does *N. scintillans* prefer heterotrophic prey more than autotrophic prey? What is its growth and feeding responses when it encounters a mixture of different prey? What are the main factors (e.g. size, motility or species-specific chemical properties) that influence its dietary choice?

In the present study, we examined *N. scintillans*' feeding preference, and the influence of prey composition, especially the importance of including diatoms in the diet, on its prey consumption and reproduction. The underlying mechanism governing its dietary choice and the possible ecological consequences of its dietary choice are also discussed. As stated above, all previous studies on *N. scintillans*' food preference use a single food source approach (Buskey 1995, Kjørboe & Titelman 1998, Nakamura 1998b). In this study, we conducted similar monospecific diet experiments, but included more diverse prey of various sizes (ESD, 4–60 μm). In addition, we also compared the ingestion and feeding preference of *N. scintillans* on mixtures of diatom (*Thalassiosira weissflogii*) and other plankton species (*Platymonas helgolandica*, *Prorocentrum dentatum* or *Lepido-*

dinium sp.) with varying ratios both in short-term feeding experiments (6 h) and longer-term growth experiments (2 d). This expanded experimental design provides a more comprehensive understanding of *N. scintillans*' feeding preference and is one of the advances of this study.

MATERIALS AND METHODS

Experimental organisms and culture conditions

Noctiluca scintillans was gently collected with a 120 μm plankton net from the pier off Port Shelter in eastern Hong Kong in October 2011. Cells were isolated and maintained as described by Zhang et al. (2015), except that they were kept in low light (20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on a 14:10 h light:dark cycle in a temperature-controlled chamber at $23 \pm 1^\circ\text{C}$.

Eleven autotrophic phytoplankton and 3 heterotrophic protozoa were used as prey in the following studies (Table 1). Five diatoms — *Thalassiosira weissflogii*, *T. pseudonana*, *Phaeodactylum tricornutum*, *Skeletonema* sp. and *Ditylum brightwellii* — were grown in seawater enriched with f/2 medium (Guillard & Ryther 1962), while 6 flagellates — *Dunaliella* sp., *Heterosigma akashiwo*, *Platymonas helgolandica*, *Rhodomonas* sp., *Prorocentrum micans* and *P. dentatum* — were grown in enriched f/2 seawater medium without silicate. The heterotrophic dinoflagellate *Lepidodinium* sp. was isolated from the same water sample as that in which *N. scintillans* occurred and was

Table 1. Equivalent spherical diameter (ESD), carbon biomass and initial concentration of phytoplankton species used as food in the feeding and growth experiments. *: chain-forming species

Species	Class	ESD (μm)	Carbon biomass (pg C cell ⁻¹)	Initial conc. (cells ml ⁻¹)
<i>Thalassiosira pseudonana</i>	Bacillariophyceae	4.5	6.6	1×10^5
<i>Dunaliella</i> sp.	Chlorophyceae	6.3	21	1.5×10^4
<i>Phaeodactylum tricornutum</i>	Bacillariophyceae	6.5	16	5×10^4
<i>Skeletonema</i> sp.*	Bacillariophyceae	7.0	18	3.5×10^4
<i>Heterosigma akashiwo</i>	Raphidophyceae	9.1	59	1×10^4
<i>Platymonas helgolandica</i>	Chlorophyceae	10.4	100	1.1×10^4
<i>Thalassiosira weissflogii</i>	Bacillariophyceae	10.6	78	1.5×10^4
<i>Uronema marinum</i>	Ciliata	10.9	131	4000
<i>Rhodomonas</i> sp.	Cryptophyceae	11.0	101	1.6×10^4
<i>Prorocentrum dentatum</i>	Dinophyceae	11.3	164	3×10^4
<i>Lepidodinium</i> sp.	Dinophyceae	14.5	319	3600
<i>Prorocentrum micans</i>	Dinophyceae	29.1	1768	5000
<i>Ditylum brightwellii</i>	Bacillariophyceae	36.9	1107	800
<i>Euplotes</i> sp.	Ciliata	52.6	14490	500

cultured with *Rhodomonas* sp. Two ciliates, *Euplotes* sp. and *Uronema marinum*, were kept in autoclaved filtered seawater with 0.005% yeast extract. *Lepidodinium* sp. and the ciliates were transferred to fresh medium every 2 wk. Autotrophic phytoplankton were maintained at $23 \pm 1^\circ\text{C}$ with $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of light; the heterotrophic dinoflagellates and ciliates were kept at low light ($20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

Feeding and growth of *N. scintillans* on monospecific diets

Monospecific diet experiments were set up to quantify the feeding and growth response of *N. scintillans* to prey of varying types and sizes. Representatives of 11 autotrophic and 3 heterotrophic taxa with sizes ranging from 4 to 60 μm were offered as prey in the growth and feeding experiments (Table 1). *N. scintillans* in stock cultures were acclimated to the experimental prey for 2–3 d before the actual experiments began. To avoid any potential effects of food carry-over, *N. scintillans* was starved for 24 h prior to the experiments to void its food vacuole.

In the feeding experiment, ~300 starved *N. scintillans* cells were inoculated into a 50 ml food suspension with a prey concentration of $\sim 1 \text{ mg C l}^{-1}$ (except *Dunaliella* sp., which was 0.32 mg C l^{-1}) and conducted in triplicate. Two bottles containing prey items without *N. scintillans* were used as controls. All cultures were incubated under dim light ($10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) with other conditions the same as described above. After a 6 h incubation, subsamples were taken from each bottle to estimate the final prey cell densities. Changes in cell density of *N. scintillans* during the incubation were neglected for the calculation of the clearance rate ($\mu\text{l Noc}^{-1} \text{d}^{-1}$) since the incubation period was only 0.25 d (i.e. <20% of its doubling time; Nakamura 1998b).

The same phytoplankton species were used as prey in the growth experiment. Approximately 100 starved *N. scintillans* cells were inoculated into 100 ml of suspended food in triplicate with the same concentration as in the feeding experiment. Triplicate bottles of 100 ml of autoclaved seawater ($0.2 \mu\text{m}$ filtered) with only *N. scintillans* (i.e. no food) were used as the control. Suspensions of each food source were prepared by diluting stock cultures with f/20 (+Si) medium. In the experiment with phototrophic prey, cultures were incubated for 3 d under the same conditions as those in the feeding experiment. In the experiments with the heterotrophic dinoflagellate and ciliates, cultures were incubated for 2 d to avoid

any possible negative effect due to starvation. Cultures were gently agitated manually 2 to 3 times a day to avoid cell aggregation and settlement. Predator and prey concentrations were determined at the beginning and end of the incubation.

Clearance ($\mu\text{l Noc}^{-1} \text{d}^{-1}$) and ingestion rates ($\text{ng C Noc}^{-1} \text{d}^{-1}$) and the average concentration of both predator and prey were calculated according to Frost (1972). The growth rate of *N. scintillans* (μ, d^{-1}) was calculated as: $\mu = \ln(n_t/n_0)/t$, where n_0 and n_t are the cell density of *N. scintillans* (cells ml^{-1}) at the beginning and end of the time interval t (d).

