Vol. 74: 59–71, 2015
doi: 10.3354/ame01729

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online January 12

Factors influencing the grazing response of the marine oligotrichous ciliate *Strombidium* cf. *sulcatum*

Jinpeng Yang*, Martin Günter Joachim Löder, Maarten Boersma, Karen Helen Wiltshire

Biologische Anstalt Helgoland, Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, PO Box 180, 27483 Helgoland, Germany

ABSTRACT: We studied the numerical and functional responses of the marine oligotrichous ciliate *Strombidium* cf. *sulcatum* on 4 prey organisms to assess which prey properties affect the grazing response and food ingestion of this taxon. The predator exhibited higher growth rates when feeding on *Dunaliella* sp. than on *Pyramimonas* sp., *Prorocentrum* sp. or a small cryptophyte species. The ingestion rate of the predator when feeding on *Dunaliella* sp. increased with increasing prey concentrations. However, no significant ingestion was detected for the other prey organisms. By measuring important properties of the prey organisms, we found that (1) cellular carbon and nitrogen contents and fatty acid composition of the prey organisms are likely unimportant factors affecting food ingestion of oligotrichous ciliates; (2) although the essential fatty acids EPA, DPA and DHA were not detected in *Dunaliella* sp., low amounts of EPA and DHA were detected in the predator, indicating that this ciliate can synthesize these fatty acids at a slow rate by itself; and (3) the swimming velocity of *Dunaliella* sp. was much slower and its swimming behaviour was different from that of the other prey organisms, indicating that the swimming motility of prey species may play a pivotal role in determining the food ingestion of oligotrichous ciliates. This study provided new insight into the mechanism of grazing response and food ingestion of oligotrichous ciliates, which aids in our understanding of how this important ciliate group shapes the composition and functioning of phytoplankton communities *in situ*.

KEY WORDS: Grazing response · C:N ratio · Fatty acid · Swimming behaviour · Microbial food web · Helgoland Roads

INTRODUCTION

It is generally recognized that planktonic ciliates are primary consumers of phytoplankton and play a pivotal role in marine ecosystems as important links from microbial food webs to higher trophic levels (Azam et al. 1983, Pierce & Turner 1992, Vargas et al. 2007, 2008). Oligotrichous ciliates are important components of the planktonic ciliate community and they often dominate the microzooplankton episodically (Agatha 2011, Löder et al. 2012). In recent years there has been increasing interest in the feeding biology of oligotrichous ciliates, as they are dominant grazers in microbial food webs (Sanders & Wickham 1993). It is important to investigate factors influencing the trophic transfer from phytoplankton to herbivores to better understand the complex trophic interactions within microbial food webs and the mechanism of phytoplankton bloom formation (Brett & Goldman 1997).

Size plays an important role in determining the grazing response between predator and prey (Fenchel 1986, Jonsson 1986, Hansen et al. 1994). Fenchel (1980) proposed that ciliates discriminate among food particles primarily on the basis of size. However, feeding and ingestion may be more complicated

*Corresponding author: jinpeng.yang@awi.de

than a simple mechanical process (Stoecker 1988, Stoecker et al. 1995). Food quality may be an important factor affecting trophic transfer in marine food webs (Boersma et al. 2008, 2009, Dutz & Peters 2008). Malzahn & Boersma (2012) investigated the effect of exposure to low and high phosphorus food on copepod growth and showed that even short term exposure to phosphorus-limited food had a lasting effect on secondary production. Meunier et al. (2013) studied the effects of food quality on a heterotrophic dinoflagellate *Oxyrrhis marina*, and showed that nutrient limitation influenced the swimming speed and escape success of the prey organisms, which in turn significantly influenced food ingestion by *O. marina*. The essential fatty acid contents in food are considered to have a significant effect on the growth efficiencies of metazoan grazers (Müller-Navarra et al. 2000). Evjemo et al. (2008) investigated the effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*, and suggested that dietary long-chain fatty acids may be essential for reproduction in this species. However, only a few studies have investigated the effects of food quality on the growth and grazing responses of oligotrichous ciliates (Chen et al. 2010). In addition, when artificial or dead prey organisms are used in studies, a strong bias may be introduced compared to results obtained with live food (e.g. Sherr et al. 1987, Christaki et al. 1998, Ichinotsuka et al. 2006). The effects of swimming behaviour on trophic interactions between copepods and ciliates have been well studied (Jonsson & Tiselius 1990, Burns & Gilbert 1993, Broglio et al. 2001). Escape behaviour of ciliates decreases predation by ambush-feeding copepods (Jonsson & Tiselius 1990) and rotifers (Gilbert 1994). However, to our knowledge, very few studies have explored the influence of prey swimming motility on the grazing response of oligotrichous ciliates. Although some progress has been made during the last a few decades, the factors that may influence grazing response and food ingestion are still largely unclear and under scientific debate.

Strombidium sulcatum, a typical oligotrichous ciliate, occurs in many marine locations (e.g. Jiang et al. 2011, Wickham et al. 2011, Xu et al. 2011). As it is relatively easy to culture and maintain compared to other ciliates, it is commonly used as a model study organism representing filter-feeding ciliates (Fenchel & Jonsson 1988). In the North Sea, a taxon identified as *Strombidium* cf. *sulcatum* due to its anterior protuberance (Montagnes et al. 1990) frequently occurs in the plankton community from January to October and reaches its maximum abundance (8.2×10^3 cells l^{-1}) in May (Yang et al. 2014). This ciliate is capable of growing on different kinds of pico- and nanoplanktonic prey (Bernard & Rassoulzadegan 1990). Its grazing activity has been investigated in previous studies using a variety of food items with a size range of 0.2 to 12 µm, including live organisms, heat-killed bacteria, fluorescently labeled cells as well as different inert particles (Fenchel & Jonsson 1988, Allali et al. 1994, Dolan & Simek 1997, Christaki et al. 1998). Bernard & Rassoulzadegan (1990) investigated the grazing response of *S. sulcatum* with 12 different types of food ranging in size from 0.6 to 11.9 µm and suggested that prey size seemed to be a major factor influencing the grazing and food uptake of this ciliate. However, this might be only a physical constraint for particular prey sizes (Thurman et al. 2010), and characteristics other than prey size may play a role in the food uptake process and determine the predator–prey relationship between ciliate predators and their algal prey.

In this study, we examined the factors that could potentially influence the grazing response and food ingestion of oligotrichous ciliates. We present the results of experiments on the numerical and functional responses of the marine oligotrichous ciliate *Strombidium* cf. *sulcatum* feeding on 4 different kinds of algal prey. By measuring the carbon and nitrogen contents and fatty acid compositions of the predator and prey organisms and observing the swimming velocity and behaviour of each prey type, we aimed to determine whether prey food quality or motility has an effect on the grazing response and food ingestion of oligotrichous ciliates.

MATERIALS AND METHODS

Isolation and culture of the predator and prey organisms

Strombidium cf. *sulcatum* was isolated from water samples collected at Helgoland Roads (54° 11.30′ N, 7° 54.00′ E) in June 2010 when the water temperature and salinity were 12.8°C and 30.2, respectively. A monoclonal culture of *S.* cf. *sulcatum* was established by 2 serial single cell isolations and grown at 15°C in 70 ml tissue culture bottles, with enriched F/2-Si seawater medium (Guillard & Ryther 1962) and *Dunaliella* sp. (from a culture collection) as food, under a 14 h light:10 h dark cycle with an illumination of ~40 µE m^{-1} s^{-1} provided by cool white fluorescent light. Three other kinds of prey organisms, *Pyramimonas* sp., *Prorocentrum* sp. and a cryptophyte sp.

were isolated from the water around Helgoland and cultured under conditions identical to those of the predator. They were offered to the predator as potential prey in the field.

