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Retention efficiency of 0.2 to 6 µm particles by the appendicularians *Oikopleura dioica* and *Fritillaria borealis*

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ABSTRACT: We used suspensions of 0.2, 0.5, 0.75, 1, 2, 3 and 6 µm fluorescent beads in combination with analytical flow cytometry to determine the efficiency of retention by small (165 µm trunk length), medium (347 µm) and large (689 and 734 µm) *Oikopleura dioica*, and by large (585 µm) *Fritillaria borealis*. Large *O. dioica* and *F. borealis* were the most efficient at retaining the 2 µm beads, and small and medium *O. dioica* were most efficient for 1 µm beads. Large *O. dioica* and *F. borealis* showed efficiencies of ca. 15% for 0.2 µm, 33% for 0.5 µm, 58% for 0.75 µm beads, 66% for 1 µm beads and 88% for 2 µm beads. However, small *O. dioica* showed higher efficiencies, measuring ca. 10% for 0.2 µm, 43% for 0.5 µm, 72% for 0.75 µm beads, 87% for 1 µm beads and 93% for 2 µm beads. The combination of our measured appendicularian particle-retention efficiency spectra with typical particle size-distribution spectra in the ocean indicates that large and small appendicularians obtain 80% of their diet from particles smaller than 15 and 7 µm respectively, and that the smallest particles represent a significant part of their diet only when they strongly dominate the biomass size spectra. Comparison with data from the literature indicates that although the appendicularian:prey length ratio is extremely high, the appendicularian:prey body-carbon ratio (14 538:1) is within the reported range for mesozooplankton (1:1 to ca. 3×10^6:1), and statistically undistinguishable from that of copepods (1603:1).

KEY WORDS: Appendicularian · Retention efficiency · Prey size · Diet · Tunicate · *Oikopleura* · *Fritillaria*

INTRODUCTION

Small particles play a crucial role in marine biogeochemistry. An important fraction of the organisms comprised within the microbial loop, which dominate in oligotrophic conditions, is within the 0.2 to 2 µm size range (Legendre & Le Fevre 1991). The marine colloidal phase, operationally defined as particles smaller than 1 µm in size (Wells & Goldberg 1994), contains more than 250 Gigatonnes of carbon (Wells 1998). Therefore, small particles represent a significant food source for filter-feeding organisms and, in turn, processes involving a significant mobilisation of these particles are potentially important for global carbon fluxes. In this context, it is important to make observations and develop models which can predict the size and quantity of particles captured by filter feeders down to the submicronic size range, and to evaluate their role as a direct transfer of carbon between the submicronic compartment and upper trophic levels. For this, it is necessary to determine the efficiency of retention of submicrometric particles.

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Appendicularians concentrate suspended matter using a mucous, external filtering device or filter house with extremely small pores (i.e. 0.24×0.07 µm in *Oikopleura dioica*, Flood 1978; see also Flood & Deibel 1998), which has led to speculation about their ability to retain submicronic particles efficiently, including colloids. Although observations confirm that appendicularians do concentrate and ingest colloids (i.e. sepia ink test, Flood et al. 1992), there are indications that their retention efficiency in the submicronic range is limited. Ultrastructural studies of the food-concentrating filter pore-size of *O. vanhoeffeni* have shown that its dimensions (Deibel et al. 1985) are smaller than the pore size of its pharyngeal filter (Deibel & Powell 1987), an internal filter that is ultimately responsible for particle retention and ingestion. According to experimental studies, *O. vanhoeffeni* reaches 100% retention efficiency for particles above 1 µm in size, while particles between 0.6 and 1 µm in size are retained with lower efficiencies of between ca. 40 and 60% (Deibel & Lee 1992), which confirms that many of these particles are not retained by the pharyngeal filter.

Deibel & Lee (1992) showed that small *Oikopleura vanhoeffeni* are more efficient than large ones in retaining submicronic particles. By extrapolation, it is thus possible to estimate that smaller appendicularian species are more efficient than larger ones in retaining small particles. While *O. vanhoeffeni* is a cryophilic species restricted to polar waters, smaller appendicularians of the genera *Oikopleura* and *Fritillaria* are nearly ubiquitous (Fenaux et al. 1998) and often dominate mesozooplankton assemblages by number. Moreover, Deibel & Lee (1992) based their study on the analysis of gut contents, thus they studied the 'Retention Efficiency of the Animal' (hereafter REA) but not the combined 'Retention Efficiency of Animal plus House' (REAH), that is, the actual effect of appendicularians on suspended particle assemblages. These efficiencies should differ substantially if there is some degree of recirculation, within the house, of particles not retained by the pharyngeal filter (as suggested by Deibel 1986, Bedo et al. 1993, Flood 1991).

In this study, we report the measurements of REA and REAH for 2 small and widespread appendicularians, *Oikopleura dioica* and *Fritillaria borealis*, of 0.2 to 6 µm fluorescent microspheres using analytical flow cytometry. This has allowed us to test the hypothesis of particle recirculation within the filter house: a flat REAH spectrum would indicate that all particles entering the house are recirculated and do not exit the house. We also wanted to test whether the small appendicularians used in our experiments retain small particles with higher efficiencies than other large oikopleurids for which particle retention efficiencies are known. We have also combined particle retention spectra with particle size distributions in the ocean to arrive at an estimation of the potential contribution of different particle sizes to the appendicularian diet. Finally, we have also compared our results with those of other mesozooplankton taxa, to elucidate whether the prey size in appendicularians does or does not depart from what we should expect from predators of a similar size.

MATERIALS AND METHODS

Experimental procedures. The REA and REAH of *Oikopleura dioica* and *Fritillaria borealis* were determined by offering mixed suspensions of phytoplankton and fluorescent microspheres to cultured organisms. Although some studies have identified discrimination against beads in some taxa (i.e. Ayukai 1987 in copepods; Ooms-Wilms et al. 1993 in rotifers), grazing experiments conducted with *O. dioica* resulted in no difference between grazing rates on fluorescently labelled beads and on natural phytoplankton in the same experimental conditions (outlined in Bedo et al. 1993). Cultures of *O. dioica* were initiated with fertilised eggs, from cultures maintained at the University of Oviedo (see Acuña & Kiefer 2000 for details), brought to the Plymouth Marine Laboratory after a 24 h trip in a 1 l glass bottle filled with natural seawater at ambient temperature. Fertilized eggs of *F. borealis* were obtained from seawater collected in buckets off Plymouth, maintained in glass jars in a walk-in controlled temperature room at 15°C, and kept in suspension by means of spiral plexiglas paddles rotating at 10 rpm (Fenaux & Gorsky 1979, 1985). Once the newly hatched *F. borealis* could be spotted inside the jars, they were transferred to fresh 30 µm filtered seawater, using wide-bore pipettes, every 3 d.

Cultures of the unicellular chlorophyte *Chlorella stigmatophora* (3.5 µm equivalent spherical diameter [ESD], reference number PCC 85 of the Plymouth Culture Collection) and the prasinophyte *Tetraselmis suecica* (5.8 µm ESD, reference number PCC 305) were kept in 2 l bottles at 15°C and used as food for the experiments. Algal cultures were centrifuged at 1500 RCF (relative centrifugal force) for 10 min, the supernatant eliminated and the pellet resuspended 3 times in filtered seawater. Final concentration of the algal stock was determined with a Coulter Multisizer II. To estimate carbon content from cell size we used the formulae of Strathmann (1967).