Feeding and growth of *N. scintillans* on mixed diets

Field studies have shown that *N. scintillans* usually reaches its peak abundance concurrently with a diatom bloom, or at least a high abundance of diatoms (Elbrächter & Qi 1998, Harrison et al. 2011). Therefore, in the mixed-diet experiments, the diatom representative *T. weissflogii* was separately paired with autotrophic flagellates *P. helgolandica* (chlorophyte) and *P. dentatum* (dinoflagellate) and a heterotrophic dinoflagellate *Lepidodinium* sp., and offered as food for *N. scintillans*. Of the 4 algal prey used in the mixed-diet experiments, *P. helgolandica* (chlorophyte) and *P. dentatum* (autotrophic dinoflagellate) represented nutritionally superior and inferior food sources, respectively, for *N. scintillans* compared with *T. weissflogii* (diatom), in terms of their ability to support the maximum asymptotic growth rate of *N. scintillans* (Zhang et al. 2015). In contrast, *Lepidodinium* sp. (heterotrophic dinoflagellate) represented poor-quality food for *N. scintillans* as it supported no or negative growth of *N. scintillans* in the range of $0.08\text{--}1.2 \text{ mg C l}^{-1}$ when offered as a sole food source (preliminary study and the above monospecific diet experiment).

To conduct the experiments, *T. weissflogii*, *P. helgolandica*, *P. dentatum* and the heterotrophic dinoflagellate *Lepidodinium* sp. were diluted to $\sim 1 \text{ mg C l}^{-1}$. *T. weissflogii* (TW) was then separately mixed with one of 3 species (*P. helgolandica*, *P. dentatum* or *Lepidodinium* sp.; X) with 7 ratios of 10:0, 2:8, 4:6, 5:5, 6:4, 8:2 and 0:10 ($C_{\text{TW}}:C_{\text{X}}$, where C is concentration), and adjusted to a total prey concentration of $\sim 1 \text{ mg C l}^{-1}$. Feeding and growth experiments were conducted as described above by offering a series of these 3 food pairings, using triplicate bottles of *N. scintillans* without any prey and duplicate bottles of only prey items as the controls. The incubations for the feeding experiment lasted 6 h, while the growth experiments continued for 2 d. Subsamples to determine predator

and prey abundance were taken at the beginning and end of the incubation and preserved in acidic Lugol's solution (final concentration 2%).

Ingestion and clearance rates and the prey consumption index of *N. scintillans* on each of the prey in the mixtures were determined both in the feeding and growth experiments. As *Lepidodinium* sp. was unable to feed on *T. weissflogii* (preliminary study), there was no trophic cascade effect when *N. scintillans* was incubated with a mixture of *T. weissflogii* and *Lepidodinium* sp. Ingestion rates, clearance rates and average prey and/or predator concentrations were also calculated as described by Frost (1972). The prey consumption index (α_i ; values from 0 to 1), an indicator of food preference, was calculated by comparing the frequency distribution of specific prey in the ambient environment and in the diet. This is similar to the calculation of the feeding selectivity index as described by Chesson (1978). When $\alpha_i = 0.5$, it indicates non-selective feeding; $\alpha_i > 0.5$ indicates a preference for a prey organism, whereas $\alpha_i < 0.5$ represents discrimination against the prey *i*.

Evacuation of prey remains in *N. scintillans'* food vacuole

In this experiment, *N. scintillans* cells in stock cultures were starved for 24 h before the experiment started. Then ~1500 vacuole-free *N. scintillans* cells were separately inoculated into 300 ml food suspensions containing 1 mg C l⁻¹ of equal cell concentrations of *T. weissflogii*, *P. helgolandica* and *P. dentatum*. After a 24 h incubation, about 15 active cells of *N. scintillans* containing 3–5 food vacuoles were separated from the prey items and transferred to 6-well plates that contained 10 ml of 0.2 µm-filtered autoclaved seawater (6 replicates). Cells were inspected under a dissecting microscope every 1.5–3.0 h for those previously reared on *T. weissflogii* and 3–6 h for those previously reared on *P. helgolandica* and *P. dentatum*. Our preliminary study found that when *N. scintillans* was fed with *T. weissflogii*, *P. helgolandica* and *P. dentatum* for 24 h, it was long enough to allow the formation of obvious food vacuoles, but also short enough to avoid cell replication of *N. scintillans* (its doubling time is usually >1 d; Nakamura 1998b, Zhang et al. 2015). We observed that food vacuoles containing algae in *N. scintillans* would merge to form one or a few large food vacuoles after their formation. Therefore, digestion was considered to be complete only when there were no traces of the food in the cell. The food vacuole defecation rate of *N.*

scintillans on different types of food was calculated as the constant of the exponential decay plot of the percentage of the cells with food vacuoles vs. starvation time. Since *N. scintillans* seldom formed visible food vacuoles when fed with *Lepidodinium* sp. for 1 d, we did not measure the evacuation of *Lepidodinium* sp. in the present study.

Estimation of biovolumes, carbon biomass and cell densities

Preserved phytoplankton biovolumes were calculated assuming appropriate geometric formulae according to Hillebrand et al. (1999) and converted to carbon according to Menden-Deuer & Lessard (2000). The carbon contents of *T. weissflogii*, *P. helgolandica* and *P. dentatum* were determined by CHN elemental analyzer reported in a previous study (Zhang et al. 2015). The carbon conversion factor for ciliate cell volume was 0.19 pg C µm³ and the volume was corrected for cells that were preserved in 2% acid Lugol's iodine following Putt & Stoecker (1989).

To determine the prey cell densities, 500–1000 µl of *D. brightwellii* and *Euplotes* sp. were transferred to 24-well plates, and 100–250 µl of the other prey samples were transferred to 96-well plates and counted. Subsamples of *N. scintillans* were settled in petri dishes and counted in duplicate.

Statistical analyses

Statistical analyses were performed with Sigma-Plot 12.5 (Systat Software). The data were checked for normality with Shapiro-Wilks test and checked for homogeneity of variance by Levene's test, both at a significance level of $\alpha = 0.05$. Student's *t*-test (2-tailed) and one-way analysis of variance (ANOVA) were performed with significance levels of $p < 0.05$. Tukey's HSD post-hoc test was used to test significance differences between groups. Linear correlations between any 2 parameters were analyzed by Pearson's correlation.

