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Molecular quantification of differential ingestion and particle trapping rates by the appendicularian *Oikopleura dioica* as a function of prey size and shape

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Abstract

To investigate whether particles are ingested or merely trapped in the house of the appendicularian *Oikopleura dioica*, we developed a real-time quantitative polymerase chain reaction (qPCR) assay and applied it in a controlled study where *O. dioica* was fed three types of algae at different concentrations. At particle concentrations ranging from 100 to 10,000 cells mL^{-1}, highest ingestion efficiencies (75–96%) were observed with the smallest algal species (*Isochrysis* sp.) compared to the larger cryptophyte *Rhinomonas* sp. (2–87%) and a similar sized but spiny diatom, *Chaetoceros calcitrans* (4–65%). When prey were mixed, the presence of *C. calcitrans* inhibited ingestion of *Rhinomonas* sp. but not the smaller *Isochrysis* sp., suggesting that *C. calcitrans* clogged the inlet filter and mechanically hindered ingestion of larger algae. *O. dioica* is adapted to feed on smaller particles at low (nonbloom) concentrations and may thus be subjected to bottom-up regulation by larger spiny or colonial prey that typically dominate late-stage phytoplankton blooms.

Zooplankton play a central role in the pelagic food web as selective predators and nutrient regenerators (Banse 1995; Gismervik et al. 1996; Verity and Smetacek 1996). The planktonic urochordate appendicularians are circumglobal organisms and second in abundance in many marine zooplankton communities after copepods (Gorsky and Fenaux 1998). Appendicularians may therefore act as key top-down regulators in the marine planktonic food web. Appendicularians have a peculiar feeding strategy; they live inside an elaborate multichambered extracellular house, complete with inlet and food-concentrating filters that they secrete from a specialized oikoplastic epithelium (Fig. 1) (Spada et al. 2001; Thompson et al. 2001). The filter in the house is able to retain particles as small as 0.2 μm. This corresponds to a prey-to-predator ratio of 1 : 10,000, which differs substantially from the 1 : 1–1 : 100 ratios usually observed for other zooplankton (Hansen et al. 1994; Gorsky and Fenaux 1998). Appendicularians are therefore able to shortcut complex food webs by channeling particles as small as bacteria directly to higher trophic levels including fish (Capitanio et al. 2005) and other large predators (Scheinberg et al. 2005; Shiganova 2005). They are efficient grazers, and their grazing pressure may even surpass that of copepods (Shiganova 2005). In addition to being ingested, particles stick to the house structures that are continuously replaced to avoid clogging and to maintain maximum filtration efficiency. The discarded houses, including trapped particles, can therefore represent a significant contribution to carbon flux in some oceanic regions (Silver and Alldredge 1981; Shiganova 2005). In order to accurately assess the trophic interactions of appendicularians, it is necessary to separately quantify ingestion and trapping in the house for all prey types. Current methodologies are not well adapted to assess this, and, in particular, in situ measurements of metazooplankton feeding suffer from methodological limitations (see

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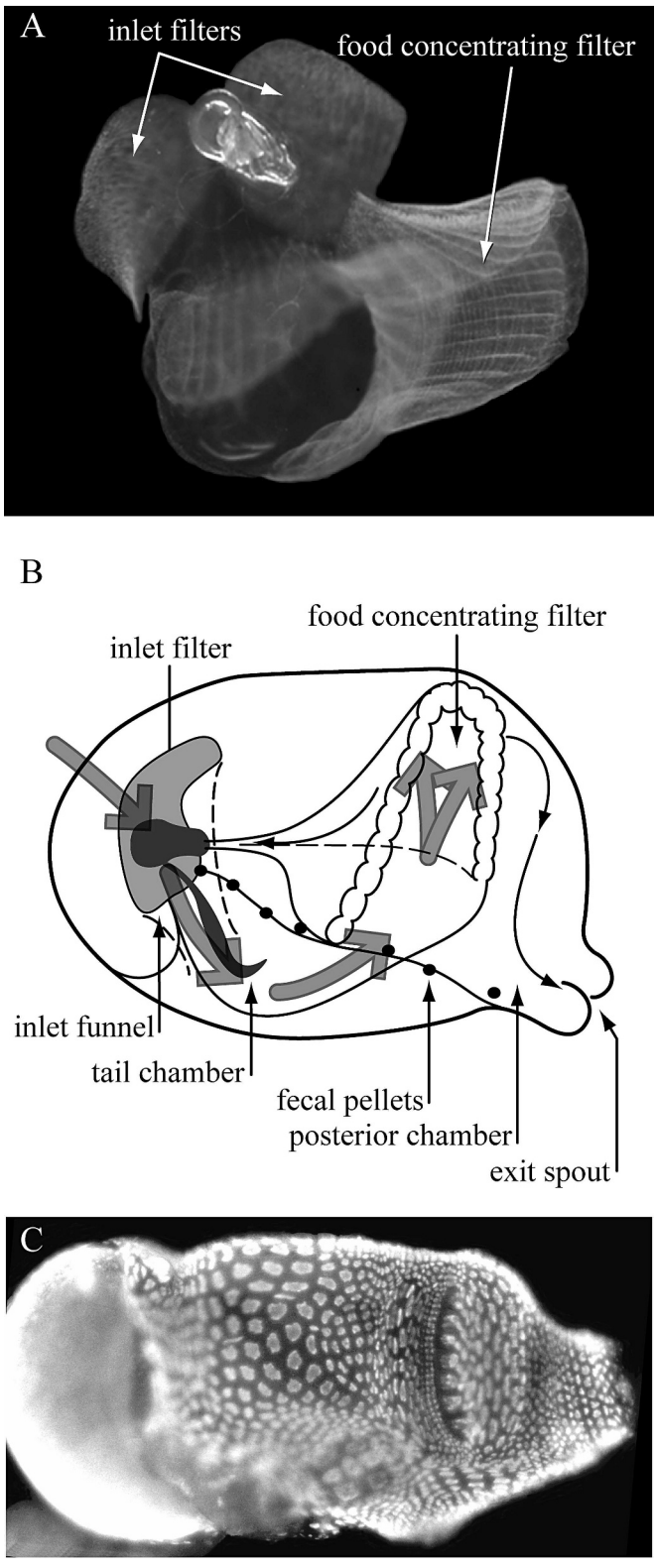

Fig. 1. The filter-feeding house of the appendicularian, *Oikopleura dioica*. (A) Live *O. dioica* inside an inflated, functional feeding house. The large food-concentrating filter is indicated. The inlet filters are more transparent and difficult to discern in this image, but their position is indicated by arrows. (B) Schematic drawing of the general organization of the oikopleurid house with the pathway of water circulation indicated by thick gray arrows (after Thompson et al. 2001). (C) Lateral whole mount view of the trunk of *O. dioica* with DNA stained by Hoechst 33342. The oikoplastic epithelium, responsible for the secretion of the filter-feeding house, shows characteristic fields of diversely shaped nuclei. The gonad, at an intermediate stage of maturation, is visible at the left.

Båmstedt et al. 2000 for review and Nejstgaard et al. 2003 for further discussion).