Fluorescent microspheres (Polysciences) measuring 0.2 (yellow green, YG), 0.5 (PC Red), 0.75 (YG), 1 (PC Red), 2 (YG), 3 (YG) and 6 µm (YG) in diameter were used. They were soaked for 24 h in bovine serum albumin (5 mg ml^{-1}) to avoid clumping (Pace & Bailiff 1987),

and resuspended in triple-filtered seawater. Preliminary experiments showed that the proportion of beads which stick together in twos and threes was always less than 1% of the total particle count for each bead size. A flow cytometer (Becton Dickinson FACSort), together with a flow cytometry data analysis program (WinMDI 2.8 software), was used to count and analyse the fluorescence and size of the microspheres. Side scatter versus green- and orange-fluorescence dot plots were used to discriminate between the different bead sizes (Fig. 1).

Experimental suspensions were prepared with triple-filtered seawater (using 0.2 µm Millipore membrane filters). Subsequently, different amounts of the algae *Tetraselmis suecica* or *Chlorella stigmatophora*, and beads of different size and fluorescence, were added in previously defined combinations. All the experiments were performed with mixtures of phytoplankton and beads, with beads representing 10% of the total volume. The target carbon concentration was 150 µg C l^{-1}, after applying the same phytoplankton volume:carbon ratio to both phytoplankton and beads. The relative number of each bead size in the experimental suspension was carefully adjusted to increase the resolution of our measurements by maximising the number of beads detected by the flowcytometer. This adjustment was done on the basis of reported filtration rates of appendicularians (King et al. 1980, Alldredge 1981), number of individuals pooled (7 to 25, depending on their size), sample volume required by the flowcytometer (300 µl) and on preliminary size-retention experiments, to ensure that at least 50 beads of each size were counted in each sample by the flowcytometer. This implies that we chose a minimum resolution of one fiftieth of the actual filtration rates. However, we routinely counted many more than 50 beads, thus, our resolution was usually much greater (i.e. the number of 6 µm beads counted in gut content analysis was 198 ± 95, mean ± SD, and ranged between 51 and 435; these were the less abundant beads). The experimental suspension with algae and beads was continuously stirred and protected from light to avoid algal growth, bead clumping or fluorescence loss.

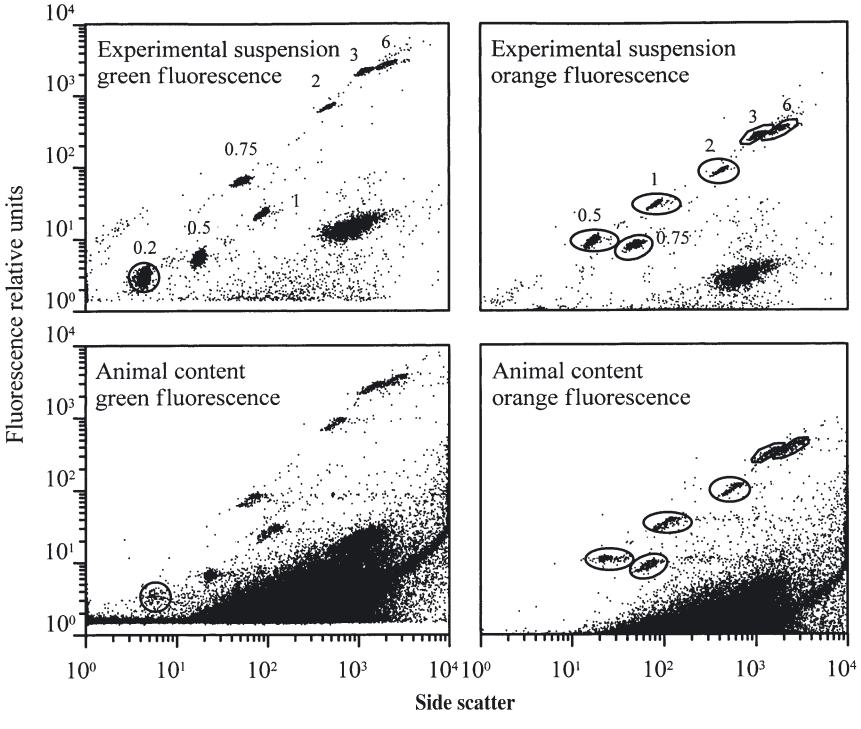

Fig. 1. Example of flowcytometer plots representing side scatter (directly related to particle size) versus green- and orange- fluorescence. Upper panels: microspheres in the experimental suspension; lower panels: microspheres in appendicularian guts. Green fluorescence was used to discriminate 0.2 µm microspheres, while orange fluorescence was used for 0.5, 0.75, 1, 2, 3 and 6 µm microspheres. Oval shapes delimit the boundaries used to discriminate the number of each bead size. Axis units are relative. The thick, small, densely packed cluster of points in the lower part of the upper panels, and the thick and much larger cluster of points in the lower part of the lower panels correspond to *Tetraselmis* sp. cells before and after being ground with a tissue grinder, respectively. Note that there is nearly no overlap between these clusters of points and the ones corresponding to microbeads

Two types of incubation were performed. In one, groups of individuals without houses were isolated for gut content analysis and analysed as a single unit, therefore these incubations allowed for measurement of the REA. In the other type, groups of individuals inside their houses were analysed as a single unit, which allowed the measurement of REAH, and showed the size spectrum of particles removed from the environment. We did not consider separate houses in our measurements, that is, houses torn apart from their animals, because it is possible that some beads are lost when the animals are forced to abandon the house, and because particle accumulation in the house can be estimated by combination of REAH and REA measurements. Prior to incubation, appendicularians were acclimatized for at least 4 h in a suspension of algae in filtered seawater at the target food concentration. *Fritillaria borealis* did not tolerate acclimatization in an algal suspension (i.e. they escaped the houses), therefore we used natural seawater to which a mixture of beads were added (10% by volume), and performed no acclimatization. In this species, it was extremely difficult to separate animals from houses, therefore only REAH were measured.

Five to fifteen individuals (depending on their size) were added to 100 ml incubation vials filled with 75 ml of experimental suspension, and incubated for 5 min, less than the gut passage time (8.6 min for *Oikopleura dioica*, López-Urrutia & Acuña 1999, 5.6 ± 0.6 min for *Fritillaria borealis*, based on video observation of the gut transit times of fluorescent beads in 5 individuals), which ensures no defecation of ingested beads and no changes in food concentration. To stop the incubation, the organisms were removed with wide-bore pipettes, rinsed twice in triple-filtered (0.2 µm Millipore) seawater, placed into cryovials and flash frozen in liquid nitrogen (preliminary experiments showed that the beads do not break or aggregate when frozen in liquid nitrogen). Samples were thawed afterwards and individuals alone or plus houses were counted and their trunk length measured under the microscope, selected individually and, because individual measurements of REA and REAH were not possible, pooled in groups of 7 to 25, depending on their size. Trunk length in *O. dioica* corresponds to the distance between the mouth and distal end of gonads if mature. Trunk length in *F. borealis* corresponds to the distance from mouth to end of the trunk, excluding the 'horns'. Trunk length of thawed animals did not significantly differ from that of living animals (*t*-test for dependent samples, $p = 0.15$). The samples were diluted to ≤1 ml with filtered seawater, ground with a tissue grinder to release gut contents, and sonicated (recommended to homogenize and avoid clumps; Deibel & Lee 1992). Preliminary experiments showed that the number of beads of each size class do not change significantly after grinding and sonication.