RESULTS

Feeding and growth of *Noctiluca scintillans* on monospecific diets

The growth and clearance rates of *Noctiluca scintillans* were measured for cells grown on various

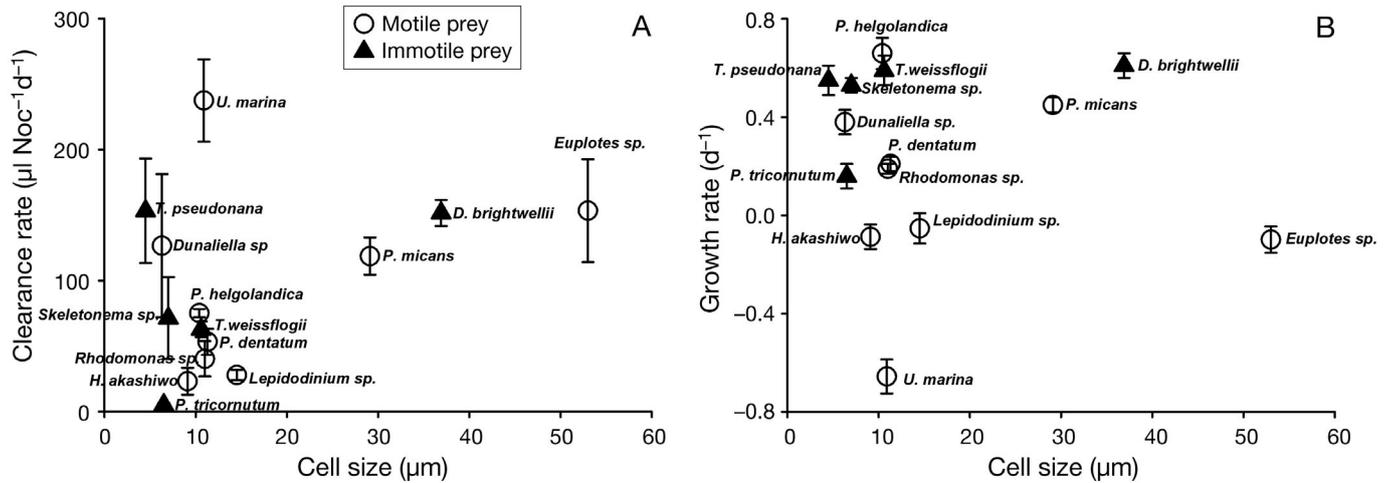


Fig. 1. (A) Clearance and (B) growth rates of *Noctiluca scintillans* (Noc) fed with various types of prey with different size (4 to 60 µm ESD) and swimming motility. See Table 1 for phytoplankton species. Error bars = ± 1 SD and $n = 3$

types of prey ranging from 4 to 60 µm (ESD) with an initial prey concentration roughly equal to 1 mg C l^{-1} . *N. scintillans* was able to feed on all the prey offered, with clearance rate ranging from 5 to $238 \text{ } \mu\text{l Noc}^{-1} \text{ d}^{-1}$ (Fig. 1A), but its clearance rate was statistically independent of prey size ($r = 0.079$, $p = 0.788$, $n = 14$). Ingested food items did not support the growth of *N. scintillans* equally at equivalent prey concentrations (Fig. 1B). *N. scintillans* was able to grow on most algal prey, but not on the raphidophyte *Heterosigma akashiwo*, the heterotrophic dinoflagellate *Lepidodinium* sp. and the ciliates *Uronema marinum* and *Euplotes* sp. Growth rates of *N. scintillans* ranged from -0.66 (for *U. marina*) to 0.66 d^{-1} (for *Platymonas helgolandica*) and showed no significant relationship with prey size ($r = 0.363$, $p = 0.202$, $n = 14$). The clearance rate and growth rate of *N. scintillans* also showed no clear relationship with prey motility, as the rates were either higher or lower on the immotile diatoms than motile flagellates and ciliates (Fig. 1).

Ingestion by *N. scintillans* on 3 mixed diets: short term vs. longer term

Comparison of the ingestion rates (Fig. 2), clearance rates (Fig. 3) and prey consumption indexes (Fig. 4) of each prey in the 3 mixed diets in both short-term feeding (6 h) and longer-term growth (2 d) experiments

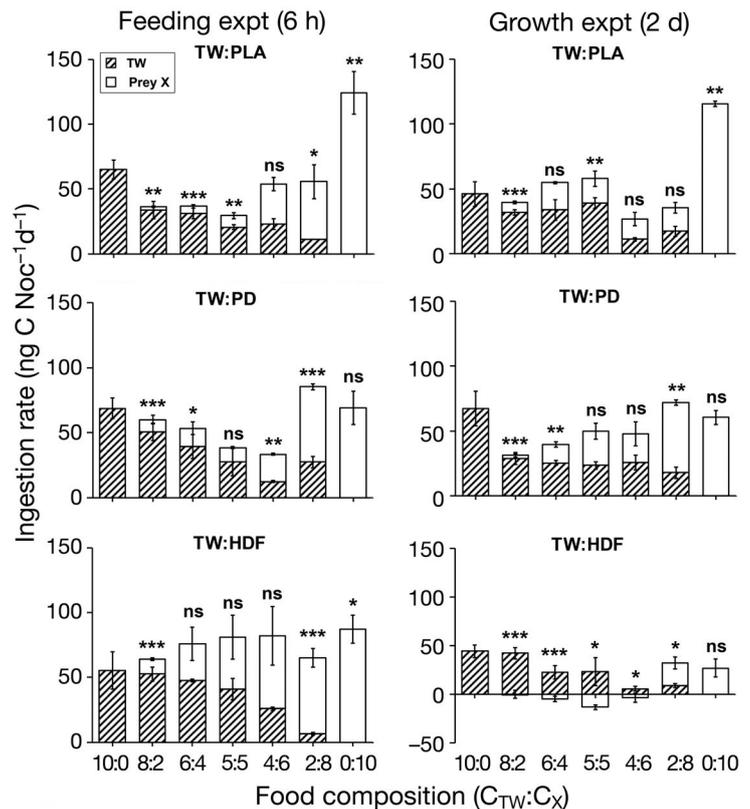


Fig. 2. Ingestion rates of *Noctiluca scintillans* (Noc) on paired prey in 3 mixed diets in the feeding (6 h) and growth experiments (2 d). Mixed diets were composed of *Thalassiosira weissflogii* (TW) and one of the 3 other phytoplankton prey designated as X (i.e. *Platymonas helgolandica* [PLA], *Prorocentrum dentatum* [PD] or the heterotrophic dinoflagellate *Lepidodinium* sp. [HDF]). $C_{TW}:C_X$: composition of *T. weissflogii* and one of the other 3 prey in terms of biomass in the experiments. Levels of significance (Student's *t*-test) for the difference between 2 prey species in the same food mixture or as the sole food source ($C_{TW}:C_X = 0:10$ and $10:0$) in the same food treatment are * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns: the difference between both prey species is not significant. Error bars = ± 1 SD and $n = 3$

