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Importance and nutritional value of large ciliates for the reproduction of *Acartia clausi* during the post spring-bloom period in the North Sea

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ABSTRACT: Shipboard experiments were performed to examine the qualitative importance of large marine microzooplankton for the reproduction of *Acartia clausi* in the North Sea. Feeding and egg production were compared in 2 treatments in which females were fed natural seston or natural seston selectively enriched with large prey (>20 µm). The mineral (C, N) and lipid contents of the food suspensions were determined for size-fractionated samples to characterize the nutritional composition of prey. Large oligotrich ciliates and Strobiliidae dominated the seston biomass. Ciliates, particularly the oligotrich *Laboea strobila*, were the preferred food and made up more than 86% of the diet (2.9 µg C female^{-1} d^{-1}) in the natural treatment. Small thecate dinoflagellates composed the remaining part. Egg production was high (>25 eggs female^{-1} d^{-1}), achieving maximum rates recorded for this species in the North Sea. Ingestion and egg production of females increased equally in the enriched treatment, largely due to ingestion of additional ciliates. This allowed calculation of the gross growth efficiency for egg production on a diet of mixed ciliates, which was high at ~30%. Measurements of the mineral and lipid content confirmed a high seston nutritional quality. The 20 to 48 µm fraction reflected the composition of female diets and indicated an enrichment of N and polyunsaturated fatty acids (PUFA) in the diet compared to total seston. The fraction >48 µm consisted mostly of ciliates (>97%) and was particularly important in providing N to copepods. Ciliates were also rich in PUFA and eicosapentaenoic acid (EPA), which is normally characteristic of diatoms. We conclude that large ciliates constitute an excellent food source for the reproduction of *A. clausi*. The occurrence of biomass peaks of large ciliates following the spring diatom bloom probably contributes substantially to the spring recruitment of the species in the North Sea.

KEY WORDS: *Acartia clausi* · Feeding · Egg production · Ciliates · *Laboea strobila* · Biochemical composition · Polyunsaturated fatty acids · North Sea

INTRODUCTION

Large hetero- or mixotrophic protists are an important component in the diet of marine pelagic copepods. Stimulated by early descriptions of copepod grazing on ciliates (Berk et al. 1977, Turner & Anderson 1983), numerous studies found ciliates and heterotrophic dinoflagellates to be a significant and often preferentially consumed prey in subtropical (Gifford & Dagg 1991, Broglio et al. 2004, Liu et al. 2005), temperate (Tiselius 1989, Fessenden & Cowles 1994) and polar ecosystems (Gifford & Dagg 1991, Levinsen et al. 2000). Since these protists are major consumers of the pico- and nanoplankton production that is unavailable to large crustacean zooplankton, dietary preference forms an important link between microbial food webs and the classical food chain (Sherr & Sherr 1988). Recent estimates indicate that on a global scale, the

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consumption of ciliates alone amounts to >⅓ of the C flux processed by zooplankton grazing on phytoplankton (Calbet & Saiz 2005). A high grazing pressure on ciliates can also temporarily control their production and have a strong influence on the composition of the microbial food web (Nielsen & Kiørboe 1994, Merrell & Stoecker 1998).

Besides the structural and functional importance of zooplankton feeding on microzooplankton in food webs, comparatively little is known about its contribution to pelagic secondary production. Microzooplankton is generally considered to be food of excellent quality that provides high levels of nitrogen or essential lipids to pelagic omnivores (Stoecker & Capuzzo 1990, Sanders & Wickham 1993, Klein Breteler et al. 1999). This is supported by studies showing that ciliates and heterotrophic dinoflagellates substantially enhance growth, egg production and survival of copepods in the laboratory (Berk et al. 1977, Stoecker & Egloff 1987, Klein Breteler et al. 1999). Recent studies also demonstrate that microzooplankton can improve the quality of nutritionally poor food and provide essential fatty acids or sterols for growth and egg production (Klein Breteler et al. 1999, Broglio et al. 2003, Tang & Taal 2005). However, the ability to upgrade the nutritional quality by de novo synthesis or modification of algal lipids is apparently limited to heterotrophic dinoflagellates. Ciliates, in contrast, reflect primarily the lipid composition of their food (Ederington et al. 1995, Broglio et al. 2003, Klein Breteler et al. 2004). This suggests that the nutritional quality of ciliates in the field is largely dependent on the variable composition and growth conditions of their food.

Despite recent progress in the characterization of microzooplankton quality in the laboratory, information on the nutritional composition and the importance of ciliates for production in the field is scarce. Microzooplankton is a major source of the mineral and biochemical composition of seston following the spring bloom in the temperate coastal ocean (Mayzaud et al. 1989, Pond et al. 1996), and feeding on heterotrophic protists may account for the apparent failure of phytoplankton ingestion to cover the energy demands of copepod egg production (Dam et al. 1994, Ohman & Runge 1994). Field studies also found correlations between copepod egg production and microzooplankton abundance (White & Roman 1992, Pond et al. 1996). However, due to generally low standing stocks of microzooplankton compared to the temporarily high phytoplankton biomass, feeding on heterotrophic protists has been suggested to contribute only a small fraction to the integrated annual production of copepods (Kiørboe & Nielsen 1994).

Estimates of the role of microzooplankton in—and their nutritional value for—the production of copepods in the field are often confounded by a high diversity of potential food particles and of copepod diets. In order to determine the biochemical composition of microzooplankton and its utilization by copepods, we took advantage of the typical size structure of pelagic food webs following the spring bloom. While the plankton community at this time often consists of smaller phytoplankton and larger hetero- or mixotrophic protists, copepods often display a typical size-based preference for larger prey (e.g. Rollwagen-Bollens & Penry 2003, Katechakis et al. 2004). Hence, we hypothesized that selective enrichment of large prey in the seston results in a simultaneous increase of its proportion in the diet and allows the characterization of its value for the reproduction of copepods. We used this approach during a cruise in the North Sea to determine (1) the contribution of microzooplankton to the diet and to the daily ration of *Acartia clausi*, (2) the efficiency with which these food items are converted into egg production, and (3) the mineral and biochemical composition of microzooplankton. Grazing and egg production of female *A. clausi* were compared in 2 treatments: one group received natural seston as food while the second group was fed natural seston in which large organisms were selectively enriched. Both food suspensions were analyzed for lipid composition and C and N contents in order to characterize their nutritional value.

MATERIALS AND METHODS

Study site. The investigation was performed onboard RV 'Heincke' (Alfred Wegener Institute, Germany) during a cruise in the North Sea from May 17 to 28, 2005. Experiments were carried out over a period of 4 consecutive days during which a central station located in the German Bight (southern North Sea, 54° 7.8′ N, 7° 15.0′ E; 38 m depth) was visited daily.

Sample collection. Each day, 80 to 100 l water samples were taken with a rosette at depths of 5 to 10 m. The water was carefully siphoned into 25 l carboys, avoiding air bubbling. Zooplankton grazers were removed by filtering through a large, submerged sieve (diameter 20 cm, mesh size 100 µm). The carboys were immediately stored at *in situ* temperature (~10°C) under dim light conditions. One batch of seawater remained unchanged (hereafter referred to as the natural treatment), while in a second batch of seawater large protists were concentrated by a factor of 4 by slow, inverse filtration via a submerged 20 µm sieve (referred to as the enriched treatment). Disruption by siphoning and sieving can cause considerable loss of ciliates (Gifford 1985). Both batches of seawater were therefore allowed to stabilize for 4 h before use in experiments or subsampling for mineral and biochem-

ical contents to avoid the inclusion of damaged organisms. Copepods were collected with a WP-2 net (100 µm mesh size) equipped with a closed cod end and towed vertically from a depth of 20 m to the surface. The catch was transferred into buckets filled with surface sea water and brought into a temperature-controlled walk-in chamber set to ambient surface water temperatures.

Experiments. Two sets of experiments were conducted in parallel and structured as follows: In the first set of experiments ('manipulation experiments', Fig. 1), grazing and egg production of *Acartia clausi* were compared in the natural and in the enriched food treatments. Females for these experiments were picked from an initial catch and transferred each day to new, freshly prepared food suspensions for a period of 4 d. On the 1st day of incubation, grazing of females was determined, followed by 2 more days for further adaptation to the experimental conditions before egg production of females was determined on the 4th day. Samples to characterize the quality of the food suspensions (lipid composition; C-, N-content) were taken on Days 1 (total seston) and 3 (size-fractionated seston), whereas samples for the analysis of protist species composition were taken each day during the incubation (Fig. 1). In the second set of experiments, *in-situ* egg production of freshly caught females was determined during each of Days 1 to 3 (Fig. 1). These experiments served as a control to evaluate how well egg production of females incubated in the natural treatment of the manipulation experiment reflects *in-situ* estimates.