Since the flowcytometer did not analyse the full size-range of beads used, 2 measurements of number of beads were conducted for each sample, one for the smallest beads (0.2, 0.5 and 0.75 µm) and another for the biggest (0.75, 1, 2, 3 and 6 µm). The number of beads of 0.75 µm did not differ when measured in either range (*t*-test for independent samples, $p = 0.66$), thus we used measurements for these beads as a common value cross-reference to combine all measurements. The number of beads in suspension was calculated as the arithmetic mean number of beads in the incubation water samples collected at the beginning and end of the incubation. The number of beads in these samples did not differ from those from the stock suspension without appendicularians, both analysed every 15 min during the experiment (matched pairs *t*-test for samples at the beginning vs at the end of the incubation, $p = 0.33$; samples at the end of the incubation vs stock suspension, $p = 0.17$).

The number of beads in each sample was divided by the number of individuals pooled to obtain mean values per individual. Clearance rates (ml h^{-1}) were calculated for each bead size, according to the average number of beads inside the animals or inside animals plus houses, and the number of beads in the seawater suspension. Differences in the clearance rate (CR) among bead sizes are attributable to differences in retention efficiency. To transform these CRs into particle retention efficiencies, we calculated mean CR for each bead size. The highest of these mean CRs was then arbitrarily assigned a retention efficiency of 1, and all of the individual measurements of CR were then scaled to estimate retention efficiencies accordingly. This procedure yields results that are indistinguishable from these obtained using more elaborate indexes of prey selection, and in some cases the result is algebraically identical.

Since we know of no reports on the CRs of *Fritillaria borealis*, or of any other fritillarid, and since these animals can be extremely abundant, we conducted an additional experiment with 6 µm beads to measure the CRs of individuals of different sizes in natural seawater, which allowed us to build a trunk-length clearance-rate relationship.

Development of empirical particle-size retention models for *Oikopleura dioica* and *Fritillaria borealis*. We used empirically determined retention efficiencies to produce quantitative, continuous models to predict the retention efficiencies of the full range of ingestible particle sizes. Typically, the retention efficiency spectrum had 2 regions: one of rapid linear increase with particle size, and another of slow increase, no increase, or even decreasing retention efficiency (see 'Results'), which was set to 1 or 0 if it intercepted the 100 or 0% retention efficiency for particles smaller than the maximum ingestible size. To determine the threshold particle size marking the transition between regions, we performed a regression analysis of CR versus bead size. We first started with data corresponding to the 3 smallest beads, and sequentially added data from the next larger bead size to recalculate new regression statistics. Whenever data from the region of rapid linear increase were added, the correlation coefficient increased. A decrease in the correlation coefficient when adding data from a larger bead size indicated that this bead size was in the region of slow increase, no change or decrease in the retention efficiency. Data in the 2 regions were simultaneously fitted by a segmented non-linear routine (SPSS statistical package). The model was extended up to the maximum ingestible bead size, which was calculated according to the pore size of the inlet filter of the house in the case of *Oikopleura dioica* (pore width = 6.82 + 0.0137 × trunk length, where trunk length includes the gonads, both in µm, from Kiefer & Acuña unpubl. data).

Modelling particle-size composition in the diet of *Oikopleura dioica*. The particle retention efficiency of a filter feeder is not a direct indication of the particle-size composition of the diet, which also depends on the

relative abundance of each particle size. Therefore, the contribution of each particle size to the diet ultimately depends on the particle-size biomass-spectrum in the ocean. It has been shown that the biomass of particles in the ocean often follows a power law of the type $\theta(\varpi) = a(\varpi)^b$, where $\theta(\varpi)$ is a biomass density function, ϖ is particle weight (hereafter we will use ϖ for the particle weight as a variable, and W with or without some subscript when particle weight is used as a limit of integration) and a and b are parameters. According to this density function, the biomass of particles whose weights are comprised within the size range from W_1 to W_2, is given by:

$$B(W_1, W_2) = \int_{W_1}^{W_2} \theta(\varpi)\, d\varpi = \int_{W_1}^{W_2} a\varpi^b\, d\varpi \quad (1)$$

Since our particle retention efficiencies are a function of particle diameter, Eq. (1) has to be set as a function of particle diameter rather than weight. If we assume that the particles are spherical, and that they all share a similar density, then we can accept that particle weight varies with particle diameter cubed, that is:

$$\varpi = \sigma\delta^3 \quad (2)$$

where σ is a scale factor and δ is the particle diameter (hereafter we will use δ for the particle diameter as a variable, and P with or without some subscript when particle diameter is used as a limit of integration). This implies that:

$$\delta = \left(\frac{\varpi}{\sigma}\right)^{\frac{1}{3}} \quad (3)$$

which, after reorganization and derivation, becomes:

$$d\varpi = 3\sigma\delta^2 d\delta \quad (4)$$

According to Eq. (3), we can calculate the particle diameters corresponding to the weights that serve as integration limits for Eq. (1) as:

$$P_1 = \left(\frac{W_1}{\sigma}\right)^{\frac{1}{3}} \text{ and } P_2 = \left(\frac{W_2}{\sigma}\right)^{\frac{1}{3}}$$

We may also substitute the ϖ and $d\varpi$ terms in Eq. (1) by the right-hand side of Eqs. (2) & (4) respectively, to arrive to an expression for the biomass of particles whose diameters are comprised within the size range from P_1 to P_2:

$$B(P_1, P_2) = \int_{P_1}^{P_2} a3\sigma^{b+1}\delta^{3b+2}\, d\delta \quad (5)$$

The ingestion rate of a filter feeder is calculated by the product of filtration rate multiplied by the particle retention efficiency and multiplied by particle concentration. It is clear that both particle concentration and particle retention efficiency are particle-size dependent. To find an equation which calculates the ingestion rate of an appendicularian in the particle diameter range from P_1 to P_2, we must include the filtration rate (F) and the term for particle-size-dependent retention efficiency, (E(δ)), into the right-hand side of Eq. (5), to arrive at:

$$I(P_1, P_2) = \int_{P_1}^{P_2} FE(\delta)a3\sigma^{b+1}\delta^{3b+2}\, d\delta \quad (6)$$

From Eq. (6), it is straightforward to calculate the cumulative proportion of the total ingestion attributable to particles smaller than a given diameter P, C(P), as:

$$C(P) = \frac{\int_{\min}^{P} FE(\delta)a3\sigma^{b+1}\delta^{3b+2}\, d\delta}{\int_{\min}^{\max} FE(\delta)a3\sigma^{b+1}\delta^{3b+2}\, d\delta} = \frac{\int_{\min}^{P} E(\delta)\delta^{3b+2}\, d\delta}{\int_{\min}^{\max} E(\delta)\delta^{3b+2}\, d\delta} \quad (7)$$

where min and max are the minimum and maximum diameters of ingestible particles, respectively.

We used Eq. (7), in combination with our developed models for the prediction of REA (in place of E(δ)), see previous section) and under different assumptions for the value of b, to examine the cumulative proportion of the total ingestion rate attributable to particles smaller than size P, with P varying within the ingestible size range of *Oikopleura dioica*. More specifically, we were interested in finding the particle sizes which delimit 10% (P_{10}) and 90% (P_{90}) of the diet of *O. dioica*, which were those values of P for which C(P) = 0.1 and C(P) = 0.9 respectively. The difference between P_{10} and P_{90} serves as an indication of diet breadth with respect to particle size, since the animal extracts most of the diet biomass (i.e. 80%) from this particle size range. We were also interested in estimating the particle size that contributes most to the diet. The first-order derivative of C(P) with respect to P, written as C′(P), indicates the instantaneous increase of C(P) with increasing P, that is, it gives an indication of the relative contribution of each particle size to the diet. Therefore by setting its second derivative equal to 0, that is, C″(P) = 0, and, solving for P, we can find the particle size that has a highest contribution to the diet of *O. dioica*, P*. The exponent of the particle size spectrum in the ocean, b, was allowed to vary between −1.4 and −0.7 in our calculations, which matches approximately the observed range for b in nature (e.g. Hobson 1988, −1.4 to −0.7 in a neritic area; Rodríguez et al. 2001, −1.2 to −0.8 in an oceanic system).

