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Living on the edge: feeding of subtropical open ocean copepods

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Abstract

The objective of this study was to provide quantitative information on environmental feeding rates of warm water oceanic epipelagic copepods. We determined clearance rates at 23 °C for various particle size ranges in shipboard studies in the western oligotrophic subtropical Atlantic Ocean for females of the calanoid species *Clausocalanus furcatus* and *Mecynocera clausii*. These *in situ* clearance rates were then applied to the various particle size ranges of environmental particle spectra of auto- and heterotrophs at different depths from three stations in the western Atlantic. After calculating the metabolic demands of each of these two copepod species and applying an assimilation efficiency of 90%, we determined that *C. furcatus* meets its metabolic demands in all six cases, and *M. clausii* in two of six cases. *Clausocalanus furcatus* would also meet its energy demands at 25 °C, where it is often found, while *M. clausii* at 20 °C, where it is regularly found, would cover its metabolic needs in four of six cases. It is hypothesized that these species, and most likely most of the other co-occurring copepod species, are limited in their abundance by food availability, or, better said, are 'living on the edge' in relation to food abundance.

Problem

The oligotrophic regions of subtropical and tropical oceans are characterized by high diversity of planktonic copepods. The number of species observed ranges from 67 to more than 200 in the epipelagial (McGowan & Walker 1979; Sameoto 1986; Amelekhina *et al.* 1990; Webber & Roff 1995a). By comparison, the copepod diversity in temperate to polar regions is far less (Mauchline 1998).

Whereas rate quantifications on copepod processes in temperate and coastal regions are numerous (*e.g.* Kleppel 1993; Harris 1996), those from warm open ocean waters are scarce (*e.g.* Petipa 1978; Webber & Roff 1995b; Gaudy *et al.* 2003). This is particularly obvious for feeding rate quantifications. Nearly all the available data on the latter cover only part of the feeding performance since almost all were based on the gut fluorescence technique (*e.g.* Dam *et al.* 1995; Rollwagen Bollens & Landry 2000).

Different methods were used by Roman & Gauzens (1997) who quantified feeding rates of zooplankton retained by 64 µm mesh with a radiolabel method. Feeding rates were also quantified with a Coulter Counter accompanied by the gut fluorescence technique (Champalbert *et al.* 2003; Gaudy *et al.* 2004). The above-mentioned radiolabel method cannot quantify the heterotrophic protists, and the Coulter Counter approach does not distinguish between particles ingested and produced during the experiment. Most planktonic copepods are omnivores (*e.g.* Marshall 1924; Mullin 1966; Kleppel 1993; Fessenden & Cowles 1994; Ohman & Runge 1994; Verity & Paffenhöfer 1996), *i.e.* they are able to ingest auto- as well as heterotroph particles.

The paucity of information on feeding and reproduction rates of abundant warm open ocean copepods had led to initial efforts to quantify such rates under laboratory conditions for females of the circumglobally occurring

Clausocalanus furcatus (Mazzocchi & Paffenhöfer 1998, 1999) and *Oithona plumifera* (Paffenhöfer & Mazzocchi 2002). Due to a limited range of food compositions available in the laboratory, these studies could only approximate environmental feeding rates. Other attempts to obtain feeding rates of warm open ocean copepods, despite being conducted in the field, mostly utilized experimentally grown food particles (e.g. Petipa *et al.* 1977; Sommer *et al.* 2002). Feeding rate quantifications on *in situ* particulate matter are few (e.g. Fessenden & Cowles 1994; Ohman & Runge 1994; Verity & Paffenhöfer 1996). Thus, we decided to quantify feeding rates of two abundantly-occurring copepods from the subtropical open ocean, offering *in situ* particle compositions and abundances.

The copepods investigated were females of the calanoids *C. furcatus* and *Mecynocera clausii*. The genus *Clausocalanus* is found abundantly in the temperate and warm zones of the open oceans and amounts to 14.2–17.6% of total epipelagic copepods (e.g. Frost & Fleminger 1968; Amelekhina *et al.* 1990; Roman *et al.* 1995; Webber & Roff 1995a). *Mecynocera clausii* occurs in the subtropical and tropical open oceans, amounting to 3.4–10.4% of total epipelagic copepods (e.g. Geinrykh 1958; Bowman 1971; Roman *et al.* 1995; Webber & Roff 1995a; Rollwagen Bollens & Landry 2000; Head *et al.* 2002; Paffenhöfer & Mazzocchi 2003). *Clausocalanus furcatus* moves fast along very convoluted tracks, does not create a feeding current (Mazzocchi & Paffenhöfer 1999) and occurs primarily in the upper 50 m where temperatures range from 23 to 28 °C; *M. clausii* moves slowly in a cruising manner, creates a feeding current and occurs abundantly at depths from 40 to 100 m where temperatures are typically between 20 and 25 °C (Paffenhöfer & Mazzocchi 2003).