Metazooplankton grazing on phytoplankton has often been estimated from gut pigment content by flourometry and high-performance liquid chromatography (Mackas and Bohrer 1976; Pandolfini et al. 2000). However, these methods are subject to pigment degradation, and numerous efforts have been made to make corrections for this error of underestimation (McLeroy-Etheridge and McManus 1999; Pandolfini et al. 2000). These methods are also restricted to prey with photosynthetic machinery and are not practical when investigating more complex interactions in the field. Alternatively, radiolabeling of prey has been used in studies of trophic interactions, including nonpigmented prey (Roman and Rublee 1981; Acuña and Kiefer 2000), but it is difficult to analyze feeding selectivity in complicated food webs with this approach since limited numbers of differentially isotope-labeled prey may be analyzed per experiment. This method includes a labeling step and will therefore deviate to some extent from undisturbed in situ conditions.

Bottle incubations are the most common method to investigate feeding on pigmented and nonpigmented prey in natural plankton (Bochdansky and Deibel 1999; Båmstedt et al. 2000; Tiselius et al. 2003). This approach has several shortcomings: (1) it involves capture and incubation of prey and predators and may not accurately reflect undisturbed in situ conditions, especially for delicate organisms such as appendicularians; (2) it is labor intensive and subject to errors due to sampling, fixation, and microscopic analysis (e.g., Verity and Paffenhöfer 1996); (3) it is prone to bottle effects (Roman and Rublee 1981); and (4) differential predation rates by various zooplankton in the natural prey suspension often yield underestimated or even significantly negative feeding rates (Nejstgaard et al. 2001). Alternatively, investigation of feeding rates and prey selection by microscopic examination of predator gut content (e.g., Verity and Paffenhöfer 1996) is labor intensive, not strictly quantitative, and unable to account for prey items with soft bodies. Finally, gut volume analysis can provide useful information in the field (López-Urrutia et al. 2003), but this type of analysis is not able to distinguish between different prey and is therefore not well suited for organisms with feeding selection.

More recently, qPCR approaches have begun to be successfully applied to investigate the diets of marine invertebrates (e.g., Nejstgaard et al. 2003). Here, we have applied this approach to investigate trophic interactions between the appendicularian *O. dioica* and several species of algae differing in size and morphology. The application

Table 1. 18S rDNA primers and expected product sizes.*

Specificity	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product (bp)
Universal**	CTCCCAGTAGTCATATGC	ACCTTGTTACGACTT	1,759
Isochrysis sp.	TCCGGTTGCGTGCTGAGTCA	TCGCCAGCGTAAAGCCGTGC	57
Chaetoceros calcitrans	GCTTCGGCGGTGTAATGGTGA	GGCAGAAACTTGAATGGTCCA	82
Rhinomonas sp.	TTCCAAGCCACATTTCTG	CACCACCGCGAGGGCTGT	61

* PCR reaction mix: 10 μL of 2XQuantitech SYBR Green, 10 μm each, forward and reverse primers, 3.5 mmol L^{-1} $MgCl_2$ for *Isochrysis* sp. and *Rhinomonas* sp. and 4.5 mmol L^{-1} $MgCl_2$ for *Chaetoceros calcitrans* and 50-ng sample DNA in a total volume of 20 μL. Amplification performed with an initial 15-min denaturation at 95°C followed by 40 cycles of 95°C for 20 s, 10 s annealing (species specific temperature), and 20 s elongation at 72°C. Annealing temperatures were 68°C, *Isochrysis* sp.; 65°C, *Chaetoceros calcitrans*; 55°C *Rhinomonas* sp.
** From Nejstgaard et al. (2003).

of the qPCR technique allowed the unique investigation of the fate of algae captured by *O. dioica*, providing estimates of the fraction of prey cells trapped in the house versus cells that were actually ingested.

Materials and methods

Culture of algae—Fresh inoculates were made from stock cultures of *Isochrysis* sp., *C. calcitrans*, and *Rhinomonas* sp. Algae were cultured in aerated 2-L plastic bags under constant light (36W/20 cool white fluorescent light tube) at 15°C in F/2 media (Guillard 1975), with silica added (5%) for the diatom *C. calcitrans*. The same strains were used throughout the experiments. Only algae in the exponential growth phase were used to minimize fluctuations in nutritional value and DNA content. The carbon content (and cell size) was 10.6 ± 0.3 pg C $cell^{-1}$ (6-μm diameter) in *Isochrysis* sp., 4.6 ± 0.3 pg C $cell^{-1}$ (4 × 3-μm length × width) in *C. calcitrans*, and 51.5 ± 3.9 pg C $cell^{-1}$ (17-μm diameter) in *Rhinomonas* sp. (J. M. Bouquet unpubl. data).

Collection and culture of animals—Appendicularians were collected and cultured in a standard food regime as previously described (Troedsson et al. 2002, 2005), except that the animals were fed an additional 1,000 cells mL^{-1} (final concentration) of *Rhinomonas* sp. every 24 h beginning 3 d postfertilization. Animals for the experiments were cultured at 15°C for 5 d postfertilization, corresponding to a total body length of 672 ± 183 μm (Troedsson et al. 2002). Further information on culturing can be obtained at http://www.sars.no/facilities/appendic.php.

Three separate feeding experiments were conducted to quantify ingestion rate and house trapping in single prey food suspensions of *Isochrysis* sp., *C. calcitrans*, and *Rhinomonas* sp., respectively, each at three concentrations of 1,000, 10,000 and 50,000 cells mL^{-1}. In a fourth experiment, we investigated the ingestion rates and house trapping of the three algae in a mix, with each algae at concentrations of 100, 1,000, and 10,000 cells mL^{-1}.

Ingestion rate—In each experiment, triplicates of 25 randomly selected day 5 animals were transferred separately to 4-L beakers (Camwear®), containing 0.2 μm filtered seawater, for 20 min to ensure complete gut evacuation (Lopez-Urrutia and Acuña 1999). Houses were not removed from the animals such that we would randomly sample house filtration efficiencies over the house lifetime (~4 h). This was done because it is known that house filtration efficiency varies with the time postinflation (Acuña and Kiefer 2000). Thus, the ingestion rates that were measured represent averaged snapshots over the lifetime of a house. The animals were then transferred to fresh 4-L beakers with known food concentration of prey for 5 min, quickly transferred to new 4-L beakers with 0.2 μm filtered seawater for rinsing, caught, removed from their houses, anesthetized in 0.25 mg mL^{-1} 3-aminobenzoic acid ethyl ester (MS222), and finally cleaned with freshly filtered seawater. The animals were pooled in 1.5-mL Eppendorf tubes and quickly centrifuged, and excess water was removed. The animals were frozen in liquid nitrogen and stored at −80°C until further analysis. Capture and transfer of animals was accomplished using a wide-bore pipette. The time from capture to anesthesia was less than 2 min for each replicate. Gut evacuation was not detected during this period. Microscopic observation of feeding animals indicated that particle passage time from the inlet filter to the mouth was 10.4 ± 2.8 s. This lag time is only 3.5% of the 5-min incubation period and will therefore not significantly impact estimations of particle ingestion.