Set-up of the experiments

Before set-up of the experiments, *S.* cf. *sulcatum* was transferred to 1, 2 and 5 l glass bottles with fresh *Dunaliella* sp. as food in order to obtain a sufficient number of grazers. Meanwhile, 4 prey organisms were transferred to 275 or 800 ml tissue culture bottles, depending on their cell densities and the volume needed for the experiments. Experiments could only be conducted when the predator was available in large numbers. The predator culture was starved for 48 h until the prey cells were almost completely grazed. Then, a 100 ml water sample was fixed with acid Lugol's solution (final conc. 2%), and triplicate Utermöhl chambers (2.973 ml) were counted to determine the initial concentration of the predator. The initial concentration of each prey was determined using a CASY particle counter (Schärfe Systems), which also measured the equivalent spherical diameter (ESD) and cell volume of each prey.

The predator and prey were transferred to each incubation bottle using an autopipette based on the known initial concentrations. The target concentration of the predator was 1 cell ml^{-1} for treatments at low prey concentrations (C_1 to C_3) and 2 cells ml^{-1} for treatments at high prey concentrations (C_4 to C_6). The target concentrations of *Dunaliella* sp. were 1.0×10^2, 2.5×10^2, 5×10^2, 1.0×10^3, 5.0×10^3 and 1.0×10^4 cells ml^{-1}. For the other prey organisms, the target concentrations were calculated according to their predetermined carbon contents (refer to the measurement of C and N contents) to provide the predator with the same amount of carbon biomass as in the treatments with *Dunaliella* sp. Triplicate 70 ml treatment bottles (a mixture of predator and prey) and triplicate control bottles (prey only) were set up for each prey concentration. Each bottle was filled to capacity with F/2-Si medium and capped. Two sets of incubation bottles were established for each prey. One set was fixed with acid Lugol's solution at the start of the experiment to determine the actual initial concentrations of the predator and prey organisms. Another set was incubated under the conditions mentioned above and fixed with acid Lugol's solution after 24 h. For the concentrations of the predator, a 25 ml sample was settled using an Utermöhl chamber (Utermöhl 1958) for each incubation bottle and all cells were enumerated under an inverted microscope (Zeiss Axiovert 135). The concentrations of the prey organisms were determined by counting cells in 1 ml Sedgwick-Rafter counting chambers (SRCs) (Jakobsen & Hansen 1997).

Measurements of C and N contents and fatty acid composition

The measurements of carbon and nitrogen contents and fatty acid composition were conducted immediately following experimental set-up. A calculated volume of experiment inoculum (according to the concentrations determined by the CASY particle counter) was filtered onto pre-combusted Whatman GF/F filters (2.37×10^4 cells $filter^{-1}$ for the predator and 4×10^6 cells $filter^{-1}$ for each prey). For each organism, 5 replicate filters were prepared for the measurement of C and N contents and 4 replicate filters were prepared for the measurement of fatty acid composition. The filters for C and N contents were dried at 60°C overnight and then wrapped in aluminum foil. The filters for fatty acid composition were put in 2 ml Eppendorf tubes and stored at −80°C. C and N contents were measured using a VARIO Micro Cube/CN-analyser (Elementar). Fatty acid composition was measured with a YXZ gas chromatograph according to the method described by Wiltshire et al. (2000).

Measurement of swimming velocity

The swimming velocities of the prey organisms in treatments and controls at the highest prey concentration were measured to investigate whether the prey cells would change their swimming velocities when under predation pressure. The films for measuring swimming velocities were converted to single frame pictures using the programme 'Avi4Bmp' (Bottomap Software). The time period found to be most suitable for our investigation was 2 s, as most of the cell tracks could be captured in the picture frames and only a few organisms crossed each other during this time. A total of 30 frames (equaling 2 s of each film) were chosen and stacked with the function 'overlay frames' in the program 'Trace' (© Heribert Cypionka 2000-2010) to create images showing the swimming path of the cells (Fischer & Cypionka 2006). The length of the swimming tracks of 40 cells (20 cells for treatment and 20 cells for control) for each prey in the stacked pictures was measured with

the software 'ImageJ'. Swimming velocity (µm s^{-1}) was calculated by dividing path length by time. For detailed information see Löder (2010). Mean values of treatments and controls for each prey were used for statistics. Two-tailed *t*-tests and 1-way ANOVA were conducted using the software SigmaPlot 11.0 (Systat Software) after testing for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test), both at $\alpha = 0.05$. Significant differences between groups were analyzed by Tukey's HSD post hoc tests. Swimming behaviour was observed by comparing the patterns of the swimming paths in the stacked pictures.

Growth and ingestion rates

The experiments were designed to measure the growth and ingestion rates of *S.* cf. *sulcatum*, as a function of geometric mean prey concentrations, when feeding on 4 different prey organisms. Growth rate of the predator, V (d^{-1}), was calculated assuming exponential growth:

$$V = [\ln(S_t) - \ln(S_0)]t^{-1} \quad (1)$$

where S_0 is the concentration of the predator at the beginning of incubation, S_t is the concentration at each sampling interval, and t is the time of the sampling interval in days.

Numerical responses, with non-zero intercepts, were established by fitting Eq. (2) to the data of growth rate using an iterative routine in SigmaPlot 11.0:

$$V = V_{max}(x - x_0)/[K_G + (x - x_0)] \quad (2)$$

where V_{max} is the maximum growth rate of the predator, x is the prey concentration, x_0 is the threshold prey concentration (where $V = 0$), and K_G is the numerical response constant (Fenton et al. 2010). The significance of the difference between V_{max}, x_0 and K_G of the different prey species was established using the pseudo standard error estimates of the parameters after iteration in pairwise *t*-tests with subsequent Bonferroni corrections for multiple testing. As the number of observations per series was 18, and 3 parameters were estimated based on these values, we used the conservative number of 29, i.e. [2 × 18 − (6 estimated parameters) − 1], as the degrees of freedom in the *t*-tests.

Ingestion rate of the predator, I (ng C ciliate^{-1} d^{-1}), was determined according to the equation by Gransden & Lewitus (2003), which accounts for depletion of prey and any change in predator number over the 24 h incubation period as:

$$I = (g \times P) \times S^{-1} \quad (3)$$

where g (specific grazing rate) = $V_n - V_w$, the difference between net prey growth rate without grazers (V_n) and with grazers (V_w), P (mean prey concentration) = $(P_t - P_0) \times [\ln(P_t \times P_0^{-1})]^{-1}$, and S (mean predator concentration) = $(S_t - S_0) \times [\ln(S_t \times S_0^{-1})]^{-1}$.

Functional responses were established by fitting Eq. (4) to the data of ingestion rate using SigmaPlot 11.0:

$$I = I_{max}(x) / (K_I + x) \quad (4)$$

where I_{max} is the maximum ingestion rate (ng C ciliate^{-1} d^{-1}), x is the prey concentration, and K_I is the half saturation constant (Fenton et al. 2010).

Gross growth efficiency

Gross growth efficiency (GGE), which is defined as the ratio of ciliate growth in terms of carbon to total ingested prey carbon as a function of geometric mean prey concentration, was calculated from estimates of measured carbon contents.