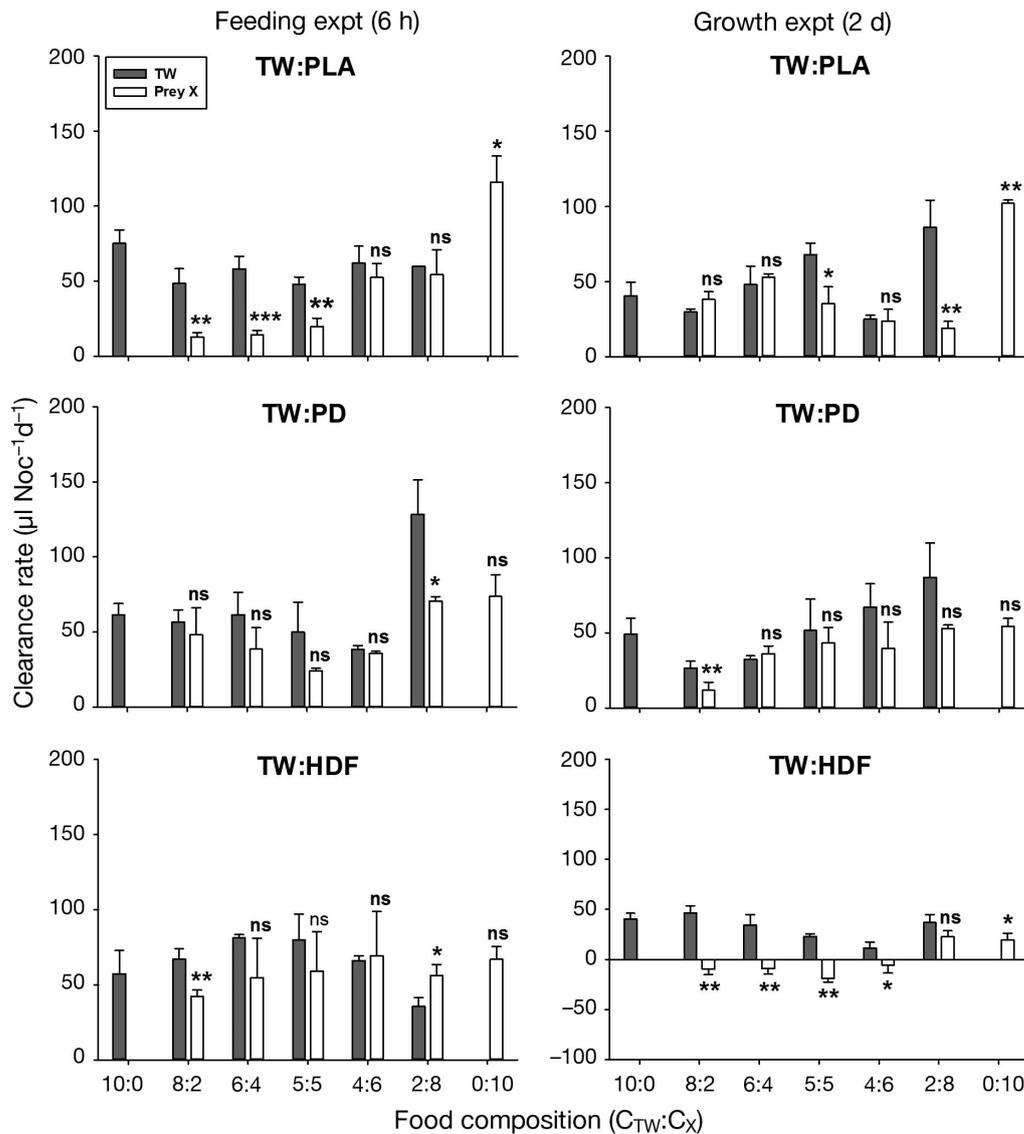


Fig. 3. Clearance rates of *Noctiluca scintillans* (Noc) on paired prey in 3 mixed diets in the feeding (6 h) and growth experiments (2 d). Details are the same as for Fig. 2

showed that prey consumption and feeding preference of *N. scintillans* not only differed among the 3 mixed diet treatments for the same incubation period, but also varied between the 2 incubation periods, even for the same food treatment.

In the short-term incubation (6 h), ingestion and feeding preference of *N. scintillans* on a given type of prey was approximately in proportion to the initial abundance of the prey in food mixtures (Table 2, Figs. 2–4); *N. scintillans* generally preferred *Thalassiosira weissflogii* in the mixed diets with high and medium diatom ratios. While in the longer-term incubation (2 d), *N. scintillans* preferentially fed on diatoms in the mixed diets of diatoms and dinoflagel-

lates, as indicated by the higher clearance rates and prey consumption index for the diatom compared with the dinoflagellates, i.e. *Prorocentrum dentatum* or *Lepidodinium* sp. (Figs. 3 & 4); its preference for *T. weissflogii* continued or was more often enhanced in the same food treatment in the 2 d incubation (Fig. 4). However, when *T. weissflogii* and *P. helgolandica* were given in combination, *N. scintillans* generally reduced its preference for *T. weissflogii*, i.e. enhanced the preference for *P. helgolandica*, and its feeding preference was negatively related to the prey abundance in food mixtures in the 2 d incubation (Table 2). Ingestion of only *T. weissflogii* ($C_{TW}:C_X = 10:0$), or only *P. helgolandica* or *P. dentatum*