House trapping—In each experiment, triplicates of 50 animals were removed from their houses and transferred separately into a 6-L beaker (Camwear) with a known concentration of algae. The animals were left in the beaker for 4 h, equivalent to the life span of an individual house (3.8 h/house at 15°C; Troedsson 2005). Fifty discarded houses per replicate were collected and gently rinsed three times in 0.2 μm filtered seawater. The houses were pooled in 1.5-mL Eppendorf tubes, frozen in liquid nitrogen, and stored at −80°C until further analysis. This yielded estimates of total number of particles trapped in a house. To estimate average rates of house trapping, it was important to consider the house renewal rate and whether this is subject to variation as a function of particle concentration. Clogging at high particle concentrations has been reported to reduce house lifetime (Selander and Tiselius 2003; Tiselius et al. 2003), though in other studies house renewal rate appears constant (Sato et al. 2001; Troedsson, 2005). Our previous measurements of house renewal rates at food concentrations ranging from 3 to 60 μg C L^{-1} showed a food-independent house renewal rate of 3.8 ± 1.1 h/house at 15°C (Troedsson

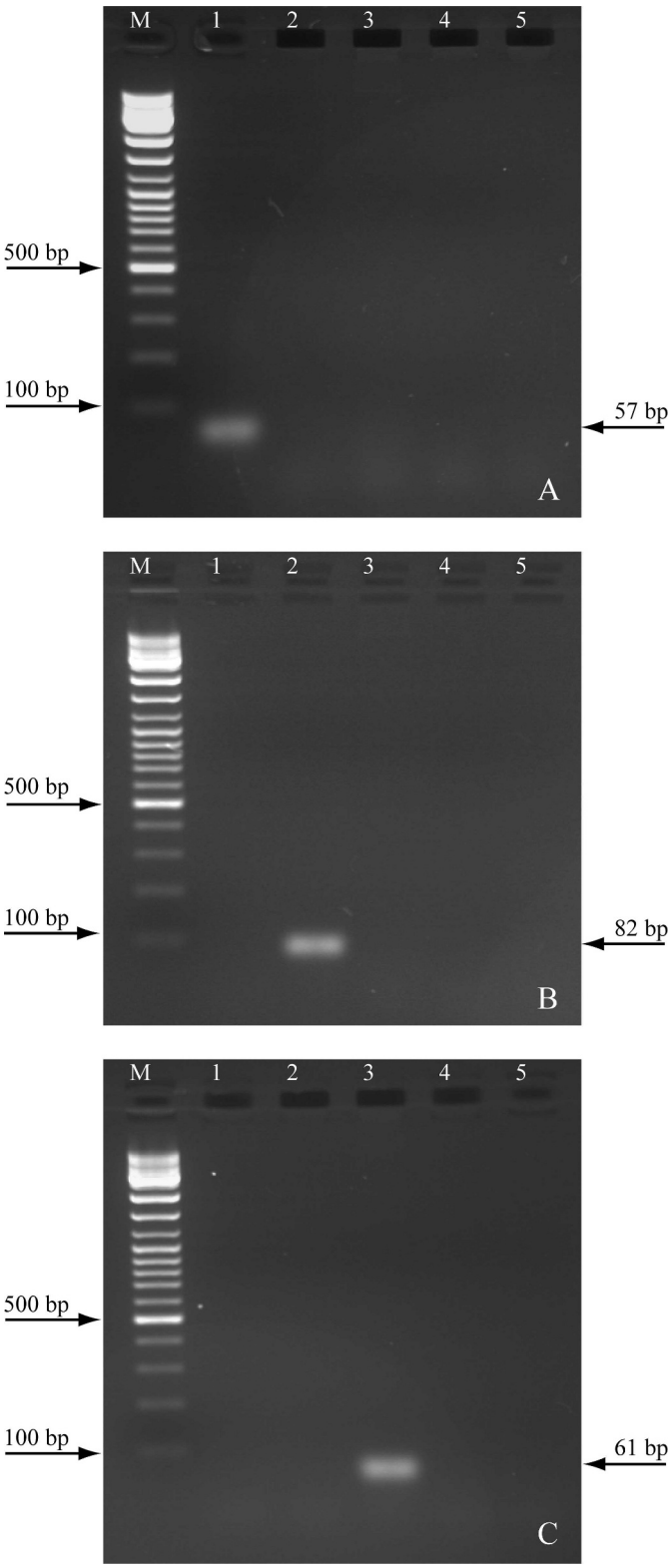

Fig. 2. Verification of species specificity of 18S rDNA primers. (A) *Isochrysis* sp., (B) *Chaetoceros calcitrans*, and (C) *Rhinomonas* sp. Wells 1, 2, 3, and 4 correspond to 50 ng of genomic DNA from *Isochrysis* sp., *C. calcitrans, Rhinomonas* sp., and *Oikopleura dioica*, respectively, with negative H_2O control in well 5.

2005), and we did not observe any deviation from this rate in this study.

DNA extraction and qPCR—DNA was extracted and purified using the DNeasy® Tissue Kit (Qiagen) according to the manufacturer's recommendations for animal tissues, with the exception that a second proteinase K digestion (final concentration 1 mg mL^{-1}) was performed for 10 min at 55°C after the RNase A treatment. This additional digestion promoted more complete homogenization of appendicularian tissues and houses (data not shown). The abundance of algal cells was estimated from qPCR of algal-specific 18S rDNA fragments. Cycle threshold (C_t) values obtained from total DNA extracted from appendicularian tissues were compared to standard curves prepared from known numbers of algal cells and from cloned algal 18S rRNA genes such that prey abundance associated with appendicularian tissues could be expressed either as cells mL^{-1} or as number of gene copies. All PCR primers and reaction conditions used in this study are provided in Table 1.

Results

Primer specificity—Species-specific primers were designed on the basis of sequences retrieved by cloning the PCR fragment that was amplified using the universal 18S rDNA primers (Table 1). Primers were designed to differentiate between the three algae species and *O. dioica*, yielding short fragments (<100 bp) (Fig. 2). The primers used in this study were designed exclusively for these controlled studies, and primer specificity was not exhaustively determined by comparison to available sequences in silico or empirically. Therefore, when investigating more complex interactions, such as a field sample, these primers should be evaluated for cross-reaction with other species-specific 18S rDNA genes.

Ingestion and house trapping rates under diets of single algal species—Ingestion of *Isochrysis* sp. by *O. dioica* was saturated at 2.7 μg C $ind.^{-1}$ d^{-1} when food concentrations exceeded 100 μg C L^{-1} (10,000 cells mL^{-1}; Table 2). In contrast, total particle removal rate increased because significantly more prey ($p < 0.005$) were trapped in the house when the concentration of *Isochrysis* sp. increased from 10,000 to 50,000 cells mL^{-1} (Table 2). This caused a drop in the percent ingested versus total particle removal at the highest prey concentration (Fig. 3). The clearance rate dropped from ~27 mL $ind.^{-1}$ d^{-1} to ~6.5 mL $ind.^{-1}$ d^{-1} when the *Isochrysis* sp. food concentration increased 5-fold from 10,000 cells mL^{-1} to 50,000 cells mL^{-1}, indicating a saturation point at very high food concentrations of *Isochrysis* sp. Nonetheless, *O. dioica* appeared to be an efficient feeder on *Isochrysis* sp. since >75% of all prey removed was ingested, even at the highest concentrations.

