

Species-specific house productivity of appendicularians

Riki Sato^{1,2,*}, Yuji Tanaka¹, Takashi Ishimaru¹

¹Department of Ocean Sciences, Tokyo University of Fisheries, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

²Present address: Louisiana Universities Marine Consortium, 8124 Highway 56, Chauvin, Louisiana 70344, USA

ABSTRACT: Appendicularians, pelagic tunicates that are common in world oceans, periodically produce new mucus houses and discard old ones. Discarded houses form macroscopic aggregates that constitute sites of biological activity in the water column and also contribute to the transport of organic matter to deeper water. In this study, we measured the house renewal rates of 10 appendicularian species cultivated in 30 µm-mesh-screened seawater at 17 to 29°C. In addition, the carbon content of the tunicates (C_B), their newly secreted houses (C_{NH}) and their discarded houses (C_{DH}) were concurrently examined for some of the oikopleurid species. House renewal rates varied from 2 houses d^{-1} for *Oikopleura cophocerca* at 20°C to 40 houses d^{-1} for *Fritillaria formica digitata* at 23°C. The C_{NH} of *O. longicauda*, *O. fusiformis*, *O. rufescens* and *Megalocercus huxleyi* were 0.16, 0.48, 1.6 and 8.8 µg, corresponding to 5.3, 9.2, 14.1 and 10.3% of C_B , respectively; the C_{DH} of the first 3 species were 0.68, 1.2 and 3.9 µg, corresponding to 17.9, 30.0 and 32.8% of C_B , respectively (C_{DH} was not measured in *M. huxleyi*). House renewal rates decreased with increasing C_{NH}/C_B ratios for all species. Our calculations indicate that at 23°C, populations of *O. longicauda*, *O. fusiformis*, *O. rufescens* and *M. huxleyi* produced new houses corresponding to 112, 217, 75 and 89% of their biomass d^{-1} , and that the first 3 species could discard houses corresponding to 380, 708 and 174% of their biomass d^{-1} , respectively. The individual lifetime production of C_{NH} and C_{DH} for all 3 species was estimated to be 1.1 to 3.6 and 2.7 to 12 times greater than the C_B of mature individuals. This high house-productivity, combined with large population sizes, indicates that appendicularians are major producers of macroscopic aggregates in marine ecosystems.

KEY WORDS: Appendicularians · House · House renewal rate · Carbon content · Macroscopic aggregates

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INTRODUCTION

Appendicularians, which are common in marine zooplankton communities, live and feed within a complex mucus structure known as their 'house' (Lohmann 1909, Alldredge 1976a). With a sinusoidal tail movement, the appendicularian draws water into its house through coarse-meshed inlet filters which exclude particles that are too large to ingest. Inside the house, fine-meshed filters sieve the food, collecting small particles such as nano- and pico-plankton which are concentrated and ingested. Some particles (comprising phytoplankton cells, ciliates, bacteria and fecal pellets of the occupying tunicate) accumulate in the house without

being ingested (Alldredge 1972, 1976a,b, Davoll & Silver 1986, Gorsky & Palazzoli 1989, Bedo et al. 1993, Hansen et al. 1996, Bochsansky et al. 1998). Houses are periodically discarded. Discarded houses are a complex of mucus, particles retained inside the house and particles attached to the outside of the house. A new house is immediately built. Each individual repeats this process 2 to 19 times per day (Flood & Deibel 1998, Sato et al. 2001), and appendicularians have thus been considered important producers of macroscopic aggregates (Alldredge 1976b, 1979, Silver & Alldredge 1981, Taguchi 1982, Alldredge & Gotschalk 1990, Hamner & Robison 1992, Hansen et al. 1996, Sato et al. 2001). There are, however, few quantitative measurements of

*Email: rsato@lumcon.edu

house renewal rate (see review by Flood & Deibel 1998). Some measurements of house carbon content have been made for *Oikopleura cornutogastra*, *O. fusiformis*, *O. intermedia*, *O. longicauda*, *O. rufescens*, *O. vanhoeffeni*, *Megalocercus huxleyi* and *Stegosoma magnum* (Alldredge 1976b, 1979, Taguchi 1982, Deibel 1986, Riehl 1992 in Flood & Deibel 1998). Most of these measurements, however, were for discarded houses (houses and associated particles). The carbon content of newly secreted, particle-free houses (the amount of carbon invested to build the house) has been reported only for *O. rufescens* (Alldredge 1976b), *O. vanhoeffeni* (Deibel 1986, Riehl 1992 in Flood & Deibel 1998) and *O. dioica* (Sato et al. 2001).