The main goal of our study was to determine the extent to which females of these two differently behaving calanoid species are able to acquire sufficient energy for metabolism and reproduction under such nutritionally dilute conditions (Conover 1968) as those found in subtropical open ocean gyres (e.g. Paffenhöfer *et al.* 2003).

Material and Methods

We conducted our study off Puerto Rico (19° N, 66° W) and off the Bahamas (25° N, 70° W) during June 2001 and 2002 onboard the R/V *Cape Hatteras*. The copepod feeding rates were evaluated by applying the particle removal method during incubation experiments. We used a 200 µm mesh net with a 4000 ml codend to collect zooplankton from within the upper 75 m while the ship was drifting. To obtain experimental water with a natural particle suspension, we collected seawater near 45 m depth with a rosette sampler equipped with 10 l Niskin

bottles. This collection depth was chosen because both selected copepod species occur there (Paffenhöfer & Mazzocchi 2003). To minimize particle damage, seawater was removed from the Niskin bottles by letting it gently flow into a cooler or a bucket by means of a large-mouth silicon tube.

Each zooplankton sample was immediately and gently diluted in a large cooler that had been previously filled with experimental water. Zooplankton were collected from the cooler with a large glass beaker (1 or 2 l) and examined by eye to identify adult females of the two species under study. The selected copepods were sorted with a large-mouth glass pipette and transferred to acclimatize into 3800 ml bottles filled with experimental seawater. Within 6 h of acclimation, the copepods were transferred by pipette to screw-cap jars filled with the experimental seawater and sealed with clingfilm over the mouth to exclude air. For each experiment, 34–50 adult females of *M. clausii* (prosome length 0.79 ± 0.04 mm SE) were placed into 960 ml jars, while 23–44 adult females of *C. furcatus* (prosome length 0.74 ± 0.03 mm SE) were placed into 1900 ml jars. The large jars were used for *C. furcatus* to limit encounters with walls during their rapid motion. We chose such high concentrations of copepods in order to produce an appreciable reduction of the initial food concentration within the chosen experimental duration (12 h). The intended particle reduction was between 15% and 40% of the initial concentrations. Previous laboratory studies with *C. furcatus* resulted in clearance rates of about $2.0 \text{ ml} \cdot \text{female}^{-1} \cdot \text{h}^{-1}$ (Mazzocchi & Paffenhöfer 1998, 1999). There was no previous information on the effects of food concentration on *in situ* clearance rates of either species, and thus no basis for predicting the magnitude of *in situ* feeding rates. Overall, the duration of the incubation time and the copepod density represent a reasonable compromise for obtaining measurable food consumption and reducing the ‘food chain effects’ (Båmstedt *et al.* 2000). We added 3 ml f/2 medium per liter of seawater resulting in $3 \mu\text{g} \cdot \text{at} \cdot \text{l}^{-1}$ of nitrate (Guillard & Ryther 1962).

Two control jars of 960 ml each, containing only natural suspension seawater, were included in each set of experiments to quantify the growth rates of the potential food particles. All jars were rotated at 0.3 rpm on a plankton wheel of 1 m diameter. All experiments were run at 23 °C, the temperature at which both species were found, and lasted from 18:00 to 06:00 hours in the dark. At the end of each experiment, all copepods were removed individually to determine mortality and prosome lengths. Subsamples of measured copepods were dried in an oven at 60 °C to quantify their dry weight, and later ashed at 500 °C to obtain their ash-free dry weight which was expressed in units of organic carbon by multiplying with a factor of 0.42 (G.-A. Paffenhöfer unpublished

results from the similar size calanoid *Paracalanus quasimodo*).

To determine the sizes and composition of food particles, water samples of 300 ml were removed from each jar at the start and end of each experiment. Sampled water was replaced with water of the same natural suspension as before. Each water sample was divided into two replicates of 150 ml. Water samples were filtered through a 0.8 μm black Nucleopore filter, after buffering with 3.75 ml glutaraldehyde (250 μl per 10 ml sample). The filters were stained with Proflavin and DAPI. Proflavin stains cellular material and fluoresces green under narrow blue light (515 nm), in contrast to chlorophyll which appears red. DAPI stains nuclear material and fluoresces under ultraviolet light (397 nm). The prepared filters were mounted on a slide with immersion oil and preserved by freezing at $-20\text{ }^\circ\text{C}$. This approach was adapted from Verity *et al.* (1996) and Paffenhöfer *et al.* (2003).