RESULTS

C and N contents of the predator and prey organisms

The carbon and nitrogen contents, also expressed as C:N ratios of the predator and prey organisms, as well as the sizes and biovolume of the prey are presented in Table 1. With regard to the sizes and biovolume, *Dunaliella* sp. and *Pyramimonas* sp. were very similar to each other, with mean ESDs of 7.06 and 6.88 µm and mean biovolumes of 192.3 and 178.9 $µm^3$, respectively. *Prorocentrum* sp. and the cryptophyte sp. were much larger, with mean ESDs of 10.75 and 9.93 µm and mean biovolumes of 679.3 and 530.5 $µm^3$, respectively. *Prorocentrum* sp. had the highest carbon and nitrogen values among the prey organisms, with a mean carbon content of 93.79 pg C cell^{-1} and a mean nitrogen content of 16.10 pg N cell^{-1}. *Pyramimonas* sp. had the lowest values among the prey organisms with 20.53 pg C cell^{-1} and 3.77 pg N cell^{-1}. The carbon and nitrogen contents of the predator were 4708 pg C cell^{-1} and 695 pg N cell^{-1}. With regard to the C:N ratios, *Prorocentrum* sp. also had the highest value (5.84) among the prey organisms while the cryptophyte sp. had the lowest value (4.61). The C:N ratio of the predator was

Table 1. Mean ± SD carbon and nitrogen contents as well as C:N ratios of the predator and prey organisms, and the sizes and biovolume of the prey organisms. ESD: equivalent spherical diameter

	Size (µm, ESD)	Cell volume (µm^3)	Carbon (pg C cell^{-1})	Nitrogen (pg N cell^{-1})	C:N ratio
Strombidium cf. *sulcatum*			4708 ± 306	695 ± 66	6.79 ± 0.29
Dunaliella sp.	7.06 ± 0.02	192.3 ± 1.5	29.62 ± 0.83	5.63 ± 0.33	5.28 ± 0.43
Pyramimonas sp.	6.88 ± 0.06	178.9 ± 5.1	20.53 ± 1.45	3.77 ± 0.35	5.47 ± 0.58
Prorocentrum sp.	10.75 ± 0.16	679.3 ± 29.0	93.79 ± 2.10	16.10 ± 0.54	5.84 ± 0.35
Cryptophyte sp.	9.93 ± 0.22	530.5 ± 33.5	51.63 ± 0.49	11.20 ± 0.33	4.61 ± 0.15

6.79 and was thus higher than the C:N ratios of the prey organisms.

Fatty acid compositions of the predator and prey organisms

The fatty acid compositions of the predator and prey organisms are presented in Table 2. Among the prey organisms, the total ω-3 polyunsaturated fatty acids (PUFAs) were highest in *Prorocentrum* sp. (69.03%) and lowest in *Dunaliella* sp. (45.35%). The total C_{18} PUFAs were highest in *Dunaliella* sp. (55.03%) and lowest in *Pyramimonas* sp. (39.53%). The total C_{20} PUFAs were highest in the cryptophyte sp. (16.56%) and the total C_{22} PUFAs were highest in *Prorocentrum* sp. (24.15%) while in *Dunaliella* sp. C_{20} and C_{22} PUFAs were not detected. With regard to the predator, the total ω-3 and C_{18} PUFAs were 23.73% and 25.92% respectively, and the total C_{20} and C_{22} PUFAs were present only in trace amounts (<1%). Based on the results, the cryptophyte sp. and *Prorocentrum* sp. seemed to be high quality food due to their high amounts of essential fatty acids (EPA, DHA). *Pyramimonas* sp. had the high amounts of DHA and DPA, but no EPA. *Dunaliella* sp. seemed to be a low quality food in terms of fatty acid composition.

Swimming velocities of the prey organisms

The swimming velocities of each prey in treatments and controls are presented in Fig. 1. There was no significant difference between treatments and controls for each prey organism (2-tailed *t*-test, $p > 0.05$). The swimming velocities between all the prey organisms (with predator) were significantly different (ANOVA $F_{3,11} = 59.845$, $p < 0.0001$). The swimming velocity of *Dunaliella* sp. (87.66 ± 1.51 [SD]) was significantly different from those of the other 3 prey organisms (*Pyramimonas* sp.: 144.43 ± 7.72; *Prorocentrum* sp.: 142.81 ± 2.16; cryptophyte sp.: 151.14 ± 10.37) (Tukey's HSD test, $p < 0.0001$) while no significant difference was detected between the other 3 prey organisms (Tukey's HSD test, $p > 0.05$).

Growth rates of the predator

The relationships between the growth rates of *Strombidium* cf. *sulcatum* and the geometric mean prey concentrations followed rectangular hyperbolic responses (Fig. 2, Table 3). V_{max} when feeding on *Dunaliella* sp. was 0.331 d^{-1}, which was significantly higher than the values obtained when feeding on *Prorocentrum* sp. (0.108 d^{-1}) and the cryptophyte sp. (0.109 d^{-1}) (Fig. 3). K_G and x_0 for *Dunaliella* sp. were 8.541 and 4.931 ng C ml^{-1}, respectively. Both values were lower than those of the other 3 prey organisms in most cases, with a significance of $p < 0.05$ after

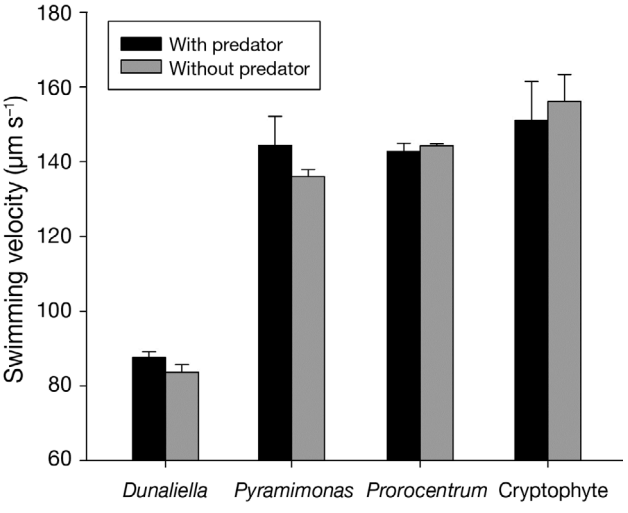

Fig. 1. Swimming velocities of the prey organisms in treatments (with predator) and controls (without predator). Error bars correspond to 1 SD, n = 3

Table 2. Fatty acid (FA) compositions (% of total FAs) of the predator and prey organisms (data as percentage of total FAs ± SD; n = 4). PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids; TU/TS: total unsaturated/total saturated; –: not detected

Fatty acid	*Dunaliella* sp.	*Pyramimonas* sp.	*Prorocentrum* sp.	Cryptophyte sp.	*Strombidium* cf. *sulcatum*
16:0	20.30 ± 4.44	24.72 ± 2.95	16.26 ± 0.78	11.47 ± 0.66	19.69 ± 3.39
16:1 ω-7	2.71 ± 0.49	6.79 ± 0.24	0.44 ± 0.03	0.31 ± 0.07	9.18 ± 1.60
16:2 ω-4	0.40 ± 0.12	0.20 ± 0.06	0.06 ± 0.01	0.01 ± 0.01	–
16:3 ω-4	–	–	0.24 ± 0.03	–	0.02 ± 0.01
17:0	3.95 ± 0.78	1.16 ± 0.27	0.48 ± 0.02	0.11 ± 0.02	2.11 ± 0.41
17:1 ω-7	–	–	1.06 ± 0.04	2.59 ± 0.22	0.93 ± 0.15
18:0	7.58 ± 2.44	10.42 ± 1.92	3.19 ± 0.68	3.91 ± 0.90	15.09 ± 2.54
18:1 ω-9	4.82 ± 0.66	2.20 ± 0.33	2.10 ± 0.12	5.49 ± 0.61	25.05 ± 1.07
18:2 ω-6	4.64 ± 0.88	0.71 ± 0.24	4.18 ± 0.22	0.31 ± 0.07	0.58 ± 0.27
18:3 ω-6 (GLA)	4.28 ± 0.75	–	0.01 ± 0.01	–	2.66 ± 0.25
18:3 ω-4	0.76 ± 0.40	6.03 ± 0.34	0.01 ± 0.01	10.24 ± 1.47	0.33 ± 0.07
18:3 ω-3 (ALA)	44.28 ± 8.53	24.66 ± 2.09	8.04 ± 0.32	29.64 ± 4.86	21.91 ± 3.55
18:4 ω-3	1.07 ± 0.28	8.13 ± 0.66	35.54 ± 0.69	8.63 ± 1.38	0.44 ± 0.10
20:0	–	–	0.29 ± 0.02	–	–
20:1 ω-9	–	0.15 ± 0.04	2.07 ± 0.08	0.31 ± 0.06	–
20:3 ω-3	–	1.19 ± 0.03	–	–	0.47 ± 0.05
20:5 ω-3 (EPA)	–	–	1.50 ± 0.04	16.56 ± 1.86	0.51 ± 0.13
22:0	–	0.09 ± 0.02	0.29 ± 0.01	0.04 ± 0.01	0.21 ± 0.07
22:1 ω-9	4.85 ± 0.46	0.12 ± 0.05	–	–	0.10 ± 0.02
22:2 ω-6	–	–	0.21 ± 0.01	–	–
22:5 ω-3 (DPA)	–	3.52 ± 0.06	0.07 ± 0.02	0.20 ± 0.03	–
22:6 ω-3 (DHA)	–	9.46 ± 0.12	23.87 ± 0.51	10.02 ± 0.98	0.07 ± 0.07
24:0	0.35 ± 0.05	0.46 ± 0.06	0.08 ± 0.01	0.15 ± 0.01	0.66 ± 0.17
Total ω-3 PUFA	45.35	46.96	69.03	65.05	23.73
Total ω-6 PUFA	8.92	0.71	4.39	0.31	3.24
Total 18 PUFA	55.03	39.53	47.78	48.82	25.92
Total 20 PUFA	–	1.19	1.50	16.56	0.98
Total 22 PUFA	–	12.98	24.15	10.22	0.07
Total SFA	32.17	36.84	20.59	15.68	37.76
Total UFA	67.83	63.16	79.41	84.32	62.24
TU/TS	2.11	1.72	3.86	5.38	1.65
Total (pg cell^{-1})	4.67	3.05	17.48	8.34	414.84

