

Functional response of the appendicularian *Oikopleura dioica*

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Abstract

Different concentrations of ¹⁴C radiolabeled cultures of the prymnesiophyte *Isochrysis galbana* (5.5 μm in size), the prasinophyte *Tetraselmis suecica* (9.5 μm), and the chlorophyte *Chlorella* sp. (3.5 μm) were offered as food to groups of 2–5 *Oikopleura dioica* to determine the response of clearance (CR) and ingestion (IR) rates to food concentration (FC). At high FCs of *I. galbana*, IR and CR of *O. dioica* decreased with age of the house. Aging had little effect on the functional response (FR) because most of the decrease in IR and CR occurred during the initial 10% of the house lifespan. The FR resembled a type II model for the IR and presented saturation and inhibition at high FC (>100 μg C L⁻¹) of *I. galbana*. Fits of FR curves of the IR of animals weighing ca. 3 μg C ind⁻¹ to a Michaelis Menten model yielded I_{\max} (maximum IR) of 198 ± 28, 498 ± 171, and 489 ± 150 ng C ind⁻¹ h⁻¹ and K_m (half saturation FC) of 38 ± 21, 225 ± 177, and 290 ± 205 μg C L⁻¹ for the prey *I. galbana*, *T. suecica*, and *Chlorella* sp., respectively. The K_m values are high and in two of three cases exceed the maximum concentration of ingestible particles found in the natural habitat, which suggests that *O. dioica* is adapted to high phytoplankton concentrations. In contrast with classical FR models, CR remained nearly constant for all concentrations. Particles cleared from suspension by *O. dioica* are ingested and transformed into fast-sinking fecal pellets at low FC, while they are mainly accumulated into slow to fast-sinking filter houses at high FC, which implies considerable shifts in the biogeochemical role of these animals with changing particle concentrations.

The functional response (FR) of a consumer describes how its feeding rate, measured as clearance (CR) or ingestion (IR), varies with food concentration (FC; symbols are defined in Table 1). The shape of the FR curve indicates the tuning between the predator's capture mechanism and its environment and determines the dynamics of predator–prey populations. There is a considerable body of knowledge on the FR of filter feeders (reviewed in Hansen et al. 1997), which contrasts with the scarce literature on the FR of appendicularians. Technical difficulties in working with these delicate zooplankton may explain why only a few controlled laboratory experiments have addressed the variations of IR and CR with FC (e.g., Paffenhöfer 1975; King 1981). In fact, only Bochsansky and Deibel (1999) have explicitly examined the shape of the FR curve of an appendicularian. Their study focused on individual, field-collected, cold water *Oi-*

kopleura vanhoeffeni, with emphasis on the behavioral component of its FR. They found a slight, twofold decrease in CR with a hundredfold increase in FC and house age, but they did not detect saturated ingestion at FCs typical of the spring bloom. However, Bochsansky and Deibel (1999) warned about high individual variability in their study and suggested that future studies should focus on the average response of populations. Moreover, appendicularians concentrate particles in the external filter house, which is secreted and discarded periodically (Fenaux 1985), and capture them in the internal pharyngeal filter (Fenaux 1986). The house accumulates noningested filtrate (Bedo et al. 1993) and becomes progressively less efficient (Bochsansky and Deibel 1999), introducing a time-sensitive component to the measurement of the appendicularian FR.

In this study we used radiolabeled algae under controlled conditions to determine the average population FR of *Oikopleura dioica*, a dioecious, coastal appendicularian typical of inshore environments and coastal embayments (Fenaux 1967). Our goals were to test the hypothesis of house age-dependent changes in feeding rates and their effect on the FR. We investigated whether feeding is saturated at high FC and determined FR parameters for comparison with other zooplankton taxa as compiled by Hansen et al. (1997). Finally, we tested the influence of variable algal diets on the shape of the FR.

Materials and methods

Animal and algal culture—Experiments were conducted and cultures of algae and animals were maintained in a 15 ± 1°C controlled temperature room. The unicellular algae *Isochrysis galbana* (5.5-μm equivalent spherical diameter, ESD; Prymnesiophyceae), *Chlorella* sp. (3.5-μm ESD; Chlorophyceae), and *Tetraselmis suecica* (9.5-μm ESD; Pra-

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Table 1. Symbols used throughout the text.

FR	Functional response
IR	Ingestion rate (ng C ind ⁻¹ h ⁻¹)
CR	Clearance rate (ml ind ⁻¹ h ⁻¹)
ESD	Equivalent spherical diameter (μm)
RAH	Rate of carbon accumulation in the filter house (ng C house ⁻¹ h ⁻¹)
PCI	Percent carbon cleared from suspension that is ingested
FC	Food concentration ($\mu\text{g C L}^{-1}$)
I_{max}	Michaelis-Menten maximum ingestion rate (ng C ind ⁻¹ h ⁻¹)
K_m	Michaelis-Menten half-saturation constant ($\mu\text{g C L}^{-1}$)
C_{max}	Michaelis-Menten maximum clearance rate (ml ind ⁻¹ h ⁻¹)
D	Measured trunk length, from mouth to distal end of the gut (μm)
TL	Total trunk length, from mouth to distal end of the trunk including gonads (μm)
IR_{max}	Theoretical ingestion rate of animals with new filter houses (ng C ind ⁻¹ h ⁻¹)
CR_{max}	Theoretical clearance rate of animals with new filter houses (ml ind ⁻¹ h ⁻¹)
IR_{ave}	Theoretical, time-averaged ingestion rate (ng C ind ⁻¹ h ⁻¹)
CR_{ave}	Theoretical, time-averaged clearance rate (ml ind ⁻¹ h ⁻¹)

sinophyceae) were cultured in 1-liter bottles under 70- μ -Einstein light with aeration and used in late-exponential growth phase for experiments. To account for the slight variability in size, carbon content of cells was calculated according to formulas by Strathmann (1967) after validation against direct CHN analysis. Appendicularian cultures were started from winter 1996 to winter-spring 1997 with animals collected at El Musel harbor in Gijón, Northern Spain, where surface temperature ranges between 10 and 18°C (Muñoz 1982). Up to 10 generations (6 d at 15°C) were cultured in 5-liter glass jars filled with seawater mixed with a Plexiglas spiral paddle rotating at 10 rpm (Fenaux and Gorsky 1979, 1985). The animals were transferred into freshly filtered (<30 μm) natural seawater every 2 d using wide bore pipettes. All experiments were performed during the fifth day of their life cycle, to minimize variability due to body size.

Measurement of house production rates—Ten to twenty appendicularians were preconditioned for 4 h in 5-liter glass beakers filled with a suspension of *I. galbana* at the target FC and transferred into similar beakers filled with a fresh food suspension. After ca. 3.5 h, appendicularians inside houses were removed, all the visible abandoned houses were collected and counted, and the entire experimental suspension filtered through a 10- μm polycarbonate filter, to reveal transparent filter houses as small bumps on the surface of the filter. Short incubation time avoided collapse and aggregation of discarded houses. House lifespan (h house⁻¹) could then be estimated as the inverse of the house production rate (houses h⁻¹). This measurement was repeated 11 times using FCs ranging from 16 to 1,352 $\mu\text{g C L}^{-1}$.