Slides were examined at $200\times$ magnification within 9–12 months after preparation, using a compound microscope with a color QRetiga digital camera connected to a computer running QImage software. For each slide, at least 50 sets of digital images, representing 50 fields of view, were captured. Each set included images viewed with Narrow Blue (NB) and ultraviolet (UV) light. Fifty fields of view at $200\times$ magnification cover *c.* 3.2% of the total filter area. Images were analyzed using Skipper (<http://mapple.skio.peachnet.edu/skipper>), an image analysis software system for scientific research developed by Ruslan Hristov at the Skidaway Institute of Oceanography. Cells ranging from 6 to 40 μm Equivalent Spherical Diameter (ESD) were counted; their ESD and biovolume were automatically estimated by Skipper, based on manual outlining of cell boundaries.

We calculated the carbon content (C) from biovolume measurements using the methods of Caron *et al.* (1999). Using the NB images, cells were determined to be autotrophic if they were red, and heterotrophic if they were green. Cells were subdivided into four size classes: 6–8, 8–10, 10–20, and 20–40 μm ESD. We decided to analyze only particles in the range from 6 to 40 μm ESD because clearance rates of similarly sized calanoids (adult females of *Paracalanus quasimodo*) on particles smaller than 6 μm ESD were difficult to quantify as only small decreases in concentrations had been observed in previous experiments (Paffenhöfer 1984).

Clearance ($\text{ml}\cdot\text{copepod}^{-1}\cdot\text{day}^{-1}$) and ingestion rates ($\text{cells}\cdot\text{copepod}^{-1}\cdot\text{day}^{-1}$) were calculated from the incubation experiments according to Frost (1972) by first calculating rates per hour, and from there as rates per day. We calculated the rates for total cells in each of the four size fractions without separating auto- from heterotroph cells because very few cells were found in the large size range.

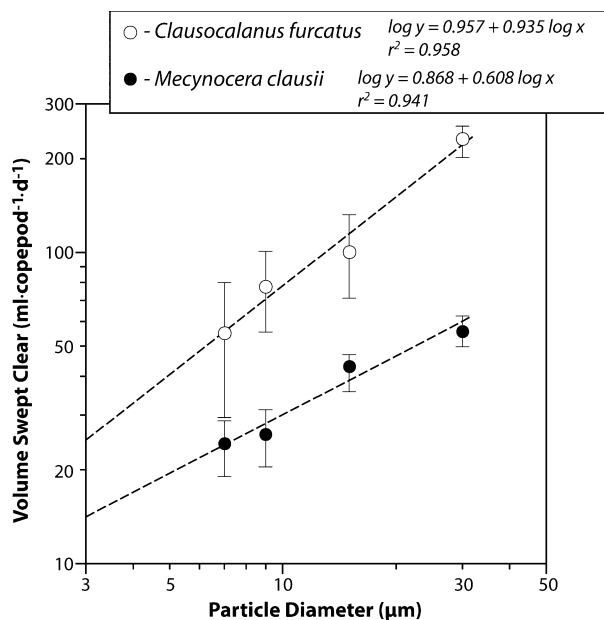


Fig. 1. Clearance rates of females of *Mecynocera clausii* and *Clausocalanus furcatus* at $23\text{ }^\circ\text{C}$ (mean \pm 1 SE).

To determine *in situ* ingestion rates we first calculated regressions ($\log y = A + B \log x$, Fig. 1) using the experimentally quantified clearance rates; y = clearance rate, x = particle diameter as ESD. We then calculated the *in situ* ingestion rates by applying the respective regression clearance rates to the *in situ* particle abundances of combined auto- and heterotroph cells observed at different stations and depths of the subtropical Atlantic Ocean (Paffenhöfer *et al.* 2003), considering cells of the average particle diameters 3, 5, 7, 9, 15 and 30 μm ESD. As *C. furcatus* mainly occurs in the upper 50 m of the water column, we applied environmental cell concentrations from 15 and 45 m depth. For *M. clausii*, which occurs mainly below 40 m depth, we applied cell concentrations from 45 and 75 m depth. The ingestion rates were calculated by multiplying each clearance rate with the respective environmental particle concentration ($\mu\text{g C}\cdot\text{l}^{-1}$). By extending the regressions to the smaller particle sizes (Fig. 1), we obtained clearance rates for the 3 and 5 μm ESD particle sizes for both copepod species.

We applied the Kruskal–Wallis test to analyze for differences between clearance rates at different particle sizes and between species. This test is a randomized block analysis of variance by ranks (Zar 1974; Conover 1980). If the null hypothesis was rejected, we followed with a multiple comparison analysis (Conover 1980).

