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Feeding ecology of the marine cladoceran *Penilia avirostris*: natural diet, prey selectivity and daily ration

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ABSTRACT: *Penilia avirostris* is an important component of the mesozooplankton of shelf waters during the stratification period. This study investigates the feeding ecology of *P. avirostris* under natural dietary conditions in the NW Mediterranean during their summer appearance. The results indicate that *P. avirostris* feeds on particles in a wide size range, mostly on nanoplankton (2 to 20 µm), including larger prey such as dinoflagellates and ciliates. Autotrophic and heterotrophic bacteria were not grazed on, but picoflagellates (<2 µm) were significant contributors to diet, indicating a narrow threshold of lower prey size. Ciliates were cleared at low rates which, at times, were not significant, probably because of their ability to escape feeding currents. Although this marine cladoceran is considered to behave like a filter feeder, our results indicate that it can display variable selectivity patterns that do not only depend on prey size. During the strict oligotrophic conditions that were present in summer NW Mediterranean waters, daily rations of *P. avirostris* averaged 82% body C d^{-1} (range: 26 to 157%). Such feeding rates seem to be an adaptation to allow them to succeed under low food conditions and outcompete other components of the marine zooplankton, such as copepods.

KEY WORDS: *Penilia avirostris* · Natural diet · Grazing rates · Selectivity · Nanoflagellates · Diatoms · Dinoflagellates · Ciliates

INTRODUCTION

The ecological role of any organism is determined by its position and significance in food webs. Decisive characteristics are body size, food spectrum and feeding strategies. Feeding is one of the most important processes for zooplankton because it supplies the requirements to maintain its production and activity. Also, it is the main route for the transfer of energy and matter from lower to higher trophic levels (Valiela 1995, Båmstedt et al. 2000). Therefore, in order to understand the dynamics of pelagic food webs, it is important to know how consumers select their food and at what rate they ingest prey.

Classically, most of the studies on feeding rates and the factors controlling them in marine planktonic systems have concentrated on copepods, the major component of the mesozooplankton. However, in recent decades, other groups like appendicularians, ctenophores, or medusae have received more attention, because of their relevance to food web dynamics (López-Urrutia et al. 2003, González et al. 2004, Maar et al. 2004). In the present study we focus on one of these other groups, the marine cladocerans.

In contrast to freshwaters, where cladocerans are the dominant mesozooplanktonic group, with >600 species recorded, cladocerans have not been ecologically successful in the marine environment (there are only 8 true marine species; Egloff et al. 1997). According to this low diversity and the overall low abundance found in the oceans, especially in cold and high-latitude ecosystems, cladocerans have been commonly neglected in marine autoecological studies. However, marine cladocerans can, in fact, build up a large fraction of the zooplankton standing stock in many coastal and even oceanic environments on a seasonal basis, and probably, on these occasions, play a major role in the dynamics of planktonic food webs (Turner et al. 1988, Kim et al. 1989). Of all marine cladocerans, only one species has been described as a true filter feeder,

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the sidid *Penilia avirostris*, which inhabits near-shore waters of tropical and warm-temperate areas all over the world (Della Croce 1964, Onbé 1985, Onbé & Ikeda 1995, Calbet et al. 2001, Marazzo & Valentin 2001).

Only a few studies have reported on the feeding of *Penilia avirostris* (e.g. Paffenhöffer & Orcutt 1986, Turner et al. 1988, 1998, Katechakis & Stibor 2004). Among those, to our knowledge, no studies encompassing the whole spectrum of naturally occurring autotrophic and heterotrophic prey types have been conducted to characterise the feeding ecology of this cladoceran. This lack of information has made it difficult to establish the relevance of *P. avirostris* in marine planktonic communities. Here, as part of a series of studies focused on the ecological role of *P. avirostris* in marine food webs, we investigated the feeding rates of *P. avirostris* under a broad range of natural diets during their seasonal peak of abundance and determined their prey size spectrum. Our aim was to describe the dietary-composition and prey-selectivity patterns of this cladoceran on different components of the microbial community and to establish the variation in their food uptake as a function of prey availability.

MATERIALS AND METHODS

We conducted 8 grazing experiments at either of 2 stations located in coastal waters (ca. 1 mile from coast) off Masnou and Barcelona (Spain) during the summers of 2002 and 2003. Both sampling sites have similar characteristics and constitute shallow waters on the open coast. Further details on hydrographic characteristics and zooplankton composition are found in Cebrián et al. (1996) and Calbet et al. (2001).

Water for experiments was collected at 1 m depth with a transparent hydrographic bottle and transported to the laboratory within 1 h. The water was gently poured into a 50 l bucket and reverse-flow filtered by gently submerging a 30 cm diameter polyvinyl chloride cylinder fitted with a 100 µm mesh bottom. Once the prey suspension was ready, it was amended with a nutrient mixture (15 µM NH_4Cl and 1 µM Na_2HPO_4) to compensate for nutrient enrichment due to zooplankton excretion.

Penilia avirostris were collected by short oblique net tows with a Juday–Bogorov net (200 µm mesh, 40 cm diameter) fitted with a 5 l plastic bag as the cod end. Once on board, the bag mouth was tied with a string to prevent *P. avirostris* from sticking to the air–water interface. The samples were transported to the laboratory in an isothermic container within 1 h of collection.

