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Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes

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ABSTRACT: Feeding of the different developmental stages of *Calanipeda aquaedulcis* on natural particles (bacterio-, phyto- and microzooplankton) was measured in a Mediterranean salt marsh (Empordà wetlands, NE Iberian Peninsula). Bottle incubations were performed in the field both in autumn and spring. The results showed differences in the diet of the different developmental stages due to both prey type and size. In general, the size of the ingested prey increased with increasing size of the *C. aquaedulcis* stage. While *C. aquaedulcis* adults had high ingestion rates and selection coefficients for large prey (micro- and nanoplankton), nauplii preferentially consumed smaller prey items (picoplankton). Copepodites showed the widest prey size range, including pico-, nano- and microplankton. Nevertheless, the lower size limit for particle capture was similar for all stages, i.e. between 1.7 and 2.1 µm. Omnivory was observed in all stages of *C. aquaedulcis*. Heterotrophic prey (picoplankton, dinoflagellates and ciliates) were the most ingested items. The ability to partition the available food among the different developmental stages could represent an advantage in times of food scarcity because it may reduce intraspecific competition. This may explain how *C. aquaedulcis* is able to predominate in the zooplankton community for several weeks during spring and summer even in situations of low food availability.

KEY WORDS: Feeding · Developmental stages · Ingestion · Omnivory · Selectivity · Zooplankton

INTRODUCTION

Due to their variations in size and bioenergetic needs during development, copepods must change their diet ontogenetically from nauplii to adult stages (Kleppel 1993, Bonnet & Carlotti 2001). Early stages invest their resources in growth while adults invest in reproduction. The morphology of the feeding appendages in nauplii differs from that of copepodites (Fernandez 1979) and, therefore, their capture of particles is also likely to be different. However, there is little evidence regarding the ontogeny of the composition of copepod diet in nature (but see Poulet 1977), most feeding studies concentrating on feeding and selectivity of adult stages. Research regarding feeding strategies in juvenile stages is still scarce, despite the fact that their abundances may equal or exceed those of adults in natural populations (Calbet et al. 2001).

Some laboratory studies have provided evidence regarding diet changes during development in various calanoid species. For instance, several authors have found differences in particle size consumed by calanoid copepodites and adults (Mullin & Brooks 1967, Paffenhöfer & Lewis 1989), while others found different optimal prey size and attributed it to differences in morphology of mouthparts at different onto-

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genic stages (Fernandez 1979, Bonnet & Carlotti 2001). Nevertheless, other authors have found similar selective behaviour and ingestion rates for all stages (Meyer et al. 2002) and concluded that copepod nauplii occupy the same feeding niche as adult stages (Conover 1982).

Some authors have stated the need to undertake feeding experiments in the field, since results in the laboratory have been different from what has been found in field conditions (Donaghay & Small 1979). The evidence from the few studies done using naturally occurring particles and freshly captured copepods show that selective feeding and diet in nature vary among naupliar, copepodite and adult stages (e.g. Poulet 1977). Such trophic niche segregation among developmental stages favours copepod populations in terms of feeding efficiency increase, intraspecific competition decrease and an increase of immature survival (Poulet 1977). Several copepods are known to be omnivorous. *Euryternora affinis,* for instance, can ingest ciliates or detritus as well as algae (Berk et al. 1977, Heinle et al. 1977). There is increasing evidence that mixed-food diets are beneficial for copepod development (e.g. Stoecker & Egloff 1987, Kleppel 1993, Bonnet & Carlotti 2001), however, information about the relative contribution of the different food sources to *in situ* ingestion is limited.

Calanipeda aquaedulcis is common in brackish and estuarine waters (e.g. Dussart & Defaye 1983). It regularly dominates the zooplankton community (e.g. Quintana et al. 1998) and its developmental stages may coexist for several weeks, especially during spring and summer (Brucet et al. 2006 and references therein). Since *C. aquaedulcis* is often found in conditions of low productivity (Brucet et al. 2006), resource partitioning among stages may be a way to reduce intraspecific competition and maintain stable populations over time. In this sense, a high intraspecific variability in amino acid composition has been found in *C. aquaedulcis*, which could indicate a gradual change in diet during the life cycle of this copepod (Brucet et al. 2005). However, to our knowledge, nothing is known about *C. aquaedulcis* feeding strategies.