Measurement of clearance and ingestion rates—Cultured algae for grazing measurements were incubated at 400 μC

L⁻¹ of ¹⁴C labeled bicarbonate and 70 $\mu\text{Einstein}$ light for 24 h, centrifuged at 1,500 R.F.C. for 10 min, resuspended in filtered seawater three times to eliminate culture medium with inorganic radiolabel, and filtered through Nuclepore polycarbonate filters (10- μm pore size for *I. galbana* and *Chlorella sp.*, 12- μm pore size for *T. suecica*) to disaggregate and remove clumps of algae. A stock solution was prepared by diluting the labeled cells in seawater filtered twice through Whatman GF/F fiberglass filters, and its concentration adjusted by in vivo fluorescence measurements using a Turner 10-005 fluorometer calibrated against cell counts with a Coulter Multisizer II particle counter. The stock was then serially diluted to the target FCs. Screw-top glass scintillation vials (20-ml) for the grazing incubations were filled with 15-ml of the suspension and covered with aluminum foil to avoid algal growth.

To start the feeding measurements, two to five animals (3.9 \pm 1.0 animals per incubation, mean \pm SD, $n = 175$ incubations) were transferred with 5 ml of the preconditioning unlabeled food suspension using a calibrated wide bore pipette to the 20-ml screw-top experimental vials. The number of filtration bouts per minute of 10 animals held in 5-liter culturing vessels was not significantly different from that of 10 animals held in 20-ml glass scintillation vials (ANOVA, $F = 3.6$, $P = 0.074$), which suggests little stress due to confinement in our incubation vials. After 4 min of incubation, the actively filtering appendicularians were transferred individually to the surface of a plastic Petri dish with a drop of water and then forced to leave their houses with the tip of a fine Pasteur pipette drawn in a Bunsen burner flame. A certain amount of material can be lost through the exit orifice when leaving the house, and therefore ours is a minimum estimate of material accumulated in the house. The end of the feeding trial was considered to be the average time of house abandonment of all the animals used in the incubation.

The appendicularians (not the houses) on the Petri dishes were anesthetized by transferring them with a fine Pasteur-pipette to a saturated (99%) solution of benzocaine in filtered seawater and fixed with a drop of formalin, both of which do not induce regurgitation of gut contents or leakage of radiolabel from phytoplankton cells at the concentrations used. Formalin was added within 7 min from the start of the incubation, less than the gut passage time of *O. dioica* (ca. 8 min; Alldredge 1981; Bedo et al. 1993). López-Urrutia and Acuña (in press) have measured gut passage times as short as 6 min for *O. dioica* at high FCs of *I. galbana*. Thus, while some (i.e., 1/7 of the total gut content) of the radiolabel could be defecated, this loss would be small compared to the FC effects measured. The animals were serially rinsed in three small microscope well slides filled with filtered seawater, their trunk length (D , μm , mouth to distal end of the gut) measured under an Olympus BH-2 stereomicroscope and transferred into a 7-ml plastic scintillation vial to which 2 ml of scintillation cocktail (Ultima Gold, Packard) were added. Houses on the Petri dish were pipetted onto a 10- μm Nuclepore Polycarbonate filter (12- μm for *T. suecica*), rinsed with filtered seawater to separate them from phytoplankton, counted, and placed in a 7-ml plastic scintillation vial to which 4 ml of scintillation cocktail were added. Our

measured clearance rates are at the upper end of published values (*see Results*), which suggests that little radiolabel was lost with this method. Last, 1 ml of the radiolabeled suspension was transferred from the incubation vial to a 7-ml plastic scintillation vial and 2 ml of scintillation cocktail added. Plastic scintillation vials with houses or animals were immersed for 10 min in an ultrasonic bath to disrupt structures and facilitate diffusion of the radiolabel, and their scintillation measured in a Wallac 1409 counter at least 3 d after the end of the experiment, which is the minimum required for stabilization of the sample activity (preliminary experiments, data not shown).

Because passive adsorption of the radiolabel could represent a source of error in the determination of IR and CR, we conducted control measurements in each experiment. Grazing measurements with control animals were conducted with unlabeled cells suspended in the supernatant of a centrifuged subsample of the experimental suspension of radiolabeled algae, which contained not more than 5% of the activity of the labeled algal suspension (preliminary experiment, data not shown) in the form of labeled inorganic bicarbonate and phytoplankton exudates. The unlabeled algae were added to ensure that the animals were experiencing the same particulate environment as in normal incubations and that they were filtering and ingesting at the same rate as in the target FCs. The unlabeled algae were added right before the grazing measurement, to avoid uptake of residual ^{14}C labeled bicarbonate. Since significant linear relationships between material adsorbed to control filter houses and phytoplankton concentration were usually found (Fig. 1), a regression relationship between passive adsorption to the filter house and FC was calculated and used to correct the activity measured in the filter house. Because passive adsorption to animals was low and unrelated to FC, activity in the animals was corrected by subtracting average activity in control animals.

To calculate IR and CR, the total activity (disintegrations per minute = dpm) in both the animals and the houses was first divided by the number of animals or houses. For incubations of time t , CR was calculated as

$$\text{CR} = \frac{\text{dpm}(\text{animal}) + \text{dpm}(\text{house})}{\text{dpm}(\text{water})t} \quad (1)$$

IR was calculated as

$$\text{IR} = \frac{\text{dpm}(\text{animal})\text{FC}}{\text{dpm}(\text{water})t} \quad (2)$$

The rate of carbon accumulation in the filter house (RAH) was calculated as

$$\text{RAH} = \frac{\text{dpm}(\text{house})\text{FC}}{\text{dpm}(\text{water})t} \quad (3)$$

and the proportion of carbon cleared from suspension that is ingested (PCI) was calculated as

$$\text{PCI} = \frac{100 \text{dpm}(\text{animal})}{\text{dpm}(\text{animal}) + \text{dpm}(\text{house})} \quad (4)$$

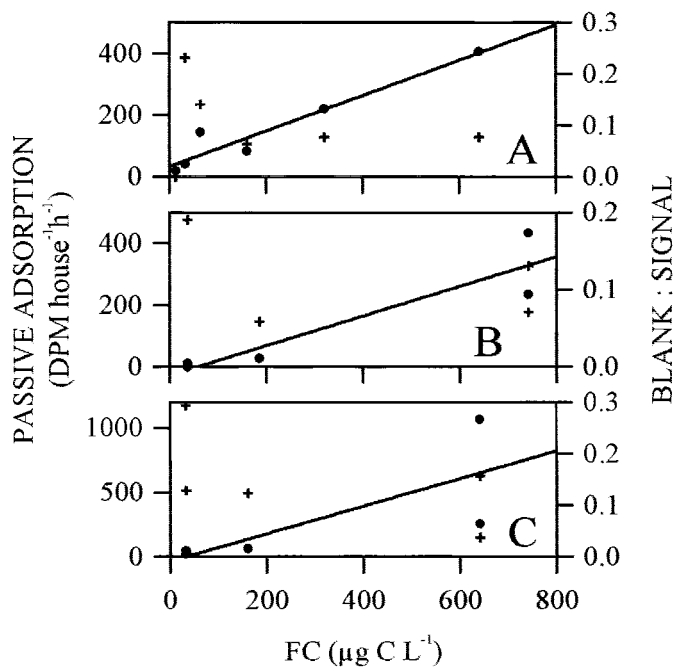


Fig. 1. Plots of the rate of passive radiolabel adsorption to control houses (circles) and the proportion of radiolabel blanks (passive adsorption) to average rate of radiolabel accumulation in houses (plus symbols) in respect to food concentration for FR experiments with (A) *I. galbana*, (B) *Chlorella sp.*, and (C) *T. suecica* as food. Lines indicate least-squares fits of passive adsorption data (PA, $\text{DPM house}^{-1} \text{h}^{-1}$) versus food concentration (FC, $\mu\text{g C L}^{-1}$) for *I. galbana* [$\text{PA} = (0.57 \pm 0.08) \text{FC} + (34 \pm 25)$, parameter estimate \pm SE, $r^2 = 0.92$, $n = 6$, $F = 48$, $P = 0.002$], *Chlorella sp.* [$\text{PA} = (0.48 \pm 0.12) \text{FC} - (27 \pm 55)$, $r^2 = 0.85$, $n = 5$, $F = 17$, $P = 0.026$], and *T. suecica* [$\text{PA} = (1.07 \pm 0.53) \text{FC} - (30 \pm 219)$, $r^2 = 0.57$, $n = 5$, $F = 4$, $P = 0.138$], which were used to correct the activity in experimental houses. Small, negative correction values for passive adsorption at very low food concentrations were assumed to be 0.