The experiments consisted of incubations in Pyrex bottles (625 ml for experiments during 2002; 1200 ml for experiments during 2003), filled with the natural microbial community (<100 µm) and added grazers *Penilia avirostris* for the experimental treatments. Groups of *P. avirostris* were sorted with a wide-mouthed pipette under the stereomicroscope and placed in the experimental bottles (33 ind. l^{-1}). In total, 4 experimental bottles and 4 control bottles (without grazers) were incubated for 24 h on a plankton wheel (0.2 rpm) in a temperature-controlled room at *in situ* temperature and photoperiod (Table 1). Three additional bottles were used to determine the initial prey concentrations. After incubation, the water was gently poured through a 135 µm sieve to collect the grazers, which were checked for activity and preserved with formalin (4% final concentration).

The microbial components analysed in the grazing experiments were heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, pico- and nanoflagellates (<2, 2 to 5 and >5 µm), dinoflagellates, diatoms (single and in chains) and ciliates.

Samples (2 ml) for heterotrophic bacteria *Prochlorococcus* and *Synechococcus* were preserved in paraformaldehyde + glutaraldehyde (1 + 0.05% final concentrations, respectively) and stored at −80°C until analysis. Flow cytometry analysis was conducted with a FACScalibur flow cytometer following the procedures in Gasol & del Giorgio (2000). Heterotrophic bacteria biomass was estimated from volume determinations (V) using the relationship µg C = 0.12 $V^{0.7}$ (Norland 1993). *Prochlorococcus* and *Synechococcus* biomasses were determined after assuming a carbon content of 0.123 pg C $µm^{-3}$ and equivalent spherical diameters (ESD) of, respectively, 0.60 and 1.0 µm (Waterbury et al. 1986).

For the assessment of pico- and nanoflagellate abundance, samples were preserved with glutaralde-

Table 1. Experimental conditions and carbon content (ind.$^{-1}$; mean ± SE) of the cladocerans incubated in the experiments conducted

	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7	Expt 8
Date (dd/mm/yy)	23/07/02	14/08/02	04/09/02	26/09/02	22/07/03	29/07/03	07/08/03	12/08/03
Temperature *in situ* (°C)	24.2	23.8	23.1	22.0	26.5	25.5	26.1	27.1
Temperature incubation (°C)	23.8	23.0	23.3	22.0	25.8	25.5	26.8	27.1
Body mass (µg C ind.$^{-1}$)	0.57 (0.06)	0.70 (0.07)	0.60 (0.01)	0.84 (0.03)	0.67 (0.05)	0.49 (0.02)	0.50 (0.01)	0.50 (0.02)

hyde (1% final concentration) for 3 to 6 h (4°C in dark), filtered onto 0.8 µm black polycarbonate membrane filters and stained with DAPI (4′,6-diamidino-2-phenylindole; 5 µg ml^{-1} final concentration) (Porter & Feig 1980). At least 300 cells were counted and classified according to size (<2, 2 to 5 and >5 µm) using epifluorescence microscopy (Olympus BX40). For the <2 µm fraction, a nominal size of 2 µm was assumed; for the other 2 categories, 40 cells were measured in each case. The carbon per cell was estimated using a factor of 0.22 pg C µm^{-3} (Børsheim & Bratbak 1987).

For the determination of dinoflagellate, diatom and ciliate concentrations, 200 ml samples were preserved with 1% acidic Lugol's solution. In 2003 we became aware of a potential source of ciliate losses due to the sieving of water to collect *Penilia avirostris* at the end of the experiments prior to withdrawing the samples. We conducted an additional experiment to investigate the effects of this standard procedure by comparing it with gentle, direct siphoning into the preservation bottles. Significant differences were found between treatments (aloricated ciliates: unsieved = 1.20 ± 0.01 SE, sieved = 0.79 ± 0.01 SE; loricated ciliates: unsieved = 2.92 ± 0.01 SE, sieved = 2.02 ± 0.07 SE; 1-way ANOVA, $p < 0.05$), the sieving process reducing the ciliate abundance by ca. 30%. Because of this result, samples were gently siphoned directly into bottles containing the required dose of acidic Lugol's. Data belonging to previous experiments (2002) were corrected accordingly. Ciliate abundance (in all experiments) was further increased by a factor of 30% to correct for losses due to Lugol's preservation (Broglio et al. 2004, Calbet & Saiz 2005). Then, 100 ml aliquots were allowed to settle in Utermöhl chambers and counted in their entirety with an inverted microscope. Digital pictures of at least 60 cells of each type were taken and sized. Cell volumes were converted into carbon content using a factor of 0.19 pg C µm^{-3} for ciliates (Putt & Stoecker 1989), the equation log (pg C cell^{-1}) = 0.811 (log V) – 0.541 for diatoms and the equation log (pg C cell^{-1}) = 0.819 (log V) – 0.119 for dinoflagellates (Menden-Deuer & Lessard 2000).

