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ORIGINAL ARTICLE

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Feeding rates and selectivity among nauplii, copepodites and adult females of *Calanus finmarchicus* and *Calanus helgolandicus*

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Abstract This study focuses on selective feeding by developmental stages of two oceanic copepods, *Calanus finmarchicus* and *Calanus helgolandicus* from nauplii to adults. A mixture of four algal species of different biochemical composition, *Prorocentrum nanum* (dinoflagellate), *Thalassiosira minima* (diatom), *Rhodomonas baltica* (cryptophyte) and *Dunaliella tertiolecta* (chlorophyte), added in an equal biovolume, was used in three different experimental set-ups. In set-up 1 the algal species were present as single cells of similar size (14 µm). In set-up 2 the diatom *T. minima* was present in chains of two or three cells and was therefore larger than the other algae, while the biovolume of all species remained the same. In set-up 3, the diatom *T. minima* was excluded from the mixture. Feeding selectivity of the copepods was assessed in relation to the quality of the algal species expressed in terms of carbon and nitrogen content, fatty acid composition, and chain length of the diatom. The results show that younger stages and adult females of *C. finmarchicus* and *C. helgolandicus* did not show a preference for an algal species when the algae were of similar size. In the feeding experiments where the diatoms were offered as chains, both copepod species showed a selective behaviour only on the basis of algal size. Individual ingestion rates increased from 0.4 to 0.7 µg C day^{-1} for nauplii of both species to 5 µg C day^{-1} for adult females of *C. helgolandicus* to 12 µg C day^{-1} for *C. finmarchicus*. Individual filtration rates ranged from 5 ml day^{-1} for *C. finmarchicus* nauplii to 70–98 ml day^{-1} for adult females, and from 3 ml day^{-1} for *C. helgolandicus* nauplii to 35–46 ml day^{-1} for adult females. Ingestion and filtration rates per unit body carbon decreased gradually in both copepod species with increasing body carbon. The daily ingested amount of food decreased for *C. finmarchicus* from 124–134% of the body carbon for nauplii to 19% of the body carbon for adult females, and for *C. helgolandicus* from 117–137% of the body carbon for nauplii to 13–26% of the body carbon of adult females.

Keywords Selective feeding · Developmental stages · *Calanus finmarchicus* · *Calanus helgolandicus*

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Introduction

In the last few decades it has been clearly demonstrated that copepods can discriminate between different types of food. For example they can selectively ingest large cells over small cells (e.g. Frost 1972), phytoplankton over plastic beads (e.g. Donaghay and Small 1979), motile over non-motile cells (e.g. Atkinson 1995) or phytoplankton over detritus (e.g. Roman 1977). Copepods also show different feeding rates on cells of similar size but of contrasting "quality", for example toxic versus non-toxic species or strains (e.g. Huntley et al. 1983; Turriff et al. 1995) or different growth states of the same species (e.g. Mullin 1963; Cowles et al. 1988).

Despite all these studies, it is still not certain how selective copepods are when faced with the wide range of food types, sizes and quality that occur in the natural environment (Huntley 1981; Turner and Tester 1989). An additional complexity is that their perception and handling of small cells can be fundamentally different to that of large cells. This has been demonstrated for *Eucalanus* (Price et al. 1983). Furthermore, it is known that feeding behaviour differs between species and even between life stages of the same species (e.g. Fernández 1979).

Time series of observations on the feeding behaviour of *C. finmarchicus* and *C. helgolandicus* in the field were carried out in the Norwegian Sea (Meyer-Harms et al. 1999) and off Plymouth (Irigoien et al. 2000a) and yielded somewhat contradictory results. For the Norwegian Sea, Meyer-Harms et al. (1999) concluded that *C. finmarchicus* selected diatoms throughout the study period and additionally dinoflagellates during pre- and post-bloom conditions. For *C. helgolandicus* off Plymouth, Irigoien et al. (2000a) observed a slight preference for diatoms, though there was generally little evidence of selective feeding on different phytoplankton.

Any attempt to produce realistic models including zooplankton feeding requires answers to some basic questions:

- Is a simple size selection behaviour enough to represent feeding or do we need to incorporate much more complex behavioural patterns into models?
- Can the behaviour of the young stages be extrapolated from that of the adults without major compromise to the results?

With the exception of bloom periods, the phytoplankton community is generally dominated by small cells. However, because of the difficulty of working with small cells, most studies on selective feeding behaviour have been oriented towards large algae (Price et al. 1983).

The aim of this study was to characterise the feeding behaviour of *Calanus* spp. during non-bloom periods and to identify whether the characteristics of the adults can generally be applied to the young stages that are usually neglected in behavioural studies. A secondary aspect of this study was to try to determine basic differences in feeding behaviour between *Calanus helgolandicus* and *C. finmarchicus*.

The present study was part of the TASC project which made an integrated trans-Atlantic study of *C. finmarchicus* and provided the opportunity to make some parallel observations on co-occurring populations of *C. helgolandicus* (Harris et al. 2000).