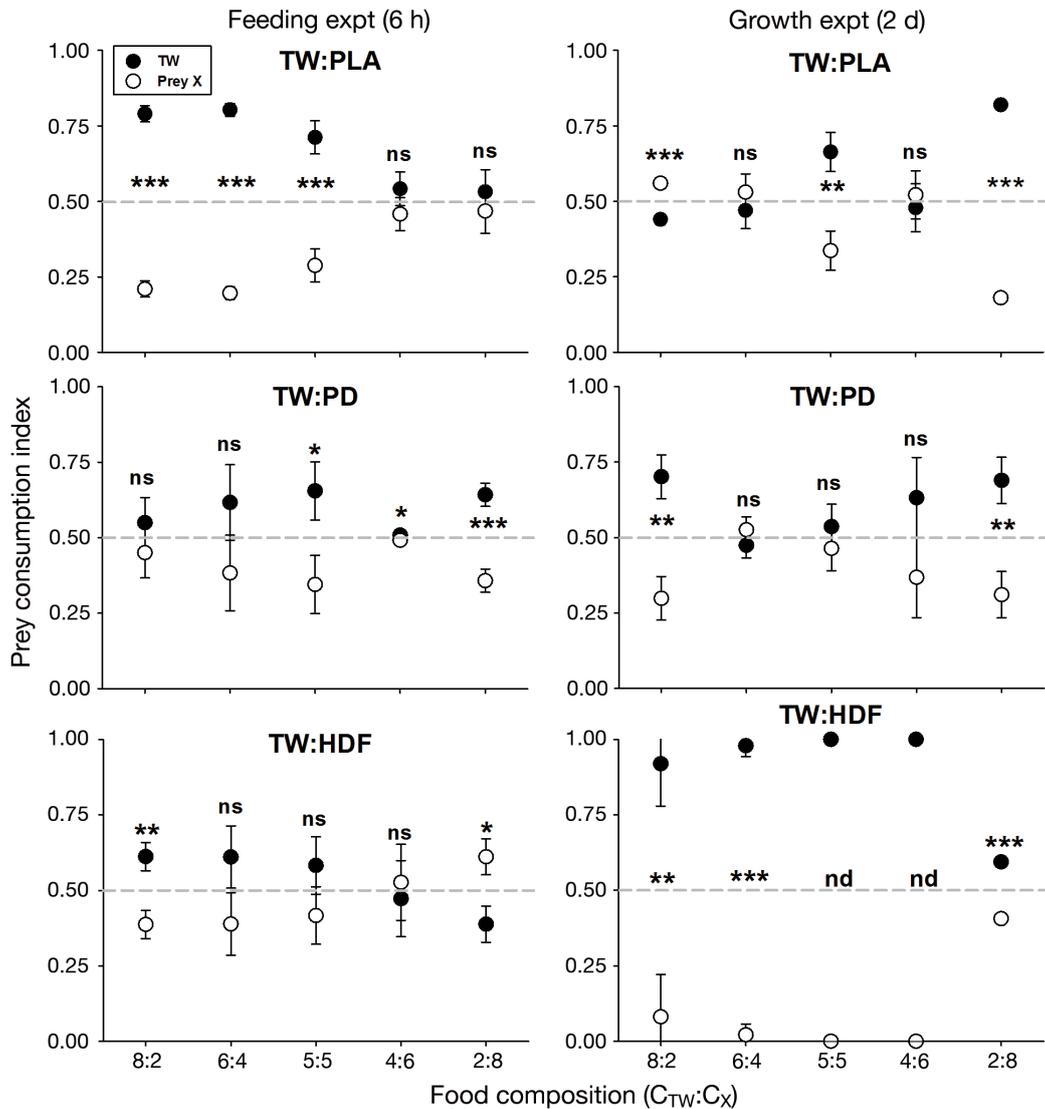


Fig. 4. Prey consumption index of *Noctiluca scintillans* on paired prey in 3 mixed diet treatments in the feeding (6 h) and growth experiments (2 d). Random feeding is shown by the horizontal line at the index value of 0.5. Levels of significance (Student's *t*-test) for differences in prey preference between 2 prey species in the same food treatment are * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns: the difference between both prey species is not significant; nd: the difference between both prey species could not be determined because for one of the prey species the deviation of prey preference was 0. Other details are the same as for Fig. 2. Error bars = ± 1 SD and $n = 3$

($C_{TW}:C_X = 0:10$) in the single species cultures showed no significant difference between short- and long-term incubations (data not shown). Due to the mortality of *N. scintillans*, *Lepidodinium* sp. was inadvertently ingested at lower rates in the 2 d incubation (Student's *t*-test, $n = 6$, $p < 0.05$).

Growth of *N. scintillans* on 3 mixed diets

N. scintillans did not grow better on the mixed diet of diatoms and one other planktonic species com-

pared with growth on the diatom-only diet. For instance, when *T. weissflogii* and a superior prey (*P. helgolandica*) were given in combination, growth of *N. scintillans* was slightly higher than that achieved on 1 mg C l^{-1} of *T. weissflogii*, but never exceeded that on *P. helgolandica* alone. Growth rates of *N. scintillans* on this food pairing showed no significant relationship with the availability of *P. helgolandica* and the ingestion of each prey, but were negatively related to the abundance of *T. weissflogii* in the food mixture (Table 3). However, the combination of *T. weissflogii* together with less satisfactory prey, i.e. *P.*

Table 2. Pearson's correlations between the initial prey concentrations (ng C ml⁻¹) and ingestion rates (ng C Noc⁻¹ d⁻¹) and clearance rates (µl Noc⁻¹ d⁻¹), as well as prey consumption indexes of *Noctiluca scintillans* on each prey in the feeding (6 h) and growth (2 d) experiments. C_X: initial concentration of phytoplankton prey *Thalassiosira weissflogii* (TW) and its counterparts (*Platymonas helgolandica* [PLA], *Prorocentrum dentatum* [PD] or *Lepidodinium* sp. [HDF = heterotrophic dinoflagellate]). N = 18 for each prey in different food treatment. Levels of significance for correlations are *p < 0.05, **p < 0.01, ***p < 0.001

Food pairings	Initial prey conc. (C _X)	Ingestion rate	Clearance rate	Prey consumption index
6 h expt				
TW+PLA	C _{TW}	0.932***	0.317	0.791***
	C _{PLA}	0.906***	0.895***	0.782***
TW+PD	C _{TW}	0.831***	-0.45	-0.25
	C _{PD}	0.922***	0.628**	-0.24
TW+HDF	C _{TW}	0.892***	0.323	0.715**
	C _{HDF}	0.923***	0.41	0.653**
2 d expt				
TW+PLA	C _{TW}	0.676**	-0.622**	-0.848***
	C _{PLA}	0.764***	0.409	-0.862***
TW+PD	C _{TW}	0.776***	-0.621**	-0.078
	C _{PD}	0.958***	0.795***	-0.162
TW+HDF	C _{TW}	0.864***	0.536*	0.513
	C _{HDF}	0.686**	0.699**	0.311

dentatum or *Lepidodinium* sp., yielded significantly lower growth rates of *N. scintillans* than on *T. weissflogii* alone (Figs. 1B & 5). Growth rates of *N. scintillans* on the diatom–dinoflagellate (either autotrophic or heterotrophic) food pairings were positively correlated with the availability of *T. weissflogii* and the ingestion of it, but negatively correlated with the biomass and ingestion of the dinoflagellates (Table 3). The heterotrophic dinoflagellate *Lepidodinium* sp.

Table 3. Pearson's correlations between the growth rates of *Noctiluca scintillans* (µ, d⁻¹) and initial prey concentrations (ng C ml⁻¹), as well as the ingestion rates of each prey (ng C Noc⁻¹ d⁻¹) in the growth experiments. C_{TW}, C_X: initial concentration (ng C ml⁻¹) of *Thalassiosira weissflogii* (TW) and its counterparts (*Platymonas helgolandica* [PLA], *Prorocentrum dentatum* [PD] or *Lepidodinium* sp. [HDF]); I_{TW}, I_X: ingestion rates of *N. scintillans* on TW (I_{TW}) and its counterparts (I_X) in growth experiments. N = 18 for each prey in different food treatment. Levels of significance for correlations are *p < 0.05, **p < 0.01, ***p < 0.001

Growth rate	C _{TW}	C _X	I _{TW}	I _X
µ _{TW+PLA}	-0.551*	0.175	-0.455	-0.245
µ _{TW+PD}	0.874***	-0.900***	0.678**	-0.896***
µ _{TW+HDF}	0.937***	-0.819***	0.715**	-0.779***

may have an inhibitory effect on *N. scintillans*, as a high proportion of *Lepidodinium* sp. in the food mixture caused its mortality.