The relation between body length (L) and dry weight (DW) of *Penilia avirostris* was also determined. Groups (10 to 15) of similar-sized individuals were measured from the tip of the head to the base of the caudal setae (Uye 1982), placed on pre-combusted and pre-weighed aluminium caps and dried at 55 to 60°C for 24 h. Dry weight measurements (µg) were made on an ATI CAHN C-35 microbalance. Fig. 1 shows the relationship between size and biomass for *P. avirostris*. The fitted equation was used to estimate the biomass of cladocerans in the grazing experiments. Carbon content was assumed to be 50% of dry weight (Uye 1982).

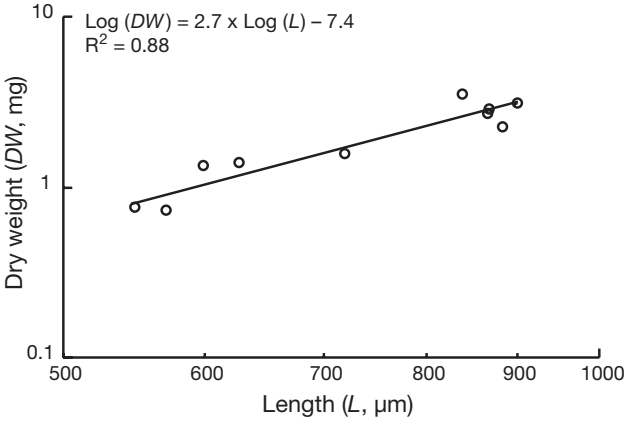

Fig. 1. *Penilia avirostris*. Length–dry weight relationship

Clearance and ingestion rates were calculated for each prey type according to Frost (1972), after verification that prey growth rates in grazing bottles were significantly different and lower than in the controls (1-way ANOVA, 2-tailed $p < 0.05$). Bacterial growth rates were never decreased by the presence of *Penilia avirostris*, in fact, on occasion, they were enhanced.

Clearance rates for diatoms were computed separately for single cells and those in chains. Diatom chains were not very long, ranging between 2 to 3 and 6 cells chain^{-1}. To take into account potential changes in chain length after grazing activity, clearance rates were computed based on the number of cells per chain at the start and end of the incubations. This procedure warranted the computation of actual ingestion rates.

Selectivity patterns of *Penilia avirostris* were determined through the normalised selectivity coefficient W' (Vanderploeg 1994). This coefficient was calculated from clearance rates (F_i) for the different groups of i prey as $W_i' = F_i / F_{pref}$, where F_{pref} is the clearance rate for the preferred prey (i.e. maximum clearance rate observed in the incubation). By definition, preferred prey have a selectivity coefficient of $W'_{pref} = 1$, whereas non-eaten prey have $W_i' = 0$. This selectivity index is independent of both the number of prey and the prey type included in the analysis (Vanderploeg 1994). The selectivity coefficients were computed within each experiment based on the average clearance values (between replicate bottles) for each prey considered. In order to determine the F_{pref} in an experiment, we initially conducted a Hsu's test for the 'best' (significance level 0.05, after previous ANOVA test; Hsu 1981) for finding the maximum clearance rates exhibited by *P. avirostris* for each prey type in that experiment. According to the results of the Hsu tests, all prey items, which were cleared at maximal rates, were allocated a selectivity coefficient of 1.

RESULTS

Table 2 shows the initial microbial community of each experiment. In terms of biomass, heterotrophic bacteria dominated the microbial community, with concentrations ranging between 17.6 and 122.2 µg C l^{-1}. Medium (2 to 5 µm) and large (>5 µm) sized flagellates were the next most important contributors (respectively, 3.6 to 15.5 and 4.3 to 29.1 µg C l^{-1}). Dinoflagellates, diatoms and ciliates were the lowest contributors to the bulk of plankton, with some exceptions (Expt 5 and Expt 6, chain-forming diatoms). Total community biomass ranged between 49.2 and 208.4 µg C l^{-1}. Clearance rates of *Penilia avirostris* were highly variable and covered a broad prey size spectrum, ranging from <2 µm up to 30 µm ESD (Table 3). Autotrophic and heterotrophic bacteria were not grazed upon in any of the experiments (in some incubations they were even significantly enhanced). Regarding the other components of the microbial community, flagellates (the 3 size classes) were regular components of the diet, with clearance rates ranging

Table 2. Initial size (equivalent spherical diameter [ESD], µm), abundance (Abund., cells ml^{-1}) and biomass (µg C l^{-1}) of the different components of the microbial community. Abundance of heterotrophic bacteria (HetBact; $\times 10^6$) (total biomass: accumulated microbial biomass for each experiment [µg C l^{-1}]; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: flagellates; Dinoflag: dinoflagellates; Single-Diat: single diatoms; Chain-Diat: chain-forming diatoms; Avg.: mean values [±SE])