Experimental design of feeding experiments—To study the effect of house age on IR and CR, five time-series experiments were conducted, each at a different FC ranging from 80 to 1,602 $\mu\text{g C L}^{-1}$. In each experiment, several appendicularians were preconditioned for at least 1 h at the target FC, transferred to a 500-ml vial filled with filtered seawater, and forced to leave their houses and rebuild new ones. For short (i.e., less than 45 min) house-age treatments the animals with new houses were transferred to a 5-liter stirred culturing beaker filled with an algal suspension at the target FC. Groups of five animals were serially retrieved from the flask at the desired house age and their grazing rates measured as above. For older (i.e., more than 45 min) house ages, groups of five animals with new houses were placed in 500-ml beakers filled with an algal suspension at the target FC, gently stirred every 5 min and, once the houses reached the desired age, retrieved from the 500-ml beaker for grazing rate measurements as above. We discarded every long house-age incubation in which at least one animal abandoned the house before the desired house age, because it was impossible to differentiate the animal with a recent house from the

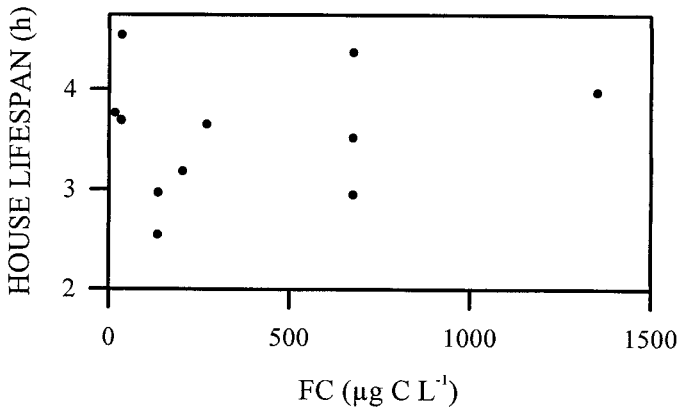


Fig. 2. House lifespan versus food concentration for animals incubated in pure cultures of *I. galbana*. Least-squares fit to a line yielded a slope estimate which was not significantly different from 0 ($n = 12$, $r^2 = 0.02$, $F = 0.23$, $P = 0.64$).

animals with old houses. Failure in house-aging incubations, restrictions in the number of animals available, time constraints, and our need of obtaining better resolution at short house ages limited the number of long (i.e., > 2 h) house-aging incubations.

Another experiment addressed the FR of animals with

newly secreted filter houses. Animals for this experiment were preconditioned at the target FC for at least 1 h, transferred to 500-ml glass beakers filled with filtered seawater, forced to leave their houses, allowed to rebuild new ones, and transferred with 5 ml of filtered seawater to the 20-ml incubation vials filled with 15 ml of labeled food suspension. A last series of experiments was conducted with batches of 2–5 animals of unknown house age, to estimate mean FR for the whole house lifespan. Animals were preconditioned at the experimental FC in 5-liter jars for at least 4 h, more than the house lifespan (see Results) and randomly selected for the grazing measurements, so that an even distribution of house ages could be expected. In all experiments, the incubations at six FCs were repeated 4 times (except for a few missing samples). The order of incubation for each FC within each of four repetitions was randomly assigned to avoid systematic biases.

Data analysis—Because our IR results did not show significant differences in explained variance between type I rectilinear (Blackman 1905; Condrey 1982) or type II hyperbolic models (following data analysis procedures by Rothhaupt 1990, results not shown; see also Mullin et al. 1975 for copepods), and to allow for comparison with parameters calculated by Hansen et al. (1997), we assumed

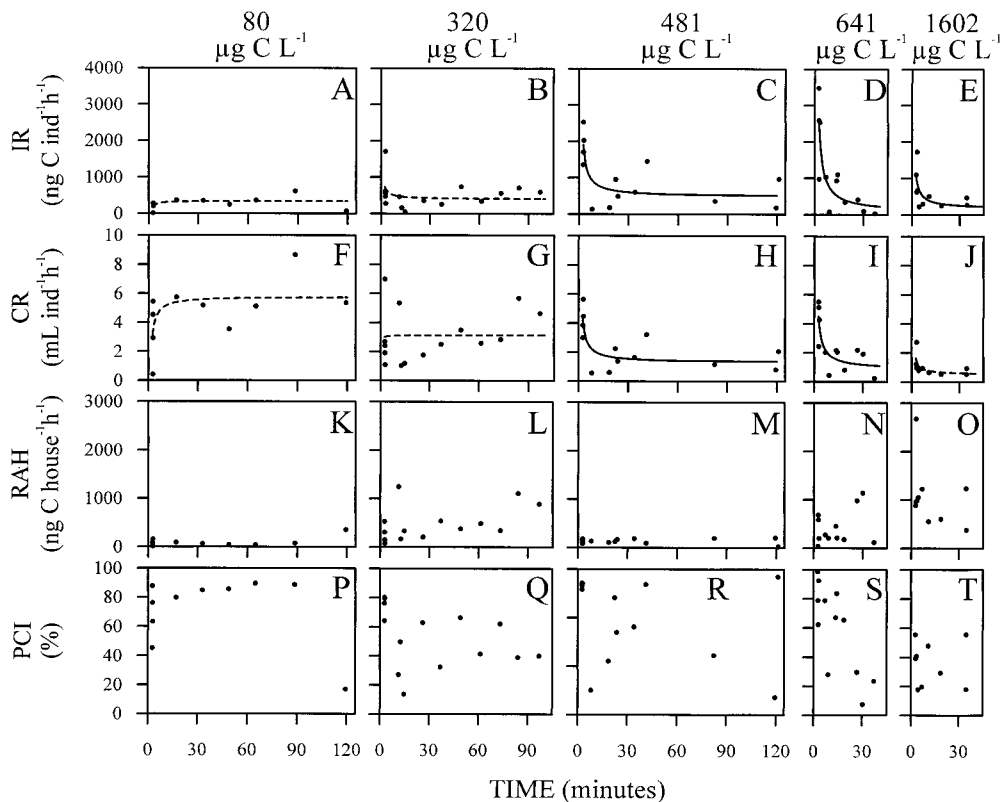


Fig. 3. Effect of house age on ingestion and clearance rates (IR and CR, respectively), rate of carbon accumulation (RAH) in the filter house and percent of carbon cleared which is ingested (PCI). Each column represents an experiment performed at a different food concentration (given at the top of the column). Dashed and solid lines represent nonsignificant and significant least-squares fits to inverse models of the type $IR = a_i + (b_i/t)$ and $CR = a_c + (b_c/t)$, where a_i , b_i , a_c , and b_c are model parameters (see Table 2 for parameter estimates) and t is house age.