Ingestion of *Chaetoceros calcitrans* appeared to be independent of food concentration (Table 3) with a large proportion of this alga trapped in the house. In Fig. 3, a clear shift toward particles trapped in the house was

Table 2. *Oikopleura dioica* particle removal rates at 15°C on monoalgal diets of *Isochrysis* sp.

FC* (μg C L^{-1})	FC* (cells mL^{-1})	Removal rate (cells $\times$ 10^3 ind.$^{-1}$ d$^-$)	Removal rate (μg C ind.$^{-1}$ d^{-1})	Clearance rate (mL ind.$^{-1}$ d^{-1})
Animals				
10.6	1,000	26 ± 11	0.28 ± 0.12	26 ± 11
106.1	10,000	259 ± 94	2.75 ± 0.99	25.9 ± 9.4
530.7	50,000	248 ± 34	2.63 ± 0.37	5.0 ± 0.7
Houses				
10.6	1,000	2.49 ± 0.20	0.03 ± 0.00	2.49 ± 0.2
106.1	10,000	11.3 ± 2.5	0.12 ± 0.03	1.1 ± 0.2
530.7	50,000	77 ± 17	0.82 ± 0.18	1.5 ± 0.3
Total				
10.6	1,000	29 ± 11	0.30 ± 0.12	28.5 ± 10.9
106.1	10,000	270 ± 94	2.87 ± 0.99	27 ± 9.4
530.7	50,000	325 ± 38	3.45 ± 0.41	6.5 ± 0.8

* FC = food concentration. Means are shown with their corresponding standard deviations.

observed as the food concentration increased. The total removal rate was lower than that of *Isochrysis* sp. (Tables 2, 3), and clearance rates were very low compared to *Isochrysis* sp. As discussed later, our data suggests that a large proportion of *C. calcitrans* was retained on the inlet filter of the house and that clearance rate was therefore not a good indicator of feeding effort.

While removal of *Isochrysis* sp. was saturated at 10,000 cells mL^{-1}, removal of *Rhinomonas* sp. increased when fed more than 10,000 cells mL^{-1} (Table 4). However, the clearance rate was reduced at concentrations greater than 10,000 cells mL^{-1} indicating a saturation point. For *Rhinomonas* sp., both the cell- and the carbon-specific ingestion rates were low compared to *Isochrysis* sp. (Tables 2, 4), while only the carbon-specific ingestion rate was higher than for *C. calcitrans* (Table 3), indicating that either size or shape is preventing maximum feeding rates by *O. dioica* on *Rhinomonas* sp. and *C. calcitrans*. Unlike *C. calcitrans, Rhinomonas* sp. is substantially larger than *Isochrysis* sp. However, most of the *Rhinomonas* sp. cells entered through the inlet filter and were then trapped in the house (Fig. 3).

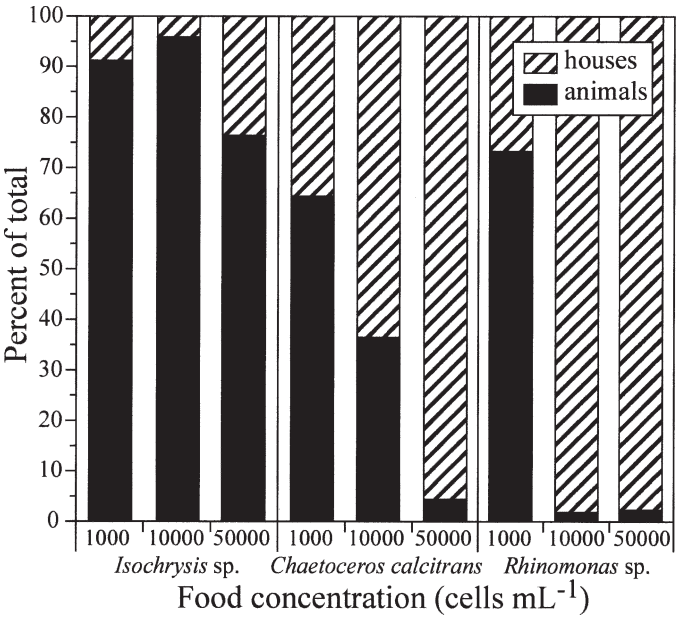

Fig. 3. Ingestion efficiency of *Oikopleura dioica* fed monoalgal diets under conditions of varying particle size/shape and concentration. Percentage of particles trapped in houses versus ingested by animals when fed *Isochrysis* sp., *Chaetoceros calcitrans*, and *Rhinomonas* sp. over a range of particle concentrations at 15°C.

Thus, at comparable concentrations, feeding on *Isochrysis* sp. resulted in higher ingestion rates than either *C. calcitrans* or *Rhinomonas* sp. (Fig. 4A). House trapping rates of *Rhinomonas* sp. were higher than either *Isochrysis* sp. or *C. calcitrans* (Fig. 4B). *O. dioica* were able to remove more particles of *Isochrysis* sp. than *C. calcitrans* at all food concentrations, while *Rhinomonas* sp. were removed at higher rates from the water column than both *Isochrysis* sp. and *C. calcitrans* at very high food concentrations (Fig. 4C). This indicates that unlike *C. calcitrans, Rhinomonas* sp. and *Isochrysis* sp. entered efficiently through the inlet filters, while only *Isochrysis* sp. were ingested efficiently by *O. dioica*.

Ingestion and house trapping rates in mixed algae food regimes—The differential ingestion, house trapping, and removal rates observed in response to the monoalgal diets described here next led us to investigate whether increasing diet complexity with a mixture of these different algal species would alter the parameters for each individual algal species in the mix. This could arise through particles of one species altering the trapping or ingestion efficiency of another. Under these mixed diet conditions, concentrations of 100, 1,000, and 10,000 cells mL^{-1} of *Isochrysis* sp. resulted in nonsaturated ingestion rates (Table 5). This was in agreement with monoalgal experiments, where saturation for ingestion of *Isochrysis* sp. required concentrations above 10,000 cells mL^{-1}. Increases in the number of cells that were trapped in the house and the total number of cells removed with increasing food concentration were also observed. Increasing the food concentration from 100 to 1,000 cells mL^{-1} also generated

Table 3. *Oikopleura dioica* particle removal rates at 15°C on monoalgal diets of *Chaetoceros calcitrans*.

FC* (μg C L^{-1})	FC* (cells mL^{-1})	Removal rate (cells $\times$ 10^3 ind.$^{-1}$ d^{-1})	Removal rate (μg C ind.$^{-1}$ d^{-1})	Clearance rate (mL ind.$^{-1}$ d^{-1})
Animals				
4.6	1,000	4.5 ± 1.7	0.02 ± 0.01	4.5 ± 1.73
46.3	10,000	3.7 ± 1.0	0.02 ± 0.00	0.37 ± 0.1
231.3	50,000	4.3 ± 1.1	0.02 ± 0.01	0.09 ± 0.02
Houses				
4.6	1,000	2.5 ± 0.9	0.01 ± 0.00	2.5 ± 1.0
46.3	10,000	6.4 ± 1.9	0.03 ± 0.01	0.64 ± 0.19
231.3	50,000	95 ± 4.4	0.44 ± 0.20	1.89 ± 0.88
Total				
4.6	1,000	7.0 ± 2.0	0.03 ± 0.01	7.0 ± 2.0
46.3	10,000	10.1 ± 2.2	0.05 ± 0.01	1.01 ± 0.22
231.3	50,000	98.9 ± 44.0	0.46 ± 0.20	2.0 ± 0.9

* FC = food concentration. Means are shown with their corresponding standard deviations.

a drop in clearance rate. Over 90% of the *Isochrysis* sp. particles were ingested versus trapped in the house at food concentrations ranging from 100 to 1,000 cells mL^{-1} (Fig. 5). However, the ingestion rate decreased to 50% of available particles at 10,000 cells mL^{-1}. This was in contrast to observations when the animals were fed a monospecific algal culture and indicated a negative influence on ingestion rate by the two accompanying algal species in the mixed diet.