	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7	Expt 8	Avg.
HetBact									
ESD	0.51	0.51	0.52	0.54	0.54	0.50	0.51	0.52	0.52 (0.01)
Abund.	1.00	0.87	1.73	0.72	2.33	0.67	0.41	0.25	0.99 (0.24)
Biomass	41.2	17.6	24.7	22.9	122.2	42.1	21.6	20.7	39.1 (12.32)
Proch									
ESD	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60[a]
Abund.	7533	2577	10937	8983	188	514	8051	1123	4988 (1530)
Biomass	0.10	0.04	0.15	0.12	0.003	0.01	0.11	0.02	0.1 (0.02)
Synec									
ESD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00[a]
Abund.	95983	70000	39139	43252	17238	30535	29442	35928	45190 (9029)
Biomass	6.2	4.5	2.5	2.8	1.1	2.0	1.9	2.3	2.9 (0.58)
<2 µm Flag									
ESD	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.00[a]
Abund.	3706	3850	1955	4770	6809	4710	3764	3644	4151 (487)
Biomass	3.4	3.5	1.8	4.4	6.3	4.3	3.5	3.4	3.8 (0.45)
2–5 µm Flag									
ESD	4.1	3.7	3.8	3.9	3.9	3.8	3.6	3.7	3.82 (0.02)
Abund.	808	2298	553	1106	2190	2408	1906	1896	1646 (254)
Biomass	6.2	13.2	3.6	7.4	14.8	15.5	10.3	11.5	10.3 (1.51)
>5 µm Flag									
ESD	8.2	8.0	7.7	8.7	8.0	7.6	7.9	7.5	7.94 (0.06)
Abund.	193	321	80	384	291	195	191	159	227 (35)
Biomass	12.1	19.2	4.3	29.1	16.9	9.7	10.7	7.6	13.7 (2.78)
Ciliates									
ESD	21.6	25.4	18.8	23.1	14.8	15.0	17.3	19.6	19.51 (0.62)
Abund.	0.8	0.6	1.9	9.1	4.4	3.4	3.9	3.0	3.4 (0.9)
Biomass	0.8	1.0	1.2	11.0	1.4	1.1	2.0	2.3	2.6 (1.22)
Dinoflag									
ESD	17.3	30.5	27.9	18.8	14.2	14.4	20.4	20.0	17.02 (1.38)
Abund.	4.7	4.7	1.4	2.0	3.0	3.2	2.0	4.5	3.2 (0.5)
Biomass	2.3	9.3	2.2	1.2	0.9	1.0	1.5	3.1	2.7 (0.99)
Single-Diat									
ESD	8.0	15.2	13.8	8.0	8.3	10.9	9.3	7.3	11.34 (0.60)
Abund.	55	12	19	20	64	33	3	2	26 (8)
Biomass	1.5	1.5	2.0	0.5	1.8	1.9	0.1	0,05	1.2 (0.29)
Chain-Diat									
ESD	12.6	19.7	20.6	1.4	13.3	17.4	11.7	9.1	16.43 (0.66)
Abund.	27	2	25	18	463	254	0.1	2	99 (60)
Biomass	2.2	0.6	6.7	1.1	43.0	45.0	0,01	0.1	12.3 (6.95)
Total biomass	76.0	70.4	49.2	80.5	208.4	122.6	51.7	51.0	

[a]Assumed values (see 'Materials and methods')

Table 3. *Penilia avirostris*. Weight-specific clearance rates (ml μg^{-1} C d^{-1}; mean ± SE). Growth: values in experimental bottles higher than controls; 0: no significant ingestion; HetBact: heterotrophic bacteria; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: flagellates; Dinoflag: dinoflagellates; Single-Diat: single diatoms; Chain-Diat: chain-forming diatoms

	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt 6	Expt 7	Expt 8
HetBact	0	0	0	0	Growth	Growth	0	Growth
Proch	Growth	Growth	Growth	0	Growth	Growth	Growth	0
Synec	Growth	0	0	0	Growth	0	0	0
<2 µm Flag	41 (4.4)	10 (0.8)	15 (0.2)	13 (0.5)	19 (1.2)	32 (1.9)	34 (1.7)	17 (1.0)
2–5 µm Flag	35 (4.3)	24 (0.9)	10 (0.7)	45 (2.4)	19 (2.1)	33 (1.8)	40 (2.4)	39 (3.5)
>5 µm Flag	23 (2.5)	39 (3.9)	7 (0.3)	19 (0.9)	17 (1.8)	43 (2.3)	30 (1.7)	33 (1.8)
Ciliates	0	0	0	0	10 (0.5)	31 (4.3)	0	8 (0.8)
Dinoflag	31 (3.3)	38 (3.4)	0	25 (1.9)	25 (1.3)	44 (3.5)	24 (3.1)	31 (1.2)
Single-Diat	19	0 (2.0)	0	19	2 (3.2)	54 (0.44)	45 (5.4)	0 (9.8)
Chain-Diat	0	0	41 (7.3)	22 (4.4)	5 (0.5)	16 (1.0)	100 (25.0)	23 (3.0)

between 7 and 45 ml μg^{-1} C d^{-1}. Dinoflagellates and diatoms, although frequently representing an important contribution to the diet, were occasionally not grazed significantly by *P. avirostris*. Ciliates were rarely consumed by *P. avirostris* (in only 3 out of 8 experiments).