Evacuation of prey remains in *N. scintillans*' food vacuole

The food vacuole defecation experiment conducted in this study allowed a rough estimation of the defecation rate and digestion time of *N. scintillans* for different prey. An exponential decay plot of the percentage of the cells with food vacuoles as a function of the starvation time was produced for each prey (Fig. 6). *N. scintillans* cells filled with *T. weissflogii* voided the food vacuoles faster, as indicated by the higher decay constant (0.55), than those filled with *P. dentatum* or *P. helgolandica* (0.10 and 0.05, respectively). *T. weissflogii* was most readily digested and

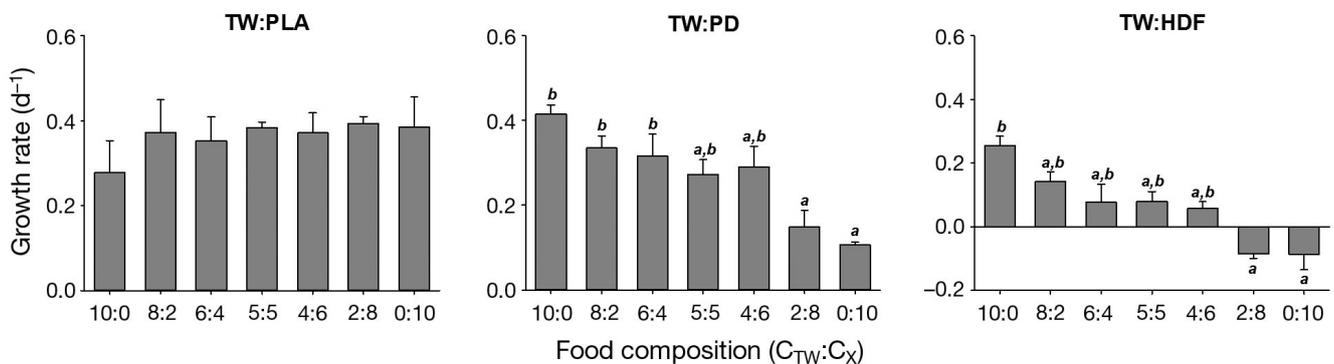


Fig. 5. Growth rates of *Noctiluca scintillans* on 3 mixed diets (*Thalassiosira weissflogii* [TW]; one of the 3 other prey, *Platymonas helgolandica* [PLA], *Prorocentrum dentatum* [PD] or the heterotrophic dinoflagellate *Lepidodinium* sp. [HDF]). C_{TW}:C_X: composition of *T. weissflogii* and one of the other 3 prey (X) in terms of biomass. Lowercase letters indicate significant differences in the growth of *N. scintillans* on the mixtures (ratios 8:2 to 2:8) compared with its growth on the 2 prey species alone (i.e. C_{TW}:C_X = 10:0 and 0:10) (ANOVA, Tukey post hoc, p < 0.05). Error bars = ±1 SD and n = 3

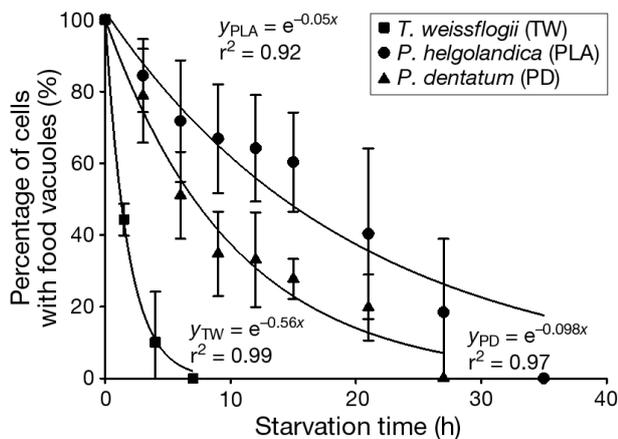


Fig. 6. Food vacuole defecation of *Noctiluca scintillans* cells that have ingested *Thalassiosira weissflogii*, *Platymonas helgolandica* and *Prorocentrum dentatum* separately for 24 h. Error bars = ± 1 SD ($n = 6$). All curves were significant at $p < 0.0001$

evacuated, as $\sim 90\%$ of the cells filled with *T. weissflogii* were able to void their food vacuoles within 4 h, while only $\sim 20\%$ of the cells containing *P. dentatum* or *P. helgolandica* voided their food vacuoles in 3 h. The time for all *N. scintillans* cells to finish the digestion was 7 h for *T. weissflogii*, 27 h for *P. dentatum* and 35 h for *P. helgolandica*.

DISCUSSION

Our study on the feeding and growth responses of *Noctiluca scintillans* on monospecific and mixed diets emphasized 2 critical aspects of the feeding interactions between *N. scintillans* and planktonic prey: (1) *N. scintillans*' food preference cannot simply be attributed to prey size or swimming motility; and (2) there was a time lag for *N. scintillans*' response to different prey in mixtures.

Factors affecting the feeding preference of *N. scintillans* on monospecific and mixed diets

N. scintillans is an obligate heterotrophic dinoflagellate that catches its prey using a clump or string of mucus that is attached to the tip of the tentacle (Omori & Hamner 1982, Metivier & Soyer-Gobillard 1986, Kiørboe & Titelman 1998). With negligible swimming capacity, *N. scintillans* uses its positive buoyancy to ascend up through the water column, allowing them to catch prey by interception, relatively without discrimination (Kiørboe & Titelman 1998, Tiselius & Kiørboe 1998). Therefore, it is not a

true 'selective' feeder actively selecting prey from mixtures (Uhlig & Sahling 1990, Kiørboe & Titelman 1998). Nevertheless, previous studies and the present study did find *N. scintillans* exhibiting some preference for certain types of prey (Buskey 1995, Kiørboe & Titelman 1998, Nakamura 1998b).

Prey size is usually considered as the primary factor influencing prey selection (Hansen et al. 1997, Jakobsen & Hansen 1997). Previous studies have shown that the optimum prey particle size for *N. scintillans* is $>10 \mu\text{m}$ based on growth experiments using various autotrophic flagellates as prey (Nakamura 1998b), or about $80 \mu\text{m}$ based on the clearance rates of latex beads in feeding experiments (Kiørboe & Titelman 1998). However, in our feeding experiments using different sized plankton as a single prey, *N. scintillans* ingested the food items indiscriminately, regardless of the prey size. For instance, *Thalassiosira pseudonana*, *Ditylum brightwellii* and *Euplotes* sp. with very different cell sizes were consumed at the same rate by *N. scintillans*, while *Dunaliella* sp. and *Phaeodactylum tricorutum* of similar size were consumed at different rates (Fig. 1A). Similarly for the growth experiments, prey with cell size around $10 \mu\text{m}$ supported a wide range of growth rates of *N. scintillans* from -0.66 (for *Uronema marina*) to 0.66 d^{-1} (for *Platymonas helgolandica*), further indicating that size alone was inadequate to explain *N. scintillans*' food preference.

Prey motility has also been considered as an important characteristic influencing a consumer's prey selection in previous studies (Kiørboe & Titelman 1998, Montagnes et al. 2008). Kiørboe & Titelman (1998) found that although motile prey had more chance to encounter *N. scintillans*, they were cleared at lower rates than non-motile diatoms, as active swimming of the motile flagellates may reduce the fatal contact with the mucus and thus avoid being entangled. We found that non-motile diatoms, e.g. *T. pseudonana* and *D. brightwellii*, were cleared at higher rates than some motile flagellates, e.g. *Heterosigma akashiwo* and *Lepidodinium* sp., whereas fast moving prey, such as the ciliates *U. marina* and *Euplotes* sp., were also consumed at similar or even higher rates than some non-motile diatoms, e.g. *T. pseudonana*, *T. weissflogii* and *P. tricorutum*. Although the swimming patterns and swimming speeds of different prey were not determined in the present study, both facts suggest that *N. scintillans*' food preference cannot be simply attributed to prey motility or encounter probability due to prey motility.