Materials and methods

General experimental set up

A mixture of four algal species at equal concentration and of similar cell size (14 µm) were fed to the copepods. The algal species used were the flagellates *Prorocentrum nanum* (dinoflagellate), *Rhodomonas baltica* (cryptophyte), *Dunaliella tertiolecta* (chlorophyte) and the diatom *Thalassiosira minima*.

In the feeding experiments three different experimental set-ups were used:

1. In the incubation mixture each algal species was present as single cells of similar size.
2. The diatom *T. minima* was present in chains of two or three cells and was therefore larger than the other algae, while the biovolume of all species remained the same.
3. The diatom *T. minima* was excluded from the mixture. This experiment was conducted as a control for set-up 1: if some diatoms were present in chains but not observed by microscopic observation, they may have influenced the feeding behaviour of the copepods.

Algal cultures

Cultures obtained from the culture collections at Plymouth and Oban (see Table 1), were grown in natural sea water enriched with F/2 nutrient levels (Guillard and Ryther 1962) in semi-continuous cultures at 10°C and 15°C in order to achieve similar conditions for the feeding experiments. Illumination was provided by white fluorescent tubes (Phillips), irradiance (12:12 h light/dark cycle) ranged from 30 to 40 µE m^{-2} s^{-1}, as measured by a Licor light meter.

The diatom *T. minima* was cultured in two different set-ups. One culture flask was bubbled and the algae were present as single cells. Another flask was only randomly swirled (four times a day) so that the diatom cells were present in chains of two or three cells. According to the cell division rate, the cultures were diluted every 1 to 2 days using a peristaltic pump to add fresh media and remove old culture. Therefore, in all the experiments, the algae were growing in log phase.

Prior to the experiments, subsamples from each algal culture were taken for the determination of carbon (C), nitrogen (N) and fatty acid composition to characterise the quality of the algae offered in the grazing experiments. In addition, subsamples were taken for microscopic observation of living algal cells to control cell size of all species and chain length of the diatom in particular.

Analysis of C, N, fatty acid composition of the algae, and body C of the copepods used for the experiments

For C and N analysis, 100 ml of the algal mixture used for the feeding experiments and 20 ml of each culture was filtered on pre-combusted glass fibre filters (GF/F) which were dried at 50°C and then analysed using a Carlo Erba CN analyser.

Estimates of body C were made on three to four replicates of 300 nauplii, 100 CI, 50 CII, 25 CIII, 10 CIV and 5 adult females of each copepod species. The animals were sorted under a stereomicroscope, filtered onto pre-combusted GF/F, dried at 50°C for 24 h and then analysed using a Carlo Erba CN analyser.

For fatty acid composition, the microalgae were filtered onto pre-combusted GF/F which were transferred into 10-ml glass vials

Table 1 Culture code in the Plymouth (*CCP*) and Oban (*CCAP*) culture collections, cell volume (*vol.*), chl *a* per cell and cellular values of carbon (*C*) to chl *a*. The C:chl *a* ratios were used for calculating feeding rates

Algal species	Culture code	Cell vol. (µm³) min.–max.	Chl *a* (pg cell^{-1}) (*n*=9)	C:chl *a* ratio (*n*=9)
Dinoflagellate *Prorocentrum nanum*	184 (CCP)	205–221	0.85±0.14	58.4±2.3
Diatom *Thalassiosira minima*	1085/8 (CCAP)	208–220	1.36±0.19	48.3±8.1
Cryptophyte *Rhodomonas baltica*	530 (CCP)	200–218	1.09±0.12	33.4±5.5
Chlorophyte *Dunaliella tertiolecta*	83 (CCP)	204–220	1.25±0.34	32.4±5.3
Phytoplankton mixture				50.7±3.2

Table 2 Biovolume of each algal species and the total algal mixture used in the three experimental set-ups 1, 2, and 3. The total algal mixture of 1.0×10^6 µm³ ml^{-1} corresponds to 120 µg C l^{-1}

Set-up	Biovolume (µm³ ml^{-1})				
	Dinoflagellate *Prorocentrum nanum*	Diatom *Thalassiosira minima*	Cryptophyte *Rhodomonas baltica*	Chlorophyte *Dunaliella tertiolecta*	Total algae mixture
1	0.25×10^6	0.25×10^6	0.25×10^6	0.25×10^6	1.0×10^6
2	0.25×10^6	0.25×10^6	0.25×10^6	0.25×10^6	1.0×10^6
3	0.33×10^6		0.33×10^6	0.33×10^6	1.0×10^6