When relating the feeding rates of *Penilia avirostris* to food availability, the inclusion of bacteria in food availability bulk estimates might lead to erroneous conclusions, since they were not grazed on. From this point on, we excluded bacteria from calculations and defined *edible food* as that microbial biomass prone to being ingested by the cladoceran. Fig. 2 shows the composition of the edible food for *P. avirostris* in the experiments conducted. The initial edible microbial community was dominated in terms of biomass by flagellates, which comprised >50% of the carbon in most of the experiments, with secondary contributions of dinoflagellates (Expt 2), diatoms (Expt 3, Expt 5 and Expt 6), or ciliates (Expt 4).

Ingestion rates in terms of daily rations ranged between 26 and 157% body C d^{-1} and were positively related to total edible prey biomass (Fig. 3). No evidence of saturation was observed over the range of edible food biomass found during the studied period.

Regarding selectivity, we found significant differences among clearance rates for the different prey types within each experiment (1-way ANOVA, $p < 0.05$; Table 3), which is an indication of selection. Fig. 4 shows the selectivity coefficients W' as a function of the relative edible prey availability. No clear recurrent pattern was identified. A positive linear relationship is suggested in Expt 2, Expt 3 and Expt 8, whereas a negative relationship seems to appear in Expt 6. In other experiments no trend was observed (Expt 1, Expt 4, Expt 5 and Expt 7). A more intriguing result of this analysis, however, is that *Penilia avi-*

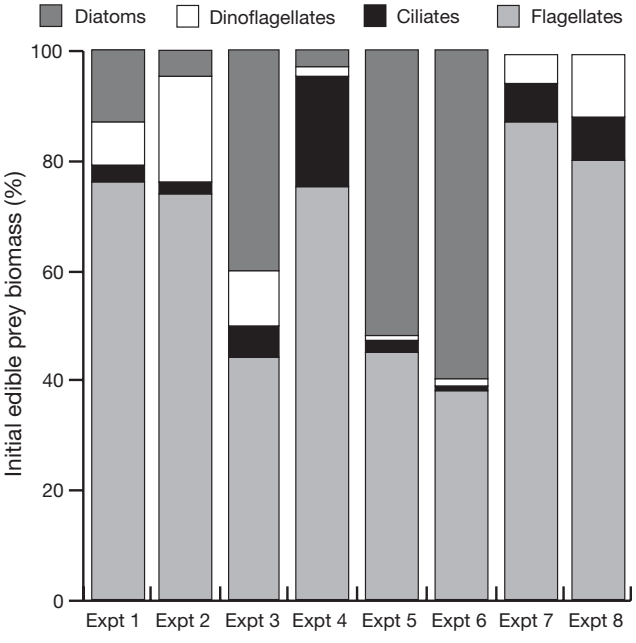

Fig. 2. Biomass contribution (as %) of the different microbial groups considered (excluding the bacterioplankton), at the beginning of the experiments

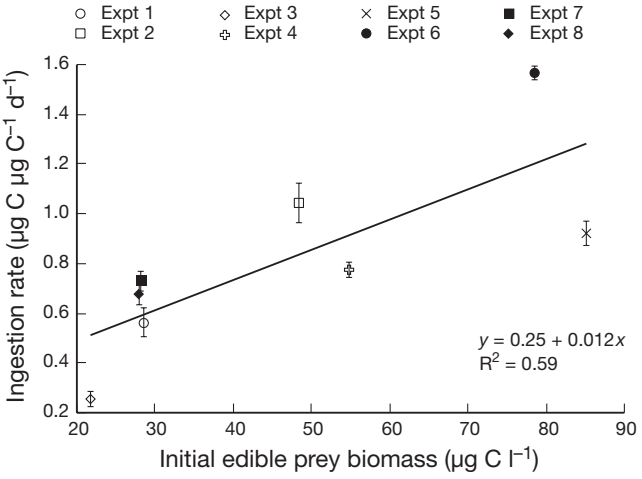

Fig. 3. *Penilia avirostris*. Relationship between ingestion rates and initial edible prey biomass. Error bars represent ±1 SE

rostris, in spite of being typically considered a passive filter feeder, displayed variability in prey preference among experiments, independent of prey type and also independent of prey contribution to available biomass. For instance, if we consider a particular prey type like the 2 to 5 µm flagellates, in 3 out of 8 experiments they constitute the preferred prey ($W' = 1$), whereas in the rest of experiments they show medium (W' between 0.6 and 0.8), or very low preference (W' between 0.2 and 0.4), independent of their relative contribution to food availability (Fig. 4). There does not seem to be dependence on the initial food composition either, as can be seen by simply comparing the patterns in W' in the selectivity graphs for Expt 1, Expt 3, Expt 7 and Expt 8 (Fig. 4), all conducted at a similar initial food concentration.

DISCUSSION

Lower prey size threshold in *Penilia avirostris*

This study is one of the few to illustrate the feeding ecology of the marine cladoceran *Penilia avirostris* under natural dietary conditions, thus estimating the grazing of *P. avirostris* confronted with a wide spectrum of natural microbial prey. The clearance rates determined in the present study lie within the same range of values as in previous reports of *P. avirostris* feeding on heterotrophic flagellates (34 to 96 ml ind.$^{-1}$ d^{-1}; Turner et al. 1988), diatoms (14 to 40 ml ind.$^{-1}$ d^{-1}; Turner et al. 1988), the Haptophyciae *Isochrysis galbana* (4 to 24 ml ind.$^{-1}$ d^{-1}; Paffenhöfer & Orcutt 1986), ciliates (4 to 33 ml ind.$^{-1}$ d^{-1}; Broglio et al. 2004), and on different components of the microbial community (20 to 30 ml ind.$^{-1}$ d^{-1}; Katechakis et al. 2004).