When presented with a mixed diet, there was a slight increase in the number of *C. calcitrans* cells ingested by *O. dioica* with increasing food concentrations. However, the general trend was similar to that seen in the single prey experiment, where ingestion of *C. calcitrans* was independent of food concentration (Table 6). Total particle removal showed similar trends to the single prey experiment, with slightly higher values at 10,000 cells mL^{-1} in the mixed experiment. In agreement with the single prey experiment, there was a continuous decrease in the percent of ingested *C. calcitrans* particles with increasing food concentration (Fig. 5).

The ingestion rate of *Rhinomonas* sp. increased with increasing food concentration and was in the same range as for the single prey experiment (Table 7). However, there were significantly less *Rhinomonas* sp. particles trapped in the house in the mixed prey experiment compared to the single prey experiment (Tables 4, 7). The total number of cleared particles was therefore lower in the mixed experiment, suggesting that the presence of other algal species influenced the entrance of *Rhinomonas* sp. through the inlet filter of the house. The clearance rate is therefore not a good indicator of feeding effort when *O. dioica* was fed this alga under these conditions. There was approximately an 80% ingestion of *Rhinomonas* sp. particles entering the house at food concentrations up to 1,000 cells mL^{-1} and then a rapid drop in ingestion efficiency at higher food concentrations (Fig. 5). This was consistent with the single prey experiment.

As in the single prey experiments, *Isochrysis* sp. was ingested at the highest rates (Fig. 6A) in mixed algal experiments. However, at high mixed food concentrations, *Isochrysis* sp. cells also exhibited the highest house trapping

Table 4. *Oikopleura dioica* particle removal rates at 15°C on monoalgal diets of *Rhinomonas* sp.

FC* (μg C L^{-1})	FC* (cells mL^{-1})	Removal rate (cells $\times$ 10^3 ind.$^{-1}$ d^{-1})	Removal rate (μg C ind.$^{-1}$ d^{-1})	Clearance rate (mL ind.$^{-1}$ d^{-1})
Animals				
51.5	1,000	4.82 ± 5.7	0.25 ± 0.3	4.8 ± 5.7
515.4	10,000	4.1 ± 0.25	0.21 ± 0.01	0.41 ± 0.03
2,577.2	50,000	12.6 ± 2.3	0.65 ± 0.12	0.25 ± 0.05
Houses				
51.5	1,000	1.8 ± 1.5	0.09 ± 0.08	1.8 ± 1.5
515.4	10,000	227 ± 27	11.7 ± 1.4	22.7 ± 2.7
2,577.2	50,000	552 ± 146	29.1 ± 7.5	11.0 ± 2.9
Total				
51.5	1,000	6.6 ± 5.9	0.34 ± 0.31	6.6 ± 5.9
515.4	10,000	231 ± 27	11.9 ± 1.4	23.1 ± 2.7
2,577.2	50,000	565 ± 146	29.1 ± 7.5	11.3 ± 2.9

* FC = food concentration. Means are shown with their corresponding standard deviations.

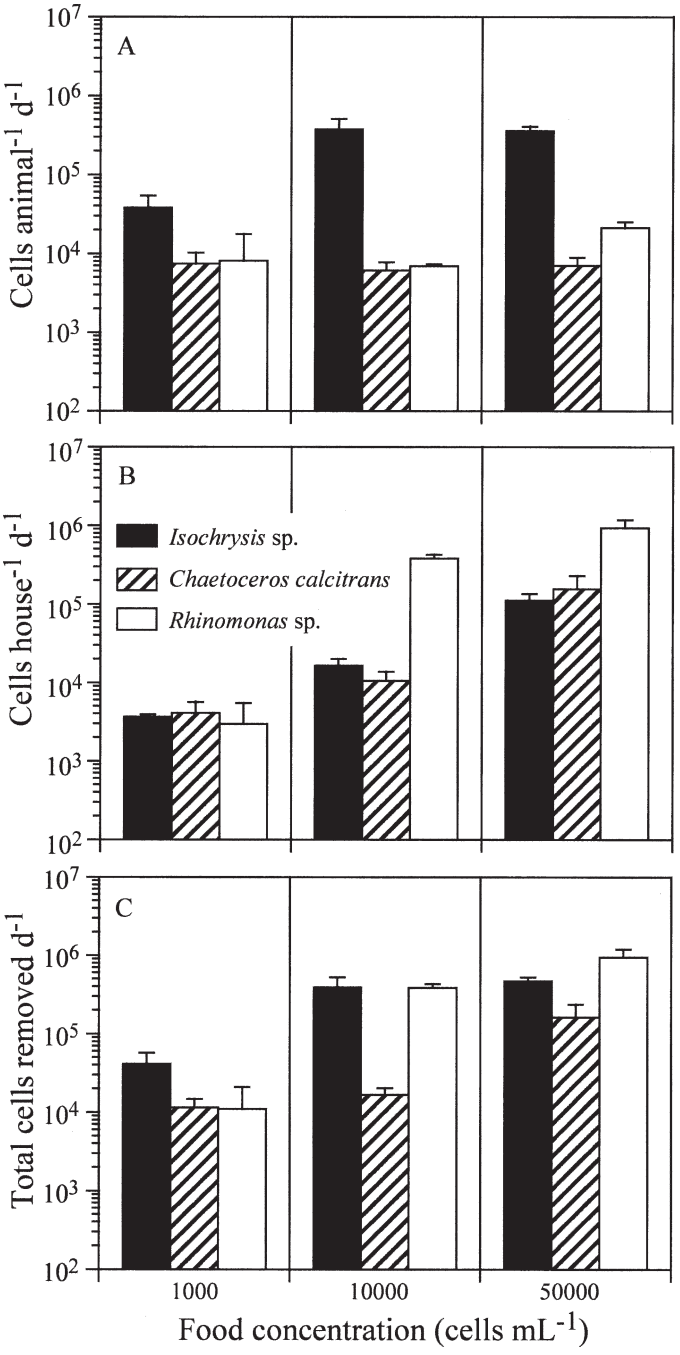

Fig. 4. Particle fate when *Oikopleura dioica* were fed monoalgal diets of varying particle size/shape and concentration at 15°C. The rates of (A) ingestion, (B) house trapping, and (C) particle removal from the water column are expressed as number of cells per individual animal per hour. Values are given as means with the corresponding standard deviations.

rates (Fig. 6B). Therefore, unlike the single prey experiment, *Isochrysis* sp. showed the highest particle removal rate of the three algal species in a mixed diet (Fig. 6C), indicating that interactions among the three particle types affected particle removal rates for *O. dioica*.