Comparison with other taxa. We have compared our optimal prey-size data — defined as the size of particle captured with 100% efficiency (Hansen et al. 1994) — of appendicularians of different sizes with those of other mesozooplankton taxa. Optimal prey size was expressed both as ESD, and as body weight (µg C), while predator size was expressed as body weight alone (µg C). Trunk length of *Oikopleura vanhoeffeni*

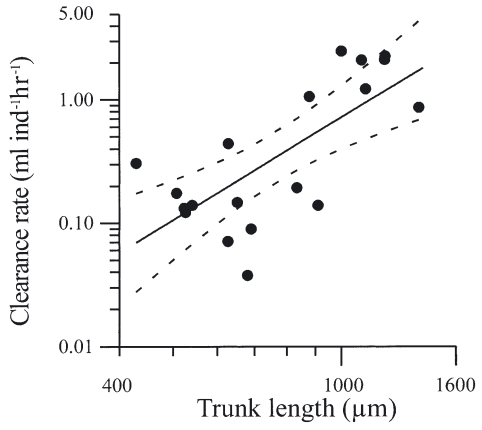

Fig. 2. *Fritillaria borealis.* Plot of clearance rate (CR, ml ind^{-1} h^{-1}) versus TL (TL, µm) data (log CR = [2.76 ± 0.65] log TL – [8.44 ± 1.88], parameter estimates ± SE, n = 19, $r^2 = 0.51$, $F_{1,17} = 18.02$, p = 0.001). The solid line represents least squares fits to power function and dashed lines indicate 95% CIs for the regression

in the size retention experiments by Deibel & Lee (1992) was transformed to biomass carbon following the equation in Deibel (1986). Trunk length of *O. dioica* was transformed to carbon following the equation in King et al. (1980). No data on the carbon content of *Fritillaria borealis* are available yet. Salp length in retention efficiency experiments by Harbison & McAlister (1979) and Kremer & Madin (1992) was transformed to carbon following the equation in Madin et al. (1981). Optimal prey sizes (ESD) compiled by Hansen et al. (1994) for dinoflagellates, nanoflagellates, ciliates, rotifers, copepodites, cladocerans and meroplankton, were first transformed to volume (µm^3) using conversion coefficients in Hansen et al. (1994), and then to carbon using conversion coefficients in Hansen et al. (1997).

RESULTS

Retention efficiency measurements

We found a significant fit of CR (ml ind^{-1} h^{-1}) versus TL (µm) data for *Fritillaria borealis* (log CR = [2.76 ± 0.65] log TL – [8.44 ± 1.88]; parameter estimates ± SE, n = 19, $r^2 = 0.51$, $F_{1,17} = 18.02$, p = 0.001, Fig. 2).

Both *Oikopleura dioica* and *Fritillaria borealis* retained all bead sizes, although retention efficiencies changed significantly with bead size (Fig. 3, Table 1; ANOVA, p < 0.001 for all 5 experiments, Table 2); thus REA and

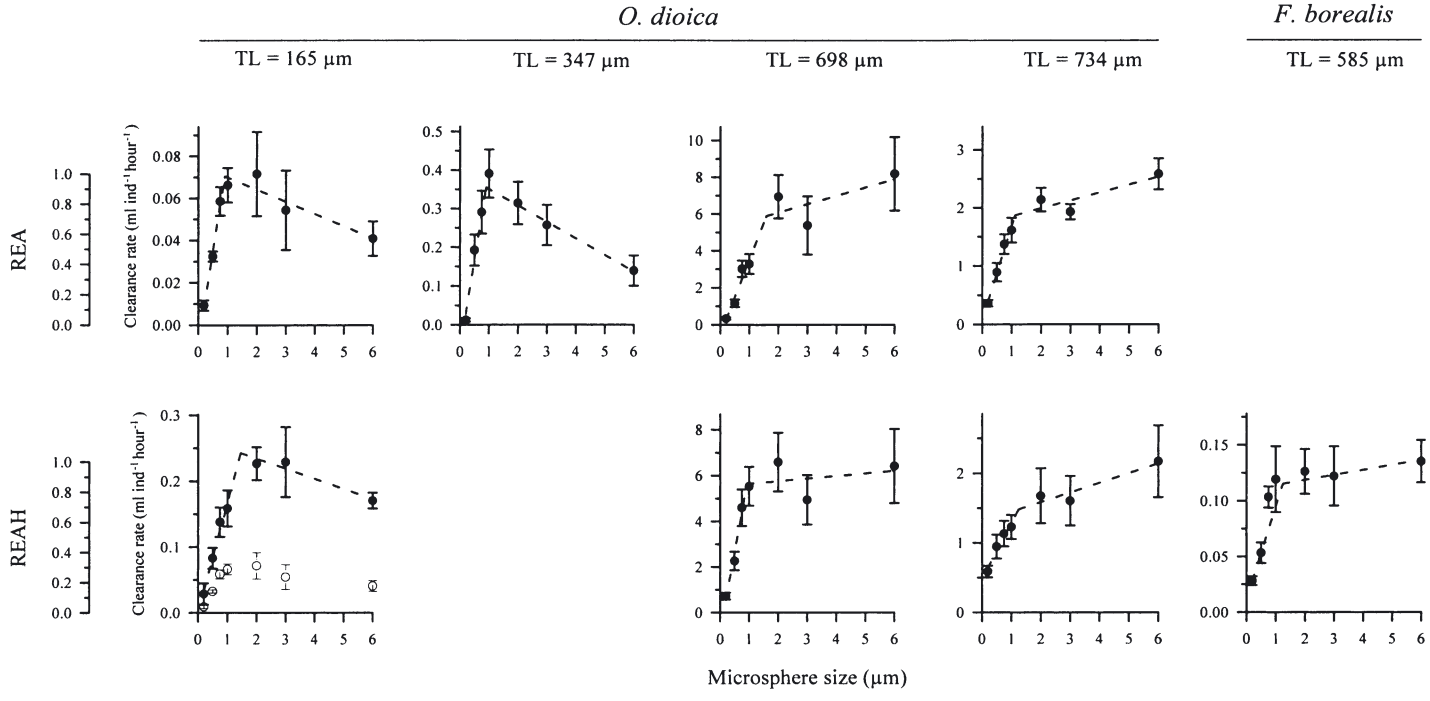

Fig. 3. *Oikopleura dioica* and *Fritillaria borealis*. Effect of different sizes of microsphere on Retention Efficiency of the Animal (REA) and Retention Efficiency of Animal plus House (REAH). Each column represents 1 experiment, performed in all cases with *Tetraselmis suecica* as the food source (except with large *O. dioica*, which was performed with *Chlorella stigmatophora*). Appendicularian trunk lengths (TL) are presented at the top of each column. To determine the REA, gut contents of organisms alone were analysed, while to determine REAH we analysed individuals plus houses. Points are means of 5 to 8 observations. Vertical lines indicate the SE. Discontinuous lines indicate the empirical model fit of retention efficiency. The bottom left-hand panel includes REA (empty circles) as a comparison