Table 3 The input (*a*) and output (*b*) matrixes of accessory pigment:chlorophyll *a* ratios calculated by CHEMTAX (*per* peridinin, *fuco* fucoxanthin, *did* diadinoxanthin, *all* alloxanthin, *lut* lutein)

	chl c_{1+2}	per	fuco	did	all	lut	chl *b*
(*a*)							
Dinoflagellate *P. nanum*	0.268	0.300	0.000	0.239	0.000	0.000	0.000
Diatom *T. minima*	0.133	0.000	0.457	0.241	0.000	0.000	0.000
Cryptophyte *R. baltica*	0.061	0.000	0.000	0.000	0.300	0.000	0.000
Chlorophyte *D. tertiolecta*	0.000	0.000	0.000	0.000	0.000	0.200	0.569
(*b*)							
Dinoflagellate *P. nanum*	0.254	0.357	0.000	0.196	0.000	0.000	0.000
Diatom *T. minima*	0.132	0.000	0.442	0.204	0.000	0.000	0.000
Cryptophyte *R.baltica*	0.571	0.000	0.000	0.000	0.259	0.000	0.000
Chlorophyte *D. tertiolecta*	0.000	0.000	0.000	0.000	0.000	0.233	0.546

with Teflon-sealed screw caps containing a mixture of chloroform/methanol (2:1; v:v) according to the method of Folch et al. (1957). The samples were stored at –30°C. Extraction of the filters was carried out with a Potter homogeniser (Braun, Melsungen) according to the method of Bligh and Dyer (1959). Polar compounds were separated by mixing with 0.88% KCl and discarded after phase separation liquid chromatographic analysis of the fatty acid aliquots of the extracted samples were taken. Methyl esters of fatty acids were prepared by transesterification with 3% concentrated sulphuric acid in methanol for 4 h at 80°C under N_2-atmosphere. After their extraction with hexane, the composition was analysed with a gas liquid chromatograph (Chrompack 9000) on a capillary column (30 m × 0.25 mm I.D.; film thickness: 0.25 µm; liquid phase: DB-FFAP) using temperature programming according to the method of Kattner and Fricke (1986). Fatty acids were identified by known standard mixtures. The data for individual fatty acids are given in mass percentage of total fatty acids.

In this study we focused on the individual 20:5ω3 and 20:6ω3 fatty acids because they are considered to determine egg production (Jónasdóttir 1994). Moreover, the fatty acid ratio ω3:ω6 has been suggested to be an important indicator in metabolic growth and reproduction processes in Crustacea (Harrison 1990 and references therein).

Cultures of *C. finmarchicus* and *C. helgolandicus*

Adult females of *C. finmarchicus* were collected in the Norwegian Sea at Station "M" (Meyer-Harms et al. 1999) and transported to the Plymouth Marine Laboratory. The females were fed ad libitum with *Prorocentrum micans*, and the eggs they produced were collected daily. Groups of eggs were set up in 10-l beakers on different dates so that, at the time of the experiment, cohorts with different stage compositions were available simultaneously. The nauplii and copepodites which developed were fed with *P. micans* before the experimentation, when they where transferred to the mixture of food to be used in the experiments. The same procedure was used with *Calanus helgolandicus* except that parental adult females were obtained from the English Channel off Plymouth.

Feeding experiments

The laboratory-reared nauplii and copepodites used in the grazing experiments were fed for 24 h prior to experimentation using food conditions identical to those of the subsequent feeding studies. Groups of 70 nauplii, 50 copepodite I (CI), 35 CII, 25 CIII, 10 CIV and 5–10 adult females of *C. finmarchicus* and *C. helgolandicus* were identified under a microscope and placed in 1-l bottles, filled with the appropriate algal mixture for the experimental set-ups 1, 2 or 3 (see above). Each algal species was added to the mixture in an equal biovolume up to a total biovolume of 10^6 µm³ ml^{-1} as estimated by a Coulter counter (see Table 2). This biovolume is equivalent to a chlorophyll *a* concentration of 2.5 µg l^{-1} and a C concentration of 120 µg C l^{-1}. Four to five replicates and three control bottles were incubated on a rotating plankton wheel at 0.2 rpm for 24 h in a 12 h dark/light cycle. Incubation temperatures were 10°C and 15°C for *C. finmarchicus* and *C. helgolandicus*, respectively.

Estimation of grazing rates

At the beginning, and after 24 h of each feeding experiment, subsamples (500 ml) of the incubation water from both the grazing and control bottles were taken for pigment analysis by HPLC. The HPLC analyses were conducted according to the method described by Barlow et al. (1997).

In general, the chl *a* concentrations express the grazing impact on the total phytoplankton biomass. The taxon-specific grazing impact on the total algae mixture was estimated by calculating the contribution of the accessory pigment distributions of each algal species to chl *a* by using the CHEMTAX program developed by Mackey et al. (1996). The input and output matrix of marker pigments/chl *a* ratio used and obtained with CHEMTAX is presented in Table 3. A detailed description of using this program in general and the input and output matrix in particular is given by Mackey et al. (1997).

The calculated chl *a* equivalents for each algal species were converted to carbon by using the C:chl *a* ratios of each algal species analysed during the study period (see Table 1). The calculated C values for each algal species from the control and grazing bottles after 24 h incubation were used to calculate the taxon-specific ingestion and filtration rates of the total algal mixture offered according to Frost (1972). A detailed description of the value of using such a pigment-based technique in grazing experiments is given by Meyer-Harms and von Bodungen (1997), who compared pigment data with cell counts, and also by Meyer-Harms et al. (1999).