Comparison of grazing and egg production in natural and enriched treatments: For each grazing treatment, 9 polycarbonate screw-cap bottles (550 ml) were filled with the respective seawater. Duplicate 200 ml samples from 3 bottles were taken immediately and fixed in 2% acidic Lugol's iodine for enumeration of the initial phytoplankton and microzooplankton composition. Three experimental bottles received 7 females each, while the remaining 3 bottles were run as controls without copepods. The bottles were incubated on a rotating plankton wheel (0.3 rpm) submerged in a temperature-controlled water bath at *in-situ* temperature (10°C) and dim light conditions (L:D 16:8). After 24 h, the contents of the bottles were carefully emptied into a large beaker containing an inner compartment with 200 µm mesh, which was slowly lifted to collect the females. Samples for the final phytoplankton and microzooplankton counts were immediately taken as described above. The females were transferred each day into fresh food suspensions during the following 2 d for further acclimation to food conditions. On the 4th day, females were incubated in spawning chambers as described for the estimates of *in-situ* egg production rates (see next section). After an additional 24 h, females were removed and eggs were concentrated on a submerged 20 µm sieve. Eggs were counted under a dissecting microscope (60×) and transferred to 250 ml glass bottles containing GF/F filtered seawater for subsequent determination of hatching success. After incubation of eggs for 5 d at *in-situ* temperature, bottle contents were concentrated and remaining eggs, empty egg shells and hatched nauplii were counted. The condition of females was inspected visually under the binocular microscope during the daily transfer of individuals. Mortality of females in the experiment was <7%; dead females were not substituted. The prosome length of all females (PL) was measured under a dissecting microscope at the end of the experiment. The C weight of females was calculated from the relation log C = −8.51 + 3.08 log PL established by Uye (1982). Egg

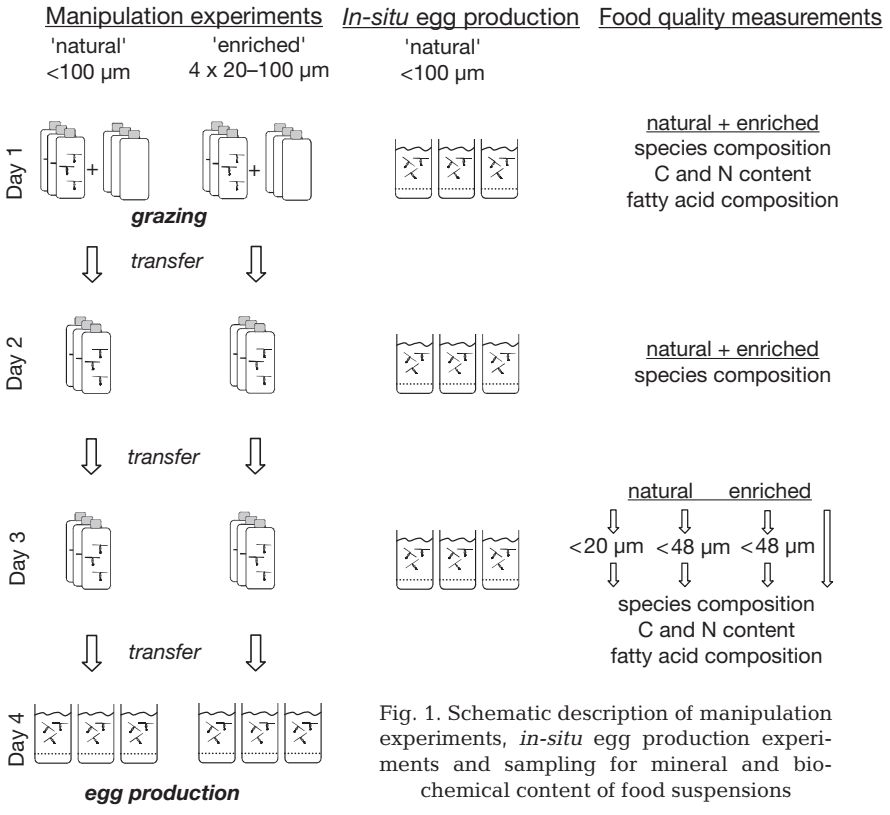

Fig. 1. Schematic description of manipulation experiments, *in-situ* egg production experiments and sampling for mineral and biochemical content of food suspensions

volume was determined from the measurement of the diameter of at least 30 eggs per treatment and egg carbon was estimated by applying a conversion factor of 1.4×10^{-6} µg C µm^{-3} (Kiørboe et al. 1985).

Estimates of in-situ egg production: *In-situ* egg production of *Acartia clausi* was determined at Days 1 to 3, parallel to the manipulation experiment. Each day, freshly collected females were incubated in triplicate groups of 10 ind. in 1 l spawning chambers, each containing an inner compartment with a bottom made of 100 µm mesh gauze to prevent copepods from feeding on their eggs. The chambers were filled with *in-situ* seawater filtered through a 48 µm sieve to remove any eggs. After 24 h, females were removed, eggs were counted and egg hatching success was determined similar to the procedure described in the manipulation experiments.

Seston biochemical and mineral composition: With the onset of the feeding experiment, duplicate samples for the determination of fatty acid, C and N concentration from the natural and enriched treatments were taken (Fig. 1). Approximately 500 to 1000 ml of seawater was gravity filtered onto pre-combusted GF/C filters (24 h, 500°C) which were immediately frozen at –80°C until analysis. On Day 3 of the incubation, samples for food quality measurements were taken from different size fractions (<20 µm, 20 to 48 µm, 48 to 100 µm) to separate distinct components of the food suspension (e.g. small phytoplankton, large heterotrophic protists) and to characterize their contribution to overall food quality. Because the natural treatment was expected to provide sufficient material only for the biochemical characterization of small protists, it was sieved into fractions of <20 and <48 µm. Samples for the measurement of larger but less abundant protists were prepared from the enriched treatment by sieving the seawater into size fractions of <48 and <100 µm. The biochemical and mineral composition of the 20 to 48 µm and the 48 to 100 µm fractions were then obtained by subtraction of the respective smaller size fraction from the larger one. Samples were also preserved with 2% acidic Lugol's iodine for enumeration of the protist concentration in the natural and enriched treatments on Days 1 to 3 and in the different size fractions on Day 3.

Table 1. Taxonomic and size composition of seston biomass and respective contribution (%) to total protist carbon, particulate organic carbon (POC) and nitrogen (PON), and C/N ratio of food suspensions used in feeding experiments at Day 1. Numbers in parentheses denote prey categories; Category (17) 'others' refers to unidentified taxonomic groups

	Natural treatment µg C l^{-1} ± SD	%	Enriched treatment µg C l^{-1} ± SD	%
Flagellates				
(1) <10 µm	4.8 ± 0.21	2.9	4.7 ± 1.05	1.1
(2) < 20 µm	0.4 ± 0.04	0.2	0.6 ± 0.08	0.1
(3) < 30 µm	0.1 ± 0.02	0.1	0.3 ± 0.07	0.1
Dinoflagellates				
(4) Athecate < 20 µm	1.0 ± 0.15	0.6	2.3 ± 0.29	0.6
(5) Thecate < 20 µm	34.7 ± 4.03	21.4	90.5 ± 5.44	21.7
(6) Athecate < 30 µm	0.6 ± 0.35	0.4	1.9 ± 0.04	0.5
(7) Thecate < 30 µm	1.7 ± 0.62	1.1	12.0 ± 3.31	2.9
(8) Athecate > 30 µm	8.6 ± 0.56	5.3	15.4 ± 3.49	3.7
(9) Thecate > 30 µm	1.9 ± 0.32	1.2	6.0 ± 0.40	1.4
Diatoms				
(10) <20 µm	0.1 ± 0.01	0.1	0.3 ± 0.02	0.1
(11) >30 µm	0.8 ± 0.65	0.5	1.6 ± 0.32	0.4
Ciliates				
(12) <20 µm	0.2 ± 0.04	0.1	0.3 ± 0.02	0.1
(13) <30 µm	0.8 ± 0.06	0.5	1.6 ± 0.12	0.4
(14) *Laboea strobila* > 30 µm	48.3 ± 2.45	29.8	121.6 ± 10.46	29.5
(15) Strobiliidae > 30 µm	54.7 ± 1.96	33.8	152.7 ± 27.90	36.6
(16) Others > 30 µm	0.9 ± 0.25	0.6	1.5 ± 0.06	0.4
Others				
(17)	2.5 ± 0.79	1.5	3.5 ± 0.58	0.8
Size classes (µm)				
5–10	4.8 ± 0.21	3.0	4.7 ± 1.05	1.1
10–20	37.3 ± 4.54	23.0	95.6 ± 5.31	23.0
20–30	3.3 ± 1.02	2.0	15.9 ± 3.54	3.8
>30	116.6 ± 5.32	72.0	300.7 ± 35.46	72.1
Total protist carbon	162 ± 11.1	100.0	417 ± 35.9	100.0
POC (µg C l^{-1})	524		743	
PON (µg N l^{-1})	96		129	
C/N (weight)	5.5		5.8	

Laboratory analysis. Microscopic enumeration of seston samples was done by the Utermöhl technique (Utermöhl 1958). Samples were concentrated by settling 10 to 100 ml of seawater and examined under phase contrast optics (Zeiss Axiovert S 100). With the exception of flagellates <10 µm which were counted in stripes, organisms were enumerated by examining the entire chamber bottom at 200 or 400× magnification. Organisms were classified into different taxonomic categories and size classes (5 to 10, 10 to 20, 20 to 30 and >30 µm, Table 1). The trophic status of ciliates and dinoflagellates was not examined as Lugol's iodine obscures chloroplasts. Protists which could not be identified were grouped in the category 'others'. This resulted in average counts (± SD) of 602 ± 77, 1729 ± 116, 62 ± 24 and 415 ± 119 cells for flagellates, dinoflagellates, diatoms and ciliates, respectively. The average counts for the size classes were 449 ± 59, 1574 ± 88, 174 ± 32 and 699 ± 173 cells for <10, 10 to 20, 20 to 30 and >30 µm, respectively. The cell volume of 30 to 40 cells of each taxonomic and size category was estimated from measurements of cell dimensions

using a digital camera, an image analysis system (Olympus DP12, DP-Soft software) and formulas for appropriate geometric shapes. Biomass was calculated from established carbon to volume relationships for flagellates (Montagnes et al. 1994), dinoflagellates and diatoms (Menden-Deuer & Lessard 2000) and ciliates (Putt & Stoecker 1989). Filtration and ingestion rates of copepods were calculated in accordance with Frost (1972). The analysis of prey preferences of *Acartia clausi* were performed according to Chesson (1978, 1983). The selection index α of Chesson compares the proportion of each food species in the diet to its proportion in the seston (in terms of carbon); Selection is positive when $\alpha > m^{-1}$, where m is the number of food species. In the present study, $\alpha > 0.06$ (17 groups of food items) indicates positive selection.