Table 2. Results of the house-aging experiments using *I. galbana* as food (Fig. 2). Each experiment was performed on animals of similar size but at a different food concentration (FC, $\mu\text{g C L}^{-1}$). Measured trunk lengths (D, mean \pm SD) and estimated body carbon weights (Weight, mean \pm SD) are indicated. For each experiment, the variation of ingestion rate (IR, $\text{ng C ind}^{-1} \text{h}^{-1}$) and clearance rate (CR, $\text{ml ind}^{-1} \text{h}^{-1}$) with time (t , seconds) was fitted to an inverse function of the kind $\text{IR} = a_i + (b_i/t)$ and $\text{CR} = a_f + (b_f/t)$, where a_i , b_i , a_f , b_f are regression parameters. Regression statistics, (n , R^2 , F , P) as well as parameter estimates (\pm SE) are given for both models. IR_{max} and CR_{max} represent regression estimates of IR and CR for $t = 2.5$ min, i.e., the predicted ingestion and clearance rates of animals with the newest houses we could incubate with our technique. IR_{ave} and CR_{ave} represent averages over the whole house lifespan (214 ± 35 min) of the inverse functions relating IR and CR with t (see results for calculations).

FC ($\mu\text{g C L}^{-1}$)	D (μm)	Weight ($\mu\text{g C ind}^{-1}$)	n	Ingestion rate				
				R^2	F_i	P_i	a_i	b_i
80	730 \pm 88	5.12 \pm 1.80	10	0.19	1.9	0.204	346 \pm 70	-400 \pm 288
320	768 \pm 89	5.92 \pm 2.01	15	0.13	2.0	0.185	403 \pm 131	820 \pm 586
481	714 \pm 67	4.74 \pm 1.33	13	0.59	15.8	0.002	488 \pm 192	3541 \pm 892
641	743 \pm 87	5.38 \pm 1.86	12	0.72	25.6	0.000	71 \pm 278	6208 \pm 1226
1602	752 \pm 81	5.55 \pm 1.72	10	0.43	6.1	0.039	181 \pm 214	2314 \pm 937

Michaelis-Menten, hyperbolic feeding kinetics for *O. dioica*. In the Michaelis-Menten model

$$\text{IR} = \frac{I_{\text{max}} \text{FC}}{K_m + \text{FC}} \quad (5)$$

where I_{max} is the saturated IR at an infinitely high FC, and K_m is the half-saturation FC. IR data were fitted to this model by iterative nonlinear regression using a Marquardt algorithm (Marquardt 1963; Mullin et al. 1975). In this model, the maximum clearance rate (C_{max}) can be calculated after assuming that all of the material cleared is ingested as (Hansen et al. 1997)

$$C_{\text{max}} = \frac{I_{\text{max}}}{K_m} \quad (6)$$

Because some of the material filtered by an appendicularian remains trapped in the filter house, it is not possible to arrive at a mathematical expression to calculate C_{max} . However, almost no material sticks to the house at very low FC (see Results), which implies that we can use Eq. 6 for *O. dioica* at low FC for comparison with C_{max} data of other zooplankton. When no a priori hypothesis on the functional relationship between a variable and FC was available, FC effects were tested by one-way ANOVA on log-transformed variables or by Kruskal-Wallis nonparametric analysis when the assumptions of parametric statistics were not met. SPSS for Windows was used in all statistical analyses. The resolution of our technique at very low FC did not allow us to test for threshold feeding sensu Frost (1975).

We compared our FR parameter estimates against the literature compilation by Hansen et al. (1997). These authors converted their source data, originally in carbon or dry weight units, to volume units by applying across-taxa averages of 0.45 gC (g dry weight) $^{-1}$ and 0.12 g C cm^{-3} . Following recommendations by Schneider (1990, 1992) for gelatinous zooplankton, we used carbon weight rather than body volume, and back-converted all of Hansen et al.'s transformed values to carbon units assuming 0.12 g C cm^{-3} . To convert trunk length (D , μm , distance between mouth and distal end of the digestive tract) for *O. dioica* to total trunk length (TL, μm ,

distance between mouth and distal gonadal end) we used an empirical relationship derived from measurements on cultured animals: $\text{TL} = (-111 \pm 38) + (1.46 \pm 0.07) D$, where $n = 33$, $r^2 = 0.94$, $F = 443$, $P < 0.001$, and parentheses denote parameter estimates \pm SE. Then we transformed TL to body carbon using equations in King et al. (1980) for *O. dioica* and Deibel (1986) for *O. vanhoeffeni*. I_{max} values of all zooplankton species reviewed by Hansen et al. (1997) were corrected to a temperature of 15°C by assuming a Q_{10} of 2.8. However, since *O. vanhoeffeni* lives at temperatures between -1.8 and 5°C, we considered it unrealistic to extrapolate its rate estimates to higher temperatures.

Results

House production rate—Mean house lifespan and house production rate for all experiments pooled were 3.57 ± 0.59 h house $^{-1}$ (mean \pm SD) and 0.28 ± 0.05 house h $^{-1}$, respectively. The slope of the linear regression of house lifespan versus FC was not significantly different from 0 (Fig. 2); thus FC had little or no effect on house lifespan.

House-aging experiments—House age had more clear effects on IR and CR than on RAH or PCI (Fig. 3). This effect was apparent only at FCs above 480 $\mu\text{g C L}^{-1}$, where both IR and CR decreased rapidly during the first 20 min (Fig. 3), which is only 10% of the house lifespan. This trend was best fitted by an inverse model (Fig. 3, Table 2), which can be extrapolated to the minimum house age attainable by our method to obtain a theoretical maximum clearance (CR_{max}) and ingestion rate (IR_{max} , Table 2). While IR_{max} clearly saturates at high FC with values above 2,000 $\text{ng C ind}^{-1} \text{h}^{-1}$, CR_{max} remains nearly constant and close to 4.5 $\text{ml ind}^{-1} \text{h}^{-1}$ for all FCs except the highest one (Table 2). The empirical, inverse functions can also be time averaged by integration, to simulate a more realistic FR. The time-averaged IR (IR_{ave}) saturates at half the saturation level of IR_{max} , whereas the time-averaged CR (CR_{ave}) is lower than the CR_{max} and decreases with increasing FC (Table 2). These calculations clearly suggest that the aging process is consistent with a saturating FR curve for the IR.

Table 2. Extended.

Ingestion rate			Clearance rate					
IR _{max} (ng C ind ⁻¹ h ⁻¹)	IR _{ave} (ng C ind ⁻¹ h ⁻¹)	R ²	F _f	P _f	a _f	b _f	CR _{max} (ml ind ⁻¹ h ⁻¹)	CR _{ave} (ml ind ⁻¹ h ⁻¹)
280±168†	280±168†	0.32	3.7	0.090	2.79±0.82	-6.52±3.38	4.70±2.13†	4.70±2.13†
528±462†	528±462†	0.00	0.01	0.911	3.14±0.66	-0.34±2.97	3.09±1.96†	3.09±1.96†
1904	556	0.59	16.1	0.002	1.31±0.39	7.33±1.83	4.24	1.45
2554	199	0.68	21.7	0.001	0.88±0.44	9.09±1.95	4.52	1.06
1106	226	0.34	4.1	0.078	0.52±0.31	2.77±1.37	1.05±0.64†	1.05±0.64†

† These estimates are simply averages of the IR and CR data for the whole house-aging experiment at that concentration. No use was made of the inverse functions here because their slope was undistinguishable from 0.