The purpose of this study was to (1) characterise the diet of the different developmental stages of *Calanipeda aquaedulcis,* and (2) evaluate the possibility of food resource partitioning among developmental stages of this copepod. The approach included using naturally occurring food particles (bacterio-, phyto- and microzooplankton), to cover the available diversity of food for the different developmental stages and to more closely approximate natural feeding conditions.

Table 1. *Calanipeda aquaedulcis.* Mean and range of size and dry weight of the different developmental stages of *C. aquaedulcis* in this study. N = 25 for each stage

Stage	Size (µm)		Dry weight (µg)	
	Mean	Range	Mean	Range
Nauplii	215	110–440	0.12	0.02–0.49
Copepodites	729	290–1750	1.71	0.02–10.2
Adults	1212	900–1900	4.71	2.38–12.3

MATERIALS AND METHODS

Experimental setup, feeding experiments and sample processing. The study was carried out in the Empordà wetlands (NE Iberian Peninsula), a set of Mediterranean shallow coastal lagoons free from tidal influence and whose hydrological regime is determined by the occurrence of floods due to meteorological disturbances in autumn and winter and the process of desiccation (Brucet et al. 2006). Samples were taken in La Pletera salt marshes, where long confinement periods lead to scarcity of inorganic nutrients and dominance of heterotrophic nano- and microplankters (López-Flores et al. 2006). Expt 1 was carried out in the autumn (November 2003) and Expt 2 in the spring (May 2004) in order to include most potential prey types of *Calanipeda aquaedulcis* in these lagoons during 2 periods of different environmental conditions (flooding and confinement) (López-Flores et al. 2006).

Copepods were collected using a plankton net (50 µm mesh size). Twenty-two Winkler bottles were filled with 250 ml of ambient water filtered through 50 µm mesh, and then different stages of *Calanipeda aquaedulcis* were added. We checked that ciliates and chain-forming diatoms were not retained in the prefilters. Due to the difficulty in separating live individuals of each stage we performed the following grouping of stages: nauplii (from NII to NVI); copepodites (from CI to CV); and adults. Size and biomass ranges of each developmental stage are shown in Table 1. Subsequent to identification under a microscope, nauplii, copepodites and adults were separated into groups of 30, 6 and 2 ind., respectively, and pipetted into distinct bottles. These proportions were equivalent to the natural densities of *C. aquaedulcis* in these lagoons. We incubated 5 bottles for each group and 5 controls. The bottles were incubated in the field for 24 h under natural conditions of temperature and light, corresponding to the sampling depth (10 to 15 cm). Two bottles without copepods were fixed immediately using Lugol's iodine. After the 24 h incubation, copepod mortality was checked. The samples were then fixed with Lugol's iodine for microplankton taxonomic identification and cell-counting with an inverted microscope, and stored in darkness prior to measurements. Bacterioplankton and autotrophic pico- and nanoplankton

samples were filtered through 50 µm mesh, fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration) and immediately deep frozen in liquid nitrogen and stored frozen at –20°C. The abundance and biovolume were calculated with a FACSCalibur flow cytometer (BD Biosciences) with laser emitting at 488 nm (see López-Flores et al. 2006 for protocol details).

For heterotrophic pico- and nanoplankton taxonomic identification and cell-counting, 1 ml of sample fixed with glutaraldehyde was mixed by inversion and left to stain for 10 min with fluorochrome 4′-6-diamidino-2-phenylindole (DAPI; final concentration of 0.5 µg ml^{-1}). Then it was carefully filtered through a 0.2 µm polycarbonate filter (Millipore, Isopore membrane filters). Subsequently, filters were mounted on a glass slide and examined by epifluorescence microscopy with a UV excitation filter block and 1000× oil immersion, and more than 300 ind. were enumerated. By using this procedure, it was possible to locate and differentiate the heterotrophic from the autotrophic pico- and nanoplankton by visualizing the DAPI-stained nuclei (blue) and the chlorophyll *a* autofluorescence (red), respectively (Porter & Feig 1980).

Biovolumes of microplankton, nano- and picoplankton were calculated from measurements of linear dimensions of cells under the inverted microscope or by means of cytometry using appropriate geometric formulae (Hillebrand et al. 1999). Carbon biomass was estimated using the equations of Lee & Fuhrman (1987) for bacterioplankton; Verity et al. (1992) for picoplankton and nanoplankton; Menden-Duer & Lessard (2000) for diatoms, chlorophytes and dinoflagellates; and Putt & Stoecker (1989) for ciliates.