In the present study, house renewal rates for 10 appendicularian species were measured. These 10 species included members of all 3 families of Appendicularia (Oikopleuridae, Fritillariidae and Kowalevskiidae). In addition, we measured the carbon contents of some oikopleurids, their newly secreted houses, and their discarded houses. These data allowed us to determine the importance of these appendicularians as producers of macroscopic aggregates.

MATERIALS AND METHODS

Live individuals were collected from surface waters 100 to 300 m off the Banda Marine Laboratory, Tokyo University of Fisheries, Japan. They were collected individually, together with their houses, in 300 ml transparent plastic vessels by a diver and brought to the laboratory immediately. They were sorted by species into 3 or 5 l plastic beakers filled with ambient seawater screened through 30 μm -mesh netting. These appendicularians were kept as stock cultures using apparatus especially designed for their cultivation through several generations (Sato et al. 1999). For all experiments, chlorophyll *a* concentration was determined by filtering 200 ml of 30 μm -mesh-screened seawater through a Whatman GF/F glass-fiber filter. These samples were extracted in 6 ml of *N,N*-dimethylformamide, and chlorophyll *a* concentrations were measured fluorometrically with a Turner design fluorometer (Suzuki & Ishimaru 1990).

Determination of house renewal rates. Individual *Oikopleura rufescens* were placed in 300 ml plastic beakers filled with seawater screened through 30 μm mesh. Beakers were then set on a motor-driven rotating table of 50 cm diameter to gently mix the water, in order to keep tunicates suspended (Sato et al. 2001). Individuals were transferred to new beakers containing fresh food medium every 4 h by use of a wide-bore pipette or a transferring apparatus (Sato et al. 1999). Discarded houses in the old beakers were counted.

Experiments were terminated by removing individuals from the beakers after 12 h. The experiments were conducted under 5 $\mu\text{E m}^{-2} \text{s}^{-1}$ irradiance at 20, 23 and 26°C, adjusted by setting the rotating table in a temperature-controlled chamber.

Other appendicularian species were so sensitive that contact of their houses with the inside walls of the small beakers caused them to prematurely discard their houses. To reduce this problem, individuals were individually (4 to 5 animals for *Oikopleura longicauda*) maintained in larger, 3 or 5 l, plastic beakers filled with seawater screened through a 30 μm mesh. The beakers were maintained on the cultivating apparatus of Sato et al. (1999). Experimental conditions such as water temperature, irradiance, feeding period and transfer interval were identical to those in the experiments using the rotating table, except that additional temperatures of 17 and 29°C were used for *O. longicauda*. Even under these conditions, *O. longicauda*, *O. fusiformis*, *Fritillaria formica digitata* and *Kowalevskia tenuis* sometimes escaped from the house while being transferred to new beakers. Experiments with these species were thus terminated after 6 h in the same beaker.

Determination of house carbon content. The carbon contents of both newly secreted, particle-free houses (C_{NH}) and discarded houses with associated particles (C_{DH}) were measured for *Oikopleura longicauda*, *O. fusiformis* and *O. rufescens*. For *Megalocercus huxleyi*, only C_{NH} was determined. Body carbon content (C_{B}) was also determined to obtain the ratios of $C_{\text{NH}}/C_{\text{B}}$ and $C_{\text{DH}}/C_{\text{B}}$.