Table 1. *Oikopleura dioica* and *Fritillaria borealis*. Summary of measured Retention Efficiencies of Animals (REA), Retention Efficiencies of Animals and Houses (REAH) (mean ± SD), and empirical models fitted to the retention efficiency versus particle size (δ) plots (see Fig. 3). *: Maximum ingestible sizes calculated using an equation for the pore size of the inlet filter of *O. dioica* (Markus et al. unpubl. data; see 'Materials and methods')

		Empirical measurements of retention efficiency								
Species	Trunk length (µm)	Retention efficiency	0.2 µm beads	0.5 µm beads	0.75 µm beads	1 µm beads	2 µm beads	3 µm beads	6 µm beads	Empirical model
O. dioica	169 ± 22	REA	0.13 ± 0.07	0.45 ± 0.07	0.82 ± 0.19	0.92 ± 0.23	1.00 ± 0.56	0.76 ± 0.53	0.57 ± 0.23	$0.20 \le \delta \le 0.90$: REA = $-0.134 + 1.245 \times \delta$ $0.90 < \delta \le 9.08^{*}$: REA = $1.058 - 0.081 \times \delta$
		REAH	0.13 ± 0.14	0.36 ± 0.14	0.60 ± 0.20	0.69 ± 0.24	0.99 ± 0.22	1.00 ± 0.46	0.74 ± 0.10	$0.20 \le \delta \le 1.45$: REAH = $-0.005 + 0.735 \times \delta$ $1.45 < \delta \le 9.08^{*}$: REAH = $1.156 - 0.067 \times \delta$
O. dioica	348 ± 33	REA	0.03 ± 0.03	0.49 ± 0.25	0.74 ± 0.35	1.00 ± 0.39	0.80 ± 0.35	0.66 ± 0.33	0.36 ± 0.25	$0.20 \le \delta \le 0.90$: REA = $-0.167 + 1.197 \times \delta$ $0.90 < \delta \le 9.22$: REA = $1.006 - 0.109 \times \delta$ $9.22 < \delta \le 11.57^{*}$: REA = 0
O. dioica	699 ± 133	REA	0.04 ± 0.02	0.14 ± 0.07	0.37 ± 0.15	0.40 ± 0.19	0.85 ± 0.41	0.66 ± 0.55	1.00 ± 0.69	$0.20 \le \delta \le 1.59$: REA = $-0.063 + 0.492 \times \delta$ $1.59 < \delta \le 6.00$: REA = $0.631 + 0.056 \times \delta$ $6.00 < \delta \le 16.38^{*}$: REA = 1
		REAH	0.11 ± 0.06	0.34 ± 0.16	0.70 ± 0.32	0.84 ± 0.34	1.00 ± 0.51	0.75 ± 0.43	0.97 ± 0.65	$0.20 \le \delta \le 0.93$: REAH = $-0.129 + 1.060 \times \delta$ $0.93 < \delta \le 6.00$: REAH = $0.839 + 0.017 \times \delta$ $6.00 < \delta \le 16.38^{*}$: REAH = 1
O. dioica	735 ± 97	REA	0.14 ± 0.06	0.35 ± 0.18	0.53 ± 0.20	0.62 ± 0.25	0.83 ± 0.24	0.75 ± 0.15	1.00 ± 0.31	$0.20 \le \delta \le 1.12$: REA = $0.030 + 0.622 \times \delta$ $1.12 < \delta \le 6.00$: REA = $0.666 + 0.052 \times \delta$ $6.00 < \delta \le 16.88^{*}$: REA = 1
		REAH	0.27 ± 0.10	0.44 ± 0.21	0.52 ± 0.22	0.57 ± 0.21	0.77 ± 0.48	0.74 ± 0.43	1.00 ± 0.63	$0.20 \le \delta \le 1.24$: REAH = $0.222 + 0.370 \times \delta$ $1.24 < \delta \le 6.00$: REAH = $0.603 + 0.064 \times \delta$ $6.00 < \delta \le 16.88^{*}$: REAH = 1
F. borealis	585 ± 207	REAH	0.21 ± 0.06	0.39 ± 0.14	0.76 ± 0.14	0.88 ± 0.44	0.93 ± 0.30	0.90 ± 0.39	1.00 ± 0.28	$0.20 \le \delta \le 1.24$: REAH = $0.085 + 0.617 \times \delta$ $1.24 < \delta \le 6.00$: REAH = $0.811 + 0.033 \times \delta$

REAH spectra are affected by bead size and not flat. This rules out particle recirculation within the filter house. Large *O. dioica* and *F. borealis* were most efficient for 2 µm particles, while submicronic particles were retained less efficiently (Fig. 3, Table 1). Smaller *O. dioica* showed maximum retention efficiencies for 1 µm beads, and efficiencies for submicronic beads were higher than those of larger individuals (Fig. 3, Table 1). The shape of the retention efficiency spectrum was unimodal for small and medium *O. dioica*, but not for large *O. dioica* and *F. borealis*, where it levelled off (Fig. 3). These differences were apparent in the retention efficiency models (Table 1). REA differed significantly from REAH in small *O. dioica* (Fig. 3; ANOVA, $p < 0.001$ and $p < 0.015$ for individuals with TL = 169 and TL = 698 µm, respectively; Table 2), but not in large *O. dioica* (Fig. 3; ANOVA, $p = 0.146$ for individuals with TL = 735, Table 2).

Model predictions for the diet of *Oikopleura dioica*

C(P) had a typical saturating-like shape, as can be expected from a cumulative proportion, with a steep, rapidly increasing section for smaller particle sizes, and a saturating zone for large particles (Fig. 4). In general, saturation was achieved more slowly for large appendicularians and for particle assemblages where large particles were abundant (i.e. for higher values of the exponent of the biomass spectrum, b, see Fig. 4). Trends in C′(P) indicate that, at low b values, particles close to the minimum size examined (0.2 µm) had the highest relative contribution to the diet of *O. dioica* (Fig. 4). For higher b values, the particles with the largest contribution to the diet were of an intermediate size, 1 to 2 µm (Fig. 4). Moreover, C′(P) varied markedly with size when particle assemblages were dominated by small particles (b = −1.4), while C′(P) values were more homogeneous when large particles dominated the assemblage (b = −0.7). Thus, when large particles dominated, there was a more diverse diet in terms of prey size (Fig. 4).

This diversity is better illustrated by the difference between P_{10} and P_{90} (Fig. 5), which delimits the size range comprising 80% of the appendicularian diet. For a highly negative slope in the biomass spectrum (b = −1.4), small *Oikopleura dioica* (TL = 165 and 347

Table 2. ANOVA for the particle retention experiments performed, including the sum of squares (SS), degrees of freedom (df), mean square (MS), and *F*- and p-values. In all cases the dependent variable is the decimal logarithm of the clearance rate. Independent variables (ind. vars.) are the microsphere size (i.e. 6 levels corresponding to each particle size), Retention Efficiencies of Animals (REA), and Retention Efficiencies of Animals and Houses (REAH) (2 levels corresponding to measurements of REA or to measurements of REAH). TL: trunk length