Grazing coefficient, selectivity coefficient and ingestion rate. The grazing coefficient, selectivity and ingestion rate were calculated for each planktonic food type and for each food size group. In the first approach, the potential planktonic food was classified into food types according to taxonomy. We only used food types that were abundant enough to calculate the grazing coefficient, selectivity and ingestion rates and discarded those food types that were only occasionally present in some replicas. In the second approach, the potential planktonic food was subdivided into 3 prey size groups following the accepted decadal size classification: picoplankton (0 to 2 µm); nanoplankton (2 to 20 µm); and microplankton (20 to 50 µm). All prey size groups were based on the longest linear dimensions of planktonic organisms.

Grazing coefficient g (d^{-1}) was calculated according to Frost (1972):

$$g = \mu - \frac{\ln C_1^{\star} - \ln C_0^{\star}}{t_1 - t_0} \quad \text{with} \quad \mu = \frac{\ln C_1 - \ln C_0}{t_1 - t_0}$$

where µ is the gross growth rate of food organisms, C_1 and C_0 are the food concentrations at the end (t_1) and at the beginning (t_0) of the experiment in the controls, and $C_1^{\star}$ and $C_0^{\star}$ are the food concentrations in treatments with copepods.

Selectivity (W') was calculated using the normalized selectivity coefficient W' defined by Vanderploeg & Scaria (1979) and modified after Vanderploeg et al. (1984):

$$W' = \frac{g_i}{g_{\max}}$$

where g_i is the grazing coefficient reached by a certain prey class and $g_{\max}$ is the grazing coefficient for the most preferred prey class ($0 < W' < 1$).

Similarly to the Katechakis et al. (2004) procedure, a *t*-test was used to test whether grazing coefficients (g) were significantly different from 0, if so, W' values were calculated. To test the possible effect of trophic cascade (Broglio et al. 2004), we compared growth rates in control and experimental bottles (*t*-test), so as to detect cases in which prey growth in control bottles was significantly lower than in experimental bottles, suggesting trophic cascade effects were masking grazing.

Clearance rate F (ml ind.$^{-1}$ d^{-1}) and ingestion rate I (pg C ind^{-1} d^{-1}) were calculated using the equations of Frost (1972). Following Nejstgaard et al. (1997), negative clearance rates were interpreted as zero ingestion.

RESULTS

Prey characteristics

Available food in the 2 experiments was relatively different (Table 2). In both experiments picoplankton was composed of bacterioplankton, auto- (APF) and heterotrophic (HPF) picoflagellates. The nanoplankton of Expt 1 included autotrophic nanoflagellates (ANF; chrysophytes and cryptophytes) and chlorophytes and diatoms of less than 20 µm in size. In Expt 2, nanoplankton included ANF and haptophytes. The microplankton of Expt 1 was composed of diatoms (*Navicula* sp., *Nitzschia* spp.) and chlorophytes between 20 and 49 µm in size. In Expt 2, microplankton was composed of autotrophic dinoflagellates (AD; *Glenodinium foliaceum*), heterotrophic dinoflagellates (HD; *Oxyrrhis marina*) and ciliates (*Strombidium* sp.).

In Expt 1, small size prey were the most dominant. Bacterioplankton, APF and HPF were the most abundant prey in terms of number cells and biomass (Table 2). In Expt 2, the most abundant prey were bacterioplankton and APF in terms of numbers of cells, and APF followed by HPF, ciliates, haptophytes and ANF in terms of biomass.

Table 2. Mean (range) of size, volume and carbon content of all food types found in ambient water and offered as food in the experiments. The density and percentage of biomass of each food type in the initial conditions are also shown. APF: autotrophic picoflagellates; HPF: heterotrophic picoflagellates; ANF: autotrophic nanoflagellates; AD: autotrophic dinoflagellates; HD: heterotrophic dinoflagellates. Methodology: C: cytometer; M: inverted microscopy; D: DAPI