Fatty acid and C/N analysis. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol (v/v:2/1) and a washing procedure with aqueous KCl solution (0.88%). For quantification of fatty acids, tricosanoic acid was added as an internal standard prior to extraction. An additional centrifugation step was carried out prior to the washing procedure to remove GF/C filter remains. For fatty acid analyses, subsamples of total lipids were hydrolyzed and fatty acids were converted to their methyl ester derivatives (FAMEs) in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h (Kattner & Fricke 1986). After cooling, 2 ml of distilled water were added, and FAMEs were extracted 3 times with 1 ml hexane. Samples were analyzed using a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) operated with a temperature programme and helium as carrier gas. Samples were injected using a programmable temperature vaporizer injector (solvent vent mode). FAMEs and free fatty alcohols were detected by flame ionization and identified by comparing retention times with those derived from standards of known composition. C and N analyses were conducted using a Euro EA (HEKAtech) element analyser.

Statistical analyses. Clearance rates on different taxonomic groups or size classes in each treatment were compared by a Kruskal-Wallis test and nonparametric multiple comparisons by step (Sokal & Rohlf 1995). A 1-way ANOVA was used to test for differences between diets in egg production and hatching, after testing for normality and homogeneity of variances. When applicable, Tukey's HSD test was run for multiple pairwise comparisons. A 2-sample *t*-test was used to compare differences in egg diameter between treatments. A 1-sample *t*-test was used to formally test the significance of positive or negative selection ($\alpha \neq 0.06$, Sokal & Rohlf 1995).

RESULTS

Composition of the seston

The biomass of the micro- and nanoplankton in the natural treatment was dominated by organisms in the size range of 10 to 20 µm and >30 µm with an average of 37.3 ± 4.54 and 116.6 ± 5.32 µg C l^{-1}, respectively, for Day 1 of the experiment (Table 1). The bimodal size distribution largely reflected the taxonomic composition of the plankton. Thecate dinoflagellates <20 µm were responsible for the biomass peak in the 10 to 20 µm size range and contributed an average of 34.7 ± 4.03 µg C l^{-1} or 21.4% to total biomass. In contrast, large ciliates caused the biomass maximum observed in the seston >30 µm and contributed >60% to total biomass. They were dominated by a single oligotrich species, *Laboea strobila* (average size 82 x 58 µm, 48.3 µg C l^{-1}), and various large Strobiliidae (cf. *Strobilidium spiralis*, *Strobilidium* spp., average size 87 µm, 54.7 µg C l^{-1}). The biomass of other groups such as diatoms and flagellates was low.

In the enriched treatment, total protist C was increased by a factor of 2.6 to 417 ± 35.9 µg C l^{-1} in comparison to the natural treatment (162 ± 11.1 µg C l^{-1}, Table 1). Irrespective of the inverse 20 µm filtration, the biomass of the thecate dinoflagellates and that of other groups of the 10 to 20 µm size range was enhanced by a factor of 1.7 to 2.6, similar to the biomass of the larger groups. Therefore, only small differences in the relative taxonomic and size composition occurred between the treatments. Detritus could have clogged the sieve and reduced its effective mesh size allowing only small flagellates to pass through the filter. The similar composition in both treatments is also reflected in the similar C/N weight ratio of 5.5 and 5.8 for the natural and the enriched treatments, respectively (Table 1).

The particulate organic carbon (POC) of 524 and 743 µg l^{-1} in the natural and in the enriched treatments was generally higher than estimates of total protist C by microscopy (Table 1). Detritus, pico- and nanoplankton not included in the microscopic analyses likely accounted for these differences. While the POC and the PON (particulate organic nitrogen) content increased by a factor of 1.4 from natural to enriched treatments, total fatty acid concentration increased from 39.4 to 89.3 µg l^{-1} or a factor of 2.3 (Table 2), similar to the increase in total protist carbon of 2.6. Apparently, protists were enriched in lipids compared to detritus or small pico- and nanoplankton. Differences in the relative composition of fatty acids between treatments were small (Table 2). The 16:0, 20:5(n-3) and 22:6(n-3) were the most important single fatty acids, followed by 14:0 and various 16- and 18-mono- and

Table 2. Fatty acid composition (% total fatty acids) and average total fatty acid concentration of the natural and enriched food suspensions used in feeding experiments (Day 1)

Fatty acid	Natural	Enriched
14:0	6.5	7.9
15:0	2.1	2.2
16:0	21.7	20.7
17:0	0.4	0
18:0	5.3	4.6
14:1(n-5)	1.5	1.4
16:1(n-5)	2.3	2.1
16:1(n-7)	4.1	4.1
16:1(n-9)	2.3	2.3
18:1(n-7)	5.0	3.9
18:1(n-9)	5.8	5.0
20:1(n-9)/18:5(n-3)	2.6	3.3
18:2(n-6)	2.3	2.3
18:3(n-3)	1.0	1.2
18:4(n-3)	3.5	4.9
20:5(n-3)	17.0	18.1
22:5(n-3)	1.0	0.8
22:6(n-3)	15.6	15.1
Saturated	36	36
Monounsaturated	24	22
Polyunsaturated	40	42
Total concentration (µg l^{-1})	39.4	89.3
EPA/DHA ratio	0.9	0.8
(n-3)/(n-6) ratio	16.8	17.3

polyunsaturated fatty acids. The ratio of (n-3) to (n-6) fatty acids and of eicosapentaenoic acid (EPA, 20:5(n-3)) to docosahexaenoic acid (DHA, 22:6(n-3)), which are an important characteristic of food quality, were similar in both treatments and ranged from 0.8 to 0.9 and 16.8 to 17.3, respectively.

Variation of food concentration during incubation

The composition of the seston fed to copepods in both experimental treatments varied only little in the course of the experiment (Fig. 2). Flagellates dominated the composition of the seston numerically throughout the experiment, with a similar abundance in the natural and enriched treatments (295 ± 24 and 331 ± 29 × 10^3 cells l^{-1}, respectively). Ciliates and dinoflagellates dominated the seston biomass with little change over time and also remained consistently enhanced in the enriched treatment throughout the experiments. Biomass averaged 52 ± 3.1 and 119 ± 21.1 µg C l^{-1} for dinoflagellates, and 92 ± 12.3 and 240 ± 33.2 µg C l^{-1} for ciliates in the natural and enriched treatments, respectively. However, in contrast to the relatively stable composition of major groups, a small shift in the composition of large ciliates was observed. While the biomass of *Laboea strobila*

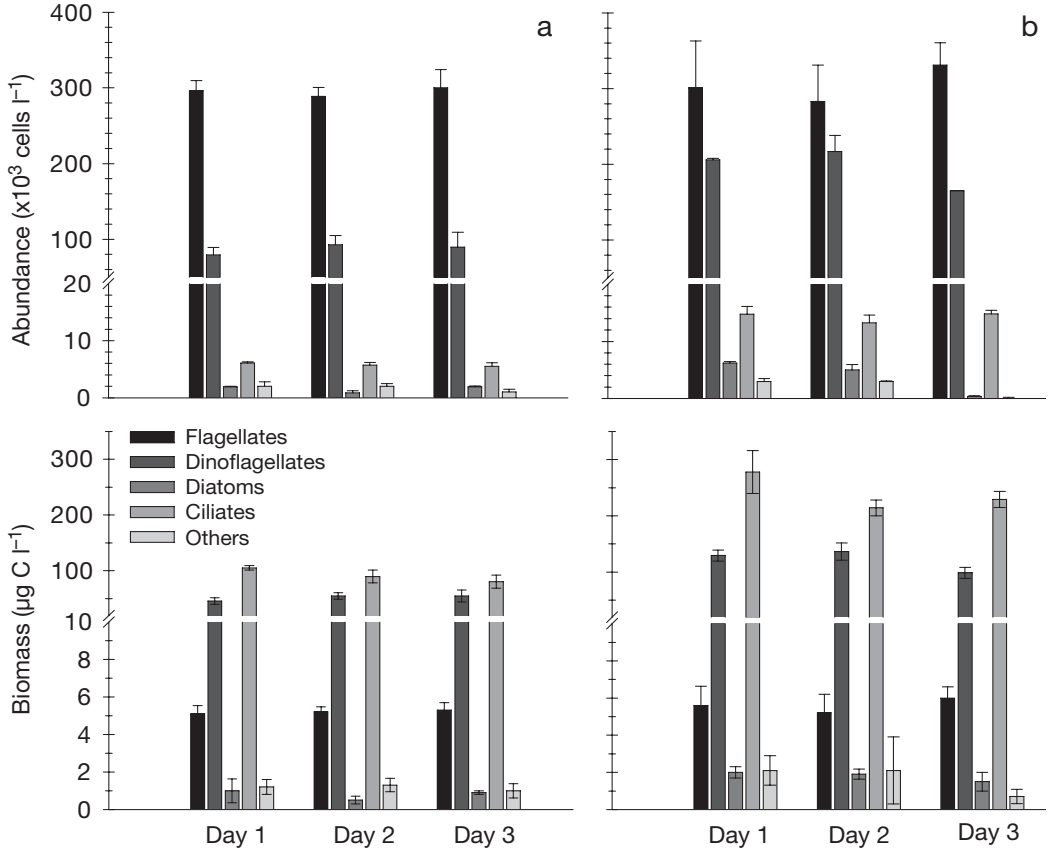

Fig. 2. Temporal variation of protist abundance (cells l^{-1} ± SD) and biomass (µg C l^{-1} ± SD) of main taxonomic groups in (a) natural and (b) enriched food suspensions over the course of the experiment

was constant (natural treatment: 48 ± 2.5 to 55 ± 1.9 µg C l^{-1}) or increased (enriched treatment: 122 ± 10.5 to 163 ± 10.1 µg C l^{-1}), the biomass of the Strobiliidae declined over the days of incubation (natural treatment: 55 ± 5.1 to 24 ± 6.8 µg C l^{-1}; enriched treatment: 153 ± 27.9 to 62 ± 3.4 µg C l^{-1}). Variations within the other groups were small.

C, N and fatty acids in size fractions

In order to break down the bulk measurements for the nutritional value of seston into its different components, the taxonomic composition, fatty acid and mineral content were determined in different size fractions (<20, <48 and <100 µm) on Day 3 of the study (Tables 3 & 4). The composition of the 20 to 48 µm and the 48 to 100 µm size fractions were obtained by subtraction of the respective smaller fraction from the larger one. As shown below, these 2 size fractions reflect the diets of *Acartia clausi* better than the bulk measurements in both treatments.