C_{NH} determination: We used the method described in Sato et al. (2001). Similar-sized individuals of each species were prodded, forcing them to abandon their old houses. While expanding their new houses, the tunicates were transferred to membrane-filtered (0.2 μm pore size) seawater to prevent particles from adhering to the houses. Because particle-free houses are almost completely transparent, the tunicates were then transferred to membrane-filtered seawater to which we added powder of a pre-ashed (480°C for 3 h) GF/C filter; the powder was taken up by the mucus and thus made the house visible. We then forced the animals to leave their houses by transferring them to a 3% KCl solution. Organisms and houses were separately pipetted onto pre-ashed 24 mm GF/C filters and dried at 60°C for 24 h. The number of organisms and houses on each filter ranged from 1–2 for *Megalocercus huxleyi* to 25–30 for *Oikopleura longicauda*. The carbon contents of these samples were determined using a Yanagimoto MT-3 CHN analyzer.

C_{DH} determination: *Oikopleura fusiformis* and *O. rufescens* were individually sorted into 300 ml plastic beakers filled with screened seawater and maintained on the rotating table. After several hours, 7 to 15 indi-

viduals were removed from the beakers, forced to abandon their houses by immersion in 3% KCl solution, pipetted onto pre-ashed GF/C filters and dried. The discarded houses in the beakers were also removed, pipetted onto pre-ashed GF/C filters and dried.

Oikopleura longicauda houses are smaller than those of *O. fusiformis* and *O. rufescens* (Allredge 1977), and therefore a larger number of their houses was required for quantitative measurements. However, simultaneous collection of large numbers of both discarded houses and occupants was difficult. Instead, we derived relationships between fecal pellet length and body size (trunk length), and between trunk length and C_B , and used these to estimate C_B from fecal pellet length. Laboratory observations showed that 70% of the discarded houses of *Oikopleura longicauda* contained fecal pellets. To develop these relationships, we used 20 similar-sized *O. longicauda*, cultivated in 5 l beakers filled with screened seawater. Discarded houses were collected from the beakers. After microscopic measurement of the length of the major axis of the fecal pellets within the discarded houses, 30 to 50 of these houses were pipetted onto pre-ashed GF/C filters. The carbon content of these samples was determined using the method described above.

Estimation of daily house production. For *Oikopleura longicauda*, *O. fusiformis*, *O. rufescens* and *Megalocercus huxleyi*, the ratio of daily new house production to appendicularian biomass was calculated by multiplying the ratio of C_{NH}/C_B and the house renewal rate. Similarly, the ratio of daily discarded house production to appendicularian biomass was calculated for each species using the C_{DH}/C_B ratio of each.

Estimation of lifetime house production. We also estimated the total number of houses and total amount of house carbon produced by an individual during its lifetime for *Oikopleura longicauda*, *O. fusiformis* and *O. rufescens*. The procedures are briefly described below (see Sato et al. 1999 for the cultivation method and Sato et al. 2001 for details of the estimation of house number and carbon content). Fertilized eggs were kept in 3 or 5 l beakers filled with 30 μm -mesh-screened seawater using the cultivating apparatus at 20, 23 and 26°C (plus 17 and 29°C for *O. longicauda*). After hatching, individuals were transferred to a fresh beaker 1 to 3 times per day. Every 1 to 3 d after fertilization, 5 to 20 individuals were sampled and fixed in 5% buffered formaldehyde seawater solution, and their trunk length (including gonad) was measured immediately under a microscope. Tail length was concurrently measured to obtain the tail–trunk length regression of each species. This allowed conversion of tail length to trunk length when gonads of mature individuals ruptured on fixation. The C_B of various-sized individuals of these 3 species were determined to

obtain the trunk length– C_B regression for each species. After measurement of trunk length, 1 to 30 individuals of similar size for each species were transferred onto pre-ashed GF/C filters and dried, and their carbon content was analyzed using the method described above. For each species, trunk length was converted to the C_B using the appropriate regression. The temporal change (C_t) of C_B for each species at each temperature was fit by the logistic curve expressed as:

$$C_t = C_\infty / (1 + e^{a-bt}) \quad (1)$$

where C_t is the C_B at t hours after fertilization, C_∞ is the maximum C_B theoretically reached, and a and b are specific constants. The period from fertilization to expansion of a specified number of houses (t_n , hours) was calculated from:

$$t_n = t_1 + t_h(n-1) \quad (2)$$

where t_1 is the period from fertilization to expansion of the first house and t_h is the house renewal interval, which is equal to the reciprocal of house renewal rate. We assumed that the house renewal rates of these 3 species would be constant through their lifetimes regardless of body size, food concentration or light condition (Sato et al. 2001). The total number of houses produced by an individual during its lifetime can be calculated by inserting the generation time into t_n of Eq. (2). Given the total house number m , the total house carbon (C_{TH}) produced by an individual during its lifetime can be calculated by combining (1) and (2) into:

$$C_{TH} = \sum_{i=t_1}^{t_n} C_i \times r \quad (3)$$

where r is the ratio C_{NH}/C_B or C_{DH}/C_B .

RESULTS

House renewal rate

The mean house renewal rates varied considerably among species, ranging from 2 houses d^{-1} for *Oikopleura cophocerca* to 40 houses d^{-1} for *Fritillaria formica digitata* (Table 1). House renewal rates tended to increase with increasing temperature.

House carbon content

The C_{NH} ranged from 0.16 μg for *Oikopleura longicauda*, the smallest of the 4 species examined, to 8.8 ± 2.2 μg (mean \pm SD) for *Megalocercus huxleyi*, the largest species examined (Table 2). The ratios of C_{NH}/C_B ranged from 5.7% for *O. longicauda* to $16.5 \pm$

Table 1. House renewal rates, H (means \pm SD), of appendicularian species at various temperatures. n: no. of individuals examined; nd: no data

Species	T ($^{\circ}\text{C}$)	Chl a ($\mu\text{g l}^{-1}$)	H (houses d^{-1})	n
Oikopleuridae				
<i>Oikopleura longicauda</i>	17	0.5–0.7	13.4 ± 1.0	20
	20	1.4–1.5	15.6 ± 0.8	20
	23	1.0–2.0	21.2 ± 1.9	20
	26	0.6–0.9	23.6 ± 1.0	20
	29	0.3	24	8
<i>O. fusiformis</i>	20	0.4–0.8	18.4 ± 2.2	9
	23	0.4–1.2	23.6 ± 2.4	9
	26	0.3–0.4	27.3 ± 3.5	11
<i>O. rufescens</i>	20	0.7	3.5 ± 0.9	8
	23	0.2–0.3	5.3 ± 1.0	17
	26	0.4–0.6	5.2 ± 1.0	28
<i>O. cophocerca</i>	20	nd	2	1
<i>O. cornutogastra</i>	23	0.3	20	1
<i>O. intermedia</i>	20	0.6	8	1
<i>Megalocercus huxleyi</i>	23	0.4	8	1
	20	0.7	6.0 ± 1.6	4
<i>Stegosoma magnum</i>	23	0.2–0.6	8.6 ± 0.9	5
	23	0.2–0.6	8	2
Fritillariidae				
<i>Fritillaria formica digitata</i>	23	0.8	40	2
Kowalevskiidae				
<i>Kowalevskia tenuis</i>	20	0.2	6	2

3.8% for *O. rufescens*. Individuals in this experiment discarded old and new houses consecutively, so C_B did not include carbon of the house rudiment (which is secreted and attached to the tunicates' trunk while they still occupy their old houses; Alldredge 1976a). To estimate life-time house production using Eqs. (1), (3) and (5) to (7) (see later subsection), we used the C_B of individuals that included the house rudiment, thus recalculating the C_{NH}/C_B ratios under the assumption that

the carbon content of the rudiment was equivalent to C_{NH} ; we thereby obtained corrected C_{NH}/C_B ($= C_{\text{NH}}/[C_B + C_{\text{NH}}]$) ratios ranging from 5.3 to 14.1% (Table 2).