Food type	Methodology	Size (µm)		Volume (µm^3)		Carbon content (pgC cell^{-1})		Initial densities (cell ml^{-1})		Biomass (%)
		Mean	Range	Mean	Range	Mean	Range	Mean	SE	
Expt 1										
Bacterioplankton	C	0.73	0.58–0.88	0.22	0.10–0.35	0.08	0.04–0.11	2807934	719772	25.1
APF	C	1.9	1.5–2.2	3.6	1.92–5.89	1.7	0.9–2.7	347124	25521	36.5
HPF	D	2.0	1.7–2.1	4.1	2.6–4.99	2.0	1.2–2.3	232475	9147	38.1
ANF	C	5.7	5.4–6.2	97.4	80.8–122	22.5	19.2–27.4	92.3	16.30	0.16
Chlorophytes	M	30	12–49	892	153–4137	125	24–538	1.45	0.30	0.02
Diatoms	M	30	6–49	1777	18–36907	104	3–1456	52.1	7.20	0.17
Expt 2										
Bacterioplankton	C	0.34	0.28–0.51	0.02	0.01–0.07	0.01	0.01–0.02	4467056	1076257	0.73
APF	C	2.6	2.4–2.7	8.8	6.9–9.9	4.2	3.2–4.7	938771	218800	77.7
HPF	D	2.0	1.7–2.1	4.2	2.6–4.9	2.0	1.2–2.3	170502	131228	8.42
ANF	C	5.9	5.5–6.4	109.9	89.3–134	25.0	20.3–29.7	6152	1324	2.37
Haptophytes	C	11.7	10.6–15.7	871.4	614–2014	148.4	110–307	347	15.1	4.36
AD	M	23	11–35	6116	611–21839	934	145–2720	60.2	0.0	0.55
HD	M	24	17–33	2956	1680–6223	522	333–973	60.2	0.0	0.40
Ciliates	M	22	12–42	3303	479–14394	1074	182–4566	360	46.5	5.44

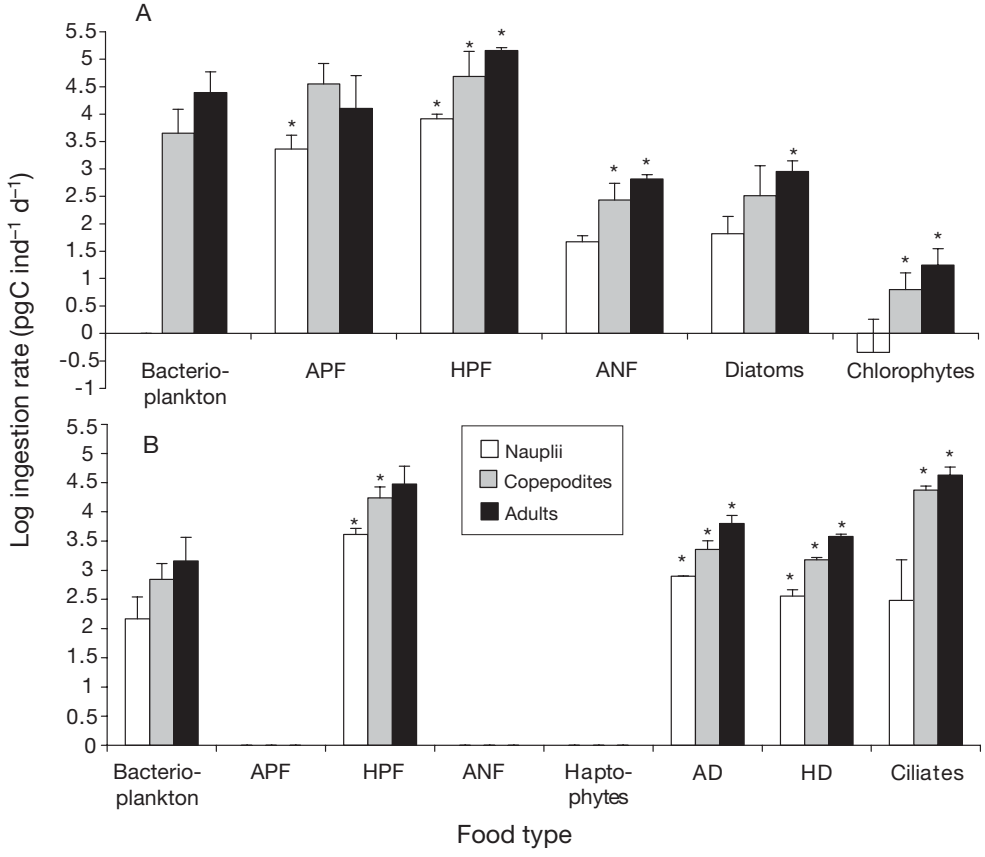

Fig. 1. *Calanipeda aquaedulcis.* Mean ingestion rates of the different developmental stages of *C. aquaedulcis* as a function of food type. (A) Expt 1, (B) Expt 2. *cases in which *g* was significantly different from 0; APF: autotrophic picoflagellates; HPF: heterotrophic picoflagellates; ANF: autotrophic nanoflagellates; AD: autotrophic dinoflagellates; HD: heterotrophic dinoflagellates, Error bars: +SD