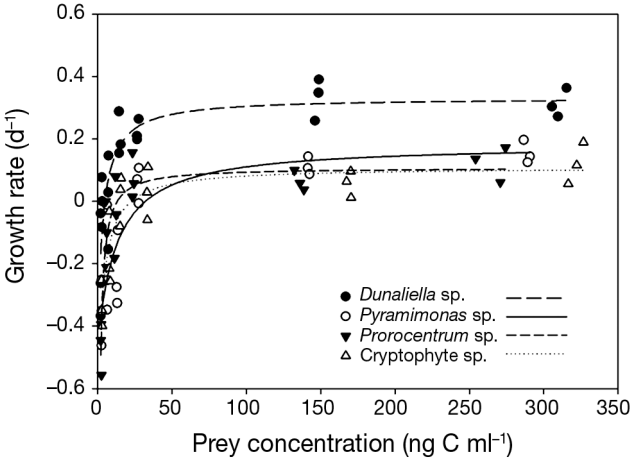

Fig. 2. Growth rate (V, d^{-1}) of *Strombidium* cf. *sulcatum* after 24 h of incubation grazing on different prey organisms as a function of geometric mean prey concentration (x, ng C ml^{-1}). The curves are fitted by Eq. (2) using all treatments in the experiment

Bonferroni correction (Fig. 3, Table 3), suggesting that in terms of growth response, *Dunaliella* sp. is the best prey for the predator compared to the other 3 prey organisms.

Ingestion rates and GGE of the predator

The ingestion rates of the predator feeding on *Dunaliella* sp. at different prey concentrations also followed a rectangular hyperbolic response (Fig. 4). The ingestion rate of the predator increased rapidly with increasing prey concentration below ca. 75 ng C ml^{-1} and slowly increased at higher prey concentrations. The I_{max} of the predator was 7.46 ng C ciliate^{-1} d^{-1} after the 24 h incubation period. For the other 3 prey organisms, no significant ingestion was detected, i.e. there was no significant difference

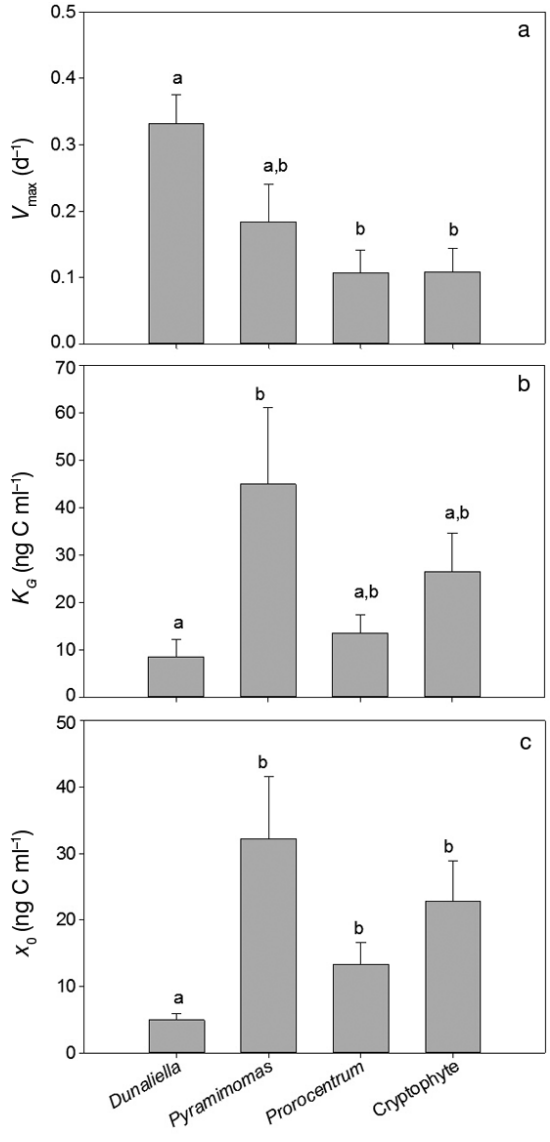

Fig. 3. Graphic representations of the estimates (+SE) of (a) the maximum growth rate (V_{max}), (b) the numerical response constant (K_G) and (c) the threshold prey concentration (x_0) for *Strombidium* cf. *sulcatum* grazing on different prey organisms. Different lower case letters indicate significant differences ($p < 0.05$) between the estimators based on Bonferroni-corrected multiple *t*-tests

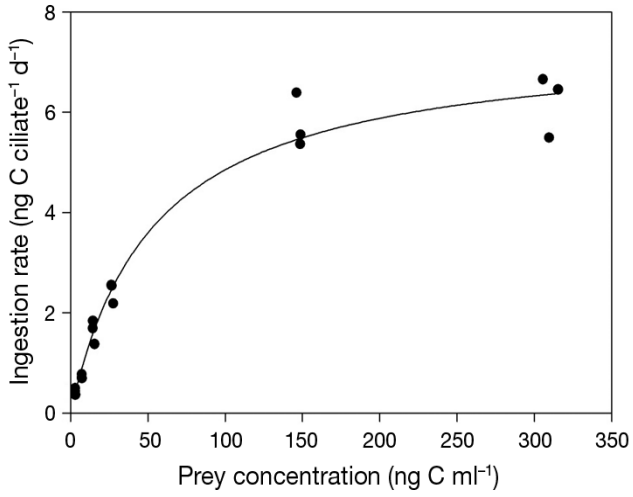

Fig. 4. Ingestion rate (I, ng C ciliate^{-1} d^{-1}) of *Strombidium* cf. *sulcatum* on *Dunaliella* sp. as a function of geometric mean prey concentration (x, ng C ml^{-1}). The curve is fitted by Eq. (4) using all treatments in the experiment. The fitted equation was $I = 7.46\ [x / (53.73 + x)]$ ($r^2 = 0.98$)

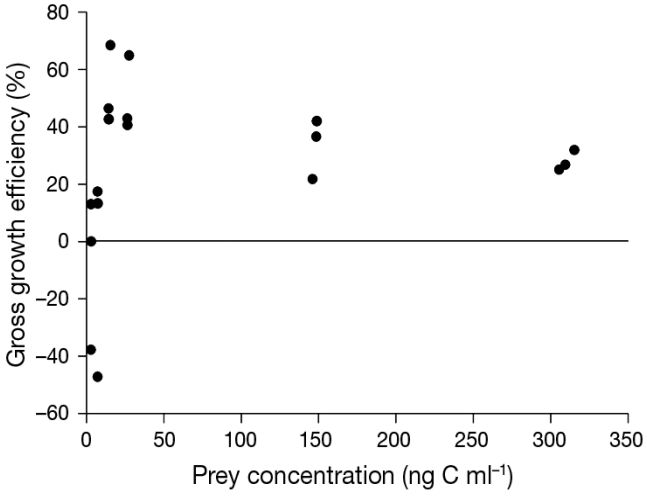

Fig. 5. Gross growth efficiency after 24 h incubation, defined as *Strombidium* cf. *sulcatum* biomass produced (+) or lost (–) per *Dunaliella* sp. biomass ingested, as a function of geometric mean prey concentration (x, ng C ml^{-1})

between the prey concentrations in treatments and controls.