In our study, ingestion of *N. scintillans* on a single prey species ($C_{\text{TW}}:C_{\text{x}} = 10:0$ and $0:10$) was the highest

on *P. helgolandica*, and showed no significant difference in the short- and longer-term incubations, except that on *Lepidodinium* sp., which caused the death of *N. scintillans*. However, when grown on the mixed diets, *N. scintillans*' ingestion and feeding preference depended on the prey abundance in short (6 h) incubations, as indicated by their significant correlations with the abundance of each prey in food mixtures. As incubation time increased (2 d), we clearly found *N. scintillans* preferentially consumed the superior prey from mixtures, i.e. *T. weissflogii* and/or *P. helgolandica*, regardless of the prey abundance. Roa (1992) has suggested that single prey experiments do not address the problem of feeding preference because no food choice is provided to the consumer. Therefore, testing selective feeding using multiple species in which the total carbon concentration is constant, but with varied prey ratios as in the present study, provides a better understanding of a grazer's feeding preference (Roa 1992). Since the feeding preference in the longer-term experiment more likely represented the dietary choice at steady state (Boenigk et al. 2001, Montagnes et al. 2008), preferential consumption of the superior prey from mixtures in the 2 d experiment suggests that nutritional quality of the prey should play a role in determining *N. scintillans*' feeding preference. There is also evidence that some prey selection in micro- and mesozooplankton is due to the variability of the nutritional quality of the prey, with discrimination of prey size, shape or motility (Sterner 1989, Landry et al. 1991, Verity 1991). For example, Verity (1991) identified nutritional value as being an important criterion affecting prey selection by ciliates, with poor prey being rejected or more slowly and incompletely digested. The variation of *N. scintillans*' food preference at different times (6 h vs. 2 d) also suggested that its dietary choice was not attained immediately in the mixed food assemblages, but was attained after a lag. Many studies of selective feeding have also noted a lag when grazers encounter new food items varying in quantity and quality (Lee 1991, Verity 1991, Boenigk et al. 2001). The actual discrimination of a poor food item, i.e. *Lepidodinium* sp., previously acceptable to the predator as less preferred prey, was an extreme example of such an effect. As suggested by Lee (1991), lags in exhibiting a foraging choice can be interpreted as the time needed for metabolic 'retooling' (e.g. adaptive enzyme formation) for the metabolic processing of diverse food items.

Moreover, good food items, such as *T. weissflogii* and/or *P. helgolandica*, were positively consumed by

N. scintillans as a supplementary food source even when they only accounted for a very small fraction in mixed prey assemblages. Such pronounced specificity of algal predation may also indicate its recognition of some chemical cues excreted by these phytoplankton. Given the nature of the prey capture mechanism of *N. scintillans*, it is unlikely to 'actively' select prey based on chemical or other cues (Kjørboe & Titelman 1998). Alternatively, different stickiness of *N. scintillans* following contact with a prey or an aggregate and sense of their characteristics (e.g. chemical cues) might also be responsible for the selection for particular types of food or marine snow (Shanks & Walters 1996, Kjørboe & Titelman 1998). Nawata & Sibaoka (1983) found that changes of ion concentration in the ambient seawater, for example SO_4^{2-} , Ca^{2+} , Mg^{2+} and Cl^{-1} , or application of an inward electric current, could affect the feeding induction time of *N. scintillans*. Perhaps variation of the ion concentrations in the microfluid environment around the prey or different cell surface properties of the prey provides information about the relative attractiveness of the prey, and simultaneously induces differential feeding of *N. scintillans*. Chemosensory ability, which is probably involved in the localization of prey patches and selection among individual particles that differ in chemical makeup (Sterner 1989, Hamels et al. 2004, Wootton et al. 2007), has been shown to exist in many protozoans (Spero 1985, Siballd et al. 1987, Bennett et al. 1988, Wootton et al. 2007). This chemosensory ability also makes evolutionary sense for organisms living in nutritionally dilute environments, but it remains unclear to what extent it is involved in the consumption of individual prey in mixed prey assemblages (Stoecker et al. 1981, Montagnes et al. 2008).

Factors affecting the growth of *N. scintillans* on monospecific and mixed diets

A comparison of the growth rates of *N. scintillans* that were achieved on different types of prey at comparable concentration in the present and previous studies (Lee & Hirayama 1992, Buskey 1995, Kjørboe & Titelman 1998, Nakamura 1998b) revealed that diatoms and chlorophytes generally support higher growth rates (average 0.4 and 0.37 d^{-1} , respectively) than other prey taxa. Cryptophytes, dinoflagellates and raphidophytes supported intermediate growth rates (0.17–0.22 d^{-1}), and ciliates were usually poor food items with low or negative growth rates (Fig. 7). These results reflect the differences in the suitability

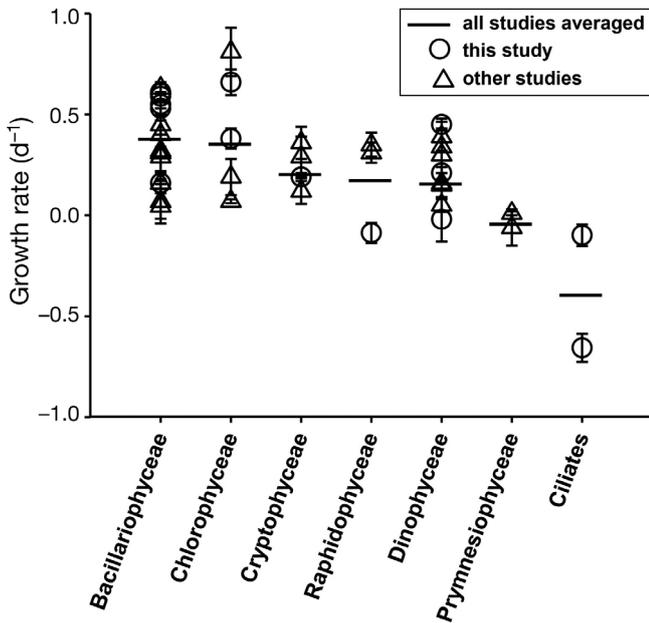


Fig. 7. Growth rates of *Noctiluca scintillans* achieved on different classes of prey. Growth rates were obtained from the present and previous studies that used 1 mg C ml^{-1} or higher food concentrations (Lee & Hirayama 1992, Buskey 1995, Kiørboe & Titelman 1998, Nakamura 1998b). Error bars = $\pm 1 \text{ SD}$ and $n = 3$

of different phytoplankton species as food for *N. scintillans*. Since the growth rates of *N. scintillans* showed no correlation with prey size or motility, species-specific chemical properties (e.g. digestibility and/or nutritional quality) of the prey may play a more crucial role in determining its feeding and food preference, and thus its growth (Verity 1991, Flynn et al. 1996, Zhang et al. 2015).