The fractionation of seawater in the natural treatment resulted in a total protist biomass of 50.8 ± 3.38 µg C l^{-1} in the <20 µm fraction and 142.3 ± 13.46 µg C l^{-1} in the <48 µm fraction (Table 3). Due to the removal of large particles by the 20 µm sieve, the size fractions differed mainly in the biomass of larger protists, while the biomass of flagellates was similar. As a consequence, the biomass of 91.4 µg C l^{-1} calculated for the 20 to 48 µm fraction by subtraction consisted mainly of *Laboea strobila* (59.6 µg C l^{-1}, 63.7% of the biomass) and Strobiliidae (12.5 µg C l^{-1}, 13.4%), but also thecate dinoflagellates <20 µm (12.4 µg C l^{-1}, 13.2%) and athecate dinoflagellates >30 µm (4.5 µg C l^{-1}, 4.8%). This composition largely resembled the diet of females in the natural treatment (see next section). The POC and PON content of the 20 to 48 µm fraction amounted to 72 µg C l^{-1} and 16 µg N l^{-1} (Table 3). The C/N weight ratio of 4.4 indicates that this fraction was enriched in nitrogen compared to the seston averages of 6.7 and 6.1 for the <20 and <48 µm fractions. Relating the POC and PON concentration to the average total protist volume of 479.5×10^6 µm^3 (data not shown)

Table 3. Taxonomic and size composition, particulate organic carbon (POC) and nitrogen (PON), and C/N ratio of size fractionated food suspensions from the natural and enriched treatments and of the calculated differences between size fractions (20 to 48 µm, 48 to 100 µm) on Day 3 of the study

	Natural treatment				Enriched treatment			
	<20 µm (µg C l^{-1})	<48 µm (µg C l^{-1})	20–48 µm (µg C l^{-1})	%	<48 µm (µg C l^{-1})	<100 µm (µg C l^{-1})	48–100 µm (µg C l^{-1})	%
Flagellates								
<10 µm	5.1 ± 0.03	4.7 ± 0.07	−0.4	–	5.0 ± 0.35	5.2 ± 0.57	0.2	0.2
<20 µm	0.8 ± 0.06	1.1 ± 0.09	0.3	0.3	0.7 ± 0.04	0.7 ± 0.02	0.0	–
<30 µm	0.1 ± 0.06	0.1 ± 0.07	0.0	–	0.1 ± 0.01	0.2 ± 0.03	0.1	0.1
Dinoflagellates								
Athecate < 20 µm	1.6 ± 0.37	3.1 ± 0.32	1.5	1.6	3.1 ± 0.09	3.5 ± 0.16	0.4	0.3
Thecate < 20 µm	24.2 ± 3.42	36.6 ± 4.52	12.4	13.2	66.0 ± 7.44	68.1 ± 8.77	2.1	1.8
Athecate < 30 µm	1.0 ± 0.09	0.8 ± 0.07	−0.2	–	1.8 ± 0.09	1.3 ± 0.04	−0.6	–
Thecate < 30 µm	1.7 ± 0.15	3.3 ± 0.82	1.5	1.6	4.0 ± 1.01	2.4 ± 0.55	−1.6	–
Athecate > 30 µm	5.7 ± 0.53	10.2 ± 1.81	4.5	4.8	14.9 ± 2.09	15.6 ± 1.87	0.8	0.7
Thecate > 30 µm	0.8 ± 0.44	1.7 ± 0.07	0.8	0.9	6.6 ± 0.15	4.9 ± 0.17	−1.7	–
Diatoms								
<20 µm	0.0 ± 0.02	0.1 ± 0.08	0.1	0.1	0.1 ± 0.05	0.0 ± 0.07	−0.1	–
>30 µm	–	0.0 ± 0.03	0.0	–	0.8 ± 0.22	1.5 ± 0.54	0.7	0.6
Ciliates								
<20 µm	0.2 ± 0.07	0.2 ± 0.05	0.0	–	0.6 ± 0.08	0.2 ± 0.03	−0.4	–
<30 µm	0.9 ± 0.12	0.5 ± 0.09	−0.4	–	1.2 ± 0.02	1.0 ± 0.13	−0.2	–
Laboea strobila > 30 µm	–	59.6 ± 6.37	59.6	63.7	71.0 ± 4.23	163.2 ± 5.56	92.2	77.2
Strobiliidae > 30 µm	4.8 ± 1.96	17.3 ± 1.96	12.5	13.4	39.1 ± 5.49	62.0 ± 0.49	22.8	19.1
Others > 30 µm	1.5 ± 0.29	1.9 ± 0.26	0.4	0.4	2.6 ± 0.37	2.8 ± 0.22	0.2	0.2
Others	2.2 ± 0.54	1.1 ± 0.45	−1.1	–	0.7 ± 0.38	0.6 ± 0.38	−0.1	–
Total	50.8 ± 3.38	142.3 ± 13.46	91.4	100	218.2 ± 9.98	331.1 ± 22.95	114.9	100
POC (µg C l^{-1})	261.2	333.4	72		499.0	645.2	146.2	
PON (µg N l^{-1})	38.3	55.0	16		80.4	110.2	29.8	
C/N	6.7	6.1	4.4		6.2	5.9	4.9	
Specific C (pg µm^{-3})	0.88	0.43	0.15		0.42	0.36	0.24	
Specific N (pg µm^{-3})	0.13	0.07	0.03		0.07	0.06	0.05	

Table 4. Composition of fatty acids (% total fatty acids), average fatty acid concentration (µg l^{-1}), volume-specific fatty acid contents (fg $µm^{-3}$), and EPA/DHA and (n-3)/(n-6) ratios in fractionated food suspensions from the natural and enriched treatments on Day 3 of the study

Fatty acid	Natural			Enriched		
	<20 µm	<48 µm	20–48 µm	<48 µm	<100 µm	48–100 µm
14:0	4.7	5.0	5.2	5.0	7.3	12.2
15:0	1.9	1.7	1.5	1.9	2.2	2.6
16:0	22.4	20.2	18.2	19.5	21.7	22.5
17:0	0.8	0.8	0.8	0	0.9	0.3
18:0	5.3	4.4	3.6	3.7	5.1	6.0
14:1(n-5)	1.5	1.3	1.1	1.4	1.0	1.5
16:1(n-5)	3.3	2.5	1.6	2.4	2.8	1.7
16:1(n-7)	4.8	4.0	3.3	4.5	4.9	3.5
16:1(n-9)	3.5	2.5	1.7	2.2	3.0	2.4
18:1(n-7)	6.8	4.4	2.2	3.8	3.3	4.1
18:1(n-9)	6.2	5.6	5.1	5.7	6.7	4.0
20:1(n-9)/18:5(n-3)	3.4	3.6	3.7	4.9	2.6	1.0
18:2(n-6)	2.6	2.5	2.5	2.4	2.3	2.2
18:3(n-3)	1.5	1.7	2.0	1.9	1.1	0.2
18:4(n-3)	3.8	5.0	6.0	7.0	3.1	1.8
20:5(n-3)	10.3	14.7	18.7	15.2	18.5	22.2
22:5(n-3)	0.5	0.5	0.5	0.8	1.0	0.9
22:6(n-3)	16.3	19.7	22.7	17.8	15.1	11.4
Saturated	35	32	29	30	37	43
Monounsaturated	30	24	19	23	22	20
Polyunsaturated	35	44	52	45	41	39
Total conc. (µg l^{-1})	14.4	30.2	15.8	50.6	71.5	20.9
Volume-specific contents (fg $µm^3$)	48	39	33	43	40	35
EPA/DHA ratio	0.6	0.7	0.8	0.9	1.2	1.9
(n-3)/(n-6) ratio	10.8	16.4	23.3	17.9	17.3	16.3

for the 20 to 48 µm fraction yielded a volume-specific C and N content of 0.15 and 0.03 pg $µm^{-3}$, respectively (Table 3). The fatty acid concentration for the <20 and <48 µm fraction were 14.4 and 30.2 µg l^{-1}, respectively, and thus amount to 15.8 µg l^{-1} for the 20 to 48 µm fraction (Table 4). The volume-specific fatty acid content of the 20 to 48 µm fraction of 33 fg $µm^{-3}$ was lower than the averages of 48 and 39 fg $µm^{-3}$ for the <20 and <48 µm fractions, respectively. However, this fraction was particularly rich in polyunsaturated fatty acids (PUFA, 52% of the total fatty acids) compared to the <20 µm (35%) and <48 µm (44%) fractions. The 22:6(n-3) and the 20:5(n-3) PUFAs were particularly enriched in the 20 to 48 µm fraction, while the contribution of 16:0 and 18:1 declined compared to the <20 and <48 µm fractions. The (n-3)/(n-6) and the 20:5/22:6 ratios were 23.3 and 0.8, respectively, and considerably higher than in the <20 and <48 µm fractions.