To relate ingestion rates to each copepod's metabolic requirements we calculated the daily metabolic requirements according to Ikeda (1985) to determine whether

the above-mentioned ingestion rates were sufficient to meet these copepods' metabolic requirements. The equation we used was $\ln y = a_0 + a_1 \ln x_1 + a_2 x_2$, where $a_0 = 0.5254$, $a_1 = 0.8354$ and $a_2 = 0.0601$ are constants, y is the oxygen consumption rate (μl oxygen individual⁻¹, expressed as $\text{mg C}\cdot\text{h}^{-1}$), x_1 the copepod's weight in units of carbon (mg C), and x_2 is the experimental temperature ($^{\circ}\text{C}$). We converted oxygen consumption into units of carbon from Winberg (1971), in Omori & Ikeda (1984) using $1 \text{ ml O}_2 \text{ respired} = 4.86 \text{ cal}$ at a Respiratory Quotient of 1.0 (Omori & Ikeda 1984), and applying $5.00 \text{ cal} = 1.0 \text{ mg ash-free} = 0.42 \text{ mg C}$ (G.-A. Paffenhöfer, unpublished results with *Paracalanus quasimodo*). Assuming an assimilation efficiency (AE) of 90%, which can be expected at very low food concentrations (Paffenhöfer & Köster 2005), when nanoflagellates are ingested (*Dunaliella tertiolecta*, Besiktepe & Dam 2002), and when heterotrophic organisms are included (Conover 1979), we calculated from the total ingested amounts the percentage of bodily carbon which could be assimilated by each of the two copepod species.

Results

We conducted a total of 11 shipboard experiments with *M. clausii* and 14 with *C. furcatus*. However, we were only able to analyze seven of the former and six of the latter because on many of the filters the various cells were not uniformly distributed, and therefore could not be counted. Changes in the abundances of auto- and heterotrophs in experimental and control jars could occur due to activities within the microbial loop. We did not quantify feeding rates of the different components of the microbial loop but obtained exponential increases or decreases of the specific particle size classes in the control jars by calculating the exponential rate $k = \ln T_0 - \ln T_t / h$ where T_0 is the initial and T_t the final respective particle concentration. The average k -value for 6–8 μm ESD particles was -0.0018 , for 8–10 μm -0.0061 , for 10–20 μm 0.0107 , and for 20–40 μm -0.0233 . These results were included in the calculation of clearance rates. Average body carbon content ($\pm\text{SE}$) was $2.74 \pm 0.05 \mu\text{g}$ for *C. furcatus*, and $1.80 \pm 0.02 \mu\text{g}$ for *M. clausii*.

The copepod mortality at the end of the experiments was $4.1 \pm 2.2\%$ (range 0–12%) of the initial concentrations for *C. furcatus*, and $10.5 \pm 3.5\%$ (range 5–16%) for *M. clausii*. The dead copepods were excluded from the calculation of clearance rates.

Clearance rates

Clearance rates of females of both copepod species increased with particle size (Fig. 1). At each of the particle

size ranges, e.g. 6–8 μm , *C. furcatus* had a significantly higher clearance rate than *M. clausii* ($P < 0.05$). However, comparing clearance rates of *C. furcatus* at 6–8, 8–10, 10–20, and 20–40 μm ESD with each other, no significant differences were found (at $\alpha = 0.05$). Also, for *M. clausii*, no significant differences were found comparing the clearance rates at the same food size ranges (at $\alpha = 0.05$). Total particle concentrations at the beginning of the experiments for cells from 6 to 40 μm ESD were on average 6.29 ± 0.75 (SE) $\mu\text{g C}\cdot\text{l}^{-1}$ for the feeding experiments with *C. furcatus*, and $7.93 \pm 1.56 \mu\text{g C}\cdot\text{l}^{-1}$ for *M. clausii* (Table 1). The data presented in Table 1 are intended to provide comprehensive information on initial food concentrations in the various size ranges. The average reductions of initial particle concentrations in the *C. furcatus* experiments ranged from 28% in the 6–8 μm ESD range to 46% in the 20–40 μm ESD range. For *M. clausii* the values were from 42% for the 6–8 μm range to 63% for the 20–40 μm range.

Ingestion rates

We used the above-mentioned shipboard clearance rates together with environmental concentrations of combined heterotrophic and autotrophic cells from different stations and depths of the subtropical and tropical Atlantic Ocean (Paffenhöfer *et al.* 2003) to determine *in situ* ingestion rates. Whereas particle concentrations from 2 to 20 μm ESD had been determined for each depth of the respective casts, those from 20 to 40 μm ESD could only be obtained from three different depths at a single station, and were averaged and then applied to each of the chosen depths (Table 2). The daily *in situ* ingestion rates, resulting from multiplying the shipboard clearance rates with the *in situ* cell abundances ($\mu\text{g C}\cdot\text{l}^{-1}$), ranged from 24.7% to 48.7% of the carbon content of a *C. furcatus* adult female, with an average of 37.9% (Table 2a). For *M. clausii*, the daily *in situ* ingestion rates ranged from 15.5% to 24.7% of the copepod's carbon content, with an average of 20.4% (Table 2b). For both species, the particle range of 10–20 μm ESD contributed the major amount of ingested material, because of particle concentrations and clearance rates.