Discussion

High feeding rates on small prey—O. dioica ingested the small (6 μm) flagellate *Isochrysis* sp. more efficiently than the larger (17 μm) flagellate *Rhinomonas* sp. or the diatom *C. calcitrans*, with a smaller cell size (4 × 3 μm), but with projecting spines. This occurred at all prey concentrations and in both monoalgal and mixed diet experiments. At abundances of 10.6–531 μg C L^{-1} of *Isochrysis* sp., ingestion varied from 0.28 to 2.6 μg C ind.$^{-1}$ d^{-1} corresponding to 7–63% daily body carbon-specific ingestion, assuming 4.1 μg C per *O. dioica* (calculated from total body length of 672 μm and a body weight per length regression y [μg C μm^{-1}] = 0.0183x − 8.1969, r^2 = 0.997; derived from table 3 in Acuña and Kiefer 2000). The ingestion, daily C-rations and house-trapping rates were within the range of comparable estimates for similar-sized *O. dioica* fed radiolabeled (^{14}C) *Isochrysis galbana* (Acuña and Kiefer 2000). Others, such as Broms and Tiselius (2003), using a similar food regime (10,000–17,000 *Isochrysis* sp. cells mL^{-1}) reported a clearance rate of 56.7 mL ind.$^{-1}$ d^{-1}, which is two times higher than detected by the qPCR method here (Table 2). The slightly lower values obtained in this study might be attributed to minor DNA degradation of the algal cells after removal from the water column. However, our data underscore previous suggestions that appendicularians have a substantial feeding potential when in favorable conditions and suggest that the qPCR method provides reliable measurement of ingestion rates.

Small prey saturation concentrations—The ingestion of *Isochrysis* sp. by *O. dioica* was saturated at 10,000 cells mL^{-1} (~100 μg C L^{-1}) in agreement with Acuña and Kiefer (2000), who reported peak ingestions between 76 and 320 μg C L^{-1}, and King (1981), reporting values of 62 to 125 μg C L^{-1} for the same grazer feeding on *I. galbana* (as cited in Bochdansky and Deibel 1999). Clearance rate was reduced at the highest concentration of *Isochrysis* sp. (50,000 cells mL^{-1} = 530 μg C L^{-1}), suggesting decreased pumping activity at prey abundance above 100–500 μg C L^{-1}. This agrees with published data showing that *Oikopleura* decreases its feeding activity with increasing food concentration (Bochdansky and Deibel 1999; Acuña and Kiefer 2000; Selander and Tiselius 2003), and the percent of cleared algae ingested decreased as *Isochrysis* sp. concentrations exceeded 100 μg C L^{-1} (Fig. 5J in Acuña and Kiefer 2000). This indicates a saturation point at ~100 μg C L^{-1}, above which an increasing amount of small prey such as *Isochrysis* sp. are still removed from the water column and trapped in the house but no longer ingested at optimal rates (Table 2; Fig. 3A).

Reduced feeding on spined or larger prey—O. dioica was not able to feed efficiently on the diatom *C. calcitrans*. The ingestion rate of *C. calcitrans* was 10–100 times lower than for *Isochrysis* sp., and 78–96% of the removed *C. calcitrans* was trapped in houses at the highest prey concentrations in both monoculture and mixed prey suspensions (Tables 2, 3,

Table 5. *Oikopleura dioica* removal rates of *Isochrysis* sp. from a mixed algal diet at 15°C.

FC* (μg C L^{-1})	FC* (cells mL^{-1})	Removal rate (cells $\times$ 10^3 ind.$^{-1}$ d^{-1})	Removal rate (μg C ind.$^{-1}$ d^{-1})	Clearance rate (mL ind.$^{-1}$ d^{-1})
Animals				
1.1	100	9.0 ± 1.3	0.1 ± 0.01	90 ± 13
10.6	1,000	31 ± 9.1	0.33 ± 0.1	31.4 ± 9.1
106.1	10,000	140 ± 48	1.54 ± 0.51	14.5 ± 4.8
Houses				
1.1	100	0.84 ± 0.15	0.01 ± 0.00	8.4 ± 1.5
10.6	1,000	1.4 ± 0.3	0.02 ± 0.01	1.4 ± 0.3
106.1	10,000	150 ± 13	1.54 ± 0.13	14.5 ± 1.3
Total				
1.1	100	9.8 ± 1.3	0.1 ± 0.01	98.1 ± 13
10.6	1,000	32.9 ± 9.1	0.35 ± 0.1	32.9 ± 9.1
106.1	10,000	290 ± 50	3.08 ± 0.53	29.0 ± 5.0

* FC = food concentration. Note that these concentrations are given for only the algae under consideration (*Isochrysis* sp.). For the total prey population from which the rates were determined, the cells mL^{-1} values were 300, 3,000, and 30,000, whereas corresponding total values in μg C L^{-1} were 6.8, 66.7, and 667.8, respectively. Means are shown with their corresponding standard deviations.

5, 6). The *C. calcitrans* cell is slightly smaller (3 $\times$ 4 μm) than *Isochrysis* sp., but they have silicate spines and can form colonies, though microscopy did not reveal any significant colony formation in our cultures. Our data suggest that the spines of *C. calcitrans* reduce entry into the house as well as increasing adherence to house structures once they have passed the inlet filter. These results support the theory that the evolution of spines and colony formation in prey may be an active defense against effective predators (e.g., Lürling et al. 1997). On the other hand, it is more difficult to evaluate feeding effort by *Oikopleura* based on studies using such algae.

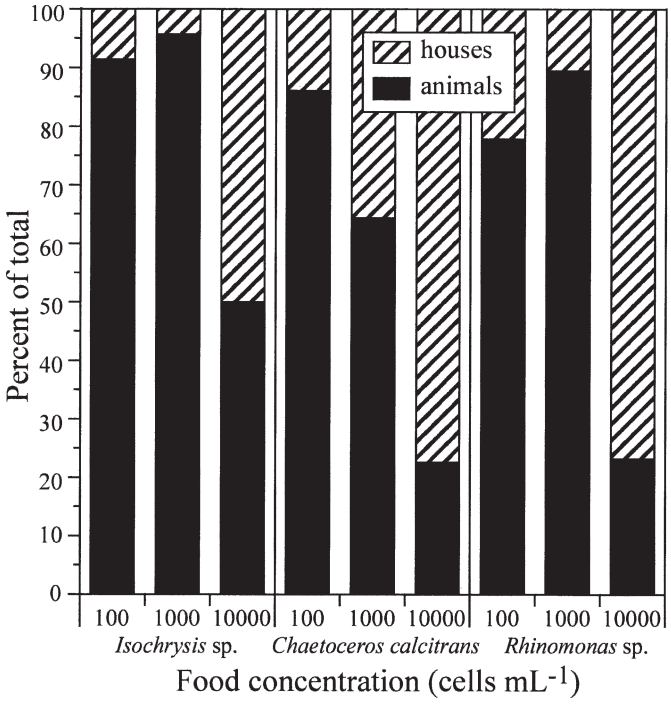

Fig. 5. Ingestion efficiency of *Oikopleura dioica* for individual algal species differing in particle size/shape when presented at varying concentrations in a mixed algal diet at 15°C. Percentage of each specific algal particle (*Isochrysis* sp., *Chaetoceros calcitrans*, and *Rhinomonas* sp.) trapped in the house versus ingested into the animal, when obtained from a mixed prey population, are indicated. In each case, particle concentrations on the x-axis are expressed only for the algal species under consideration, and the total mixed prey concentrations are therefore triple these values.