Random samples of faeces were analysed by HPLC, as the residual pigment remaining in faecal pellets may influence calcula-

tion of grazing rates (Head and Harris 1994). A detailed description of this approach is given by Meyer-Harms et al. (1999). In all of the samples analysed, both chlorophylls and marker pigments were almost completely transformed into degradation products. Hence, the faecal pellets, which were produced during incubation and collected on the filters together with the residual phytoplankton cells at the end of the experiments, did not influence the calculated grazing rates.

Estimation of selective feeding behaviour

Selective feeding behaviour was characterised using the chi-squared (χ^2) goodness-of-fit test, as described in detail by Sokal and Rohlf (1969) and indicated by a significant difference between the distribution (in terms of C) of the different algae in the diet and the distribution (in terms of C) of the different algae in the food mixture (Kleppel et al. 1996). Student's *t*-test was used to test the significance of differences in filtration rates between the different algae offered in a mixture of equal biovolume of each algae.

Results

Food quality

The diatom *T. minima* had the highest C and N content per cell followed by the dinoflagellate *P. nanum*, the chlorophyte *D. tertiolecta* and the chlorophyte *R. baltica* (Fig. 1). The largest percentages of 20:5ω3, 22:6ω3 fatty acids and ω3:ω6 ratio were also found in *T. minima* (Fig. 1).

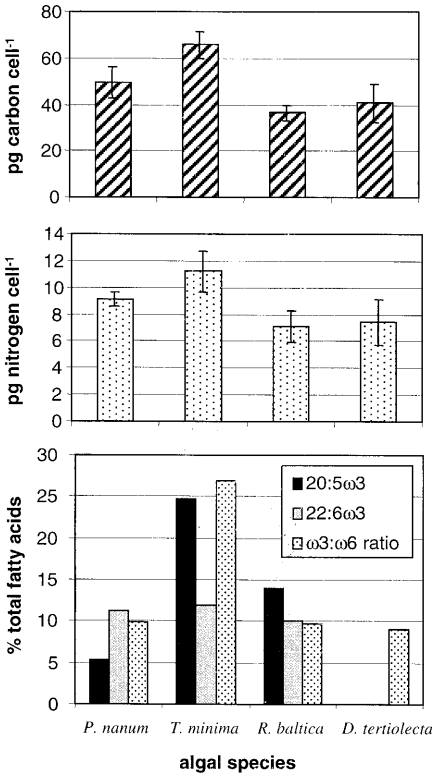

Fig. 1 Cellular mean values (pg) of carbon and nitrogen with standard deviation (*n*=5) as well as percentage (%) 20:5ω3, 22:6ω3, and ω3: ω6 ratio of total fatty acids from the algal species used in the feeding experiments

Ingestion and filtration rates

Individual ingestion rates increased for *C. finmarchicus* from 0.7 µg C day^{-1} for nauplii to 12 µg C day^{-1} for adult females, and for *C. helgolandicus* from 0.4 µg C day^{-1} for nauplii to 5 µg C day^{-1} for adult females (Table 4, Fig. 2). Individual filtration rates ranged from 5 ml day^{-1} for *C. finmarchicus* nauplii to 81–98 ml day^{-1} for adult females and from 3 ml day^{-1} for *C. helgolandicus* nauplii to 36–46 ml copepod^{-1} day^{-1} for adult females (Table 4).

In all the experimental set-ups, ingestion and filtration rates of both copepod species per unit body C were very similar and decreased gradually with increasing body C (Figs. 3, 4 and 5). Differences in weight-specific ingestion between both species were tested by using an ANCOVA test of covariance on the C weight-specific ingestion versus C body weight regressions after log – log transformation. Neither the intercepts nor the slopes were significantly different (P>0.05, Fig. 3). The daily ingested amount of food decreased for *C. finmarchicus* from 124–134% of body C of nauplii to 19% of the body C of adult females, and for *C. helgolandicus* from 117–137% of the body C of nauplii to13–26% of the body C of adult females.

Due to the high C content per cell of dinoflagellates and diatoms compared with the other algae offered, the taxon-specific feeding rate per unit C showed highest values on dinoflagellates and diatoms in experimental

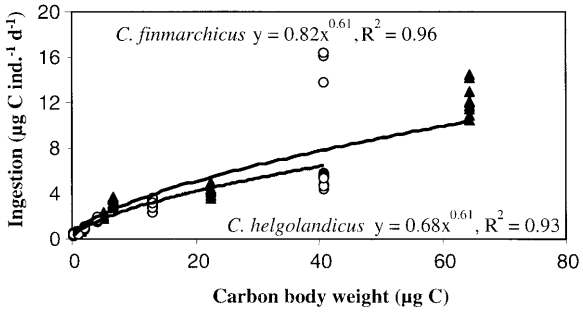

Fig. 2 Regression of experimental set-ups 1, 2, and 3 between body C and ingestion per individual and day for *C. finmarchicus* (*triangles*, *n*=63) and *C. helgolandicus* (*circles*, *n*=64)