The GGE of the predator feeding on *Dunaliella* sp. is shown in Fig. 5. The value was quite high (40 to 68%) when the prey concentrations ranged between 7 and 27 ng C ml^{-1}, and then decreased linearly to ca. 28% at the highest prey concentration.

Table 3. Parameters for the numerical responses of *Strombidium* cf. *sulcatum* growing on 4 different prey organisms as depicted in Figs. 2 & 3

Prey	V_{max} (d^{-1})	K_G (ng C ml^{-1})	x_0 (ng C ml^{-1})	SE	Adjusted r^2
Dunaliella sp.	0.331	8.541	4.931	0.104	0.755
Pyramimonas sp.	0.183	44.934	32.229	0.108	0.750
Prorocentrum sp.	0.108	13.550	13.314	0.088	0.835
Cryptophyte sp.	0.109	26.462	22.774	0.082	0.774

DISCUSSION

This study focused on prey-related factors that could potentially influence the grazing response and food ingestion of oligotrichous ciliates. We investigated the numerical and functional responses of the oligotrichous ciliate *Strombidium* cf. *sulcatum* feeding on 4 different prey species. Compared to any of the other 3 prey organisms, the predator exhibited significant ingestion and attained a higher growth rate when fed with *Dunaliella* sp. According to the responses of the predator, it was evident that *Dunaliella* sp. was the best prey in terms of growth and ingestion compared to the other 3 prey organisms.

The role of prey size and food quality

Two different size groups of prey organisms were used in our study. *Prorocentrum* sp. (10.75 µm) and the cryptophyte sp. (9.93 µm) might be too large for the predator to effectively ingest. Bernard & Rassoulzadegan (1990) reported that *S. sulcatum* exhibited no ingestion of the algae *Hymenomonas elongata* (11.85 µm), and that the optimum prey size was below 6.6 µm. However in our study, although *Dunaliella* sp. (7.06 µm) and *Pyramimonas* sp. (6.88 µm) were nearly the same size, the former was effectively ingested by the predator while the other was not. Similarly, Bernard & Rassoulzadegan (1990) found that *S. sulcatum* could ingest *Dunaliella minuta* (6.40 µm) while exhibiting no ingestion of the similarly-sized algae *Platymonas* sp. (6.55 µm). Meunier et al. (2013) observed significant selectivity of the heterotrophic dinoflagellate *Oxyrrhis marina* for *Rhodomonas salina* when 2 similar sized algae, *R. salina* and *Teleaulax* sp., were offered together as prey to *O. marina*. These findings, together with our results, show that the grazing response observed in our study was probably not just a result of prey size preference alone, and that other factors influenced the prey uptake by the predator.

The 'quality' of phytoplankton in terms of nutrient and metabolic composition can be a major factor influencing the growth of grazers (John & Davidson 2001, Montagnes et al. 2008, Montagnes et al. 2011) and also the selection for prey (Malzahn et al. 2010, Löder et al. 2011). In this study, we used the carbon and nitrogen contents and fatty acid compositions as proxies for food quality of the prey organisms, and investigated whether these can determine the grazing response and food ingestion of oligotrichous ciliates. The stoichiometric balance of cell carbon and nitrogen is often chosen as an index of prey quality (Montagnes et al. 2011), whereby the predator benefits the most if the prey has a similar ratio of carbon to nitrogen as the predator itself. In terms of cellular carbon and nitrogen contents, *Prorocentrum* sp. showed the highest ratio among the prey organisms while the cryptophyte sp. had the lowest. The C:N ratio of the predator was higher than any of the prey. We thus could not explain the food ingestion of the predator on *Dunaliella* sp. based on the results of cellular carbon and nitrogen contents. Similarly, Hantzsche & Boersma (2010) fed starved *O. marina* with nutrient replete, nitrogen depleted and phosphorus depleted *R. salina* and observed no effect of food quality on the food uptake of this protozoan predator. These findings suggest that the differences in grazing responses we observed were not due to the carbon and nitrogen contents of the prey organisms.

Fatty acid composition is an important factor that affects food quality (Becker & Boersma 2003, Boersma et al. 2008, 2009, Chen et al. 2010). Many studies have shown the importance of essential fatty acids in promoting the growth and reproduction of metazoan zooplankton in both limnitic (Brett & Müller-Navarra 1997, Boersma et al. 2001) and marine systems (Hagen et al. 1995, Peters et al. 2007). However, studies on the potential role of fatty acids in trophic transfer from phytoplankton to microzooplankton in marine systems are still very rare. Klein Breteler et al. (2004) reported that the fatty acid compositions of *S. sulcatum* and its prey *Dunaliella* sp. were similar, and that long-chain, highly-unsaturated fatty acids (HUFAs, such as EPA and DHA) were absent, indicating that *S. sulcatum* could not produce these HUFAs by itself and thus cannot upgrade the low food quality of phytoplankton for higher trophic levels. In this study, although there was no EPA, DPA or DHA detected in *Dunaliella* sp., low amounts of EPA and DHA were detected in the predator, indicating that this ciliate can synthesize these fatty acids at a very low rate by itself. Sul & Erwin (1997) reported that the marine scuticociliate *Parauronema acutum* can produce EPA and DHA at low rates when shorter-chain ω-3 fatty acids occur in the medium. We found that the dominant fatty acid in *Dunaliella* sp. was 18:3 ω-3 (linolenic acid, 44.28%), which is consistent with the findings of Klein Breteler et al. (2004). However, although conversion of linolenic acid into HUFAs has been shown in some marine ciliates (Sul & Erwin 1997, Klein Breteler et al. 2004), the presence of this building block for HUFAs could not explain the grazing response on *Dunaliella* sp. we observed, since the

other 3 prey organisms also have high amounts of linolenic acid.

It is known that *Dunaliella* sp. is an unsuitable food source for copepods as it contains no HUFAs (Klein Breteler et al. 2004), whereas cryptophyte species represent a good food source for copepods due to their high amounts of essential fatty acids (Boersma et al. 2008, Chen et al. 2012), as is the case in our study. Interestingly, we found that the 'bad food' for copepods could be a 'good food' for this ciliate, indicating that the grazing response observed in our study was not related to the presence of HUFAs in the prey. The fatty acid compositions of microalgae are substantially dependent on environmental conditions, especially different salinity and nutrient concentrations (Siron et al. 1989, Mendoza et al. 1999). Therefore, the variability of fatty acid composition due to the change of environmental conditions can influence the trophic conditions in the marine microbial food webs (Schoo et al. 2013). However, our findings indicate that the prey fatty acid composition could not explain the grazing response we observed. The 'quality' of prey organisms in terms of C:N ratio and fatty acid composition might influence the growth of grazers after the prey cells are effectively ingested, but it does not seem to be an important factor influencing the grazing response and food ingestion of oligotrichous ciliates, since in comparison to metazoan predators, they seemingly do not need to differentiate the prey organisms with different C:N ratios and fatty acid composition (Klein Breteler et al. 1999, Tang & Taal 2005).