Functional response of animals with newly secreted houses—The IR of *O. dioica* with new houses increased with FC, but this increase decelerated at high concentrations of *I. galbana* (Fig. 4A). The CR remained nearly constant up to 800 $\mu\text{g C L}^{-1}$ and decreased above that FC, although this trend was not significant (one-way ANOVA, $P = 0.07$, Fig. 4C). This is qualitatively consistent with the mathematical extrapolation based on the house-aging experiments (IR_{max} and CR_{max}, Table 2). Michaelis-Menten estimated I_{max} and K_m were $1,708 \pm 232 \text{ ng C ind}^{-1} \text{ h}^{-1}$ and $306 \pm 152 \mu\text{g C L}^{-1}$, respectively (Table 3). RAH increased as a 1.22 power of FC (Fig. 4E). PCI decreased significantly with increasing FC (one-way ANOVA on arcsin-transformed data; $F_{5,14} = 14.6$, $P < 0.001$, Fig. 4G).

Average functional response—IR of animals weighing $4.03 \pm 1.22 \mu\text{g C ind}^{-1}$ was saturated and even inhibited (sensu Rothhaupt 1990) at high concentrations of *I. galbana*, which implies a peak in the ingestion rate between 76 and 320 $\mu\text{g C L}^{-1}$ (Fig. 4B). Therefore, the Michaelis-Menten model was unsuitable to describe this relationship. CR decreased with FC ($F_{5,18} = 16.8$, $P < 0.001$, Fig. 4D). These results are in qualitative agreement with time-averaged trends from the house-aging experiments (IR_{ave} and CR_{ave} versus FC in Table 2). RAH increased with increasing FC according to a power function with an exponent of 1.32 (Fig. 4F). PCI decreased with increasing FC (ANOVA on arcsin-transformed data; $F_{5,18} = 17.3$; $P < 0.001$) from ca. 95% to ca. 18% (Fig. 4H).

Effect of algal species on the average functional response—The average IR of *O. dioica* when exposed to a narrower range of FC of *I. galbana* was also saturated or even slightly inhibited at the highest FC (Fig. 5A), but this trend was not apparent with *Chlorella sp.* (Fig. 5B) or *T. suecica* (Fig. 5C). These differences affected the parameter estimates of the Michaelis-Menten model. K_m was 38 ± 21 , 290 ± 205 , and $225 \pm 177 \mu\text{g C L}^{-1}$, and I_{max} was 198 ± 27 , 489 ± 150 , and $498 \pm 171 \text{ ng C ind}^{-1} \text{ h}^{-1}$ for *I. galbana*, *Chlorella sp.*, and *T. suecica*, respectively (Table 3). Thus,

the IR for *I. galbana* had a steeper initial slope and lower saturation level than for *Chlorella sp.* and *T. suecica*.

There was a slight, twofold decrease after an initial peak in CR with increasing FC when *I. galbana* was used as food (ANOVA, $F_{5,19} = 2.94$, $P = 0.039$, Fig. 5D), which is consistent with results from the house-aging experiments (CR_{ave} versus FC, Table 2). This trend did not appear when using *Chlorella sp.* ($F_{5,18} = 1.11$, $P = 0.18$, Fig. 5E) or *T. suecica* ($F_{5,15} = 1.33$, $P = 0.31$, Fig. 5F). CR averaged 2.14 ± 1.36 , 1.51 ± 0.59 , and $2.15 \pm 1.42 \text{ ml ind}^{-1} \text{ h}^{-1}$ when feeding on *I. galbana*, *Chlorella sp.*, and *T. suecica*, respectively. Since CRs were similar for all three algae but IRs were lower for *I. galbana*, a greater proportion of filtered material must remain trapped within the house and not ingested when *I. galbana* was used as food.

RAH increased with FC faster than linearly, according to power curves that explained more than 80% of the total variance for all three algae (Fig. 5G,H,I). Therefore the balance of cleared particles shifted from ingestion to accumulation in the filter house at high FCs. PCI decreased significantly with increasing FC for *I. galbana* (Kruskal-Wallis one-way analysis, $P < 0.001$, Fig. 5J) but not for *Chlorella sp.* ($P = 0.091$, Fig. 5K) or *T. suecica* ($P = 0.159$, Fig. 5L), which suggests that the house fouls faster when feeding on *I. galbana*.

Across-taxa comparison—In *O. dioica*, the estimated I_{max} are slightly above the 95% confidence bounds for the across-taxa allometric regression line of I_{max} versus body carbon (Fig. 6A), while K_m are close to the median K_m ($240 \mu\text{g C L}^{-1}$) in Hansen et al. (1997) (Fig. 6B). An exception was the FR experiment on *I. galbana* (Fig. 5A, Table 3), when K_m was in the lower quartile (Fig. 6B). C_{max} values for *O. dioica* are also above the 95% confidence intervals for the regression line of C_{max} on body size, a departure that is comparable to that of some copepods (Fig. 6C,D). The low variability of CR within natural FCs (Fig. 5D,E,F; Bochdansky and Deibel 1999), suggest that field CR measurements should be close to the physiological maximum, or C_{max} (Eq. 2). For comparison, we have included literature data on field CR measurements of appendicularians in Fig. 6D. C_{max} cal-