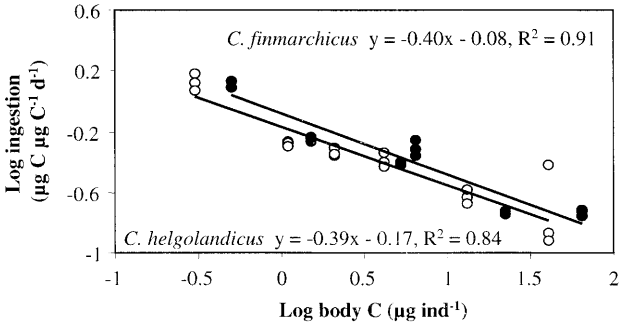

Fig. 3 Regressions of the log-transformed C weight-specific ingestion versus C body weight for *C. finmarchicus* (*filled circles*) and *C. helgolandicus* (*empty circles*). *Each point* represents the average ingestion for each stage in the different experimental set-ups

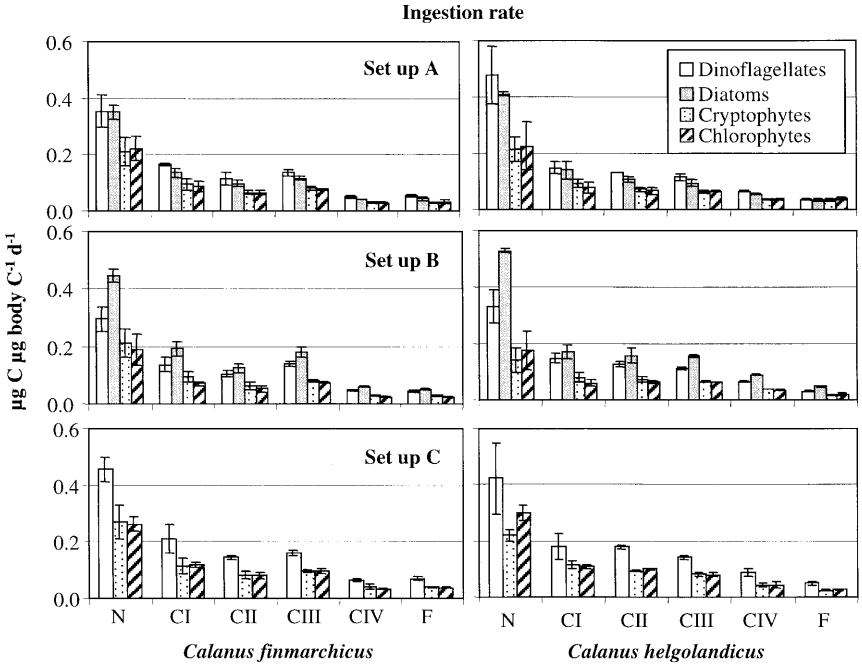

Fig. 4 Ingestion rates (n=5) from experimental set-ups 1 (*A*), 2 (*B*) and 3 (*C*) of nauplii (*N*), copepodite I (*CI*), *CII*, *CIII*, *CIV*, and adult females (*F*) of *C. finmarchicus* (*left*) and *C. helgolandicus* (*right*) expressed in µg carbon per µg body carbon per day on a mixture of the dinoflagellate *P. nanum*, the diatom *T. minima*, the cryptophyte *R. baltica*, and the chlorophyte *D. tertiolecta*

Table 4 Body weight, ingestion and filtration rate on total algal biomass in experimental set-ups 1, 2, and 3 expressed as µg carbon per individual of developmental stages (*N* nauplii, *C* copepodites) and adult females (*F*) of *Calanus finmarchicus* and *C. helgolandicus* (± standard deviation of all experiments, *C. finmarchicus* n=22, *C. helgolandicus* n=24)

Stages	Weight (µg body C)[a]	Ingestion rate (µg C ind^{-1} day^{-1})			Filtration rate (ml ind^{-1} day^{-1})		
		Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
C. finmarchicus (±<24%)							
N	0.5	0.67	0.68	0.62	4.76	4.89	5.08
CI	1.5	0.84	0.86	0.77	5.98	6.12	7.02
CII	5.2	2.09	2.18	1.95	14.84	14.84	15.74
CIII	6.5	3.12	3.55	2.85	22.15	25.18	23.48
CIV	22.4	3.99	4.21	3.59	28.28	29.84	30.20
F	64.5	12.25	11.43	11.71	87.73	81.01	98.00
C. helgolandicus (±<13%)							
N	0.3	0.41	0.40	0.35	2.92	2.84	2.76
CI	1.1	0.59	0.58	0.55	4.21	4.08	4.42
CII	2.1	0.90	1.03	0.95	6.38	7.27	8.38
CIII	4.2	1.68	1.94	1.56	11.93	13.77	13.32
CIV	13.1	3.01	3.45	2.76	21.37	24.44	25.86
F	40.7	5.25	5.49	5.17	35.79	38.93	46.19

[a] ±<15% n=5 for *C. finmarchicus*, ±<12% n=4 for *C. helgolandicus*

set-up 1, and on dinoflagellates in experimental set-up 3 (Fig. 4), while the taxon-specific filtration rates on all algal species present in the mixture of experiments 1 and 3 were similar (Fig. 5). In experiment 2, where the diatom *T. minima* was present in chains of two or three cells, and therefore larger than the other species, both copepods showed highest ingestion and filtration rates on the diatom (Figs. 4 and 5).