Source of variation	SS	df	MS	*F*	p
***Oikopleura dioica* TL = 169 µm**					
2-way ANOVA: ind. vars. = microsphere size and REA/REAH					
Microsphere size	5.36	6	0.90	20.55	<0.001
REA/REAH	3.27	1	3.27	75.27	<0.001
Microsphere size × REA/REAH	0.24	6	0.040	0.91	0.496
Error	1.83	42	0.043	–	–
***Oikopleura dioica* TL = 347 µm**					
1-way ANOVA: ind. var. = microsphere size					
Microsphere size	10.85	6	1.81	34.13	<0.001
Error	1.85	35	0.053	–	–
***Oikopleura dioica* TL = 698 µm with *Tetraselmis suecica* as food**					
2-way ANOVA: ind. vars. = microsphere size and REA/REAH					
Microsphere size	14.56	6	2.43	35.71	<0.001
REA/REAH	0.42	1	0.42	6.11	0.015
Microsphere size × REA/REAH	0.67	6	0.11	1.63	0.146
Error	6.18	91	0.068	–	–
***Oikopleura dioica* TL = 734 µm with *Chlorella stigmatophora* as food**					
2-way ANOVA: ind. vars. = microsphere size and REA/REAH					
Microsphere size	5.32	6	0.89	26.11	<0.001
REA/REAH	0.073	1	0.073	2.15	0.146
Microsphere size × REA/REAH	0.42	6	0.07	2.06	0.065
Error	3.33	98	0.034	–	–
***Fritillaria borealis* TL = 585 µm**					
1-way ANOVA: ind. var. = microsphere size					
Microsphere size	1.53	6	0.25	9.14	<0.001
Error	0.59	21	0.028	–	–

µm) obtained 80% of their diet from a very narrow range of particle sizes, from 0.2 to 2.5 µm, while for less negative slopes (b = –0.7) the range was from ca. 0.5 to 6–7 µm (Fig. 5). Large *O. dioica* (TL = 698 and 734 µm) had wider ranges in diet in terms of prey size, with P_{10} and P_{90} ranging from 0.5 to ca. 6 µm for highly negative slopes of the biomass spectrum (b = –1.4), and from ca. 2 to 15 µm for less negative slopes (b = –0.7, Fig. 5). In all cases, the particle size with the highest contribution to the diet was close to P_{10} or even below (Fig. 5).

Comparison with other taxa

Although small appendicularians are able to capture the smallest prey (1 µm) of all the mesozooplankton compared (Fig. 6), predator:prey carbon ratios for appendicularians (14 538:1) are within the range of ratios for several groups of mesozooplankton (1:1 to 3×10^6:1), and are statistically undistinguishable from those of copepods (1603:1; regressions of log prey-carbon on log predator-carbon for appendicularians and copepods did not differ significantly in slope and intercept; ANCOVA, p = 0.48 and p = 0.24, respectively).

DISCUSSION

Our use of flow cytometry, in combination with a suspension of 7 different sizes of fluorescently labelled beads (0.2 to 6 µm), has allowed us to measure the particle-retention efficiency spectrum of 2 common, warm water appendicularians with unprecedented resolution. The retention efficiencies of between 3 and 27% for 0.2 µm beads in our experiments (Table 1) are consistent with the low, ca. 10% efficiencies that were

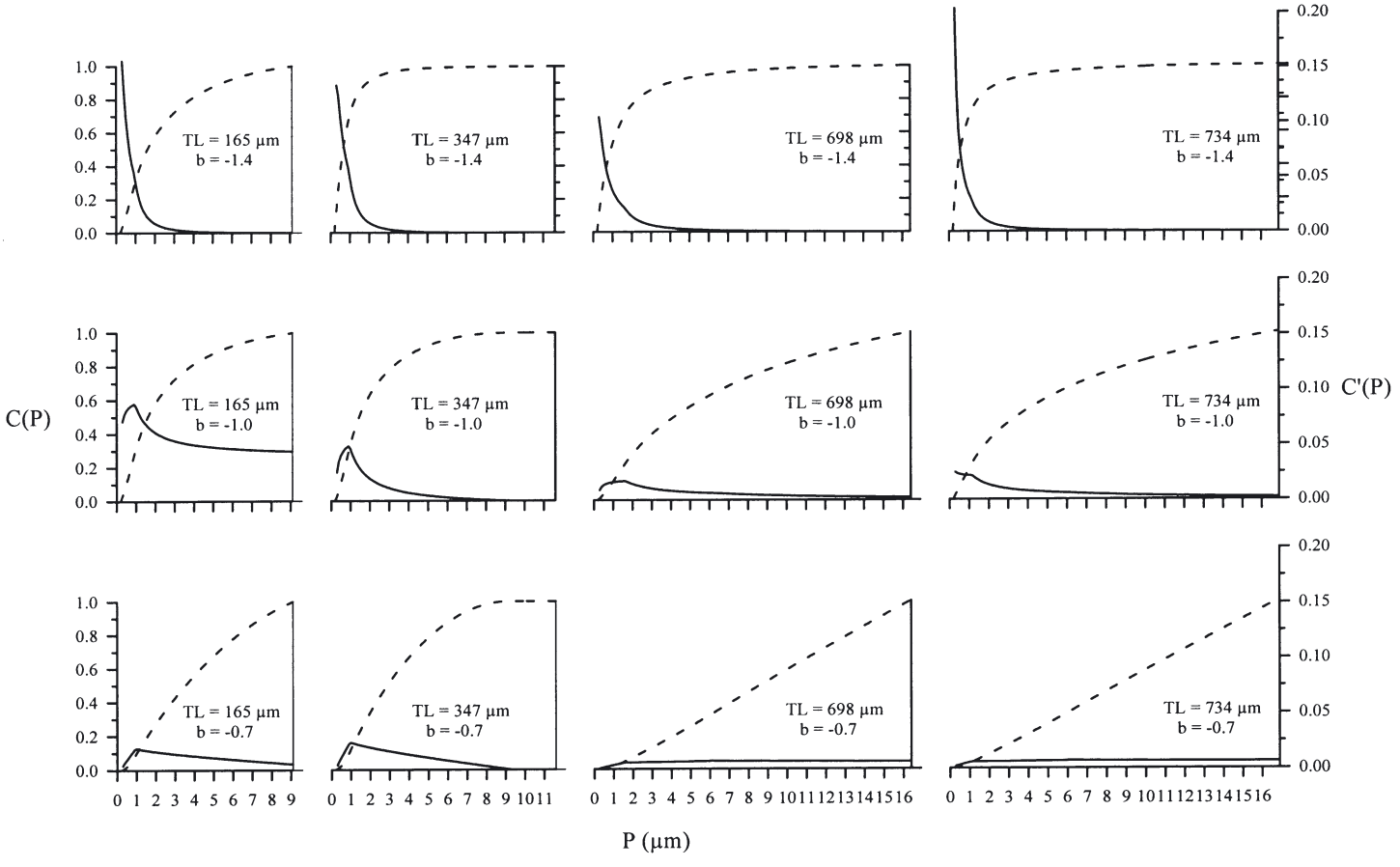

Fig. 4. *Oikopleura dioica*. Cumulative proportion of the diet, C(P) (discontinuous line), and relative contribution to the diet, C′(P) (continuous line), versus particle size, P (see methods for definitions of these functions). C(P) was calculated according to Eq. (7), where we used equations for the prediction of Retention Efficiency of the Animal (REA) from Table 1 in place of the particle-size-dependent retention efficiency E(δ), and where the exponent of the biomass spectrum, b, was set to –1.4 (upper row), –1.0 (middle row) and –0.7 (lower row). We present model calculations for each of the 4 retention efficiency experiments performed on *O. dioica* (each of the 4 rows), indicated by their average trunk length (TL)