One of the intriguing and ecologically relevant questions about the feeding of *Penilia avirostris* is whether or not they are capable of grazing on bacterial and picoflagellate communities and therefore whether or not they directly impact the microbial loop, similar to their freshwater relative *Daphnia*. Daphnids are important consumers of natural bacteria and small flagellates (Jürgens 1994), their ability to feed on small particles being determined by the morphology of the filtering apparatus (mesh size of the filter; Lampert 1987, Gerritsen et al. 1988). Impacts on picoplanktonic communities of about 50% due to daphnids are frequent in freshwater planktonic food webs (Degans et al. 2002, Zollner et al. 2003).

In the case of *Penilia avirostris*, there has been controversy about its ability to feed on picoplankton. Some studies have suggested that bacteria could be an important source of carbon for this cladoceran (Pavlova 1967, Paffenhöfer & Orcutt 1986, Lipej et al. 1997). However, Turner et al. (1988) concluded that this organism could ingest relatively large or clumped bacteria, but not natural bacterioplankton (of a smaller size). This latter conclusion agreed with the observations of Gore (1980), who reported preferential ingestion for *P. avirostris* on plastic beads >1 µm. Our results confirm the observations made by Turner et al. (1988), showing that natural heterotrophic and phototrophic bacteria cannot be grazed by *P. avirostris*. However, our study also evidences that the gap for the lower prey size threshold is very narrow, because <2 µm flagellates were significantly consumed in all experiments (albeit, overall, at lower clearance rates). Hence, the threshold for the minimum prey size for *P. avirostris* seemed to fall between the size of *Synechococcus* (1 µm) and the <2 µm flagellates. The disagreement between our results and the recent work of Katechakis & Stibor (2004), where no grazing on <2.5 µm prey was observed, is probably due to the methodology used by those authors to preserve and quantify bacteria and small flagellate samples. Our conclusions are further confirmed by the observations of intrasetular distances (1 to 2 µm) under light microscopy for our Mediterranean Sea specimens, which are also in agreement with the ≤2 µm values under scanning electron microscopy reported by Turner et al. (1988) for West Atlantic *P. avirostris*.

One might expect, however, that there would be genetic and ontogenetic differences in the intersetular gap, which could explain some of the variability observed and the discrepancies among studies reported. The fact that the intrasetular distance increases with body size in daphnids (Brendelberger & Geller 1985) suggests that perhaps younger (smaller) *Penilia avirostris* than the individuals used in our experiments might extend their prey size spectrum to smaller items