Our results showed that the combination of *T. weissflogii* together with an inferior prey (*P. dentatum* or *Lepidodinium* sp.) yielded significantly lower growth rates of *N. scintillans* than on a single diatom diet. However, when *T. weissflogii* was combined with the superior prey (i.e. *P. helgolandica*), *N. scintillans* grew slightly faster on the mixtures compared with the single diatom diet, but with no significant differences among prey rations and never exceeding the growth on *P. helgolandica* alone. Such food dependence of *N. scintillans* growth further emphasizes the importance of the nutritional value of the prey in governing its growth. Taghon (1981) suggested that for a predator (such as *N. scintillans*) that has both limited ability to select food items and a limited ability to seek out new food patches, varying the gut passage time and ingestion rate as a function of the available food's nutritional quality would be the prin-

cipal method of maximizing the net rate of energy intake. The digestion time of *N. scintillans* on different mixed diets was not determined in the present study. However, based on the estimations from the functional response experiments (Zhang et al. 2015), the handling time (which includes the time to pursue, capture, ingest and digest the prey; Kiørboe 2008) of *T. weissflogii* is shorter ($0.38 \text{ min prey}^{-1}$) than *P. dentatum* and *P. helgolandica* (0.71 and $1.31 \text{ min prey}^{-1}$, respectively). In addition, the digestion time of *T. weissflogii* was faster than other algal food items or copepod eggs (Nikishina et al. 2011, the present study). Since *N. scintillans* defecated at an exponentially declining rate (Fig. 6), ingested food items were not treated in a first-in, first-out manner, but rather were mixed at some point (Dolan & Coats 1991, Dolan & Šimek 1997). Therefore, the availability of *T. weissflogii* may govern vacuole passage time and digestion efficiency when combined with a refractory food item such as *P. dentatum* and *P. helgolandica* as food for *N. scintillans*. It is well known that preferential feeding is an evolutionarily selected tendency to maximize energy intake per unit time (Schoener 1987) and consequently it should improve the predator's growth and survival in a given prey assemblage (Mayer & Wahl 1997, Montagnes et al. 2008). Obviously, it is energetically efficient for a consumer to feed preferentially on the prey with high nutritional value, but a short handling time or an efficient digestion process is also important and was evident in *N. scintillans*' growth and ultimate dietary choice (e.g. the food choice in the 2 d experiment). For example, *N. scintillans* fed preferentially on *T. weissflogii* when it was given in combination with a dinoflagellate that required a longer handling or digestion time, and consequently supported a lower growth rate of *N. scintillans* relative to the diatom-only diet and thus its growth increased with the increase in the proportion of diatoms in the mixed diets. Nevertheless, *N. scintillans* preferentially consumed the superior food item *P. helgolandica* when *T. weissflogii* was abundant in the mixtures, but it preferred the more easily digested or handled prey, i.e. *T. weissflogii*, when *P. helgolandica* was abundant in food mixtures. Thus, there may be a trade-off between the synergistic nutritional benefit from feeding on multiple species and the energy cost of handling or digesting the different prey. Gentleman et al. (2003) suggested that preferentially consuming high-quality food resources is advantageous only when their improved nutritional content outweighs any cost of that foraging choice, such as that due to time lost distinguishing among resources and digesting diverse

food items. Therefore, more subtle differences in maximizing nutritional quality from a mixed food source and minimizing the energy cost in handling food, or more specifically digestion, may determine the ultimate dietary choice of *N. scintillans*.

Ecological implications

Phytoplankton are considered to be the main food items for *N. scintillans* in the field, due to their relatively high abundance and growth rates relative to microzooplankton, i.e. heterotrophic dinoflagellates and ciliates (Elbrächter & Qi 1998, Harrison et al. 2011). As stated above, *N. scintillans* preferentially fed on diatoms and generally benefited from an increase in diatoms in the mixed diets of diatoms and dinoflagellates, both of which are major groups of phytoplankton in many coastal waters. This feeding habit may account for the numerous reports that a high abundance of *N. scintillans* is usually associated with diatom blooms in the field (Elbrächter & Qi 1998, Harrison et al. 2011). Also food resources with a high nutritional value (i.e. N- and P-sufficient prey with high amounts of $\Sigma\alpha$ -linolenic acid [ALA] + eicosapentanoic acid [EPA]), such as *P. helgolandica*, also stimulated *N. scintillans* growth and had the potential to trigger a bloom (Zhang et al. 2015, the present study).

Typically, actively growing *N. scintillans* cells are found well below the surface, close to their food supply of phytoplankton. It feeds on phytoplankton—a process that leads to its own bloom, while at the same time its active feeding suppresses the bloom formation of other phytoplankton, especially diatoms (Harrison et al. 2011). Other phytoplankton groups, especially dinoflagellates that are usually avoided by *N. scintillans*, may take advantage of the preferential grazing on diatoms and consequently outgrow diatoms and become dominant. Bloom succession usually follows the sequence of diatoms then *N. scintillans* then other dinoflagellates (either phototrophic or heterotrophic) and has been observed in many coastal areas, such as the Seto Inland Sea (Nakamura 1998a) and South China Sea (Elbrächter & Qi 1998, Weston et al. 2008, Harrison et al. 2011). Preferential feeding of *N. scintillans* regulates not only the biomass, but also the composition of the prey community, and simultaneously affects its population dynamics.

Furthermore, phytoplankton contain diverse biochemical compounds among different taxonomical groups that are delivered to higher trophic levels

(Brett & Müller-Navarra 1997, Müller-Navarra et al. 2000, Dalsgaard et al. 2003, Arendt et al. 2005). For example, dinoflagellates are rich in docosahexaenoic acid (20:6 ω 3, DHA), while diatoms and chlorophytes contain larger amounts of EPA (20:5 ω 3) and ALA (18:3 ω 3), respectively (Dalsgaard et al. 2003, Arendt et al. 2005, Zhang et al. 2015). Preferential consumption of prey with high concentrations of ALA and EPA (e.g. *T. weissflogii* and *P. helgolandica*) by *N. scintillans* would modify the quantity and distribution of n-3 polyunsaturated fatty acids in the ambient environment (Zhang et al. 2015), thereby altering the amount and ratio of DHA and EPA available to higher trophic levels, such as copepods, fish larvae or hydromedusae.

CONCLUSIONS

Noctiluca scintillans' food preference seems to depend neither on the cell size nor on the swimming motility of the prey. Our study suggests a time lag for *N. scintillans*' response to the different prey in mixed diets. Its feeding preference depends on the prey abundance in the short term, but in the long run, its dietary choice is more likely a result of the trade-off between maximizing food and nutrient intake and minimizing the energy cost of handling food, or specifically food digestion. *N. scintillans*' feeding preference for the diatom *T. weissflogii*, especially in the diatom and dinoflagellate paired diet, should have significant ecological consequences, such as influencing the population dynamics of both *N. scintillans* and other trophic compartments, causing bloom succession, and even altering the whole food web structure and biogeochemical cycling. This is the first comprehensive study to determine the ingestion rates and growth rates of *N. scintillans* using monospecific and mixed diets, and its feeding preferences in both short-term feeding experiments (6 h) and longer-term growth experiments (2 d). However, to better understand *N. scintillans*' feeding preference in natural systems, more research into the behavioral and/or metabolic mechanisms that govern lags in the response of consumers' preference to changes in prey composition and density is required.

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