In the enriched treatment, the biomass of the <48 and <100 µm fractions was 218.2 ± 9.98 and 331.1 ± 22.95 µg C l^{-1}, respectively (Table 3). Both size fractions showed a similar biomass for flagellates, dinoflagellates, diatoms and small ciliates, but differed strongly in the biomass of large ciliates. Hence, the difference of 114.9 µg C l^{-1} between them (the 48 to 100 µm fraction) consisted of *Laboea strobila* (92.2 µg C l^{-1}, 77.2% of the biomass in this fraction), Strobiliidae (22.8 µg C l^{-1}, 19.1%) and very few dinoflagellates (combined 3.3 µg C l^{-1}, 2.5%). Again, this composition resembled the diet of females in the enriched treatment (see next section). The differences in the mineral and biochemical content of the size fractions can, therefore, largely be attributed to ciliates. The average POC and PON contents were 499.0 and 645.2 µg C l^{-1}, and 80.4 and 110.2 µg N l^{-1} for the <48 and <100 µm fractions, respectively. This yielded 146.2 µg C l^{-1}, 29.8 µg N l^{-1} and a C/N weight ratio of 4.9 for the 48 to 100 µm fraction. Based on a protist volume of 603.5×10^6 $µm^3$ for this fraction, the volume-specific C and N contents were 0.24 and 0.05 pg $µm^{-3}$, respectively. The difference in the fatty acid content between the <48 and <100 µm fraction was 20.9 µg l^{-1} and the calculated volume-specific fatty acid content for the 48 to 100 µm fraction amounted to 35 fg $µm^{-3}$ (Table 4). The composition of the fatty acids in this fraction differed strongly from the bulk composition of all other size fractions. PUFAs contributed only 39% to the total fatty acids while the contribution of saturated fatty acids was enhanced to 43%. Among PUFAs, 20:5(n-3) was enriched (22%), but the contribution of the 18:3(n-3), 18:4(n-3) and 22:6(n-3), in contrast, was lower than in the <48 and <100 µm fractions. The (n-3)/(n-6) and the 20:5/22:6 ratios were 16.3 and 1.9, respectively.

Grazing and egg production of *Acartia clausi*

The composition of the seston in controls showed only small changes during the incubation (Fig. 3). In the natural treatment, the biomass of flagellates and ciliates decreased from an average of 5.1 ± 0.5 to 3.7 ± 0.3 µg C l^{-1}, and 105.4 ± 5.3 to 96.9 ± 12.9 µg C l^{-1}, respectively, while no change was observed in the other groups. In the enriched treatment, the biomass of ciliates decreased from an average of 276.5 ± 23.9 to 236.2 ± 12.6 µg C l^{-1}. Again, no major change was

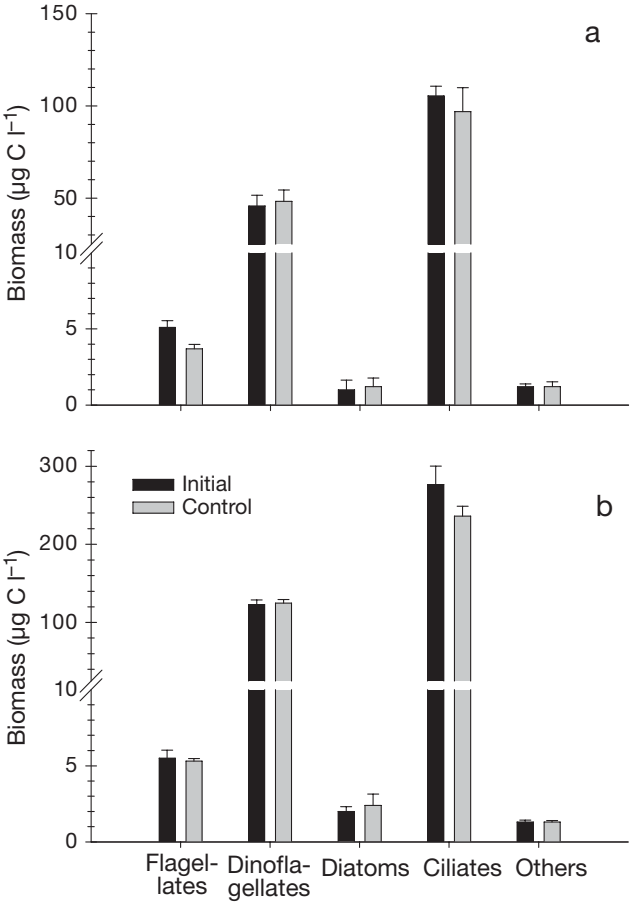

Fig. 3. Biomass of main taxonomic groups (µg C l^{-1} ± SD) in the initials and controls of (a) natural and (b) enriched food suspensions in the grazing experiments

observed in the other groups. Thus, realistic clearance rates were obtained in both treatments.

Total clearance rates of females were 0.77 ± 0.17 and 0.55 ± 0.10 ml female^{-1} h^{-1} for the natural and the enriched treatments, respectively (Fig. 4). Clearance was related to prey size in both treatments, but no clear relationship to prey biomass was observed. While negative clearance was recorded on cells of 5 to 10 µm, average clearance rates on cells of 10 to 20 µm size (0.3 and 0.1 ml female^{-1} h^{-1} for the natural and the enriched treatments) were significantly lower than those on cells of 20 to 30 µm (1.5 and 0.4 ml female^{-1} h^{-1}) and >30 µm (0.6 and 0.7 ml female^{-1} h^{-1}) within each treatment (Kruskal-Wallis test, $p < 0.001$). Furthermore, clearance rates on major size and prey groups tended to be lower in the enriched treatment than in the natural treatment, particularly for diatoms and protists of 20 to 30 µm size (Fig. 4). Clearance rates on the major prey groups closely followed those of the size classes in which they were dominating; e.g. Rates on flagellates, dinoflagellates, diatoms and ciliates resembled those of the 5 to 10, 10 to 20, 20 to 30 and >30 µm size classes, respectively (Fig. 4). Thus, comparably high, but quite variable clearance rates (0.5 to 1.9 ml female^{-1} h^{-1}) were recorded on diatoms and ciliates while those on dinoflagellates and other protists tended to be lower (0 to 0.4 ml female^{-1} h^{-1}), which was significant for diatoms in the natural treatment and for ciliates in both the natural and the enriched treatments (Kruskal-Wallis test, $p < 0.001$). However, clearance rates on single prey items were very heterogeneous with regard to their size, taxonomic group or biomass (Fig. 5). For instance, athecate dinoflagellates >30 µm were cleared at a lower rate than the smaller thecate dinoflagellates <30 µm although available at a similar biomass. On the other hand, they were cleared at a similar rate than thecate dinoflagellates <20 µm or Strobiliidae >30 µm although available at a much lower biomass. Also, clearance rates on *Laboea strobila* were considerably higher than on thecate dinoflagellates <20 µm or Strobiliidae >30 µm which had a similar or higher biomass.

Females ingested an average of 2.87 ± 0.36 µg C female^{-1} d^{-1} in the natural treatment, equivalent to a daily ration of 55%. Ciliates were the most important single dietary group (Fig. 6). *Laboea strobila* (prey category 14, 1.65 ± 0.54 µg C female^{-1} d^{-1}) and diverse Strobiliidae (prey category 15, 0.82 ± 0.23 µg C female^{-1} d^{-1}) together contributed more than 86% to the diet (Figs. 6 & 7). Apart from the large ciliates, small thecate dinoflagellates (10 to 20 µm) also con-

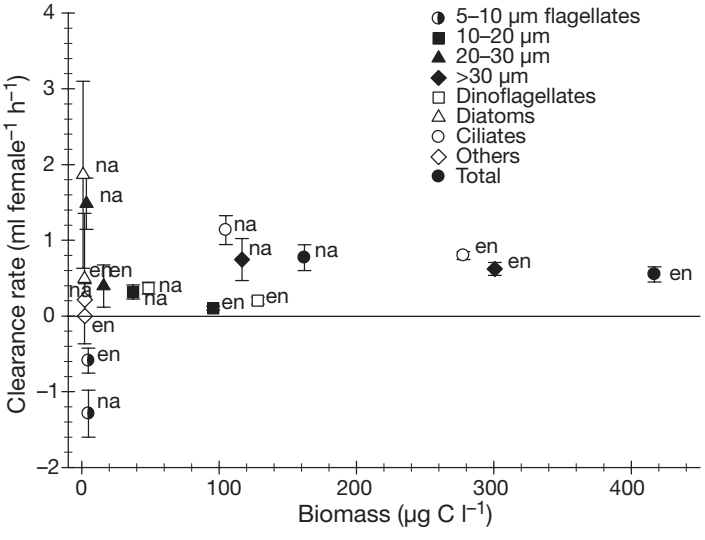

Fig. 4. *Acartia clausi.* Clearance rates (ml female^{-1} h^{-1} ± SD) vs. prey biomass for different prey size classes (filled symbols) or prey categories (open symbols) in natural (na) or in enriched (en) food suspensions