The role of prey swimming motility

Several studies have investigated the effects of prey swimming motility on trophic interactions between ciliates and copepods as well as rotifers (Archbold & Berger 1985, Burns & Gilbert 1993, Jack & Gilbert 1993). However, very few studies have explored the influences of prey motility on trophic interactions between microalgae and ciliates. Löder (2010) investigated the effect of prey swimming motility on the growth and grazing responses of the dinoflagellate *Gyrodinium dominans* and the tintinnid *Favella ehrenbergii*, and found that prey immobilized by *F. ehrenbergii* enhanced the growth and grazing of the smaller predator *G. dominans*. Meunier et al. (2013) found that phosphorus-replete *R. salina* that swam at moderate speed was ingested at significantly higher rates by *O. marina* in comparison to slow swimming phosphorus deplete *R. salina* and fast swimming *Teleaulax* sp. In this study, we found that *Dunaliella* sp., which could be significantly ingested by the predator and support better growth as a result, was significantly slower than the other 3 prey organisms. In addition, although we only measured the prey swimming velocities at the highest prey concentration, the extent of congestion does not seem to affect the swimming velocities based on our observations at different prey concentrations.

The results of the above-mentioned studies show that prey swimming velocity might be an important factor affecting the grazing response and food ingestion. Swimming velocity is species-specific and influenced by environmental conditions (Kamykowski et al. 1988) and nutrient supplies (Meunier et al. 2013). Jeong et al. (2004b) reported that the elongated *Protoperidinium bipes* was much faster than spherically-shaped *Protoperidinium* spp., and suggested that prey cell shape might have a marked effect on the swimming speed. This could not be proven based on our results since *Dunaliella* sp. has a similar elongated shape to the cryptophyte sp., while *Pyramimonas* sp. and *Prorocentrum* sp. have almost spherical shapes. With regards to swimming behaviour, *Dunaliella* sp. always swam slowly while the other 3 prey organisms normally drifted in the water and could suddenly speed up when they met disturbance, indicating that they potentially possess the abilities to detect predators via swimming currents and try to escape from predation. Although we did not directly observe and measure the escape behaviour of prey organisms in this study, some studies have reported that escape behaviour can decrease predation rates (Jonsson & Tiselius 1990, Gilbert 1994, Broglio et al. 2001). Meunier et al. (2013) observed that *Teleaulax* sp. increased its swimming speed and changed direction randomly after an encounter with *O. marina*, resulting in actively induced escape from the predator. On the other hand, the enhancement of swimming speed can increase predator–prey encounter rates (Gerritsen & Strickler 1977) and thereby also food uptake and higher growth rates.

Although the mechanism of the grazing response and food ingestion might be much more complex, and some other factors still remain to be discovered, the results of this study as well as others have shown that the swimming motility of the prey could be an important factor determining food uptake by protozoan grazers, and plays a pivotal role in grazing response and food ingestion.

Methodology

Some points concerning our methodology have to be discussed and clarified here.

First, the growth rates of the predator reported in this study are lower than those reported in most related studies with this ciliate (e.g. Fenchel & Jonsson 1988, Allali et al. 1994, Christaki et al. 1998). This is probably due to the relatively low temperature used in our study (15°C), since it is known that growth rate is closely related to temperature (Macek et al. 1996, Montagnes 1996). Montagnes et al. (2003) suggested that the growth rates of free-living protists usually increase linearly with temperature. In our study, the maximum ingestion rate of the predator feeding on *Dunaliella* sp. was higher than those reported for dinoflagellate grazers (Jeong et al. 2003, 2004b, 2005), but lower than those reported for other oligotrichous ciliates. For example, Jeong et al. (2004a) reported a maximum ingestion rate of 108 ng C ciliate^{-1} d^{-1} for the large-sized ciliate *Strombidinopsis jeokjo* (150 × 70 µm) feeding on *G. dominans* at 18.5°C. Chen et al. (2010) reported a maximum ingestion rate of 167 ng C ciliate^{-1} d^{-1} for an oligotrichous ciliate, *Strobilidium* sp. (diameter 40 µm), feeding on *Nannochloropsis* sp. at 26°C. The difference in ingestion rates between oligotrichous ciliates might be related to the size of grazers and the temperature used in the studies. Li et al. (2011) reported that the maximum ingestion rate of *Condylostoma spatiosum* generally increased with increasing temperature.

Another factor that probably had a diminishing impact on the growth rate of the predator was that the ciliates used in our study were starved before the start of the experiments, and had to gain energy before they could divide again. Due to the pre-starving method used in this experiment, all numerical and functional response data are potentially biased and should not be used for direct comparison with field data or for parameterising pelagic ecosystem models.

Another important remark is that although the predator did not significantly ingest the other 3 prey organisms, it still exhibited positive growth at high prey concentrations. This is probably due to bacteria in the treatments, as it is known that this ciliate is capable of growing on bacteria as a food source (Rivier et al. 1985, Bernard & Rassoulzadegan 1990). Since our cultures were not axenic, bacteria were present in all the experimental treatments, as in most of the studies applying grazing experiments. One possibility to circumvent the presence of bacteria would be to keep the microalgal and predator cultures axenic using antibiotics, but this could also affect the conditions of the predator and prey organisms (Turner & Lloyd 1971, Hagenbuch & Pinckney 2012) and thus potentially lead to an even higher bias than with the presence of bacteria alone. Although we did not measure their abundance, it must be assumed that the bacterial concentrations would have been similar in the different treatments. Thus, the presence of bacteria should not have fundamentally changed the qualitative outcome of our study, i.e. the grazing responses to the 4 prey organisms.

Finally, we did not pre-acclimate the predator to the other 3 prey organisms before setting up the experiment because the predator could only grow at a high rate when fed with *Dunaliella* sp. and we needed a large number of ciliates to set up the experiments and measure the carbon and nitrogen contents. However, Hamels et al. (2004) found no significant difference between pre-acclimated and untreated predators concerning growth and ingestion.

Implications and perspective

Despite the limitations concerning methodologies that all laboratory grazing experiments may have, we investigated the numerical and functional responses of an oligotrichous ciliate, *S.* cf. *sulcatum*, feeding on 4 different prey organisms with very different characteristics. Our results showed that *Dunaliella* sp. was obviously the best prey choice for the predator in terms of ingestion and growth, whereas the other 3 prey organisms could not be effectively ingested and failed to support the growth of the predator. We measured key physical and chemical properties of the prey organisms, and the results indicated that the cellular carbon and nitrogen contents and fatty acid composition of the prey organisms might not be important factors influencing the food ingestion of oligotrichous ciliates. Rather, prey swimming motility might play a key role in determining the trophic interactions between ciliates and their prey organisms. The 'quality' of prey organisms is affected by different nutrient conditions and can influence the swimming ability of prey organisms, and therefore could indirectly affect the food ingestion of the predators. The feeding process and mechanism of ciliated protozoa are so complex that our data are not sufficient to completely reveal the answers. However, the results of our research provide important clues for future studies, and are very important for a better understanding of the predator–prey relationships

between oligotrichous ciliates and their microalgal prey in pelagic ecosystems.

Meunier et al. (2013) found that *O. marina* ingested significantly less phosphorous-replete *Teleaulax* sp. compared to phosphorous-deplete *Teleaulax* sp. even though they swam at the same speed, indicating that prey swimming motility is not the only factor that can influence ingestion. It is likely that a combination of factors determines the overall process of food ingestion. Characteristics such as the presence of feeding receptors (Wootton et al. 2007), the motility of the prey and its hydrophobicity (Matz & Jürgens 2001) appear to play a role in food ingestion and selection as well. There are indications that ciliates possess chemosensory receptors at the cytosome which may function in the process of prey ingestion (Wilks & Sleigh 2004). Molecular detection is considered to be important in searching, capture, processing and ingestion of prey (Montagnes et al. 2008). However, the mechanism by which ciliates ingest and select individual prey cells is still unclear at present and deserves more efforts in future studies (Montagnes 2013). Thus, further investigations using other oligotrichous ciliates are necessary to support the findings of our study, and to determine which combination of mechanisms and factors control the food ingestion and selection processes of oligotrichous ciliates and the trophic transfer from phytoplankton to microzooplankton in microbial food webs.