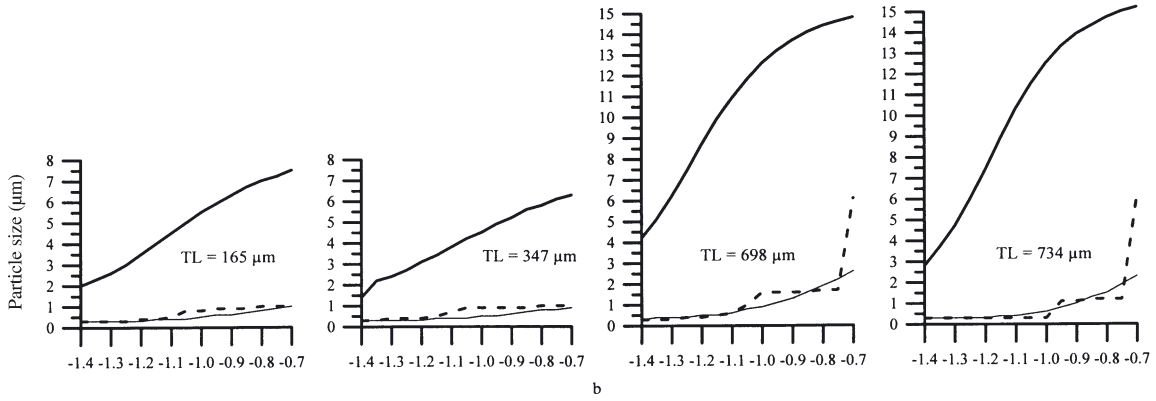

Fig. 5. *Oikopleura dioica*. Variation of P_{90} (thick continuous line) and P_{10} (thin continuous line; these 2 parameters define a particle size range that encloses 80% of the diet, see methods), and P* (dashed line; particle size with the highest contribution to the diet) versus the exponent of the biomass spectrum, b (see 'Materials and methods' for calculation of these parameters). Each panel corresponds to each of the 4 retention efficiency experiments performed on *O. dioica*, indicated by their average trunk length (TL)

observed for *Oikopleura vanhoeffeni* feeding on 0.13 µm colloids (Flood et al. 1991). Intermediate efficiencies between 14 and 80% for beads measuring 0.5 and 0.75 µm (Table 1) also compare well with a retention efficiency of 44% measured by Deibel & Lee (1992) for *O. vanhoeffeni* feeding on 0.6 µm plastic beads, or approximately 30 to 40% efficiencies measured by Bedo et al. (1993) for *O. dioica* using algal cultures and plastic beads. However, retention efficiencies of 58% for *O. vanhoeffeni* (Deibel & Lee 1992) feeding on 1 µm beads seem somewhat lower than our average retention efficiency of 73% measured for *O. dioica* for the same bead size (Table 1), which is suggestive of coarser pores in the pharyngeal filter of the latter species. Thus, our initial hypothesis that smaller species should have finer pharyngeal filters and therefore retain smaller particles has been confirmed. We have also shown that smaller individuals tend to retain smaller prey than larger individuals of the same species, a pattern that has already been described for other appendicularians (*O. vanhoeffeni*, Deibel & Lee 1992), which is probably due to age-related shifts in the pore size of the pharyngeal filter. Finally, we have shown that particles larger than the pore size of the

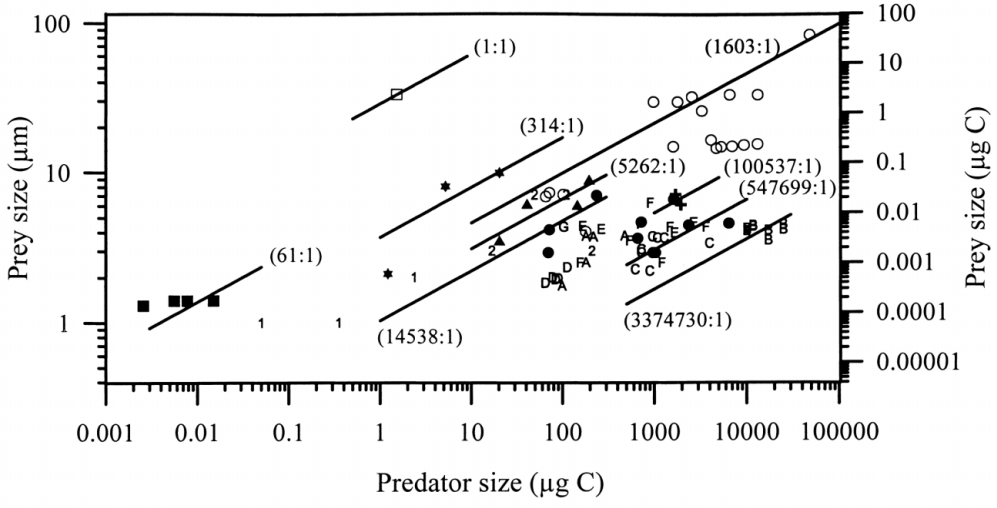

Fig. 6. Prey size in carbon units (right vertical axis), and in equivalent spherical diameter (left vertical axis) versus predator size, in carbon units. Symbols correspond to data compiled by Hansen (1994), while numbers and letters represent our compilation of data from the literature on appendicularians and salps, respectively: (■) nanoflagellates; (□) dinoflagellates; (*) ciliates; (▲) rotifers; (✚) cladocerans; (●) meroplankton larvae; (○) copepods. 1, *Oikopleura dioica*, present study; 2, *Oikopleura vanhoeffeni*, Deibel & Lee (1992); A, *Cyclosalpa floridana* solitary; B, *C. affinis* solitary; C, *C. polae* solitary; D, *C. floridana* aggregate; E, *C. affinis* aggregate. Data for A to E from Harbison & McAlister (1979). F, *Pegea bicaudata* aggregate; G, *P. confoederata* aggregate. Data for F and G from Kremer & Madin (1992). Solid lines have slopes = 1, therefore they join points with the same predator:prey body carbon ratio (between brackets). Their intercepts were calculated by fitting data for each taxonomic category to linear functions with fixed slope = 1 (using SPSS non-linear regression routine)

food-concentrating filter of *O. dioica* (0.2 µm, Flood 1978) are retained with less than 100% efficiency, thus the pharyngeal filter must have coarser pores. A similar situation occurs in the cold-water appendicularian *O. vanhoeffeni*, which reaches its maximum retention efficiency for particles 3 µm in size (Deibel & Lee 1992) and has coarser pore widths in the pharyngeal (3.26 × 6.35 µm, Deibel & Powell 1987) than in the food-concentrating filter (0.22 × 1.04 µm, Deibel et al. 1985).

An interesting and unexpected result was the decrease in retention efficiency with the largest beads in small- and medium-sized *Oikopleura dioica* (see experiments with 165 and 347 µm *O. dioica* in Fig. 3). The simplest explanation for this pattern is particle-size selection by the inlet filters, which are prescreening filters present on the surface of the house of most oikopleurids, and that prevent the entrance of large particles inside the house (Flood & Deibel 1998). Either by classical sieving or by direct interception, the inlet filter will retain particles with an efficiency that should decrease with decreasing particle size. Note that in our experiments we were counting beads that passed through the inlet filters, that is, we were observing the inverse of the particle retention spectrum of these inlet filters. This would explain why we observed decreasing filtration rates with increasing size for the largest particles (3 to 6 µm). It is also logical that we detected this pattern only in the smallest animals, since their inlet pore widths (9 and 12 µm for *O. dioica* measuring 165 and 347 µm, respectively, obtained from relationship from Kiefer & Acuña [unpubl. data, see 'Materials and methods']) are much closer to our largest bead size (6 µm) than the inlet pore width of the largest animals (16 and 17 µm for *O. dioica* measuring 698 and 734 µm).