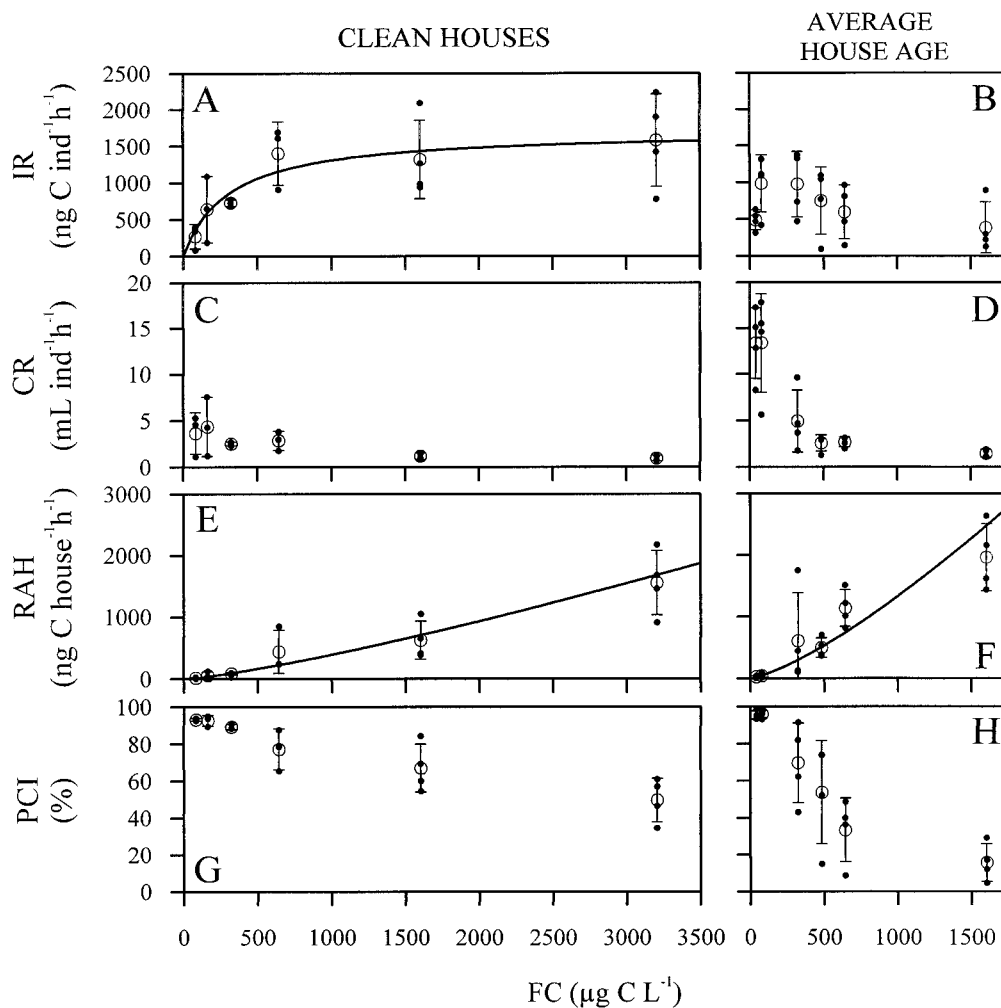


Fig. 4. Feeding functional response of (A) and (B) ingestion rate (IR), (C) and (D) clearance rate (CR), (E) and (F) rate of carbon accumulation in the filter house (RAH), and (G) and (H) proportion of carbon cleared that is ingested (PCI), to food concentration (FC) of animals with newly secreted filter houses (left column) and animals of average house age (right column) feeding on *I. galbana*. Empty dots and vertical lines represent means \pm SD. Solid line in (A) represents the best fit to a Michaelis-Menten (type II) FR (see Table 3 for parameter estimates). Solid lines in (E) and (F) represent least-squares fits between RAH ($\text{ng C house}^{-1} \text{h}^{-1}$) and FC ($\mu\text{g C L}^{-1}$) to a power function, which is $\log(\text{RAH}) = (-1.08 \pm 0.32) + (1.22 \pm 0.11) \log(\text{FC})$ for the experiment with new houses ($n = 20$, $r^2 = 0.86$, $F = 115$, $P < 0.001$), and $\log(\text{RAH}) = (-0.83 \pm 0.29) + (1.32 \pm 0.12) \log(\text{FC})$ for the experiment with houses of average age ($n = 24$, $r^2 = 0.85$, $F = 126$, $P < 0.001$, parameter estimate \pm SE between parenthesis).

Table 3. Measured trunk length (D, mean \pm SD), estimated body carbon weight (weight, mean \pm SD) and parameter estimates (\pm SE) for the four experiments to which a Michaelis-Menten model could be fitted (see method for equation).

Experiment	D (μm)	Weight ($\mu\text{g C ind}^{-1}$)	I_{max} ($\text{ng C ind}^{-1} \text{h}^{-1}$)	K_m ($\mu\text{g C L}^{-1}$)	C_{max} ($\text{ml ind}^{-1} \text{h}^{-1}$)
Clean houses (<i>I. galbana</i>)	741 \pm 100	5.41 \pm 2.03	1708 \pm 232	306 \pm 152	5.58
Average house (<i>I. galbana</i>)	637 \pm 61	3.37 \pm 0.88	198 \pm 27.6	38.9 \pm 21.1	5.09
Average house (<i>Chlorella sp.</i>)	579 \pm 38	2.47 \pm 0.47	489 \pm 150	290 \pm 205	1.69
Average house (<i>T. suecica</i>)	641 \pm 93	3.55 \pm 1.56	498 \pm 171	225 \pm 177	2.21

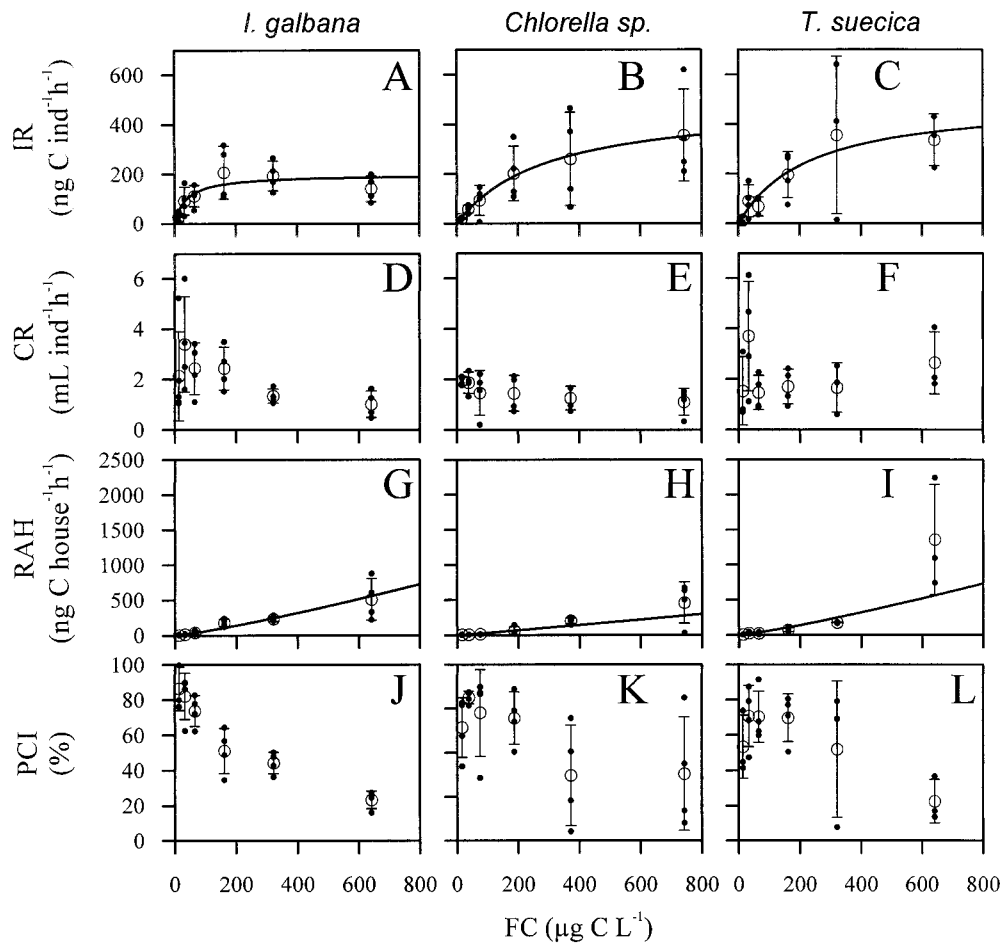


Fig. 5. Ingestion rate [IR; (A), (B), and (C)], clearance rate [CR; (D), (E), and (F)], rate of carbon accumulation in the filter house [RAH; (G), (H), and (I)] and proportion of carbon cleared which is ingested [PCI; (J), (K), and (L)] versus food concentration (FC) of groups of animals with houses of average ages and feeding on *I. galbana* (left column), *Chlorella sp.* (center) and *T. suecica* (right column). Symbols as in Fig. 4. See Table 3 for parameter estimates of the FR model in (A), (B), and (C). Least-squares fits for power functions of RAH ($\text{ng C house}^{-1} \text{h}^{-1}$) versus FC ($\mu\text{g C L}^{-1}$) are $\log(\text{RAH}) = (-0.56 \pm 0.20) + (1.18 \pm 0.09) \log(\text{FC})$ for *I. galbana* ($n = 23$, $r^2 = 0.89$, $F = 161$, $P < 0.001$), $\log(\text{RAH}) = (-0.56 \pm 0.24) + (1.05 \pm 0.11) \log(\text{FC})$ for *Chlorella sp.* ($n = 24$, $r^2 = 0.80$, $F = 86$, $P < 0.001$) and $\log(\text{RAH}) = (-0.57 \pm 0.23) + (1.18 \pm 0.11) \log(\text{FC})$ for *T. suecica* ($n = 21$, $r^2 = 0.86$, $F = 112$, $P < 0.001$).

culated for our experiments fall in the upper end of published CR under a variety of conditions.