Ingestion rates

Calanipeda aquaedulcis showed an omnivorous feeding strategy, with bacterioplankton, autotrophic and heterotrophic phytoplankton and ciliates occurring in their diet (Figs. 1 & 2). Individual clearance rates ranged from 1.1 ml d^{-1} for nauplii to 119 ml d^{-1} for adults (Table 3). In some cases, the high variability between replicates resulted in clearance and ingestion rates higher than 0 but grazing coefficients not significantly different from 0 (Tables 3 & 4). Consequently, although represented in Figs. 1 & 2, we opted not to consider these ingestion rates in the analyses.

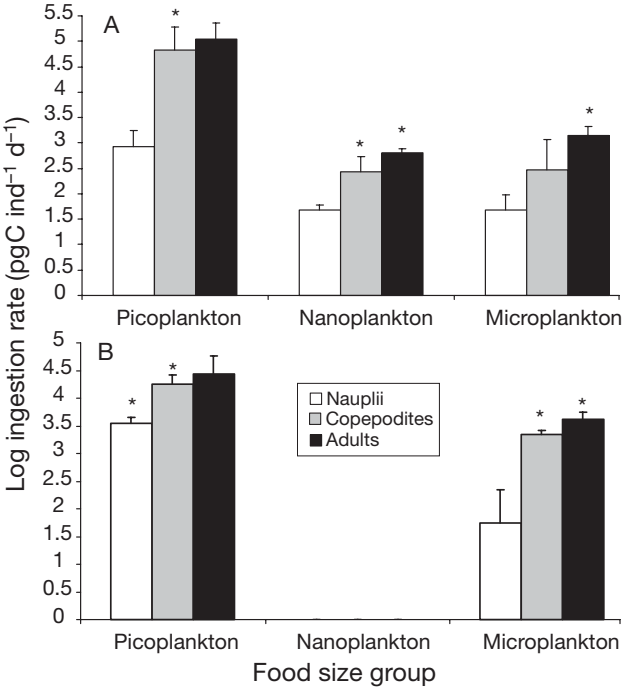

Fig. 2. *Calanipeda aquaedulcis.* Mean ingestion rates in the different treatments as a function of food size groups. (A) Expt 1, (B) Expt 2. *cases in which *g* was significantly different from 0

In Expt 1 (Fig. 1A), all developmental stages had their highest ingestion rates on HPF (up to 0.14 µgC ind.$^{-1}$ d^{-1}). Nauplii were the only stage that consumed APF while copepodites showed some ingestion of ANF and chlorophytes. The width of the prey size spectra tended to increase with developmental stage, with adults having the widest prey size spectrum: apart from HPF, they showed also high ingestion of ANF, diatoms and chlorophytes.

In Expt 2 (Fig. 1B), nauplii again presented maximum ingestion rates of HPF, but copepodites and adults showed their maximum ingestion rates for ciliates. Nauplii did not consume ciliates. Copepodites had the widest prey size spectrum indicated by high ingestion rates for HPF, whereas adults did not consume HPF in this experiment. All stages consumed AD and HD and none of the stages consumed APF, ANF or haptophytes. Indeed, in most of these cases (Table 3) the growth rate of APF, ANF and haptophytes in the treatment bottles was significantly higher than in the control bottles.

Results of ingestion rates based on food size groups (Fig. 2A,B) showed that adults consumed mainly large

Table 3. *Calanipeda aquaedulcis.* Mean (SE) grazing coefficient *g* (d^{-1}), selectivity coefficient *W′* and clearance rate *F* (ml ind.$^{-1}$ d^{-1}) for each food type and for each *C. aquaedulcis* developmental stage in both experiments. Only values significantly different from 0 are indicated. *$p < 0.05$; **$p < 0.01$. Acronyms as in Table 2