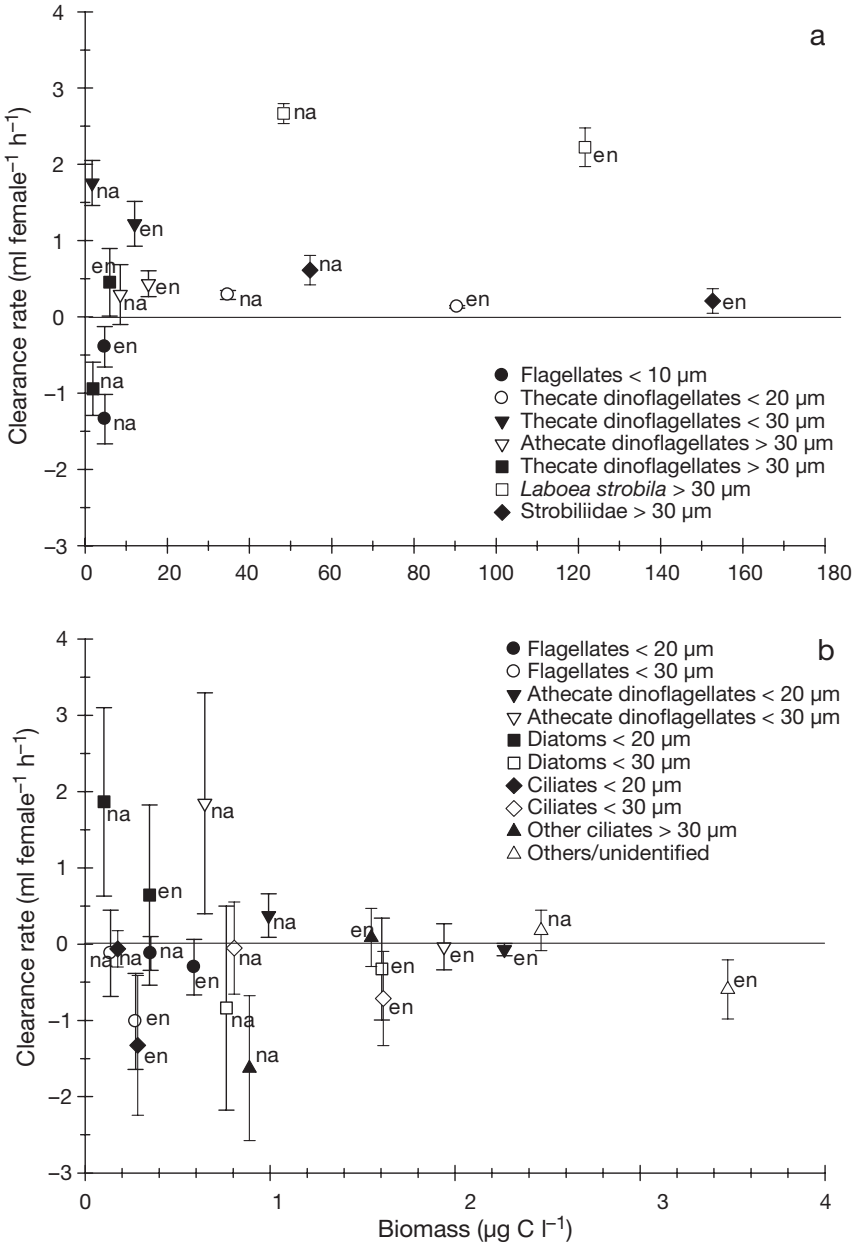

Fig. 5. *Acartia clausi*. Clearance rates (ml female^{-1} h^{-1} ± SD) vs. biomass of protist prey in the natural (na) and in the enriched (en) treatments with a biomass (a) >4 µg C l^{-1} and (b) <4 µg C l^{-1}

tributed substantially to the diet (prey category 5, 0.23 ± 0.05 µg C female^{-1} d^{-1}), while other dinoflagellates and other groups combined were of minor importance (0.16 ± 0.06 µg C female^{-1} d^{-1}). *Laboea strobila* and thecate dinoflagellates of 20 to 30 µm size (prey category 7) were significantly positively selected food items (1-sample *t*-test, $p < 0.01$ and $p < 0.05$, respectively), but not the small thecate dinoflagellates <20 µm nor the Strobiliidae (Fig. 8). In the enriched treatment, females ingested an average of 4.81 ± 0.71 µg C female^{-1} d^{-1}, which is equivalent to a daily ration of 92%. Similar to the natural treatment, large ciliates dominated the diet (Fig. 6). *Laboea strobila* and Strobiliidae were ingested at rates of 3.59 ± 0.29 and 0.74 ± 0.21 µg C female^{-1} d^{-1}, respectively, and together accounted for more than 90% of the diet (Fig. 7). The ingestion of dinoflagellates combined amounted to 0.46 ± 0.07 µg C female^{-1} d^{-1}, with various athecate and thecate dinoflagellates (prey categories 5, 7 to 9) contributing about equally to the ingestion rate (0.05 to 0.14 µg C female^{-1} d^{-1}). *Laboea strobila* was the only food item which was positively selected in the enriched treatment. (1-sample *t*-test, $p < 0.01$) (Fig. 8). Dinoflagellates >20 µm, ciliates >20 µm and diatoms <30 µm were ingested in proportion to their availability, while all other groups were selected against (1-sample *t*-test, $p < 0.05$ and $p < 0.01$).

Egg production of females collected from the field displayed little variation over the 3 consecutive days of sampling and ranged from an average of 25 ± 3.9 to 27 ± 4.5 eggs female^{-1} d^{-1} (Fig. 9). The hatching success of eggs was invariably high and ranged from 77 ± 11.4 to 85 ± 2.8%. Egg production and hatching success in the natural treatment were 25 ± 1.1 eggs female^{-1} d^{-1} and 86 ± 2.9%, respectively, and not significantly different from the *in-situ* rates (1-way ANOVA; $p > 0.05$). In contrast, egg production in the enriched treatment increased significantly to 43 ± 3.6 eggs female^{-1} d^{-1} (1-way ANOVA $F_2 = 57.1$, $p < 0.001$, Tukey HSD $p < 0.05$), while hatching rates remained high (83 ± 11.4%). The egg diameter was similar in both treatments ($t_s = 1.1$, $p > 0.5$); The C-specific egg production was therefore calculated from the overall average diameter of 81 ± 2.1 µm. The specific ingestion and egg production rates had averages of 0.55 ± 0.07 and 0.21 ± 0.012 d^{-1}, and 0.92 ± 0.141 and 0.32 ± 0.029 d^{-1} for the natural and the enriched treatments, respectively (Table 5). The calculated efficiency of egg production (EPE) of 0.39 and 0.35 did not differ between the natural and the enriched treatments (Table 5).

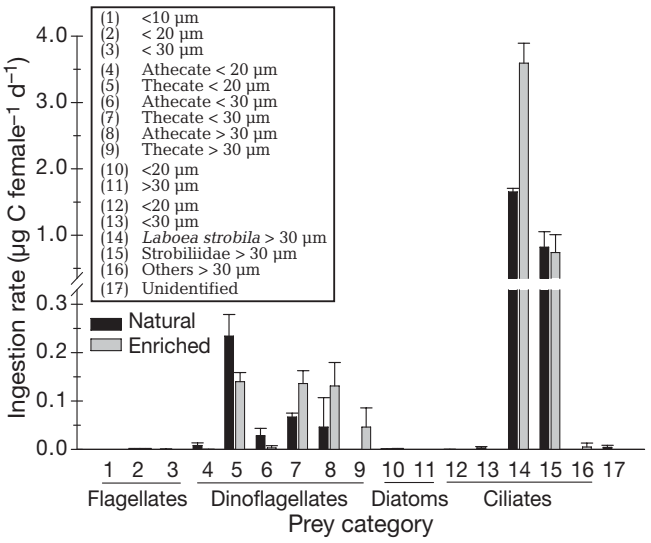

Fig. 6. *Acartia clausi*. Ingestion rates (µg C female^{-1} d^{-1} ± SD) on different prey categories in the natural and enriched treatments

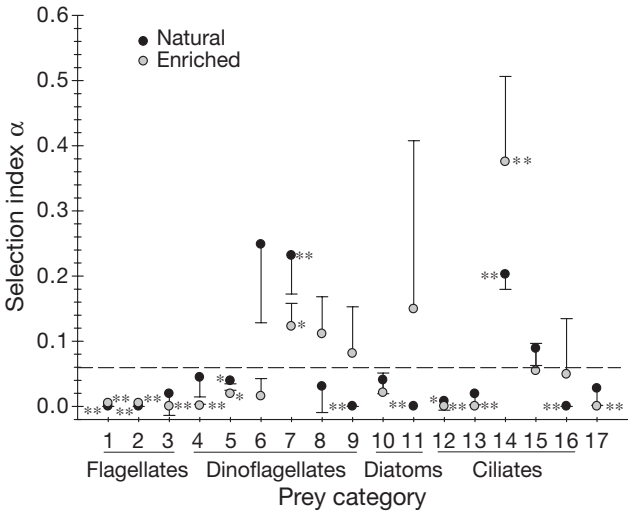

Fig. 8. Selectivity indices α calculated for prey in the natural and in the enriched treatments. Numbers indicate prey categories as denoted in Fig. 6; *: significant selection for prey items (*t*-test): *p < 0.05, **p < 0.01. Dashed line indicates α = 1/(number of prey categories) = 0.06, at which feeding is non-selective. Error bars are SD

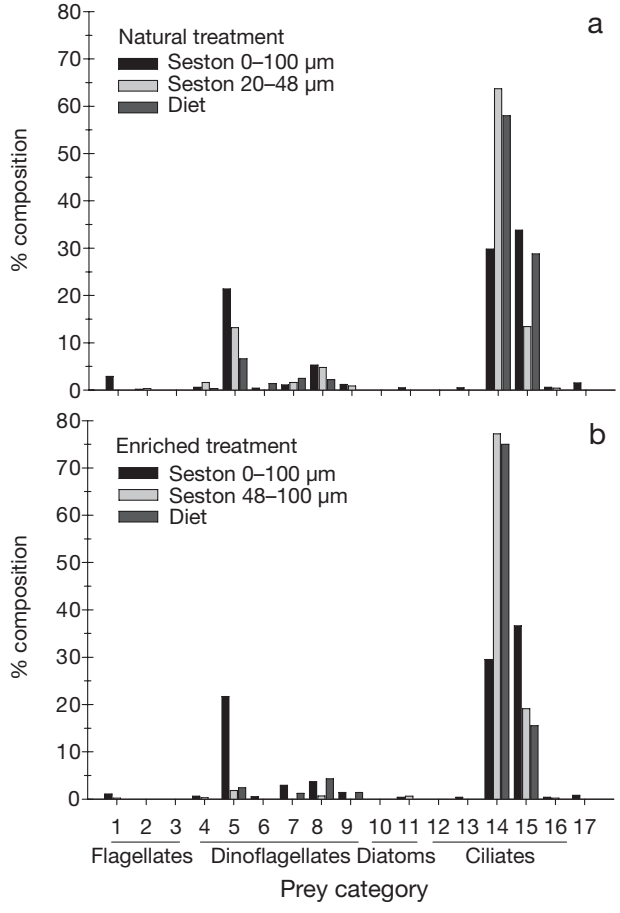

Fig. 7. Average composition (%) of (a) bulk seston (0 to 100 µm), the size fraction 20 to 48 µm and the diet of female *Acartia clausi* in the natural treatment, and (b) bulk seston (0 to 100 µm), the size fraction 48 to 100 µm and the diet of females in the enriched treatment. Numbers indicate prey categories as denoted in Fig. 6

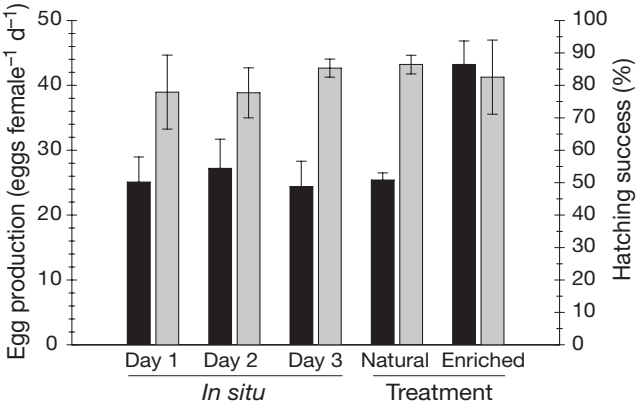

Fig. 9. *Acartia clausi*. Egg production (eggs female^{-1} d^{-1} ± SD; black bars) and hatching success of eggs (% ±SD; grey bars) during the 3 consecutive days in the field and after 4 d of incubation in the natural and enriched treatments

DISCUSSION

During our investigation which was conducted after the spring phytoplankton bloom, large ciliates were the dominant component of the seston biomass and the preferred food source for *Acartia clausi*. This dominance together with an excellent nutritional quality of the diet composed of ciliates, particularly *Laboea strobila* and to a minor degree, of dinoflagellates, enabled a high reproductive success of females. This suggests that the spring recruitment of *Acartia clausi* might be partly related to the peak abundance of large hetero- and mixotrophic ciliates occurring subsequent to the spring diatom bloom.