Selectivity

In experimental set-ups 1 and 3, where the algae were of similar size, no significant difference was found between the distribution of the algae in the food mixture and their contribution to the diet for any of the developmental stages (Table 5). In experimental set-up 2, where the diatom was present in chains of two or three cells, the frequency distribution of the algae in the food mixture was significantly different from that of the diet for all the stages in both species (P<0.05, Table 5). In general, there do not seem to be large differences in selectivity between stages (Table 5).

The taxon-specific filtration rates on all species present in the mixture were similar in experiments 1 and 3. In experiment 2, where the diatom *T. minima* was present in chains of two or three cells and therefore larger than the other species, both copepods showed highest filtration rates on the diatom (Fig. 4). This was significantly different for all stages of both copepod species (P<0.05) from the filtration rates on the other algae present in the incubation medium.

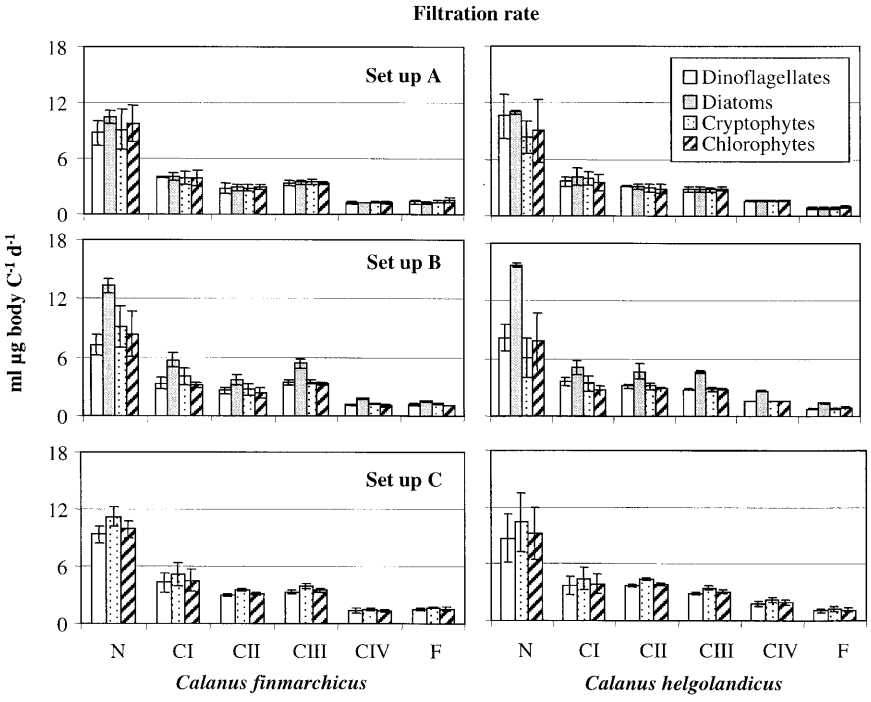

Fig. 5 Filtration rates (n=5) from experimental set-ups 1 (*A*), 2 (*B*) and 3 (*C*) of nauplii (*N*), copepodite I (*CI*), *CII*, *CIII*, *CIV*, and adult females (*F*) of *C. finmarchicus* (*left*) and *C. helgolandicus* (*right*) expressed in ml per µg body carbon per day (on a mixture of the dinoflagellate *P. nanum*, the diatom *T. minima*, the cryptophyte *R. baltica*, and the chlorophyte *D. tertiolecta*

Table 5 Chi-square (χ^2) goodness-of-fit test for the frequency distribution of the algae in the diet of that in the control bottles of the three experimental set-ups 1, 2, and 3 of *Calanus finmarchicus* and *C. helgolandicus* nauplii (*N*), copepodite I (*CI*), *CII*, *CIII*, *CIV* and adult females (*F*)

Stages	Experimental set-up					
	1		2		3	
	C. finmar.	*C. helgol.*	*C. finmar.*	*C. helgol.*	*C. finmar.*	*C. helgol.*
N	1.26	3.38	18.50**	34.25**	0.97	1.24
CI	1.96	0.97	19.56**	15.47**	1.36	1.11
CII	2.23	2.29	12.77*	15.39**	1.11	1.08
CIII	1.94	2.38	16.34**	18.10**	1.21	1.22
CIV	1.95	2.22	15.98**	18.10**	1.15	1.21
F	3.24	3.94	9.81*	21.08**	1.16	1.15

**P<0.001, *P<0.05

Discussion

Our results show that when feeding on algal cells without a clear distinction in external morphology the main selection factor for *Calanus finmarchicus* and C. *helgolandicus* is cell size.