Acknowledgements. This study is part of a PhD thesis within the Food Web Project at the Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, and we are grateful for the funding and financial support from the China Scholarship Council. We thank the anonymous reviewers for their helpful suggestions. Special thanks to Julia Haafke and Bettina Oppermann for their technical support. Many thanks to Silvia Peters for her help in the laboratory, and last but not least, the whole team of the AWI Food Web Project for their helpful discussions.

LITERATURE CITED

Agatha S (2011) Global diversity of aloricate Oligotrichea (Protista, Ciliophora, Spirotricha) in marine and brackish sea water. PLoS ONE 6:e22466

Allali K, Dolan J, Rassoulzadegan F (1994) Culture characteristics and orthophosphate excretion of a marine oligotrich ciliate, *Strombidium sulcatum*, fed heat-killed bacteria. Mar Ecol Prog Ser 105:159–165

Archbold JHG, Berger J (1985) A qualitative assessment of some metazoan predators of *Halteria grandinella*, a common freshwater ciliate. Hydrobiologia 126:97–102

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Becker C, Boersma M (2003) Resource quality effects on life histories of *Daphnia*. Limnol Oceanogr 48:700–706

Bernard C, Rassoulzadegan F (1990) Bacteria or microflagellates as a major food source for marine ciliates: possible implications for the microzooplankton. Mar Ecol Prog Ser 64:147–155

Boersma M, Schöps C, McCauley E (2001) Nutritional quality of seston for the freshwater herbivore *Daphnia galeata* × *hyalina*: biochemical versus mineral limitations. Oecologia 129:342–348

Boersma M, Aberle N, Hantzsche F, Schoo K, Wiltshire KH, Malzahn A (2008) Nutritional limitation travels up the food chain. Int Rev Hydrobiol 93:479–488

Boersma M, Becker C, Malzahn A, Vernooij S (2009) Food chain effects of nutrient limitation in primary producers. Mar Freshw Res 60:983–989

Brett MT, Goldman CR (1997) Consumer versus resource control in freshwater pelagic food webs. Science 275: 384–386

Brett MT, Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic food web processes. Freshw Biol 38:483–499

Broglio E, Johansson M, Jonsson PR (2001) Trophic interaction between copepods and ciliates: effects of prey swimming behaviour on predation risk. Mar Ecol Prog Ser 220:179–186

Burns CW, Gilbert JJ (1993) Predation on ciliates by freshwater calanoid copepods: rates of predation and relative vulnerabilities of prey. Freshw Biol 30:377–393

Chen BZ, Liu HB, Lau MTS (2010) Grazing and growth responses of a marine oligotrichous ciliate fed with two nanoplankton: Does food quality matter for micrograzers? Aquat Ecol 44:113–119

Chen M, Liu H, Chen B (2012) Effects of dietary essential fatty acids on reproduction rates of a subtropical calanoid copepod, *Acartia erythraea*. Mar Ecol Prog Ser 455: 95–110

Christaki U, Dolan JR, Pelegri S, Rassoulzadegan F (1998) Consumption of picoplankton-size particles by marine ciliates: effects of physiological state of the ciliate and particle quality. Limnol Oceanogr 43:458–464

Dolan JR, Simek K (1997) Processing of ingested matter in *Strombidium sulcatum*, a marine ciliate (Oligotrichida). Limnol Oceanogr 42:393–397

Dutz J, Peters J (2008) Importance and nutritional value of large ciliates for the reproduction of *Acartia clausi* during the post spring-bloom period in the North Sea. Aquat Microb Ecol 50:261–277

Evjemo JO, Tokle N, Vadstein O, Olsen Y (2008) Effects of essential dietary fatty acids on egg production and hatching success of the marine copepod *Temora longicornis*. J Exp Mar Biol Ecol 365:31–37

Fenchel T (1980) Suspension feeding in ciliated protozoa: structure and function of feeding organelles. Arch Protistenkd 123:239–260

Fenchel T (1986) Protozoan filter feeding. Prog Protistol 1: 65–113

Fenchel T, Jonsson PR (1988) The functional biology of *Strombidium sulcatum*, a marine oligotrich ciliate (Ciliophora, Oligotrichina). Mar Ecol Prog Ser 48:1–15

Fenton A, Spencer M, Montagnes DJS (2010) Parameterising variable assimilation efficiency in predator-prey models. Oikos 119:1000–1010

Fischer JP, Cypionka H (2006) Analysis of aerotactic band formation by *Desulfovibrio desulfuricans* in a stopped-

flow diffusion chamber. FEMS Microbiol Ecol 55: 186–194

Gerritsen J, Strickler JR (1977) Encounter probabilities and community structure in zooplankton: a mathematical model. J Fish Res Board Can 34:73–82

Gilbert JJ (1994) Jumping behaviour in the oligotrich ciliates *Strombilidium velox* and *Halteria grandinella*, and its significance as a defense against rotifer predators. Microb Ecol 27:189–200

Gransden SG, Lewitus AJ (2003) Grazing of two euplotid ciliates on the heterotrophic dinoflagellates *Pfiesteria piscicida* and *Cryptoperidiniopsis* sp. Aquat Microb Ecol 33: 303–308

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Husteda and *Edtonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Hagen W, Kattner G, Graeve M (1995) On the lipid biochemistry of polar copepods: compositional differences in the Antarctic calanoids *Euchaeta antarctica* and *Euchirella rostromagna*. Mar Biol 123:451–457

Hagenbuch IM, Pinckney JL (2012) Toxic effect of the combined antibiotics ciprofloxacin, lincomycin, and tylosin on two species of marine diatoms. Water Res 46: 5028–5036

Hamels I, Mussche H, Sabbe K, Muylaert K, Vyverman W (2004) Evidence for constant and highly specific active food selection by benthic ciliates in mixed diatoms assemblages. Limnol Oceanogr 49:58–68

Hansen B, Bjornsen PK, Hansen PJ (1994) The size ratio between planktonic predators and their prey. Limnol Oceanogr 39:395–403

Hantzsche FM, Boersma M (2010) Dietary-induced responses in the phagotrophic flagellate *Oxyrrhis marina*. Mar Biol 157:1641–1651

Ichinotsuka D, Ueno H, Nakano S (2006) Relative importance of nanoflagellates and ciliates as consumers of bacteria in a coastal sea area dominated by oligotrichous *Strombidium* and *Strobilidium*. Aquat Microb Ecol 42:139–147

Jack JD, Gilbert JJ (1993) Susceptibilities of different-sized ciliates to direct suppression by small and large cladocerans. Freshw Biol 29:19–29

Jakobsen HH, Hansen PJ (1997) Prey size selection, grazing and growth response of the small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate *Balanion comatum* — a comparative study. Mar Ecol Prog Ser 158:75–86

Jeong HJ, Kim JS, Yoo YD, Kim ST and others (2003) Feeding by the heterotrophic dinoflagellate *Oxyrrhis marina* on the red-tide raphidophyte *Heterosigma akashiwo*: a potential biological method to control red tides using mass-cultured grazers. J Eukaryot Microbiol 50:274–282

Jeong HJ, Yoo YD, Kim JS, Kang NS, Kim TH, Kim JH (2004a) Feeding by the marine planktonic ciliate *Strombidinopsis jeokjo* on common heterotrophic dinoflagellates. Aquat Microb Ecol 36:181–187

Jeong HJ, Yoo YD, Kim ST, Kang NS (2004b) Feeding by the heterotrophic dinoflagellate *Protoperidinium bipes* on the diatom *Skeletonema costatum*. Aquat Microb Ecol 36:171–179

Jeong HJ, Kim JS, Kim JH, Kim ST and others (2005) Feeding and grazing impact of the newly described heterotrophic dinoflagellate *Stoeckeria algicida* on the harmful alga *Heterosigma akashiwo*. Mar Ecol Prog Ser 295: 69–78