In monocultures, *O. dioica* removed more cells of *Rhinomonas* sp. than *Isochrysis* sp. per unit time at the highest food concentrations (Fig. 4). At food concentrations up to 1,000 cells mL^{-1}, 70–80% of the removed *Rhinomonas* sp. cells were ingested, though at concentrations $\geq$10,000 cells mL^{-1}, most *Rhinomonas* sp. cells were trapped in the house after passing the inlet filter (Tables 4, 7). Consequently, clearance rates from such algae can be misleading when assessing secondary production and energy budgets. On the other hand, *Rhinomonas* sp. showed house trapping rates of 10–30 μg C ind.$^{-1}$ d^{-1} (approximately 10 times the body C d^{-1}), which again stress the ecological significance of appendicularians in studies of food web energy transfer, sedimentation, and nutrient regeneration (e.g., Silver and Alldredge 1981; Capitano et al. 2005; Scheinberg et al. 2005 and references therein).

In contrast to the other two algae, feeding on *Rhinomonas* sp. was highly influenced by other particles in the mixed algae experiments. Significantly less cells of *Rhinomonas* sp. were trapped in the house in the mixed experiment compared to the single prey experiment (Tables 4, 7). In the single prey experiment, *O. dioica* ingested more cells of *Isochrysis* sp. than *Rhinomonas* sp., while more cells of *Rhinomonas* sp. were trapped in the house than of *Isochrysis* sp. (Tables 2, 4). This suggests that both *Isochrysis* sp. and *Rhinomonas* sp. enter the house easily in single prey suspensions, while *C. calcitrans* was retarded at the inlet filter in all experiments. Thus, it seems that *C. calcitrans* clogs the inlet filter and therefore blocks larger particles, such as *Rhinomonas* sp., from entry, while *Isochrysis* sp. seems to be small enough to still pass relatively unimpeded. Further, because *C. calcitrans* was

Table 6. *Oikopleura dioica* removal rates of *Chaetoceros calcitrans* from a mixed algal diet at 15°C.

FC* (μg C L^{-1})	FC* (cells mL^{-1})	Removal rate (cells $\times$ 10^3 ind.$^{-1}$ d^{-1})	Removal rate (μg C ind.$^{-1}$ d^{-1})	Clearance rate (mL ind.$^{-1}$ d^{-1})
Animals				
0.5	100	5.4 ± 1.3	0.02 ± 0.01	54.0 ± 13.3
4.6	1,000	4.7 ± 1.2	0.02 ± 0.01	4.7 ± 1.2
46.3	10,000	9.4 ± 3.8	0.04 ± 0.02	0.94 ± 0.38
Houses				
0.5	100	0.88 ± 0.08	0.00 ± 0.00	8.8 ± 0.8
4.6	1,000	2.6 ± 1.0	0.01 ± 0.00	2.6 ± 1.0
46.3	10,000	32 ± 5.5	0.15 ± 0.03	3.2 ± 0.6
Total				
0.5	100	6.3 ± 1.3	0.03 ± 0.01	62.8 ± 13.4
4.6	1,000	7.3 ± 1.5	0.03 ± 0.01	7.3 ± 1.5
46.3	10,000	42 ± 6.6	0.19 ± 0.03	4.2 ± 0.7

* FC = food concentration. Note that these concentrations are given for only the algae under consideration (*Chaetoceros calcitrans*). For the total prey population from which the rates were determined, the cells mL^{-1} values were 300, 3,000, and 30,000, whereas corresponding total values in μg C L^{-1} were 6.8, 66.7, and 667.8, respectively. Means are shown with their corresponding standard deviations.

not observed at higher abundance in the house, we suggest that *C. calcitrans* is not trapped within the house but is caught on the inlet filter and held there by the negative pressure exerted by *O. dioica* pumping. When pumping is arrested, as, for example, when the house is discarded, this mat is shed.

Ecological significance—The significant feeding rates even at the lowest food concentrations (1–10 μg C L^{-1}) suggest that *O. dioica* is adapted to oligotrophic or nonbloom conditions. Indeed, even under suboptimal conditions, they show large rates of population increase (Troedsson et al. 2002). For small prey such as *Isochrysis* sp., saturation of ingestion is reached at about 100 μg C L^{-1}. This C-concentration corresponds to moderate bloom conditions in eutrophic coastal waters, while seasonal phytoplankton blooms can often reach several hundreds to over a thousand μg C L^{-1} in Norwegian fjords and coastal waters (e.g., Paasche and Østergren 1980; Erga and Heimdal 1984). Furthermore, seasonal phytoplankton blooms are often dominated by larger chain-forming spiny diatoms or colony forming *Phaeocystis* spp. (e.g., Paasche and Østergren 1980; Erga and Heimdal 1984; Erga 1989). As indicated in this study (and Tiselius et al. 2003), such algae will most likely block the entry of prey into the house of *O. dioica*. This suggests that *O. dioica* populations may be bottom-up regulated by large or spiny particles, which typically dominate late phytoplankton blooms, and instead act as a mediator of smaller prey such as algae and bacteria-sized prey (Fernández et al. 2004) to higher trophic levels in periods of dominance by small prey particles (Capitano et al. 2005; Shiganova 2005). In accordance, dense populations of *O. dioica* have been reported early in the season before large particles are thought to dominate in the southern Sea of Japan (Tomita et al. 2003). Interestingly, other appendicularian species dominate at other

Table 7. *Oikopleura dioica* removal rates of *Rhinomonas* sp. from a mixed algal diet at 15°C.

FC* (μg C L^{-1})	FC* (cells mL^{-1})	Removal rate (cells $\times$ 10^3 ind. $^{-1}$ d^{-1})	Removal rate (μg C ind. $^{-1}$ d^{-1})	Clearance rate (mL ind. $^{-1}$ d^{-1})
Animals				
5.2	100	3.5 ± 1.8	0.18 ± 0.09	34.5 ± 17.6
51.5	1,000	4.1 ± 3.2	0.21 ± 0.16	4.1 ± 3.2
515.4	10,000	14.0 ± 3.3	0.7 ± 0.17	1.4 ± 0.3
Houses				
5.2	100	1.0 ± 0.6	0.05 ± 0.03	9.9 ± 6.4
51.5	1,000	0.48 ± 0.05	0.02 ± 0.00	0.5 ± 0.1
515.4	10,000	45.0 ± 3.2	2.3 ± 0.2	4.5 ± 0.3
Total				
5.2	100	4.4 ± 1.9	0.23 ± 0.1	44.4 ± 18.8
51.5	1,000	4.6 ± 3.2	0.24 ± 0.16	4.6 ± 3.2
515.4	10,000	58.2 ± 4.6	3.0 ± 0.24	5.8 ± 0.5

* FC = food concentration. Note that these concentrations are given for only the algae under consideration (*Rhinomonas* sp.). For the total prey population from which the rates were determined, the cells mL^{-1} values were 300, 3,000, and 30,000, whereas corresponding total values in μg C L^{-1} were 6.8, 66.7, and 667.8, respectively. Means are shown with their corresponding standard deviations.