These findings confirm previous results by Richman and Rogers (1969) with single and paired cells of *Ditylum brightwellii*. These authors reported that in the feeding experiments *C. helgolandicus* actively selected the paired cells and passively filtered the single ones. These results also support the observations by Price et al. (1983) showing a clear difference in the capture of small ("filtered") and large ("handled") cells. In our experiments *Calanus* did not show any selectivity due to the intrinsic nutritional quality of the cells, measured by either lipid composition or C:N ratio. Several studies have shown that copepods are able to select between particles of the same size but different nutritive value: plastic beads versus phytoplankton (Fernández 1979), fast-growing versus senescent cells or different growing states of the same species (Mullin 1963; Cowles et al. 1988), or toxic versus non-toxic strains of the same or similarly sized algae (Huntley et al. 1983; Turriff et al. 1995). However, two factors have to be considered: most of those studies were conducted with large cells that are handled by the copepods (Price et al. 1983), and furthermore it is likely that in all the cases the quality difference between the particles is chemically expressed in the external surface of the cell. In fact, for healthy non-toxic cells, without clear physical or chemical signals in the cell surface, there is no reason why a copepod should be able to detect the internal composition of the cells and therefore there is no reason to expect any selection.

On the other hand, size selection is a behaviour that offers a good compromise between reducing handling times and optimising food quality and quantity. As discussed in Irigoien et al. (2000a), no large cell can be expected to be suspended in the water column for a long time without having some sort of buoyancy mechanism, and therefore being "alive", whereas small detrital particles are more likely to remain in suspension. Furthermore, for spherical or semi-spherical particles, a small increase in diameter represents a large increase in the

cytoplasm volume. Therefore, size selection is a mechanism that optimises both food quality and quantity without a significant increase in the handling times.

This study also shows a similar selective behaviour for all *Calanus* stages. Recent work (Richardson and Verheye 1999) has suggested that the adult copepod production could be food limited, whereas in the same environment nauplii of the same species would not be limited because they were able to ingest smaller cells. On the other hand, another recent study (Campbell et al. 2001) shows that under conditions of food limitation, nauplii of *C. finmarchicus* are more strongly affected than the older stages. The results are in apparent agreement with our experiments. The relation between ingestion of small cells and copepod size (I=aW$^{0.6}$) seems to be in the range of the decrease in weight-specific ingestion with increasing size that can be expected from other metabolic rates such as respiration or excretion (Ikeda et al. 2000). Furthermore, if there was a difference in the ability to capture small cells depending on the stage, one would expect to find an increase in the selectivity (χ^2 value) of the older stages when presented with larger cells (diatom chains) that we did not detect (Table 5). Our results suggest that the behaviour and ingestion rates of nauplii can be extrapolated reasonably well from those of the adults. However, such an assertion requires further investigation, especially in view of possible differences in the minimum food needed for maximum growth (Hygum et al. 2000).

Our results also point out the potential importance of small cells in the diet of *Calanus* spp. It has generally been assumed that small cells are not consumed by *Calanus*. Nevertheless, *C. helgolandicus* has been successfully raised from egg to adult on a diet of *Isochrysis galbana* (Irigoien et al. 2000c). In our experiments the total food concentration offered (~120 µg C l^{-1}) is far from being saturating for *C. finmarchicus* or *C. helgolandicus* (Meyer-Harms et al. 1999; Irigoien et al. 2000a). However, the ingestion rates, >100% of the C body weight for nauplii and about 20% for adults, are considerable and are likely to cover the metabolic requirements for all the stages.

In the North Atlantic, *C. finmarchicus* dominates north of the Gulf Stream, from the North Sea to the Barents Sea, whereas *C. helgolandicus* dominates south of the Gulf Stream, being present from the North Sea to the Mediterranean (Planque and Fromentin 1996). In these areas both species are confronted not only with a temperature gradient but also very probably to a food size distribution gradient, with smaller cells becoming dominant towards the south of the *C. helgolandicus* distribution (Gibb et al. 2001). One could expect *C. helgolandicus* to be better adapted to capturing small cells, and this could have important consequences because the food composition could change more radically with shifts in the climatic conditions such as temperature (Irigoien et al. 2000b). However, in our experiments there was no significant difference in the specific ingestion and filtration rates of developmental stages between both species studied. Further experiments with mixed sizes will be required to determine whether the feeding rates and behaviour of both species are as similar as is suggested by our results.

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References

Atkinson A (1995) Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea. Antarctica. ICES J Mar Sci 52:385–396

Barlow RG, Cummings DG, Gibb SW (1997) Improved resolution of mono- and divinyl chlorophylls *a* and *b* and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC. Mar Ecol Prog Ser 161:303–307

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 27:911–917

Campbell RG, Runge JA, Durbin EG (2001) Evidence for food limitation of *Calanus finmarchicus* production rates on the southern flank of Georges Bank during April 1997. Deep-Sea Res II 48:531–549

Cowles TJ, Olson RJ, Chisholm SW (1988) Food selection by copepods: discrimination on the basis of food quality. Mar Biol 100:41–49

Donaghay PL, Small LF (1979) Food selection capabilities of the estuarine copepod *Acartia tonsa*. Mar Biol 52:137–146

Fernández F (1979) Nutrition studies in the nauplius larva of *Calanus pacificus* (Copepoda: Calanoida). Mar Biol 53:131–147

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification on total lipids from animal tissues. J Biol Chem 226:497–509