Food type	Nauplii			Copepodites			Adults		
	g (d^{-1})	*W′*	*F* (ml ind.$^{-1}$ d^{-1})	*g* (d^{-1})	*W′*	*F* (ml ind.$^{-1}$ d^{-1})	*g* (d^{-1})	*W′*	*F* (ml ind.$^{-1}$ d^{-1})
Expt 1									
Bacterioplankton	0	0	0	0	0	0	0	0	0
APF	0.13* (0.04)	0.21	1.06 (0.36)	0	0	0	0	0	0
HPF	0.60* (0.18)	1.00	5.02 (1.57)	0.98** (0.27)	1.00	50.2 (26.5)	0.95** (0.04)	1.00	119 (11.9)
ANF	0	0	0	0.67** (0.15)	0.69	31.8 (10.8)	0.42* (0.11)	0.50	51.5 (18.3)
Diatoms	0	0	0	0	0	0	0.93* (0.31)	0.97	86.2 (22.8)
Chlorophytes	0	0	0	0.70** (0.21)	0.92	24.4 (7.38)	0.87* (0.30)	0.96	70.0 (22.2)
Expt 2									
Bacterioplankton	0	0	0	0	0	0	0	0	0
APF	0	0	0	0	0	0	0[a]	0	0
HPF	0.27* (0.04)	0.31	3.93 (0.54)	0.43* (0.11)	0.70	21.5 (6.93)	0	0	0
ANF	0[a]	0	0	0[a]	0	0	0[a]	0	0
Haptophytes	0[a]	0	0	0[a]	0	0	0[a]	0	0
AD	0.85* (0.23)	1.00	10.7 (2.90)	0.48* (0.13)	0.78	22.2 (8.81)	0.52* (0.16)	0.89	63.5 (20.9)
HD	0.64* (0.26)	0.66	7.07 (1.48)	0.84* (0.00)	1.00	28.0 (1.47)	0.84* (0.00)	1.00	70.0 (6.36)
Ciliates	0	0	0	0.35* (0.02)	0.58	15.7 (1.37)	0.23* (0.04)	0.38	25.2 (4.48)

[a]Prey number in the control bottles is significantly lower than in the experimental bottles (*t*-test, $p < 0.05$), suggesting possible trophic cascade effects

Table 4. *Calanipeda aquaedulcis.* Mean (SE) grazing coefficient g (d^{-1}), selectivity coefficient W', and clearance rate F (ml $ind.^{-1}$ d^{-1}) for each food size group and for each *C. aquaedulcis* developmental stage in both experiments. Only values significantly different from 0 are indicated. $^{*}p < 0.05$; $^{**}p < 0.01$

Food size groups	Nauplii			Copepodites			Adults		
	g (d^{-1})	W'	F (ml $ind.^{-1}$ d^{-1})	g (d^{-1})	W'	F (ml $ind.^{-1}$ d^{-1})	g (d^{-1})	W'	F (ml $ind.^{-1}$ d^{-1})
Expt 1									
Picoplankton	0	0	0	0.24* (0.06)	0.03	13.86 (6.27)	0	0	0
Nanoplankton	0	0	0	0.61** (0.14)	1.00	31.79 (10.8)	0.42* (0.11)	0.45	51.5 (18.3)
Microplankton	0	0	0	0	0	0	0.92** (0.30)	1.00	312 (64.1)
Expt 2									
Picoplankton	0.25* (0.05)	1.00	3.15 (0.60)	0.38* (0.08)	1.00	18.68 (5.07)	0	0	0
Nanoplankton	0[a]	0	0	0[a]	0	0	0[a]	0	0
Microplankton	0	0	0	0.36* (0.03)	0.95	16.14 (1.87)	0.25* (0.04)	1.00	27.7 (5.28)

[a]Prey number in the control bottles is significantly lower than in the experimental bottles (*t*-test, $p < 0.05$), suggesting possible trophic cascade effects

prey: the highest ingestion rates in both experiments were for microplankton, and they also consumed nanoplankton in Expt 1. Copepodites showed the highest ingestion rates for picoplankton in both experiments but, while in Expt 1 they fed also on nanoplankton, in Expt 2 they ate microplankton. Nauplii preyed on the smallest sizes (picoplankton) in Expt 2, while in Expt 1 g was not significantly different from 0.

Selectivity

The W' selective coefficients were different among nauplii, copepodites and adults and also between the 2 experiments (Table 3). During Expt 1, all stages showed maximum selectivity coefficients for HPF. Adults and copepodites had also high selectivity coefficients for chlorophytes (between 20 and 49 µm) and ANF. Adults also selected diatoms. Nauplii selected HPF and to a lesser extent, APF. In Expt 2, all stages had higher selectivity coefficients for HD than for HPF and only nauplii and copepodites showed some selection for HPF. Indeed, the most preferred prey for copepodites and adults were HD while nauplii selected mainly AD. Ciliates were selected by copepodites and adults but not by the nauplii.