Ingestion rates and metabolic requirements

According to Ikeda's (1985) equation, *C. furcatus* would metabolize daily 18.4% and *M. clausii* 19.7% of its body carbon at 23 $^{\circ}\text{C}$. Assuming an assimilation efficiency of 90%, which can be expected at very low food concentrations (Paffenhöfer & Köster 2005), and also with heterotrophic food organisms (Conover 1979) or flagellates

Table 1. (a) *Clausocalanus furcatus* and (b) *Mecynocera clausii*. Initial cell and carbon concentrations of food particles during feeding experiments at 23 °C.

(a)						
Range of cell diameter (μm ESD)		6–8	8–10	10–20	20–40	
cell diameter (geom. mean, μm)		6.9	8.9	14.1	28.2	
cell volume (μm^3)		172	369	1,467	11,736	
pg C-cell ⁻¹		31	68	268	1,455	
Experiment						Total ($\mu\text{g C-l}^{-1}$)
C.f. 3	cells·ml ⁻¹	25.9	10.8	7.5	0.19	
29♀♀	$\mu\text{g C-l}^{-1}$	0.80	0.73	2.01	0.78	= 4.32
C.f. 4	cells·ml ⁻¹	22.3	8.5	4.7	0.58	
37♀♀	$\mu\text{g C-l}^{-1}$	0.69	0.58	1.26	0.84	= 3.37
C.f. 11	cells·ml ⁻¹	36.3	15.5	13.2	0.83	
27♀♀	$\mu\text{g C-l}^{-1}$	1.13	1.05	3.54	1.22	= 6.94
C.f. 13	cells·ml ⁻¹	50.8	19.8	14.0	0.83	
23♀♀	$\mu\text{g C-l}^{-1}$	1.57	1.35	3.75	1.21	= 7.88
C.f. 16	cells·ml ⁻¹	34.7	14.0	13.4	0.83	
44♀♀	$\mu\text{g C-l}^{-1}$	1.02	0.84	2.30	1.06	= 6.83
C.f. 18	cells·ml ⁻¹	36.5	13.6	16.9	1.24	
25♀♀ in 1900 ml	$\mu\text{g C-l}^{-1}$	1.13	0.92	4.53	1.80	= 8.38
Mean		1.07	0.93	3.11	1.18	6.29
n		6	6	6	6	6
SE		0.12	0.10	0.45	0.14	0.75
Range		0.69–1.57	0.58–1.35	1.26–4.53	0.58–1.80	3.37–8.38
(b)						
Range of cell diameter (μm ESD)		6–8	8–10	10–20	20–40	
cell diameter (geom. mean, μm)		6.9	8.9	14.1	28.2	
cell volume (μm^3)		172	369	1,467	11,736	
pg C-cell ⁻¹		31	68	268	1,455	
Experiment						Total ($\mu\text{g C-l}^{-1}$)
M.c. 5	cells·ml ⁻¹	22.4	8.6	5.6	0.24	
45 ♀♀ in 960 ml	$\mu\text{g C-l}^{-1}$	0.69	0.58	1.50	0.35	= 3.12
M.c. 9	cells·ml ⁻¹	38.3	26.8	18.7	1.83	
38♀♀	$\mu\text{g C-l}^{-1}$	1.19	1.82	5.01	2.61	= 10.68
M.c. 10	cells·ml ⁻¹	45.4	49.1	25.9	3.05	
50♀♀	$\mu\text{g C-l}^{-1}$	1.41	3.34	6.94	4.44	= 16.13
M.c. 14	cells·ml ⁻¹	27.7	16.5	9.3	0.41	
31♀♀	$\mu\text{g C-l}^{-1}$	0.86	1.12	2.49	0.60	= 5.07
M.c. 15	cells·ml ⁻¹	36.5	33.9	17.8	0.71	
48♀♀	$\mu\text{g C-l}^{-1}$	1.13	2.31	4.77	1.03	= 9.24
M.c. 19	cells·ml ⁻¹	21.1	10.7	7.6	1.24	
34♀♀	$\mu\text{g C-l}^{-1}$	0.65	0.73	2.04	1.80	= 5.22
M.c. 20	cells·ml ⁻¹	34.3	18.0	12.8	0.21	
50♀♀	$\mu\text{g C-l}^{-1}$	1.06	1.22	3.43	0.31	= 6.02
Mean		1.00	1.59	3.74	1.60	7.93
n		7	7	7	7	7
SE		0.10	0.34	0.68	0.53	1.56
Range		0.65–1.41	0.58–3.34	1.50–6.94	0.31–4.44	3.12–16.13

ESD, equivalent spherical diameter.