The C_{DH} values ($0.68 \pm 0.15 \mu\text{g}$ for *Oikopleura longicauda*, $1.2 \pm 0.26 \mu\text{g}$ for *O. fusiformis* and $3.9 \pm 1.1 \mu\text{g}$ for *O. rufescens*; Table 3) were higher than the C_{NH} values (Table 2). The relationship between fecal pellet length (L_{Fi} ; μm) and trunk length (L_{TR} ; μm) of *O. longicauda* was expressed as:

$$L_{\text{TR}} = 3.15 \times L_{\text{Fi}} + 109 \quad (4)$$

$$(r^2 = 0.868, n = 55, p < 0.001)$$

Using this regression and the trunk length– C_B regression (see subsection 'Lifetime house production'), we obtained the C_B of *O. longicauda* individuals which had discarded their houses. The ratios of C_{DH}/C_B were $17.9 \pm 8.4\%$ for *O. longicauda*, $30.0 \pm 6.6\%$ for *O. fusiformis* and $32.8 \pm 4.4\%$ for *O. rufescens* (Table 3), equivalent to 3.4, 3.3 and 2.3 times the C_{NH}/C_B ratios, respectively.

Daily house production

On a carbon basis, daily new house production by *Oikopleura longicauda* corresponded to 71–127% of the organism biomass, and daily discarded house production corresponded to 240–430% of the biomass at 17 to 29°C (Table 4). The daily new and discarded house production by *O. rufescens* corresponded to

Table 2. *Oikopleura* spp. and *Megalocercus huxleyi*. Carbon content (means \pm SD) of newly secreted, particle-free house (C_{NH}). Since organisms discarded 2 houses successively in this experiment, corrected ratios (P) of C_{NH}/C_B (body carbon) were calculated as $P = 100 \times C_{\text{NH}}/(C_{\text{NH}} + C_B)$, see 'Results'; n: no. of samples

Species	C_B ($\mu\text{g ind.}^{-1}$)	C_{NH} ($\mu\text{g house}^{-1}$)	Ratio (%) C_{NH}/C_B	Corrected ratio (P , %)	n
<i>O. longicauda</i>	3.1	0.16	5.7	5.3	2
<i>O. fusiformis</i>	4.7 ± 0.3	0.48 ± 0.01	10.2 ± 0.8	9.2 ± 0.6	3
<i>O. rufescens</i>	10.4 ± 4.0	1.6 ± 0.6	16.5 ± 3.8	14.1 ± 2.8	7
<i>M. huxleyi</i>	79.0 ± 25.4	8.8 ± 2.2	11.5 ± 2.5	10.3 ± 2.0	5

Table 3. *Oikopleura* spp. Carbon content of discarded house (C_{DH}). Also shown are chlorophyll a concentrations in water used for maintaining tunicates during collection of discarded houses. C_B : body carbon; n: no. of samples

Species	Chl a ($\mu\text{g l}^{-1}$)	C_B ($\mu\text{g ind.}^{-1}$)	C_{DH} ($\mu\text{g house}^{-1}$)	Ratio (%) C_{DH}/C_B	n
<i>O. longicauda</i>	0.3–0.4	4.3 ± 1.9	0.68 ± 0.15	17.9 ± 8.4	3
<i>O. fusiformis</i>	0.4	4.1 ± 1.2	1.2 ± 0.26	30.0 ± 6.6	3
<i>O. rufescens</i>	0.7–1.0	11.8 ± 2.3	3.9 ± 1.1	32.8 ± 4.4	3

Table 4. *Oikopleura* spp. and *Megalocercus huxleyi*. Ratio of daily new house production (C_{NHP}) and daily discarded house production (C_{DHP}) by appendicularians to their biomass (C_{Bio}) (on carbon basis) at various temperatures. nd: no data

Species T (°C)	Ratio (%)	
	$C_{\text{NHP}}/C_{\text{Bio}}$	$C_{\text{DHP}}/C_{\text{Bio}}$
<i>O. longicauda</i>		
17	71	240
20	83	279
23	112	380
26	125	422
29	127	430
<i>O. fusiformis</i>		
20	169	552
23	217	708
26	251	819
<i>O. rufescens</i>		
20	49	115
23	75	174
26	73	171
<i>M. huxleyi</i>		
20	62	nd
23	89	nd

49–73% and 115–171% of the organism biomass at 20 to 26°C, respectively. These values were lower than those for *O. longicauda*. For *O. fusiformis*, the daily production of new and discarded houses corresponded to 169–251% and 552–819% of the organism biomass, the highest production of the 4 species. Daily new house production by *Megalocercus huxleyi* corresponded to 62 and 89% of the organism biomass at 20 and 23°C. These ratios increased with increasing tem-

perature concordant with the increase in house renewal rates with increasing temperature (Table 1).