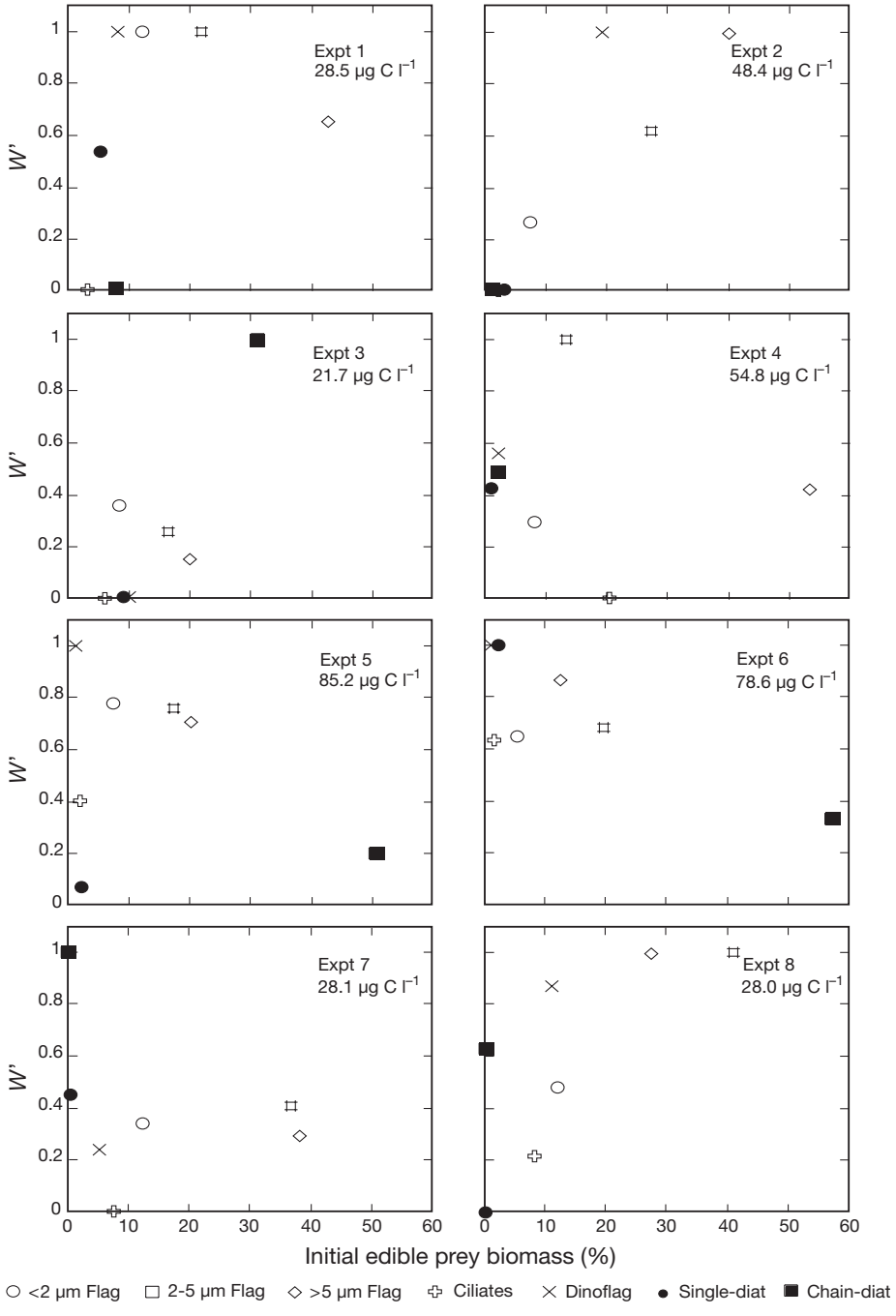

Fig. 4. *Penilia avirostris*. Relationship between selectivity coefficient *W'* and prey contribution to initial edible biomass. Flag: flagellates; Dinoflag: dinoflagellates; Single-Diat: single diatoms; Chain-Diat: chain-forming diatoms

than the ones reported here. Other factors could also affect the lower prey size thresholds reported here. For example, changes in the hydrophobicity or electrostatic charges of the cell membrane (see 'Discussion') could affect the retention efficiency of the phyllopods. Due to the narrow gap for the lower prey size threshold observed, slight variations in the average size of the picoplanktonic fraction would result in variability in the clearance rates for <2 µm flagellates displayed by *P. avirostris*.

From an ecological point of view, the fact that *Penilia avirostris*, in contrast to *Daphnia*, cannot graze on heterotrophic and autotrophic bacteria indicates that this marine cladoceran cannot exert direct control on bac-

terial production. But the ability of *P. avirostris* to feed on <2 µm flagellates leads to a trophic shortcut to the microbial loop very close to its base, resulting in more efficient transfer of energy towards the upper consumers compared to other marine zooplankters like copepods. In addition, *P. avirostris* may have indirect effects on bacterial production by grazing on the first-order bacterivorous grazers (<2 µm flagellates).

Prey selectivity by *Penilia avirostris*

Penilia avirostris is a typical filter feeder that, similar to daphnids, is expected to largely exhibit passive and mechanical selection, wherein phyllopods form a mesh that retains particles (Paffenhöfer & Orcutt 1986, Turner et al. 1988). Our observations seem to contradict, or at least do not fully confirm, such a theory.

In the present study we found relatively high clearance rates for flagellates, diatoms and dinoflagellates, whereas other prey were either not grazed upon because of their size (i.e. bacteria) or were grazed at low rates probably because of their ability to escape from feeding currents (ciliates; Jakobsen 2001, 2002). Our results partially contrast with those of Katechakis & Stibor (2004) and Katechakis et al. (2004), who reported maximum ingestion rates by *Penilia avirostris* in the prey range between 15 and 70 µm (equivalent to 6–28 µm ESD), corresponding to diatoms and dinoflagellates. However, the comparison is difficult because their investigation was conducted under very enriched conditions (up to 575 µg C l^{-1}), which were not representative of the natural microbial community where *P. avirostris* is found in the Mediterranean.

The 3 major dietary components in our study (flagellates, dinoflagellates and diatoms) alternated their role as preferred prey from experiment to experiment. Flagellates were the preferred prey on 6 occasions, and dinoflagellates and diatoms constituted the preferred prey, respectively, 4 and 3 times. It is also important to note that, often, there was >1 preferred prey for a given experiment. These variations in *Penilia avirostris* preferences are puzzling, and do not seem to be related to either prey size (for instance in Expt 1) or prey abundance (either in relative or absolute terms). It is possible that part of this preference for certain prey could be artifactual. For instance, the feeding activity of *P. avirostris* may break diatom chains apart, resulting in a higher selection for diatom chains compared to single cells (diatom chains disappear from incubation bottles, whereas single diatom cells increase their abundance). Trophic cascade effects could also mask real patterns. Feeding on both <2 and 2 to 5 µm flagellates by *P. avirostris* may result in a release of grazing pressure on <2 µm flagellates by heterotrophic and mixotrophic 2 to 5 µm flagellates, therefore masking the feeding of *P. avirostris* on those smallest flagellates. This hypothesis does not seem to hold, however, because in 5 out of the 8 experiments W' was the same for <2 and 2 to 5 µm flagellates (Fig. 4). There is also a possibility that variations in *P. avirostris* body size among experiments could explain the variations in W' patterns. However, we explored this possibility by measuring the intrasetular distance of 2 animals from each experiment and found no difference. Changes in surface properties, such as hydrophobicity or the electrostatic charge of the cell membranes of both prey and cladoceran phyllopods, have also been considered plausible explanations for such changes in selectivity (Lampert 1987, Vanderploeg 1994).