(Besiktepe & Dam 2002), we calculated from the total ingested amounts the percentage of bodily carbon which would be assimilated per day by each of the two copepod

species (Table 2a and b). In all six cases, *C. furcatus* would ingest sufficient amounts to cover its metabolic needs, whereas *M. clausii* would do so in two of the six

Table 2. Daily *in situ* rations and assimilated percentages of females of (a) *Clausocalanus furcatus* and (b) *Mecynocera clausii*.

(a)		2–4	4–6	6–8	8–10	10–20	20–40	Total	(1) Daily ingestion as % of body C (2) Assimilated C (at 90% assim.eff., as % of body C)
Range of all diameter (μm ESD)		2–4	4–6	6–8	8–10	10–20	20–40		
Clearance rate ($\text{ml}\cdot\text{copepod}^{-1}\cdot\text{day}^{-1}$)		25	41	56	71	114	218		
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Cast 11a 15 m depth									
Particle conc. ($\mu\text{g C}\cdot\text{l}^{-1}$)		1.61	1.61	0.93	1.02	2.00	1.02	= 8.19	(1) 24.7%
Ingestion rate ($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		40	64	52	72	228	222	= 678	(2) 22.3%
45 m depth									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.41	1.30	0.51	1.46	3.11	1.02	= 8.84	(1) 29.1%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		35	53	29	104	355	222	= 798	(2) 26.2%
Cast 19 15 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		2.14	2.50	1.91	1.21	5.06	1.02	= 13.84	(1) 41.9%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		54	103	107	86	577	222	= 1,149	(2) 37.7%
45 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.74	1.80	1.49	0.95	6.75	1.02	= 13.75	(1) 46.0%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		44	74	83	67	770	222	= 1,260	(2) 41.4%
Cast 35 15 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.74	1.84	1.44	1.24	7.25	1.02	= 14.50	(1) 48.7%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		44	75	81	88	826	222	= 1,336	(2) 43.9%
45 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.36	2.10	1.08	0.87	4.86	1.02	= 11.29	(1) 37.2%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		34	86	60	62	554	222	= 1,018	(2) 33.4%
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(b)									
Range of all diameter (μm ESD)		2–4	4–6	6–8	8–10	10–20	20–40	Total	(1) Daily ingestion as % of body C (2) Assimilated C (at 90% assim.eff., as % of body C)
Clearance rate ($\text{ml}\cdot\text{copepod}^{-1}\cdot\text{day}^{-1}$)		14	19	24	28	39	59		
Cast 11a 45 m depth									
Particle conc. ($\mu\text{g C}\cdot\text{l}^{-1}$)		1.44	1.30	0.51	1.46	3.11	1.02	= 8.84	(1) 15.5%
Ingestion rate ($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		20	25	12	41	121	60	= 279	(2) 14.0%
75 m depth									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.42	1.51	1.04	0.46	5.85	1.02	= 11.30	(1) 20.8%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		20	29	25	13	228	60	= 375	(2) 18.8%
Cast 19 45 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.74	1.80	1.49	0.95	6.75	1.02	= 13.75	(1) 24.7%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		24	34	36	27	263	60	= 444	(2) 22.2%
75 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.57	1.98	1.73	1.29	5.76	1.02	= 13.35	(1) 23.4%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		22	38	41	36	225	60	= 422	(2) 21.1%
Cast 35 45 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.36	2.10	1.08	0.87	4.86	1.02	= 11.19	(1) 19.9%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		19	40	26	24	190	60	= 359	(2) 18.0%
75 m									
($\mu\text{g C}\cdot\text{l}^{-1}$)		1.26	1.40	0.74	1.35	4.10	1.02	= 98.7	(1) 17.9%
($\text{ng C}\cdot\text{♀}^{-1}\cdot\text{day}^{-1}$)		18	27	18	38	160	60	= 326	(2) 16.1%

ESD, equivalent spherical diameter.

cases. Even at 25 °C, a temperature at which it is often found, *C. furcatus* would meet its metabolic needs (20.8%) in all cases, whereas *M. clausii* at 20 °C, where it is mainly found, would cover metabolic needs in four of the six cases (16.5%).

Discussion

In subtropical gyres the concentrations of living particulate matter in the epipelagial are largely determined by activities of the microbial loop (e.g. Caron *et al.* 1999), and do not

show much variability during short periods of time. Thus, the numerous species of copepods existing in the epipelagial depend on a food source which persists in concentration over time. It was our goal to find out to what extent females of two abundant calanoid species could obtain sufficient food for metabolism and also reproduction.