Frost BW (1972) Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815

Gibb SW, Cummings DG, Irigoien X, Barlow RG, Mantoura RFC (2001) Phytoplankton pigment chemotaxonomy of the northeastern Atlantic. Deep-Sea Res II 48:795–823

Guillard RR, Ryther J (1962) Studies of marine planktonic diatoms: *Cyclotella nana* (Hustedt) and *Detonula confervacea* (Cleve.). Can J Microbiol 8:229–239

Harris RP, Irigoien X, Head RN, Rey C, Hygum BH, Hansen BW, Niehoff B, Meyer-Harms B, Carlotti F (2000) Feeding, growth and reproduction in the genus *Calanus*. ICES J Mar Sci 57:1708–1726

Harrison KE (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. J Shellfish Res 9:1–28

Head EJH, Harris LR (1994) Feeding selectivity by copepods grazing on natural mixtures of phytoplankton determined by HPLC analysis of pigments. Mar Ecol Prog Ser 110:75–83

Huntley M (1981) Nonselective, nonsaturated feeding by three calanid copepod species in the Labrador Sea. Limnol Oceanogr 26:831–842

Huntley M, Barthel KG, Star JL (1983) Particle rejection by *Calanus pacificus*: discrimination between similarly sized particles. Mar Biol 74:151–160

Hygum BH, Rey C, Hansen BW (2000) Growth and development rates of *Calanus finmarchicus* nauplii during a diatom spring bloom. Mar Biol 136:1075–1085

Ikeda T, Torres JJ, Hernández-León S, Geiger SP (2000) Metabolism. In: Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley (eds) ICES zooplankton methodology manual. Academic Press, London, p 455–520

Irigoien X, Meyer-Harms B, Head RN, Harris RP, Cummings D, Harbour D (2000a) Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel. Limnol Oceanogr 45:44–54

Irigoien X, Harris RP, Head RN, Harbour D (2000b) North Atlantic Oscillation and spring bloom phytoplankton composition in the English Channel. J Plankton Res 22:2367–2371

Irigoien X, Obermüller B, Head RN, Harris RP, Rey C, Hansen B, Hygum B, Heath M, Durbin EG (2000c) The effect of food on the determination of sex ratio in *Calanus* spp.: evidence from experimental studies and field data. ICES J Mar Sci 57:1752–1763

Jónasdóttir SH (1994) Effects on food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. Mar Biol 121:67–81

Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J Chromatogr 361:263–268

Kleppel GS, Burkart CA, Carter K, Tomas C (1996) Diets of calanoid copepods on the West Florida continental shelf: relationships between food concentration, food composition and feeding activity. Mar Biol 127:209–217

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144:265–283

Mackey MD, Higgins HW, Mackey DJ, Wright SW (1997) CHEMTAX users manual: a program for estimating class abundances from chemical markers application to HPLC measurements of phytoplankton pigments. (Marine Laboratories Report 227) CSIRO, Australia

Meyer-Harms B, Bodungen B von (1997) Taxon-specific ingestion rates of natural phytoplankton by calanoid copepods in an estuarine environment (Pomeranian Bight, Baltic Sea) determined by cell counts and HPLC analyses of marker pigments. Mar Ecol Prog Ser 153:181–190

Meyer-Harms B, Irigoien X, Head R, Harris R (1999) Selective feeding on natural phytoplankton by *Calanus finmarchicus* before, during and after the 1997 spring bloom in the Norwegian Sea. Limnol Oceanogr 44:154–165

Mullin MM (1963) Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnol Oceanogr 8:239–250

Planque B, Fromentin JM (1996) *Calanus* and environment in the eastern North Atlantic. I. Spatial and temporal patterns of *C. finmarchicus* and *C. helgolandicus*. Mar Ecol Prog Ser 134:101–109

Price HJ, Paffenhöfer GA, Strickler JR (1983) Modes of cell capture in calanoid copepods. Limnol Oceanogr 28:116–123

Richardson AJ, Verheye HM (1999) Growth rates of copepods in the southern Benguela upwelling system: the interplay between body size and food. Limnol Oceanogr 44:382–392

Richman S, Rogers JN (1969) The feeding of *Calanus helgolandicus* on synchronously growing populations of the marine diatom *Ditylum brightwellii*. Limnol Oceanogr 14:701–709

Roman MR (1977) Feeding of the copepod *Acartia tonsa* on the diatom *Nitzschia closterium* and brown algae (*Fucus vesiculosus*) detritus. Mar Biol 42:149–155

Sokal RR, Rohlf FG (1969) Biometry. WH Freeman, San Francisco

Turner JT, Tester PA (1989) Zooplankton feeding ecology: nonselective feeding by copepods *Acartia tonsa* Dana, *Centropages velificatus* de Oliveira, and *Eucalanus pileatus* Giesbrecht in the plume of the Mississippi River. J Exp Mar Biol Ecol 126:21–43

Turriff N, Runge JA, Cembella AD (1995) Toxin accumulation and feeding behaviour of the planktonic copepod *Calanus finmarchicus* exposed to the red-tide dinoflagellate *Alexandrium excavatum*. Mar Biol 123:55–64