Discussion

Adaptation of the direct radiolabel technique for incubations involving several individuals and careful control of the experimental conditions allowed us to observe and model the population FR of an appendicularian. We have also confirmed that house aging depends on FC (Fig. 3), although trends in time-integrated IR versus FC (Table 2) are consistent with a type II FR, for which we also provide empirical support (Figs. 4A,B and 5A,B,C). However, trends in CR, which is nearly nonresponsive to FC (Figs. 4C,D and 5D,E,F), are at variance with any known FR model. Our results also suggest that the FR may show signs of saturation

or inhibition (Figs. 4B and 5A) and that the FR parameters are similar to those calculated for other zooplankton organisms of the same carbon content (Fig. 6).

At high FC, a filter house experiences an aging process involving accumulation of noningested filtrate (Fig. 3; see also Bedo et al. 1993) associated with decreasing IRs and CRs not only in *O. dioica* (Fig. 3C,D,E,H,I), but also in the cold water appendicularian *O. vanhoeffeni* (Bochdansky and Deibel 1999). Although this decrease could be due to a change in the behavioral pattern, purely mechanical reasons offer a more likely explanation. For example, a sudden drop in pumping rate during the first minutes of operation is a typical characteristic of manmade tangential flow membranes at relatively high feed concentrations, e.g., small-sized high molecular weight compounds (Baker and Strathmann 1970), large cultured cells (Zahka and Leahy 1985),

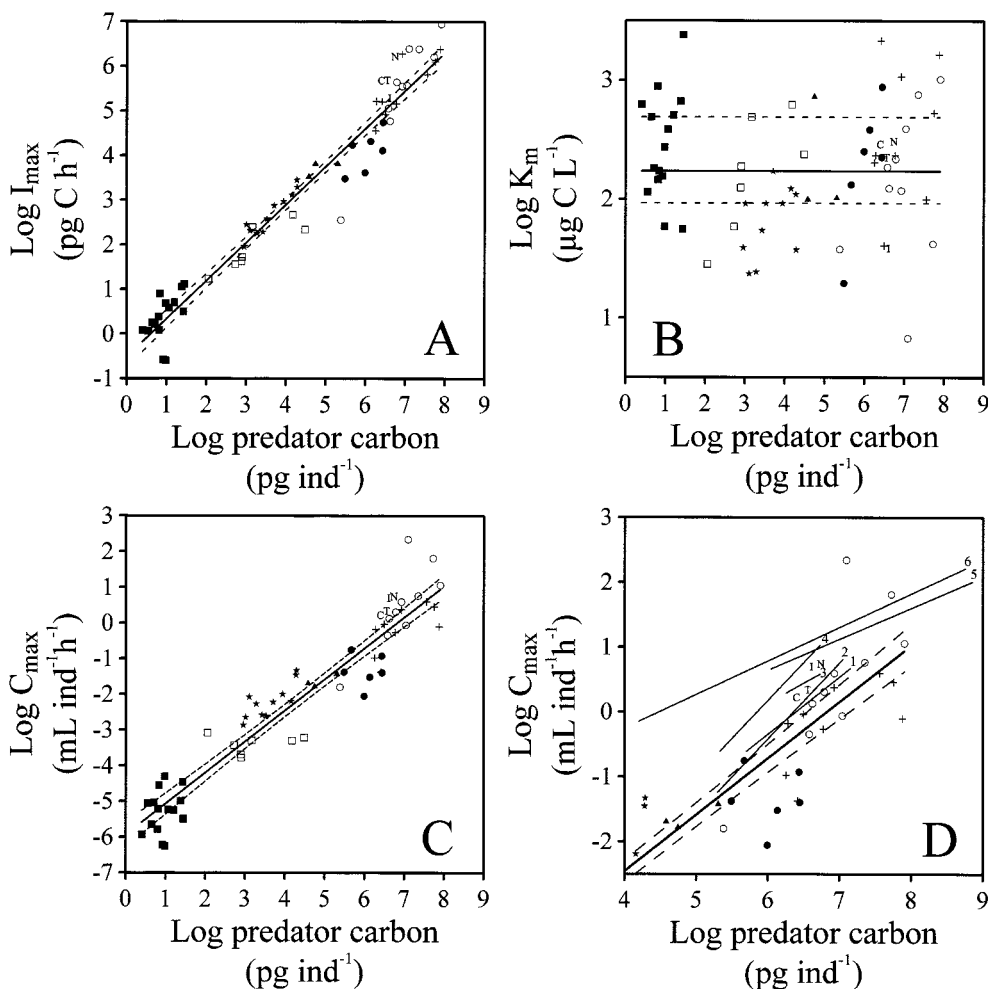


Fig. 6. Comparison of the Michaelis-Menten FR parameters (A) I_{\max} , (B) K_m , and (C) and (D) C_{\max} of *O. dioica* against the compilation by Hansen et al. (1997), which includes nanoflagellates (filled square), dinoflagellates (empty square), ciliates (star), rotifers (triangle), meroplankton larvae (filled circle), copepods (empty circle), and cladocerans (cross). Letters correspond to experiments with *O. dioica* with new filter houses feeding on *I. galbana* (N), and with average house age feeding on *I. galbana* (I), *T. suecica* (T), and *Chlorella sp.* (C) (see Table 3 for parameter estimates). Thick solid lines in (A), (C), and (D) represent least-squares fits to power functions relating I_{\max} ($\text{pg C ind}^{-1} \text{ h}^{-1}$) and C_{\max} ($\text{mL ind}^{-1} \text{ h}^{-1}$) to predator carbon (PC, pg ind^{-1}), $\log(I_{\max}) = (0.51 \pm 0.12) + (0.85 \pm 0.03) \log(\text{PC})$ ($n = 61$, $r^2 = 0.95$, $F = 1,196$, $P < 0.001$) and $\log(C_{\max}) = (-5.94 \pm 0.18) + (0.87 \pm 0.04) \log(\text{PC})$ ($n = 61$, $r^2 = 0.95$, $F = 574$, $P < 0.001$), and dashed lines indicate 95% CI for the regressions (appendicularian data excluded from regressions). Solid and dashed lines in panel B are median and 25–75% quartiles for K_m , respectively. We have compiled regression equations relating clearance rate (CR, $\text{mL ind}^{-1} \text{ h}^{-1}$) and predator carbon for appendicularians and superimposed these equations as thin lines on (D) for comparison with C_{\max} values, although they do not represent actual C_{\max} measurements. The equations correspond to data from *O. dioica* (Bedo et al. 1993, $\text{CR} = 2.29 \times 10^{-6} \text{ PC}^{0.88}$, line labeled 1; King et al. 1980, $\text{CR} = 3.47 \times 10^{-8} \text{ PC}^{1.17}$, line 2; Allredge 1981, $\text{CR} = 2.75 \times 10^{-4} \text{ PC}^{0.62}$, line 3; Paffenhöfer 1975, $\text{CR} = 1.41 \times 10^{-7} \text{ PC}^{1.17}$, line 4) and *O. vanhoeffeni* (Knoechel and Steel-Flynn 1989, $\text{CR} = 5.89 \times 10^{-3} \text{ PC}^{0.48}$, line 5; Deibel 1988, $\text{CR} = 4.79 \times 10^{-3} \text{ PC}^{0.52}$, line 6).

or seawater natural seston (Whitehouse 1990). The presence of variable amounts of phytoplankton cells attached to the food concentrating filter (pers. obs.), lower PCI at higher FCs (Figs. 4H and 5J,K,L), and experimental results by Bochdansky and Deibel (1999), suggest fouling as a most likely cause for the aging process.