Jiang Y, Xu HL, Al-Rasheid KAS, Warren A, Hu XZ, Song WB (2011) Planktonic ciliate communities in a semi-enclosed bay of Yellow Sea, northern China: annual cycle. J Mar Biol Assoc UK 91:97–105

John EH, Davidson K (2001) Prey selectivity and the influence of prey carbon:nitrogen ratio on microflagellate grazing. J Exp Mar Biol Ecol 260:93–111

Jonsson PR (1986) Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Mar Ecol Prog Ser 33: 265–277

Jonsson PR, Tiselius P (1990) Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. Mar Ecol Prog Ser 60:35–44

Kamykowski D, McCollum SA, Kirkpatrick GJ (1988) Observations and a model concerning the translational velocity of a photosynthetic marine dinoflagellate under variable environmental conditions. Limnol Oceanogr 33:66–78

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135:191–198

Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. Mar Ecol Prog Ser 274:199–208

Li C, Xu K, Lei Y (2011) Growth and grazing responses to temperature and prey concentration of *Condylostoma spatiosum*, a large benthic ciliate, feeding on *Oxyrrhis marina*. Aquat Microb Ecol 64:97–104

Löder MGJ (2010) The role of heterotrophic dinoflagellate and ciliate grazers in the food web at Helgoland Roads, North Sea. PhD dissertation, Jacobs University Bremen

Löder MGJ, Meunier C, Wiltshire KH, Boersma M, Aberle N (2011) The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. Mar Biol 158: 1551–1580

Löder MGJ, Kraberg A, Aberle N, Peters S, Wiltshire KH (2012) Dinoflagellates and ciliates at Helgoland Roads, North Sea. Helgol Mar Res 66:11–23

Macek M, Šimek K, Perthaler J, Vyhnálek V, Psenner R (1996) Growth rates of dominant planktonic ciliates in two freshwater bodies of different trophic degree. J Plankton Res 18:463–481

Malzahn AM, Boersma M (2012) Effects of poor food quality on copepod growth are dose dependent and non-reversible. Oikos 121:1408–1416

Malzahn AM, Hantzsche F, Schoo KL, Boersma M, Aberle N (2010) Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. Oecologia 162:35–48

Matz C, Jürgens K (2001) Effects of hydrophobic and electrostatic cell surface properties of bacteria on feeding rates of heterotrophic nanoflagellates. Appl Environ Microbiol 67:814–820

Mendoza H, Martel A, Jim nez del Río M, Reina GG (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J Appl Phycol 11:15–19

Meunier CL, Schulz K, Boersma M, Malzahn AM (2013) Impact of swimming behaviour and nutrient limitation on predator-prey interactions in pelagic microbial food webs. J Exp Mar Biol Ecol 446:29–35

Montagnes DJS (1996) Growth response of planktonic ciliates in the genera *Strobilidium* and *Strombidium*. Mar Ecol Prog Ser 130:241–254

Montagnes DJS (2013) Ecophysiology and behaviour of tintinnids. In: Dolan JR, Montagnes DJS, Agatha S, Coats DW, Stoecker D (eds) The biology and ecology of tintinnid ciliates: models for marine plankton. Wiley-Blackwell, Chichester, p 85–121

Montagnes DJS, Taylor FJR, Lynn DH (1990) *Strombidium inclinatum* n. sp. and a reassessment of *Strombidium sulcatum* Claparède and Lachmann (Ciliophora). J Protozool 37:318–323

Montagnes DJS, Kimmance SA, Atkinson D (2003) Using Q_{10}: Can growth rates increase linearly with temperature? Aquat Microb Ecol 32:307–313

Montagnes DJS, Barbosa AB, Boenigk J, Davidson K and others (2008) Selective feeding behaviour of key free-living protists: avenues for continued study. Aquat Microb Ecol 53:83–98

Montagnes DJS, Lowe CD, Martin L, Watts PC and others (2011) *Oxyrrhis marina* growth, sex and reproduction. J Plankton Res 33:615–628

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–77

Peters J, Dutz J, Hagen W (2007) Role of essential fatty acids on the reproductive success of the copepod *Temora longicornis* in the North Sea. Mar Ecol Prog Ser 341:153–163

Pierce RW, Turner JT (1992) Ecology of planktonic ciliates in marine food webs. Rev Aquat Sci 6:139–181

Rivier A, Brownlee DC, Sheldon RW, Rassoulzadegan F (1985) Growth of microzooplankton: a comparative study of bacterivorous zooflagellates and ciliates. Mar Microb Food Webs 1:51–60

Sanders RW, Wickham SA (1993) Planktonic protozoa and metazoa: predation, food quality and population control. Mar Microb Food Webs 7:197–223

Schoo KL, Malzahn AM, Krause E, Boersma M (2013) Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. Mar Biol 160:2145–2155

Sherr BF, Sherr EB, Fallon RD (1987) Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. Appl Environ Microbiol 53: 958–965

Siron R, Giusti G, Berland B (1989) Changes in the fatty acid composition of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* during growth and under phosphorus deficiency. Mar Ecol Prog Ser 55:95–100

Stoecker DK (1988) Are marine planktonic ciliates suspension-feeders? J Protozool 35:252–255

Stoecker DK, Gallager SM, Langdon CJ, Davis LH (1995) Particle capture by *Favella* sp. (Ciliata, Tintinnida). J Plankton Res 17:1105–1124

Sul D, Erwin JA (1997) The membrane lipids of the marine ciliated protozoan *Parauronema acutum*. Biochim Biophys Acta 1345:162–171

Tang KW, Taal M (2005) Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. J Exp Mar Biol Ecol 318:85–98

Thurman J, Parry JD, Hill PJ, Laybourn-Parry J (2010) The filter-feeding ciliates *Colpidium striatum* and *Tetrahymena pyriformis* display selective feeding behaviour in the presence of mixed, equally-sized, bacterial prey. Protist 161:577–588

Turner G, Lloyd D (1971) The effect of chloramphenicol on growth and mitochondrial function of the ciliate protozoan *Tetrahymena pyriformis* strain ST. J Gen Microbiol 67:175–188

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Plankton-Methodik. Mitt Int Ver Theor Angew Limnol 9: 1–38

Vargas C, Martínez R, Cuevas L, Pavez M and others (2007) The relative importance of microbial and classical food webs in a highly productive coastal upwelling area. Limnol Oceanogr 52:1495–1510

Vargas CA, Martínez RA, González HE, Silva N (2008) Contrasting trophic interactions of microbial and copepod communities in a fjord ecosystem, Chilean Patagonia. Aquat Microb Ecol 53:227–242

Wickham SA, Steinmair U, Kamennaya N (2011) Ciliate distributions and forcing factors in the Amundsen and Bellingshausen Seas (Antarctic). Aquat Microb Ecol 62: 215–230

Wilks SA, Sleigh MA (2004) Lectin binding sites on *Euplotes mutabilis* (Tuffrau, 1960) and the implications for food particle selection. Eur J Protistol 40:153–162

Wiltshire KH, Boersma M, Möller A, Buhtz H (2000) Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). Aquat Ecol 34: 119–126

Wootton EC, Zubkov MV, Jones DH, Jones RH, Martel CM, Thornton CA, Roberts EC (2007) Biochemical prey recognition by planktonic protozoa. Environ Microbiol 9: 216–222

Xu KD, Choi JK, Lei YL, Yang EJ (2011) Marine ciliate community in relation to eutrophication of coastal waters in the Yellow Sea. Chin J Oceanology Limnol 29:118–127

Yang JP, Löder MGJ, Wiltshire KH (2014) A survey of ciliates at the long-term sampling station 'Helgoland Roads', North Sea. Helgol Mar Res 68:313–327

Editorial responsibility: Klaus Jürgens, Rostock, Germany

Submitted: April 7, 2014; Accepted: October 22, 2014
Proofs received from author(s): December 5, 2014