These results suggest that particle removal experiments may underestimate CRs if the particles used are not of the appropriate size, and would explain why calculation of filtration rates by alternative approaches, like the gut content technique, tends to yield higher figures (López-Urrutia et al. 2003). It is symptomatic that CRs calculated for those particles retained with maximum efficiency in our experiments (ca. 2, 10, 192 and 60 ml d^{-1} for individuals measuring 165, 347, 698 and 734 µm, calculated from the data in Fig. 3) are among the highest in the literature (compare with Fig. 5 of López-Urrutia et al. 2003), but correspond well with CRs measured by the gut pigment technique (for individuals measuring 165, 347, 698 and 734 µm in size, these rates are 0.4, 9, 156 and 192 ml d^{-1} when based on gut volume content, or 0.9, 10, 102 and 121 ml d^{-1} when based on gut pigment content; from Fig. 5 of López-Urrutia et al. 2003).

Our results lead to the rejection of our initial null hypothesis of a flat REAH spectrum (Fig. 3; ANOVA in Table 2), with smaller particles being retained with lower efficiency by the animal plus house system, which is a clear indication that these particles exit the house and are not recirculated. This is consistent with the idea that particles not retained by the pharyngeal filter flow through the spiracles to the house exit chamber (Fenaux 1986), and is at variance with a process of recirculation from the spiracles to the tail chamber, and then back to the food concentrating filter (Deibel 1986 and Flood 1991). Bedo et al. (1993) determined that *Oikopleura dioica* remove submicronic beads from suspension at rates that do not differ from those of >1 µm cells measured in separate experiments. They interpreted this as an indication that particle removal by *O. dioica* is non-selective and highly efficient down to 0.2 µm particles, as predicted by Flood (1978), according to the pore size of the food-concentrating filter (ca. 0.1 µm). Measurement of particle removal is conceptually and methodologically similar to measurement of REAH in our experiments, thus Bedo et al. (1993) were reporting essentially flat REAH spectra, which is in contrast with our results. We must note, however, that our approach has several advantages over that of Bedo et al. (1993). Our experiments were intended for a statistical test of the 'flatness' of the REAH spectrum (ANOVA in Table 2), we used particles of the same type for all experiments, and measured retention efficiencies for all 6 particle sizes simultaneously in the same experiment. Bedo et al.'s (1993) comparison was not statistical, and not all particle sizes were offered simultaneously, thus their comparison of micronic and submicronic particles was done in separate experiments. This is particularly problematic given the enormous variances in CRs among experiments, which renders any comparison very difficult.

Here we have combined continuous, size dependent, power-biomass distributions with empirical continuous, size-dependent particle-retention spectra, to explore the consequences of different shapes of biomass distributions on the diet composition of appendicularians. We know of no other similar attempts to do this in the published literature, although ours is a rather simple extension of biomass spectra theory (see Blanco et al. 1994 for a lucid account of methodological and theoretical aspects). We must note, however, that our model calculations are based on the assumption of power particle biomass distributions, which may not apply to some pelagic ecosystems that are far from the steady state, i.e. under a strong phytoplankton bloom where cells of a particular species become dominant. Our extrapolation of power spectra for the submicronic range is based on empirical evidence and mathematical convenience. It has been suggested on the basis of experience (e.g. Wells & Goldberg 1991), and demonstrated empirically (e.g. Cavender-Bares et al. 2001),

that power-law particle size distributions of living matter in the ocean can be extended down to ca. 0.20 µm in size, which approximately coincides with the minimum size to which our empirical models of retention efficiency can and have been extended (see 'Results'). Additional evidence suggests that power distributions describe the distribution of submicronic particles reasonably well, and that their exponents overlap the range of exponents for particles >1 µm. For example, Wells & Goldberg (1994) found good size-distribution fits of colloids <200 nm in size to the power function $N = kD^{-\beta}$ where N is the number of particles having diameter >D, k is a constant (particles cm^{-3}) and β is a non-dimensional constant. In their study, β ranged from 3.3 in coastal areas, to 7.4 in oligotrophic areas. Using some simple mathematical derivations and assumptions not presented here, it can be shown that $-\beta = 3b$, where b is the exponent of the biomass density function, thus their range in β is equivalent to a range from ca. –2.4 to –1.1 for b. Although this overlaps partially with the range of b observed in nature for particles >1 µm (from –1.4 to –0.7, Hobson 1988, Cavender-Bares et al. 2001, Rodríguez et al. 2001), lower b's indicate that the actual biomass of the smallest submicronic particles is probably higher than could be expected from extrapolation of the biomass density function obtained for particles >1 µm. This, in combination with the fact that power laws do not necessarily apply in all conditions (e.g. Cavender-Bares et al. 2001), calls for caution in interpreting our simple, power law modelling exercise. However, power law biomass distributions are widespread enough in aquatic systems to serve as a starting point for the exploration of the implications of continuous particle-size retention-efficiency spectra. In any case, our study illustrates a pressing need to generate continuous descriptions of particle distributions in the ocean that are consistent with a continuous description of the retention-efficiency spectrum of filter feeders, and to develop a theoretical framework to combine the two.

We must also point out that food assimilation by appendicularians depends not only on the efficiency with which particles are retained, but also on the efficiency with which they are digested. Our predicted particle size spectra in the diet do not necessarily match particle assimilation spectra, as our experiments have addressed retention but not digestion efficiencies. Submicronic particles may make a substantial fraction by volume of the ingested particles (Fig. 5), but their contribution to the appendicularian's diet may be limited given their refractory nature (Koike et al. 1990). Gorsky et al. (1999) have reported a dominance of undigested cyanobacteria cells in fecal pellets of the appendicularian *Megalocercus huxleyi* after passage through the digestive system. This contrasted with an apparently good assimilation of *Prochlorococcus* and picoeucariotes, which indicates that digestion efficiencies vary greatly with particle size and quality. Since size and taxon dependence of assimilation efficiencies were out of the scope of our work, caution should be applied in extrapolating our results to the actual role that different particle sizes play in the energy budget of appendicularians.

Our model calculations indicate that not only the retention efficiency, but also the shape of the particle size distribution, are crucial in defining the size composition of the diet of an appendicularian. Although appendicularians present peak retention efficiencies for particles >1 µm, their diet will be dominated by either micronic or submicronic particles, depending on the value of the exponent of the biomass spectrum (Fig. 4). Therefore, maximum retention efficiency does not necessarily indicate the 'optimum' or prevailing particle size in the diet, particularly in environments where values of the exponent of the biomass density function, b, are highly negative (i.e. values of b between –1.4 and –1), i.e. where small particles are very abundant, which is the case in vast areas of the ocean.

Appendicularians exhibit feeding and respiration rates that do not depart allometrically from reported ranges for other mesozooplankton when both organism size and physiological rates are expressed in carbon units (see Schneider 1992, Acuña & Kiefer 2000). However, a predator:prey length ratio of nearly 10^3 for appendicularians clearly falls out of the range reported by Hansen et al. (1994) for a variety of mesozooplanktonic organisms, which justifies the current perception of appendicularians as organisms with an unusual biology. The conclusion would be essentially similar if predator:prey size ratios were considered in volume, rather than linear units. However, when size is measured in carbon units, the predator:prey size ratio for appendicularians does not depart from that of copepods, which seems to imply that, although appendicularians have large, gelatinous bodies, their physiology and ecology is in agreement with what we should expect according to their body carbon content.

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