When analysing W' coefficients by means of the food size groups (Table 4), differences among copepod stages increased. In both experiments, adults showed the highest preference for microplankton and in Expt 1 they also selected nanoplankton. Copepodites selected mainly nanoplankton but also picoplankton in Expt 1 and picoplankton and microplankton in Expt 2. Nauplii did not select any prey size in Expt 1 even though they showed a high selection for picoplankton in Expt 2.

DISCUSSION

Results of this study show that the diet of *Calanipeda aquedulcis* is diverse, which is in accordance with previous findings for other calanoid species (Kleppel 1993). Furthermore, differences in the ingestion rates of certain prey between the 2 experiments support the hypothesis of a flexible feeding behaviour that may be modified with variability in the food environment. For example, in the presence of ciliates and HD and AD, adults did not ingest HPF.

Omnivory was observed in all stages of *Calanipeda aquaedulcis*. HPF were the most ingested and one of the most preferred prey items. Ciliates were also ingested in large quantities by copepodites and adults, and HD were selected by all stages. Previous findings also reported that copepods can ingest ciliates and HD at higher rates than phytoplankton, and may preferentially select the former (Stoecker & Egloff 1987, Sanders & Wickham 1993, Nejstgaard et al. 1997). This fact has been attributed to the high nutritional value of ciliates and heterotrophic flagellates since they can produce essential copepod growth compounds (unsaturated fatty acids and/or sterols) that are not always found in phytoplankton (Oman & Runge 1994) and they are relatively rich in nitrogen and phosphorous (Sanders & Wickham 1993). The mixture of phytoplankton and ciliates has been found to be the most favourable for development, growth and egg production for some copepods (Bonnet & Carlotti 2001); however, this is not always the case, since the presence of ciliates and heterotrophic dinoflagelates in the diet may sometimes not provide copepods with an adequate nutrition for long-term survival (Koski et al. 1998). Our results showed that in the absence of cili-

ates and HD, *C. aquaedulcis* ingested high amounts of HPF. However, when different types of heterotrophic prey were present, adults of *C. aquaedulcis* preferred ciliates and HD to HPF, while copepodites ingested all 3 at similar rates.

According to our results, none of the developmental stages of *Calanipeda aquaedulcis* significantly prey on bacteria. Usually, bacterioplankton was considered too small to be efficiently ingested by most adult copepods, but nauplii of some species have been shown to feed upon bacterioplankton (Turner & Tester 1992) and some feeding on free-living bacteria has been recorded in adults (Boak & Goulder 1983).

In Expt 1, significant ingestion of APF and ANF was recorded, while in Expt 2 an increase of these prey items in experimental bottles with respect to control bottles was observed. This could be due to trophic cascade effects, which have been observed in other feeding behaviour studies dealing with the whole size spectrum (Broglio et al. 2004, López-Flores et al. 2006). Grazing of *Calanipeda aquaedulcis* on ciliates and dinoflagellates, both of which consume small particles (Broglio et al. 2004), could result in a decrease in the grazing mortality of APF, ANF and haptophytes with a consequent increase in their growth rate. The fact that the increase in APF, ANF and haptophytes was only observed in Expt 2 when ciliates and dinoflagelates were present supports this hypothesis.

This study shows differences in the diet of the developmental stages of *Calanipeda aquaedulcis* due both to the size and type of prey. In general, the size of the ingested prey increases with an increasing size of the developmental stage, which is in accordance with other studies (Poulet 1977, Berggreen et al. 1988). While *C. aquadulcis* adults had high ingestion rates and selection coefficients for large prey (micro- and nanoplankton), nauplii preferentially removed small prey (picoplankton). Copepodites showed the widest prey size range, including pico-, nano- and microplankton. Nevertheless, the lowest size limit of captured particles was similar for all stages (between 1.7 and 2.1 µm), and is similar to what has been found for other species: e.g. between 2 and 4 µm for all developmental stages of *Acartia tonsa* (Bergreen et al. 1988), 1 µm for *Eurytemora affinis* copepodites and adults (Burkill & Kendall 1982), 3 µm in *Pseudodiaptomus marinus* nauplii and copepodites (Uye & Kasahara 1983) and 1.5 µm in *Pseudocalanus minutus* copepodites and adults (Poulet 1977).