Our results also suggest that the algal species, rather than

their size, caused differences in the shape of the FR of *O. dioica*. The animals had nonsaturated, similar FRs when feeding on algae of different size (*Chlorella sp.*, 3.5 μm and *T. suecica*, 9.5 μm ESD; Fig. 5B,C) and different FRs when feeding on similarly sized algae belonging to different taxonomic groups (*I. galbana*, prymnesiophyte, saturated FR; *Chlorella sp.*, chlorophyte, nonsaturated FR; Figs. 4B and

5A,B). During a seasonal field study of feeding rates of zooplankton, Landry et al. (1994) found symptoms of saturation in the gut pigment contents of warm-water *Oikopleura* spp., whereas Bochsansky and Deibel (1999) found that saturation is generally not reached even at the highest natural particle concentrations in *O. vanhoeffeni*. These contrasting results could be due to differences in surface properties and filter clogging potential between varying particle types, which is supported by differences in PCI between phytoplankton species in the present work (Fig. 5J,K,L). In fact, steady state flux conditions in manmade tangential membranes are known to change according to solute surface properties (Cherydan 1986).

The CR of *O. dioica* decreased slightly with increasing FC when feeding on *I. galbana* (only twofold, Fig. 5D) or remained nearly constant when feeding on *Chlorella* sp. or *T. suecica* (Fig. 5E,F). This low variability in CR is in agreement with experimental work by Bochsansky and Deibel (1999) on *O. vanhoeffeni* and by Paffenhöfer (1975) and King (1982) on *O. dioica*, but is different from the decreasing trend with increasing FC expected from classical models. Our experiments show that the discrepancy is due to material accumulating in the filter house. Although other filter feeders may also lose material, it is much more apparent in appendicularians where it remains in an external filter device and is not released into the environment. Back calculation of CR from IR would therefore considerably underestimate the effort to extract particles from the environment.

Inhibition in the IR of *O. dioica* at high FCs of *I. galbana* (Figs. 4B and 5A) is not described by classical FR models either, and can be due to mechanical interference or accumulation of metabolites at the high FC at which it was observed. Inhibition happens also in some copepods (Mullin 1963) and rotifers (Rothhaupt 1990) and implies the existence of an optimal FC, which for *O. dioica* feeding on *I. galbana* is ca. $100 \mu\text{g C L}^{-1}$ (Figs. 4B and 5A). Interestingly, King (1981) found maximum growth and survival rates for *O. dioica* when feeding at FCs between 62 and $125 \mu\text{g C L}^{-1}$ of *I. galbana* (as cited in Bochsansky and Deibel 1999). Just how frequently inhibition will happen in the field will depend on the type of particle and the concentration, which reaches values up to $170 \mu\text{g C L}^{-1}$ in the Cantabrian Sea. If appendicularians are frequently exposed to these high concentrations in the field, then we will need to incorporate inhibition in FR models, perhaps by assimilating production-irradiance models with photoinhibition that have been used for decades to describe the photosynthetic response of phytoplankton to light intensity (e.g., Platt et al. 1980).

The K_m values for *O. dioica* feeding on *I. galbana*, *Chlorella* sp., and *T. suecica* are 38, 290, and $225 \mu\text{g C L}^{-1}$, respectively (Table 3, Fig. 6B), which suggests adaptation to high particle loads. Chlorophyll concentrations reach $7 \mu\text{g L}^{-1}$ in our collection site at the El Musel harbor (Muñoz 1982), and ca. $4 \mu\text{g L}^{-1}$ during coastal upwelling, spring and autumn blooms in the Cantabrian coast (Botas et al. 1989, 1990; Fernández et al. 1993), all situations where *O. dioica* is abundant (Acuña and Anadón 1992; Fernández et al. 1993). Assuming a Carbon: Chlorophyll ratio of 50, maximum carbon concentrations in the area would range between 200 and $350 \mu\text{g C L}^{-1}$, which is substantially lower than the

maximum FCs used in our experiments. Except during early stages of the spring bloom which are dominated by nano-plankton producers (Fernández et al. 1993), nearly 50% of the suspended particles during bloom situations in this area are attributable to noningestible netplankton (Bode et al. 1994). Therefore the above figures would drop to 100–175 $\mu\text{g C L}^{-1}$ of ingestible phytoplankton. However, we must be cautious in extrapolating our FR to bloom conditions, since large diatoms may have a negative effect on the feeding rates of appendicularians (Knoechel and Steel-Flynn 1989; Acuña et al. 1999), and the cell type ingested may have a great influence on the parameters and shape of the FR (Table 3, compare Fig. 5A with Figs. 5B,5C).

The nearly constant CR at FCs from 0 to $700 \mu\text{g C L}^{-1}$ (Figs. 4B and 5D,E,F) suggests the great clearance potential of *O. dioica* both during periods of low particle abundance and during phytoplankton blooms, given appropriate particle size and quality. Because the small particles on which appendicularians feed sink slower than their large houses and fecal pellets, appendicularians can affect the residence time of photosynthetically fixed CO_2 in the upper layers of the ocean (Fortier et al. 1994). In our FR experiments, particles cleared from suspension by *O. dioica* were preferentially ingested and transformed into fecal pellets at very low FCs and accumulated in the filter house at high FCs (Figs. 4H and 5J,K,L). Although fecal pellets sink quickly ($25\text{--}166 \text{ m day}^{-1}$, Gorsky et al. 1984), sinking velocities of filter houses span a range from fast enough to sediment to deep layers ($26\text{--}157 \text{ m day}^{-1}$, Gorsky et al. 1984) to slow enough to become remineralized within the euphotic layer (i.e., using a water-jacketed settling column stabilized during 24 h inside a 15°C cold room we detected no sinking of filter houses shed by *O. dioica* fed the coccolithophore *Emiliania huxleyi* or newly secreted, clean filter houses; J. L. Acuña and R. P. Harris, unpubl. data). Thus, changes in the concentration of available particles could imply drastic qualitative and quantitative shifts in the role of appendicularians on vertical particle fluxes in the ocean.

In conclusion, the FR of the IR of *O. dioica* resembles that of other coastal zooplankton typical of mesoeutrophic, coastal habitats. Energy demands should be similar for poikilotherms of a similar body carbon content, which might explain why empirical allometric extrapolation of multispecific trends gives good approximations to the FR of the IR of *O. dioica*. However, the unresponsiveness of CR to FC, and the potential inhibition of IR at high particle loads, depart from the typical features of a filter-feeding, classical FR. Since the relative proportion of material trapped in the house in relation to material released as fecal pellets is a function of food quality and concentration, these factors need to be taken into account when describing the role of appendicularians in the biogeochemical cycle.

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