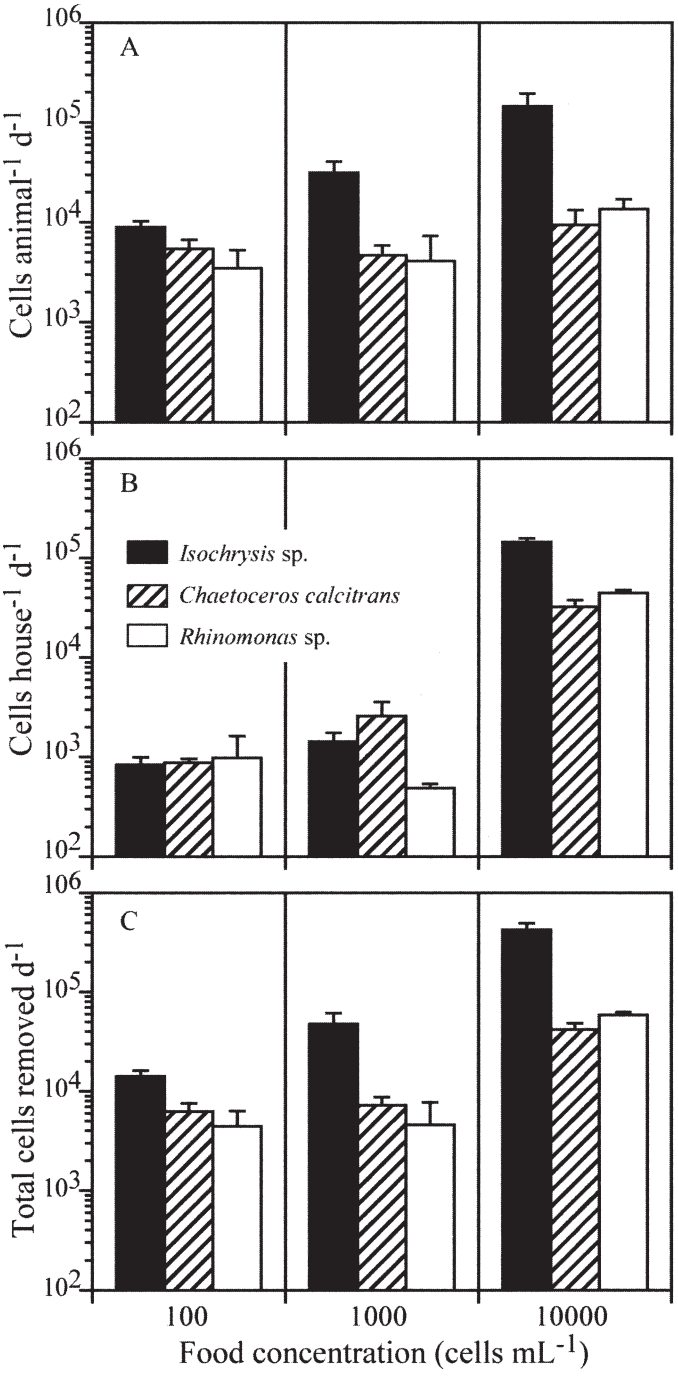

Fig. 6. Particle fate for each individual algal species when *Oikopleura dioica* were fed a mixed algal diet of varying particle size/shapes and concentrations at 15°C. For each algal species, the rates of (A) ingestion, (B) house trapping, and (C) particle removal from the water column are expressed as number of cells per individual animal per hour. Values are given as means with the corresponding standard deviations. In each case, particle concentrations on the x-axis are expressed only for the algal species under consideration, and the total mixed prey concentrations are therefore triple these values.

times of the year. As particles of different sizes can have a detrimental effect on appendicularians, this suggests that the evolution of different morphologies of the filters might explain the succession of appendicularians throughout the year. This may also partly explain the adaptation of different appendicularians to different regions of the ocean. Thorough investigations of filter morphologies, combined with population dynamics and in situ feeding studies of the different species, are required to test these hypotheses. The assay detailed in this work makes these types of feeding studies possible.

The notion that appendicularians also trap particles in the house has important consequences for studies of nutrient regeneration cycles in the ocean. Given the abundance of appendicularians (Tomita et al. 2003), they could represent a significant source of transport of nutrients from surface to bottom, as has been reported earlier (e.g., Alldredge 1976; Silver and Alldredge 1981; Hansen et al. 1996). A major route of nutrient flux in the ocean involves organisms with shells made of $CaCO_3$ (Feely et al. 2004). As these organisms die, the $CaCO_3$ shells sink to the bottom and trap lighter nutrient-rich particles in a process called ballasting, making larger aggregates as they sink. However, the increasing partial pressure of CO_2 observed in oceans in recent years drives the dissolution of $CaCO_3$ and therefore reduces nutrient flux (Feely et al. 2004). Appendicularians, on the other hand, aggregate particles independently of the ballasting mechanism and therefore represent an alternative mechanism. With the increasing pCO_2 of the oceans, the appendicularian route may become increasingly important for export of organic material out of the euphotic zone. The qPCR method developed here can be instrumental in assessing the amount and type of prey particles that appendicularian houses can remove from the surface layers of the ocean.

Assessing the methodology and the potential of the qPCR approach—Several studies have reported clearance rates for *Oikopleura* spp., both in the field and in the laboratory (e.g., Bochdansky and Deibel 1999; Selander and Tiselius 2003; Scheinberg et al. 2005). We are aware of only one previous study on ingestion rate versus house trapping of different algal species by *O. dioica* (Acuña and Kiefer 2000). Using similar-size animals and temperature, both studies indicate that different prey particles may be ingested and trapped in the house at different rates. This study also indicates that shape and size of the different prey, as well as interactions between them, may change these rates over several orders of magnitude. This clearly demonstrates the need to adequately assess these processes separately to understand and quantify the trophic interactions with appendicularians in complex natural food webs. The method used by Acuña and Kiefer (2000) has some practical limitations in quantifying these processes in situ. Organisms need to be manipulated during radiolabeling and incubation, and the number of prey types that can be differentially labeled simultaneously is limited. In comparison, the qPCR method is a highly sensitive and a potentially efficient way of analyzing a multitude of specific food

particles in a relatively short time. Unlike gut volume estimates (López-Urrutia et al. 2003), qPCR is able to distinguish the quantity of prey in the gut at the species level.

Although, the qPCR method depends on the development of unique primers for each prey type, once such primers are defined, this is a very fast and powerful method that can assess any prey type containing amplifiable DNA. This study is to our knowledge the first to adopt a qPCR approach to amplify prey-specific amplicons in the gut of a predator. Although our laboratory experiment is subject to some of the shortcomings of bottle incubations, it demonstrates that we can quantify species-specific prey types in the gut of *O. dioica*. This method can easily be adapted to the field for in situ analysis of prey in the gut of any zooplankton. Similar molecular approaches have already proven very promising for copepods (Nejstgaard et al. 2003) and in a number of other biological fields (reviewed by Symondson 2002).

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