Besides these hypothetical explanations for our results, there is evidence in daphnids that prey-selectivity patterns can be modified by the individual to some extent, as a function of size and quality. Vanderploeg (1994) reviewed such particle-selection mechanisms in freshwater cladocerans. Very large particles (e.g. filamentous algae) can be rejected by the abdominal claw or just by decreasing the carapace gap. Particles can be rejected even when contained in boluses. Further evidence is given by Lampert & Brendelberger (1996), who reported that *Daphnia* can adjust the area of the filter screens and the appendage beat rate as a function of food concentration. The application of these behaviourally driven mechanisms of prey selection in the closely related *Penilia avirostris* seems plausible. In fact, Turner et al. (1988) observed that *P. avirostris* mostly ingested small heterotrophic flagellates (2 to 5 µm) and diatoms (4 to 12 µm), but was unable to graze upon the intermediate-sized *Pseudoisochrysis paradoxa* (5 to 6 µm). Paradoxically, Paffenhöfer & Orcutt (1986) reported ingestion of the similarly sized *Isochrysis galbana*. Therefore, we feel that the variability in prey preference observed in our experiments reflect true changes in selectivity by *P. avirostris*, although the mechanisms underneath are not fully clear.

Feeding performance of *Penilia avirostris*

In the present study, *Penilia avirostris*' maximal daily food rations (157% body C d^{-1}) were higher than those reported previously (Pavlova 1967, Paffenhöfer & Orcutt 1986), but similar to the maximal value (151%) reported by Broglio et al. (2004) at food concentrations (phytoplankton and ciliate) of 125 µg C l^{-1}. Our results also agree with estimates for the freshwater cladoceran *Daphnia* (52 to 115% body C d^{-1}, Burns & Schallenberg 2001; 100 to 157% body C d^{-1}, DeMott et al. 1998; 148 to 312% body C d^{-1}, Sterner et al. 1993). More notable is the efficient performance of

Table 4. Comparison of daily food rations (*DR*, % body C d^{-1}) at low food availability (*C*, µg C l^{-1}) for *Penilia avirostris* and other zooplankters

Penilia avirostris[a]		*Oikopleura dioica*[b]		*Oikopleura dioica*[c]		Calanoid copepods[d]	
C	*DR*	*C*	*DR*	*C*	*DR*	*C*	*DR*
22	26	80	131	40	87	20–40	17
28	73	320	214	80	198	40–70	24
28	68	481	281	150	347	70–100	27
29	56	641	88				
48	104	1602	97				
55	78						
79	157						
85	92						

[a]Present study, field data
[b]Acuña & Kiefer (2000), laboratory experiments
[c]Selander & Tiselius (2003), laboratory experiments
[d]E. Saiz & A. Calbet (unpubl. data), field data obtained from a review of literature

P. avirostris at the low food concentrations that characterise its natural habitat, with daily rations up to 73% at concentrations <30 µg C l^{-1}. This high efficiency of *P. avirostris* under oligotrophic conditions may explain its fast blooming and dominance in coastal zooplankton during the stratification period, where oligotrophic conditions prevail. However, it poses the question why *P. avirostris* does not spread to the oligotrophic open-ocean waters. Very likely the dependence on resting embryos in the sediment for the successful development of a new seasonal cohort in the following year restricts its habitat to shelf waters. This geographic restriction also constrains *P. avirostris* on a seasonal basis to the stratification period, because the richer conditions of shelf waters during the rest of the year may have deleterious effects (clogging) on their filter-feeding appendages (Paffenhofer & Orcutt 1986). At similarly low food concentrations, the daily food rations of copepods are <30% body C d^{-1} (Table 4). Copepods are less efficient feeding on small prey (<5 µm, Bergreen et al. 1988), which are the major contributors to seston biomass under such oligotrophic conditions (Agawin et al. 2000). We can compare *P. avirostris* to another important filter feeder in coastal waters as well. The appendicularians perform similarly well at equivalent food concentrations (Table 4), with the peculiarity that they also can do well in richer waters and ingest bacterioplankton (Scheinberg et al. 2005). Having a similar or even better feeding performance than *P. avirostris*, it is not clear why appendicularians do not dominate summer coastal waters in the NW Mediterranean (Calbet et al. 2001).

In this study we have characterised the feeding ecology of *Penilia avirostris*, the only filter-feeding cladoceran in the marine environment. *P. avirostris* ingests a broad spectrum of prey types, their diet being constituted mainly of flagellates, dinoflagellates and diatoms. In contrast to a typical, passive filter feeder, *P. avirostris* shows behaviourally driven plasticity in their prey selection by mechanisms not fully understood. The species seems to be optimally adapted to oligotrophic environments, exhibiting relatively high daily rations compared to the most common marine zooplankters (copepods).

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