Table 5. *Acartia clausi*. Mean (±SD) carbon-specific ingestion (IR, µg C µg C^{-1} d^{-1}) and egg production rates (EPR, µg C µg C^{-1} d^{-1}) of females and the efficiency of egg production (EPE) calculated for the natural and enriched treatments and for the difference between both treatments (enriched minus natural)

	Natural	Enriched	Difference
IR	0.55 ± 0.070	0.92 ± 0.141	0.37
Flagellates	–	–	–
Dinoflagellates	0.07 ± 0.018	0.09 ± 0.014	0.01
Diatoms	<0.001	<0.001	<0.001
Ciliates	0.48 ± 0.018	0.83 ± 0.128	0.36
Others	<0.001	<0.001	<0.001
EPR	0.21 ± 0.012	0.32 ± 0.029	0.11
EPE (± range)	0.39 ± (0.32 – 0.44)	0.35 ± (0.26 – 0.40)	0.30

Methodology

Ciliates are very fragile organisms sensitive to handling and fixation (Gifford 1985, Stoecker et al. 1994). The manipulation of water samples by size fractionation and inverse filtration in our study, therefore, bears the risk of severe underestimation of ciliate abundance and consumption by copepods. Strongly reduced cell concentrations with losses exceeding 70% of the initial number have been recorded for sieved oligotrich ciliates, whereas the abundance of tintinnids was found to be unaffected (Gifford 1985). Tiselius (1989), in contrast, did not observe a pronounced effect of sieving on seston composition, but reported some severe mortality of ciliates of over 80% during subsequent incubation. Since no initial samples from the collecting bottles without any additional handling were taken in our study, the reported abundance and biomass of ciliates should be regarded as conservative. However, the increase of total ciliate abundance by a factor of 2.6 (range for different ciliate size classes: 1.7 to 2.7) in the enriched treatment compared to the natural treatment compares well with the similar increase in the abundance of total dinoflagellates by a factor of 2.6 (range: 1.9 to 5.9), which are expected to be more robust. This suggests that the inverse filtration did not exert a strong detrimental effect on the abundance of ciliates, which might be related to the dominance of large species in the seston.

Although Lugol's acidic solution has been considered as one of the least detrimental preservatives for estimates of ciliate abundance (Leakey et al. 1994, Stoecker et al. 1994), losses due to fixation can range from 0 to 30% (Vincent & Hartman 2001, Broglio et al. 2003, 2004) and cause an additional underestimate of ciliate consumption by copepods. Since losses are species-specific, and no estimates of the effect of Lugol's solution on the cell integrity of large *Laboea strobila* and various Strobiliidae yet exist, we did not correct ciliate counts for potential fixation artifacts. Applying a maximal loss factor of 30%, average total ingestion rates would rise from 2.9 to 3.6 µg C $female^{-1}$ d^{-1} and from 4.8 to 6.1 µg C $female^{-1}$ d^{-1} for the natural and the enriched treatments, respectively. As a consequence, the efficiency of egg production (EPE) would decrease from 0.39 to 0.31 and from 0.35 to 0.27. However, the correction would only slightly change the overall dominance of large ciliates in the seston and their high contribution to the diet of *Acartia clausi* with a strong preference for *Laboea strobila*. The absolute values for volume-specific mineral and biochemical contents of the size fractions should be regarded as potentially overestimated, but again the conclusions on the biochemical composition of the size fractions is not affected by the lack of a correction for ciliate biomass.

As outlined by Nejstgaard et al. (1997, 2001), under certain conditions, food rations of copepods can also be severely underestimated in grazing experiments that involve more than 2 trophic levels. Due to carnivorous feeding by copepods and the subsequent relaxation of ciliate grazing pressure, any direct ingestion of small cells by copepods is masked by enhanced growth in experimental bottles relative to controls, and needs to be corrected. Nejstgaard et al. (2001) suggested adjusting the grazing coefficient for copepods by applying a correction factor (k_p) derived from microzooplankton abundance and grazing coefficients, which are obtained in dilution experiments. Due to the lack of such simultaneous experiments, published maximum C-specific grazing coefficients for heterotrophic ciliates and dinoflagellates were used to correct copepod clearance rates. This, however, lead to unrealistically high copepod feeding rates on flagellates of 4.5 to 6.4 µg C $female^{-1}$ d^{-1}, which considerably exceeded feeding rates on the much more abundant ciliates. Such severe overestimates may not only result from the application of maximum rates from the literature, but also from the inclusion of mixotrophic ciliates such as *Laboea strobila* in the potential grazer biomass, which were dominant microzooplankton and potentially displayed much lower feeding rates. This is supported by only small differences in the abundances of flagellates in control bottles between the natural and the enriched treatment, despite the large increase in ciliate biomass in the latter. Furthermore, *Acartia* spp. are known to switch their feeding mode from suspension to ambush feeding in response to a high availability of motile food (Kiørboe et al. 1996, Gismervik & Andersen 1997). Due to the dominance of large ciliates in our study, suspension feeding and, thus, high clearance rates of females on flagellates are rather unlikely. Because of the ab-

sence of evidence for a substantial grazing control of flagellates by microzooplankton and the observed preference of *A. clausi* for particles larger than 20 µm (see below), clearance rates were not corrected.

Prey selection patterns and daily ration

Clearance rates of 0.5 to 3 ml female^{-1} h^{-1} for *Acartia clausi* feeding on various prey items are within the range observed for this species or its congeners in various natural ecosystems (Gifford & Dagg 1991, Pérez et al. 1997, Rollwagen-Bollens & Penry 2003). Higher clearance of ciliates in relation to co-occurring phytoplankton has been commonly reported in field studies and has often been attributed to motility, size and nutritional quality of microheterotrophs (e.g. Gifford & Dagg 1991, White & Roman 1992, Ohman & Runge 1994, Nejstgaard et al. 1997, Vincent & Hartman 2001, Broglio et al. 2004). In contrast, a higher clearance of small flagellates compared to larger protists has sometimes been observed and related to their high abundance and copepod prey preferences (e.g. Rollwagen-Bollens & Penry 2003, Vargas & Gonzáles 2004).

Our results do not support a generally higher clearance for ciliates. In both treatments, ciliates were cleared at a similar rate to diatoms despite large differences in the biomass of both groups (Fig. 4). In contrast, dinoflagellates were cleared at a lower rate, although their availability was in the range of the biomass of ciliates. Because diatoms and ciliates dominated in the size range of 20 to 30 and >30 µm, but dinoflagellates dominated in the 10 to 20 µm range, which was also cleared at a significantly lower rate than the larger size classes, the observed differences were presumably size-based. The reduced clearance of cells <20 µm agrees well with results for *Acartia* spp. from other field studies (Rollwagen-Bollens & Penry 2003, Katechakis et al. 2004) and size-dependent clearance of phytoplankton observed in the laboratory (Nival & Nival 1976, Berggreen et al. 1988). Also, other studies indicate that large phytoplankton is cleared at the same rate as ciliates (Tiselius 1989, Ohman & Runge 1994, Vargas & Gonzáles 2004). This suggests that the often observed preference for ciliates might in fact reflect a size bias caused by the comparison of clearance rates of generally large microzooplankton with those of small phytoplankton or of chlorophyll *a*, which integrates over a wide range of particle sizes.

The more detailed taxonomic analysis of feeding by *Acartia clausi*, however, emphasized that conclusions regarding the clearance of taxonomic or size groups should be drawn with caution because highly different clearance rates and preferences for diverse prey are in fact integrated (see Fig. 5). Among the dinoflagellates, thecate dinoflagellates <30 µm were cleared at high rates and positively selected by females, whereas various other groups including the small, but abundant thecate dinoflagellates <20 µm were cleared at much lower rates. Similarly, the clearance rate for ciliates combined the strong preference for *Laboea strobila* with the apparent reluctance to feed on Strobiliidae and other ciliates. The diverse clearance rates of *A. clausi* for various particles indicates that the feeding response is complex rather than dependent on a single but still important factor such as prey size or abundance. Apart from motility and escape behaviour in ciliates (Broglio et al. 2001, Jakobsen et al. 2005), feeding mode (Jonsson & Tiselius 1990), morphological cell features or distaste for specific algae (Wolfe 2000) may be responsible for shaping the feeding behaviour on various other groups.