Clearance rates

Despite the occurrence of numerous calanoid species in the epipelagial of warm open oceans, attempts to obtain feeding rates of such species had been limited to the Soviet expeditions (e.g. Petipa *et al.* 1977) which, however, were mostly conducted with experimentally grown food particles, similar to such attempts in the Gulf of Aqaba (Sommer *et al.* 2002).

The magnitude of clearance rates is a function of the food concentration (e.g. Corner *et al.* 1972) and signal strength (e.g. Price & Paffenhöfer 1985). As the food concentration decreases, the clearance rate increases because the copepod, to obtain sufficient food, increases its perception performance to compensate for the lower food abundance, as shown by Paffenhöfer & Lewis (1990). The clearance rates of *C. furcatus* and *M. clausii* should have been at their maximum because of the continuously low food levels in the North Atlantic Subtropical Gyre (e.g. Paffenhöfer *et al.* 2003). In our studies, the high experimental concentrations of copepods resulted in decreases of the initial food concentrations of 28–63%. Only the highest decreases could have resulted near the end of a 12 h experiment in a reduction of those maximum clearance rates as observed by Corner *et al.* (1972, their Fig. 1C) for *Calanus helgolandicus*, because in most of our experiments the final food concentrations were in the range of those found *in situ*. Should such a reduction have occurred, then our average hourly clearance rates would have been slightly lower than the rates during most of the 12 h. In contrast to Corner *et al.* (1972), Paffenhöfer & Lewis (1990, their Fig. 3) found no significant reduction in clearance rates for the subtropical copepod *Eucalanus pileatus* when food concentrations decreased from 8 to 2.4 $\mu\text{g C}\cdot\text{l}^{-1}$, the latter being in the range of our lowest final experimental concentrations.

Similar to earlier results on clearance rates of planktonic copepods in relation to food particle size (e.g. Bartram 1981; Bergreen *et al.* 1988), we observed an increase of those rates with particle size, both in *M. clausii* that creates a feeding current, and in *C. furcatus* that captures cells by direct interception (Mazzocchi & Paffenhöfer 1999). This occurs because the percentage of encountered phytoplankton cells, which are actively perceived by a planktonic copepod using a feeding current, increases with the cell volume, *i.e.* for small cells only a small

percentage, and for large cells a high percentage of all cells encountered is actively perceived (Price & Paffenhöfer 1985). Clearance rates of each, *C. furcatus* as well as *M. clausii*, on *in situ* cells did not significantly differ among the three smaller particle ranges (6–8, 8–10, 10–20 μm ESD). This should be related to the variability of the individual rates which is documented in the rather large range of the standard errors (Fig. 1). The variability and paucity of our feeding rate data for *C. furcatus* and *M. clausii* does not allow a meaningful statistical analysis comparing rates on heterotrophs *versus* autotrophs. Simultaneous feeding by calanoids on auto- and heterotrophs has been observed several times (e.g. Kleppel 1993; Verity & Paffenhöfer 1996).

How do the clearance rates of *C. furcatus* and *M. clausii* compare with those from other studies of calanoids of similar weight? The only feeding rate quantifications of planktonic copepods on *in situ* living particles of different size, *i.e.* similar to ours, were those by Bartram (1981) on females of the neritic *Paracalanus parvus*. Adult females of this copepod species move slowly while creating a feeding current. Judging from Fig. 2 of Bartram (1981), the range of clearance rates of this species, obtained at 17–18 °C, are in the range of the rates of *M. clausii* and *C. furcatus* of our study. A closer look reveals that *C. furcatus*' average clearance rates are near the maximum of those of *P. parvus* in all size ranges (Bartram 1981, his Fig. 2E) whereas those of *M. clausii* are close to *P. parvus*' minimum values. While females of *C. furcatus* should be slightly lower in body weight than *P. parvus* (c. 4 $\mu\text{g C}\cdot\text{female}^{-1}$), those of *M. clausii* should be far lighter. The variability in Bartram's results is probably partly due to varying particle concentrations, which ranged from about 10–50 $\mu\text{g C}\cdot\text{l}^{-1}$ (Bartram 1981, Figs 9 and 11). This comparison also reveals that clearance rates of two abundant to dominant calanoids of very different behavior in their mode of particle acquisition can be similar, *i.e.* *P. parvus* perceives food particles via its feeding current whereas the fast-swimming *C. furcatus* does so by direct interception.

Ingestion rates and metabolic requirements

Ingestion rates were obtained by multiplying clearance rates with the respective environmental cell concentrations (Table 2). These *in situ* cell concentrations were chosen as average cell concentrations because the metazooplankton do not affect their abundance *in situ*, largely because of the metazooplankton's low abundance (Webber & Roff 1995a; Paffenhöfer & Mazzocchi 2003), and therefore low grazing effect on environmental cell abundances.