Studies dealing with the size spectra that each stage is capable of capturing have obtained contradictory results. Some authors have stated that nauplii are unable to capture prey effectively at the extreme ends of the size spectrum (Fernandez 1979, Paffenhöfer & Lewis 1989) since they do not develop mature feeding appendages until copepodid stage Cl (Björnberg 1986). For example, nauplii of *Eucalanus* sp. were not able to capture small prey as efficiently as later stages (Paffenhöfer & Lewis 1989) and nauplii of *Calanus helgolandicus* could not consume large diatoms that were fed upon by adults (Mullin & Brooks 1967). In contrast, nauplii of *Acartia tonsa* were more efficient than adults in capturing small prey (Bergreen et al. 1988). Additionally, some studies have documented ingestion of protozoan microplankton by copepod nauplii (Fessenden & Cowles 1994). For example, nauplii of *Acartia tonsa* and *Eurytemora* may ingest ciliates up to 40 µm (Stoecker & Egloff 1987) and 35 µm (Merrell & Stoecker 1998) in size, respectively. According to our results, *Calanipeda aqueadulcis* nauplii were not able to capture larger prey (chlorophytes, diatoms and ciliates) which were readily consumed by adults, but they did consume dinoflagellates between 11 and 35 µm in size. Both copepodites and adults ingested prey within the same range, i.e. 1.7 to 49 µm. Although it is generally reported that copepods tend to be inefficient at filtering particles smaller than 5 to 10 µm (e.g. Berggreen et al. 1988), several authors have documented a significant ingestion of particles <5 µm by adults and copepodites (Boak & Goulder 1983, Nejstgaard et al. 1997, Broglio et al. 2004). Nevertheless, the high preference for small cells found in copepodites and adults in this study appears not to be previously documented. It might be explained by the fact that, as already reported in other costal waters (Gasparini & Castel 1997), in the Empordà wetlands, the largest particles were scarce compared to the large amount of smaller particles. In such conditions, Richman et al. (1977) demonstrated that copepods graze predominantly on small size prey, probably because they shift their grazing pressure to the size where the peak concentration of particles occurs (Poulet 1977). This could explain the high ingestion of picoplankton in Expt 1 where larger particles were almost non-existent.

The different *Calanipeda aquaedulcis* life stages showed a different selective behaviour since the size of the selected prey increased with increasing size of the stage. According to our selectivity metrics, prey type also determined selective behaviour since, in general, all stages showed a preference for heterotrophic forms among preys of similar size. These results would confirm the previous studies that show that adult copepods are able to select between particles of the same size but different nutritive value: plastic beads versus phytoplankton (Fernandez 1979), fast-growing versus senescent cells or different growing states of the same species (Koski et al. 1998) or toxic versus non-toxic strains of the same or similarly sized algae (Turriff et al. 1995). In contrast, Meyer et al. (2002) showed similar

selection behaviour among stages of *Calanus* spp. and only depending on size.

These differences in feeding among developmental stages of *Calanipeda aquaedulcis* might be related to the changes in the amino acid composition during the life cycle of this copepod found in a previous study in the same lagoons (Brucet et al. 2005). Indeed, several studies have shown a high variation in the elemental composition during the life cycle of calanoids (e.g. Carrillo et al. 2001), which would agree with ontogenic changes in their diet. On the other hand, the ability to partition the available food among the different developmental stages would represent an advantage when food is scarce since it reduces intraspecific competition. This could be the reason why *C. aquaedulcis* is able to dominate the zooplankton community for several weeks during spring and summer even in situations of low nutrient content (Quintana et al. 1998, Brucet et al. 2006 and references therein). The dietary differences among developmental stages have already been described to be important for reducing competition in environments where there is a scarcity or high temporal variability of food resources (Poulet 1977).

In summary, *Calanipeda aquaedulcis* is feeding omnivorously on a wide spectrum of natural food particles and its diet can change throughout ontogeny and also with food availability. As previously reported for other copepod species (e.g. Poulet 1977), early stages of *C. aquaedulcis* can have feeding niches partially separated from the adults. Hence, further studies should take into account the intraspecific variability in the feeding behaviour of copepod species in order to fully understand the mechanisms that structure pelagic food webs.

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