In both treatments, ciliates were major food items and dominated the composition of the diet by more than 86%, while other food particles contributed only a small fraction (Table 5). These values agree well with a high proportion of ciliates in copepod diets of more than 60% when they dominate the potential prey (Broglio et al. 2004, Calbet & Saiz 2005). However, the daily ration obtained from feeding on ciliates is often low and rarely exceeds 20 to 30% of body weight (Calbet & Saiz 2005). This obvious contrast shows that ciliates regularly dominate the diet under oligotrophic conditions when food availability is low or large parts of the phytoplankton production are unavailable for copepod consumption due to size constraints (Gifford & Dagg 1991, Fessenden & Cowles 1994). As a consequence, the ingested ration is often small because of low ciliate biomass. The daily consumption of *Acartia clausi* of 2.87 to 4.81 µg C female^{-1} d^{-1}, which was equivalent to 55–92% of body C, associated with the high dietary contribution of ciliates deviates considerably from the generally low rations obtained by copepods feeding under oligotrophic and post-bloom conditions, but falls well within the range of total rations recorded for this species (Pagano et al. 2003, Katechakis et al. 2004). This high daily ration likely resulted from the high ciliate biomass and the dominance of large species recorded in our study. Although an abundance of 4 to 6 cells ml^{-1} fits well with the range of 1 to 15 cells ml^{-1} observed for ciliates in various field studies, a biomass of more than 100 µg C l^{-1} appears exceptionally high (compare to Calbet & Saiz 2005).

Our attempt to study the feeding and egg production of *Acartia clausi* in response to the manipulation of the seston size structure failed regarding the intended selective increase of the biomass of larger protists by inverse fractionation. Instead, the concentration of all organism groups except flagellates, was similarly enhanced (Table 1). Nevertheless, the increase in the

specific ingestion rates of females of 0.37 µg C µg C^{-1} d^{-1} in the enriched treatment compared to the natural treatment can be largely attributed to feeding on large ciliates which amounted to 0.36 µg C µg C^{-1} d^{-1} (Table 5). This likely reflects the strong preference of *Acartia clausi* for *Laboea strobila* associated with the dominance of this species and other large ciliates in the seston. The consumption of dinoflagellates, in contrast, remained similar in the 2 treatments although small changes in contribution of the various groups to the diet were observed. A decrease in the ingestion of small thecate dinoflagellates of 10 to 20 µm was compensated by an increase in the clearance and ingestion of dinoflagellates >30 µm (Figs. 5 & 6). This might reflect an increase in the time females spent on ambush feeding in response to the increased availability of larger prey.

Reproductive success, nutritional quality of diet and ciliates

The egg production and egg hatching success of *Acartia clausi* were high during our study. The observed rates of 25 eggs $female^{-1}$ d^{-1} are close to the maximum egg production of 25 to 33 eggs $female^{-1}$ d^{-1} recorded for the North Sea during late spring (Halsband & Hirche 2001) and indicate that females met with favourable conditions for high reproductive success. This is supported by the high gross efficiency of egg production of 39% for the natural treatment which is in the upper range reported for various copepods (Kiørboe et al. 1985, Straile 1997).

Estimates of the biochemical and mineral content of the bulk seston generally corroborate a high quality of the potential prey ingested by females. The low C/N ratio of <6 and the composition of fatty acids with a high proportion of C_{18}- and C_{22}-PUFAs are typical for the post-spring bloom conditions composed of flagellates, dinoflagellates and microzooplankton (Mayzaud et al. 1989, Pond et al. 1996). However, such bulk estimates inadequately characterize the diet of copepods when the dietary composition deviates from that of seston due to selective feeding. For instance, detritus and small flagellates not consumed by *Acartia clausi* were generally included in our biochemical analysis of total seston. In this respect, the fractionation of the seston in size fractions of <20, 20 to 48 and 48 to 100 µm eliminated some of these under-utilized food sources and thus appeared more suitable in characterizing the nutritional quality of the ingested prey in general and of the preferred ciliates in particular.

The 20 to 48 µm size fraction described the diet composition of females in the natural diet better than the bulk seston composition (Fig. 7). In addition to the elimination of flagellates and presumably a high amount of detritus, the contribution of small thecate dinoflagellates was reduced in favour of a higher contribution of ciliates compared to the bulk seston, similar to the diet of *Acartia clausi*. *Laboea strobila,* Strobiliidae and dinoflagellates contributed 58, 29 and 14% to the diet and 63, 13 and 21% to the 20 to 48 µm size fraction, but contributed 30, 34 and 30% to the seston biomass. The mineral and biochemical characterization of seston revealed that the composition the 20 to 48 µm size fraction and, thus, of the diet differed from bulk seston. The C/N ratio of 4.4 indicated enrichment in N in comparison to the seston average of 5.5. The diet also included a disproportionately high amount of essential long-chain PUFA of 52% compared to the 40% of the total fatty acids in bulk seston. It was particularly rich in C_{18}-PUFA, EPA, DHA and (n-3) fatty acids, which are favourable for high egg production and hatching success and cannot be synthesized de novo (Jónasdóttir et al. 1995, Broglio et al. 2003, Shin et al. 2003). Apparently, larger protists are particularly important sources of essential fatty acids in the nutrition of copepods.

The 48 to 100 µm size fraction in the enriched treatment corresponded to the diet of *Acartia clausi* in this treatment (Fig. 7). While *Laboea strobila*, Strobiliidae and dinoflagellates respectively contributed 75 to 77, 16 to 19 and <5% to the size fraction and the diet, their share of about 30, 37 and 22% in total seston was considerably different. Moreover, due to the dominance of *Laboea strobila* and Strobiliidae of more than 97% of total protists, the measurements for the mineral and biochemical content of the 48 to 100 µm fraction characterize not only the diet of females in the enriched treatment, but can also be used to address the nutritional composition of large ciliates. These measurements showed that the volume-specific C and N contents of protists in the 48 to 100 µm size fraction of 0.24 and 0.05 pg $µm^{-3}$ were higher than those for the protists in the 20 to 48 µm size range of 0.15 and 0.03 pg $µm^{-3}$. On the other hand, protists in the 48 to 100 µm size fraction contained considerably less PUFA (39%) than protists in the 20 to 48 µm size range (52%) at a similar volume-specific fatty acid content of 33 to 35 fg $µm^{-3}$. This indicates that ciliates apparently provided a disproportionately high amount of N to females, while the dinoflagellates, which were more abundant in the 20 to 48 µm fraction, were the more important source for essential fatty acids. Hence, the biochemical content of ciliates does not always appear to be superior compared to other prey, as has been concluded in the literature (e.g. Stoecker & Capuzzo 1990, Sanders & Wickham 1993, but see Broglio et al. 2003).

The biochemical measurements also revealed substantial compositional differences in fatty acids

between the bulk seston and the ciliates dominating the 48 to 100 µm fraction. The ciliates were characterized by a higher proportion of 14:0, 16:0, 18:1(n-7) and EPA, but a lower proportion of particularly polyunsaturated fatty acids such as 18:3(n-3), 18:4(n-3) and DHA. This further suggests that dinoflagellates were in fact the major source of these important C_{18}-PUFAs and DHA, which agrees well with measurements of their composition in the laboratory (Broglio et al. 2003, Dalsgaard et al. 2003, Tang & Taal 2005). In contrast, comparatively little is known of the composition of marine ciliates. The dominance of EPA and the lack of major quantities of C_{18}-PUFAs in *Laboea strobila* and the Strobiliidae contrast with the high proportions of C_{18}-PUFA recently determined for *Mesodinium pulex* or *Strombidium sulcatum* (Broglio et al. 2003, Klein Breteler et al. 2004), but agree well with composition of a natural assemblage dominated by tintinnids in the Mediterranean (Claustre et al. 1988/89). Arendt et al. (2005) also observed higher levels of EPA following the spring bloom, which coincided with the occurrence of *L. strobila* (S. Jónasdóttir pers. comm.). The fatty acid composition of ciliates, however, can be quite variable and is largely determined by their food because of an apparent inability to modify dietary sources (Broglio et al. 2003, Klein Breteler et al. 2004). In this respect, the origin of EPA in ciliates is interesting. Trophic marker studies identified diatoms or Eustigmatophyceae as major pelagic sources of this fatty acid (Dalsgaard et al. 2003). However, these were rare during our investigation. Whether the specific fatty acid composition of the ciliates reflects a long-term feeding history on specific phytoplankton or alternatively, a potentially mixotrophic physiology known for the dominant oligotrich *Laboea strobila* remains to be elucidated.

CONCLUSIONS

Our evidence for high egg production of *Acartia clausi* supported largely by oligotrich ciliates contrasts considerably with the general idea of a minor role for ciliates in egg production and recruitment of copepods. From their analysis of seasonal variation in egg production of several copepod species in relation to seston composition, Kiørboe & Nielsen (1994) concluded that ciliate biomass is generally too low to contribute significantly to annual egg production. This is corroborated by the fact that the daily ration of copepods feeding on ciliates is generally minor (Calbet & Saiz 2005). In this respect, our results appear to reflect a small-scale event during a biomass peak of ciliates, which is well known to occur irregularly and unpredictably in comparison to phytoplankton blooms (e.g. Smetacek 1981, Nielsen & Kiørboe 1994). However, biomass peaks of large oligotrich ciliates like *Laboea* spp. have been frequently observed following the spring diatom bloom (Smetacek 1981, Nielsen & Kiørboe 1994, Nejstgaard et al. 2001), which suggests that such events are a regular phenomenon in the seasonal succession of the pelagic system. Moreover, in comparison to other small coastal copepod species such as *Temora longicornis* or *Pseudocalanus* spp., the seasonal peak in egg production of *A. clausi* or *A. tonsa* is delayed and occurs in May to June rather than April to May (Kiørboe & Nielsen 1994, Halsband & Hirche 2001). This suggests that spring recruitment of *A. clausi* may be related to the successional occurrence of large ciliates. The strength of this link and the potential correlation of the succession of diatoms and large ciliates as indicated by the specific fatty acid pattern require further investigation. Nevertheless, high daily rations of copepods feeding on microzooplankton appear to be primarily realized in the presence of large ciliates rather than due to an exceptionally high abundance (Gifford & Dagg 1991, Ohman & Runge 1994, Liu et al. 2005) making them particularly important for the recruitment of marine zooplankton populations.

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