At these cell concentrations, the *C. furcatus* females can cover their metabolic expenses readily (18.4% of its body

C·day⁻¹ at 90% AE) and have considerable amounts of carbon left over for reproduction (Table 2a). Many *C. furcatus* females were producing an egg-mass in the experimental jars. *Clausocalanus furcatus* was often found at temperatures of 25 °C and higher. At a temperature of 25 °C when daily metabolic expenditures would be 20.8% of body C, *C. furcatus* would ingest sufficient amounts for metabolism but with limited reproduction. These findings support the fact that the genus *Clausocalanus* fast-moving is among the dominant copepod taxa in the subtropical and tropical open oceans. At 75% AE, *C. furcatus* ingested sufficient amounts in all but one of the six cases to meet its metabolic demands (data not shown).

Mecynocera clausii, however, which would require 19.7% of its body C daily for metabolic purposes, would cover its metabolic expenditures at only two of the six *in situ* cell concentrations (Table 2b). At 20 °C, a temperature at which it is often found, it would meet metabolic needs at four of the six concentrations. Little if any energy would be left over for reproduction, which may partly explain why it is far less abundant in the epipelagial than genera like *Clausocalanus*, *Oithona* and *Calocalanus* (e.g. Roman *et al.* 1995; Webber & Roff 1995a; Paffenhöfer & Mazzocchi 2003). At an AE of 75% and 20 °C, *M. clausii* could not meet its metabolic needs in any of the six cases (data not shown).

Implications of persistently low food abundance for the copepod community

Our results reveal that *M. clausii* barely obtains sufficient food for metabolic expenditures and *C. furcatus* obtains sufficient amounts for metabolism but only limited amounts of assimilated matter for reproduction. Separate quantitative oceanographic observations (using 63 µm mesh) had revealed that these and other planktonic copepod species always appear to occur in low to very low abundances in subtropical to tropical open ocean waters (Roman *et al.* 1995; Webber & Roff 1995a; Paffenhöfer & Mazzocchi 2003). To what extent would food abundance or, possibly, predation lead to such continuously low abundances? The probability of predation should be relatively low because concentrations of omnivorous copepods in the subtropical open ocean epipelagial (<0.5 l⁻¹) are about an order of magnitude lower than on subtropical shelves when comparing quantitative samples from both environments (southeastern U.S. shelf, Paffenhöfer 1983; Sargasso Sea, Paffenhöfer & Mazzocchi 2003). That implies the probability of encounter with predatory zooplankton (e.g. Gerritsen & Strickler 1977) is most likely very low in the Sargasso Sea.

The continuously low abundances of nanoplankton in subtropical gyres throughout much of the year (except

during the spring bloom, Menzel & Ryther 1961) imply a steady state of food particle availability. While early copepodid stages of *Clausocalanus* spp. and *Oithona plumifera* experience high growth rates, such rates decrease with increasing stage in experiments using natural particulate matter (Webber & Roff 1995b). One may assume, as shown by Bergreen *et al.* (1988), that here also the particle sizes which are perceived as food increase with increasing copepod stage/size from nauplius to adult female. Since the abundance of particles decreases with increasing size in the Sargasso Sea, particles which can be readily perceived by late copepodids and females are scarce, leading to the observed reduced growth rates. This should also manifest itself in a rather low reproductive output, as noted for *Clausocalanus* spp. (Webber & Roff 1995b), and for *C. furcatus* (Mazzocchi & Paffenhöfer 1998). From these rate quantifications one may conclude that available food concentrations and compositions do not affect so much the early juvenile stages but impede growth of the later stages, and also reproduction. Thus it is hypothesized that the continuously low particle abundance is the main variable governing the abundance of the various copepod species by limiting reproduction. Since particle concentrations in subtropical gyres rarely change, and adult females appear to barely obtain sufficient food for metabolic needs and reproduction, many if not most of the epipelagic species seem to live 'on the edge'. Evidence for thin layers of higher levels of nanoplankton for the epipelagial of subtropical and tropical open oceans is scarce (Vinogradov & Shushkina 1976). The most recent results for the open ocean are from Cowles (2003), who found at the Oregon shelf break layers of 1 to several m thickness of chlorophyll levels exceeding those above and below by about 50%.

Since at times environmental food concentrations appear to be insufficient to support reproduction, these copepod species ought to have developed mechanisms permitting them to overcome such periods of food limitation. The mechanisms by which the various copepod species overcome temporary shortages of food in the epipelagial of warm oligotrophic oceans remain unsolved.

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