Lifetime house production

We determined the generation time as the period from fertilization until >50% of the survivors became mature. The generation time of *Oikopleura longicauda* ranged from 4 d at 26 and 29°C to 10 d at 17 and 20°C (Table 5). Generation times were 3 d at 26°C and 4 d at 23°C for *O. fusiformis*, and 6 d at 26°C and 8 d at 23°C for *O. rufescens* (Table 5). At 20°C, large individuals of *O. fusiformis* and *O. rufescens* could grow and spawn, but laboratory-hatched individuals of both species died within several days.

The trunk length of each species was converted to C_{B} , using trunk length (L_{TR} ; μm)– C_{B} (μg) regression equations

$$C_{\text{B}} = 3.29 \times 10^{-8} L_{\text{TR}}^{2.85} \quad (5)$$

($r^2 = 0.948$, $n = 21$, $p < 0.001$) for *O. longicauda*

$$C_{\text{B}} = 7.79 \times 10^{-9} L_{\text{TR}}^{3.00} \quad (6)$$

($r^2 = 0.989$, $n = 12$, $p < 0.001$) for *O. fusiformis*

$$C_{\text{B}} = 8.20 \times 10^{-8} L_{\text{TR}}^{2.70} \quad (7)$$

($r^2 = 0.923$, $n = 24$, $p < 0.001$) for *O. rufescens*

and was fit by logistic curves as shown in Figs. 1 to 3. After the first day of spawning, mean C_{B} sometimes appeared to decrease because several large individuals had already spawned before the sampling time and their fragile tails were damaged by struggling or being trapped on the water surface, resulting in the lack of

Table 5. *Oikopleura* spp. Total number and carbon content of newly secreted and discarded houses produced by different appendicularian species during their lifetime at various temperatures. Period (h): period from fertilization to first house expansion; Gen time (d): generation time; mC_{B} : body carbon of mature individual, i.e. body carbon of individual at generation time (mean \pm SD, μg); Total houses: total no. of houses (mean \pm SD houses ind.⁻¹); Total C_{NH} , Total C_{DH} : total amount of carbon in new and old houses, respectively ($\mu\text{g ind.}^{-1}$)

Species T (°C)	Period (h)	Gen time (d)	mC_{B}	Total houses	Total C_{NH}	Total C_{DH}	Total $C_{\text{NH}}/mC_{\text{B}}$	Total $C_{\text{DH}}/mC_{\text{B}}$
<i>O. longicauda</i>								
17	14	10	8.5 \pm 3.3	127 \pm 10	21.4	72.3	2.5	8.5
20	12	10	7.3 \pm 2.3	149 \pm 8	16.2	54.8	2.2	7.5
23	10	7	6.4 \pm 3.4	141 \pm 13	22.9	77.3	3.6	12.0
26	7.5	4	2.3 \pm 1.0	88 \pm 4	4.1	13.8	1.8	6.1
29	5	4	2.1 \pm 0.4	92	6.4	21.4	3.1	10.4
<i>O. fusiformis</i>								
23	9	4	3.5 \pm 1.6	86 \pm 9	7.7	25.0	2.2	7.2
26	7.5	3	5.6 \pm 0.8	74 \pm 9	8.8	28.8	1.6	5.1
<i>O. rufescens</i>								
23	14	8	13.0 \pm 4.2	40 \pm 7	21.4	49.7	1.6	3.8
26	12	6	11.5 \pm 5.2	30 \pm 6	13.2	30.7	1.1	2.7

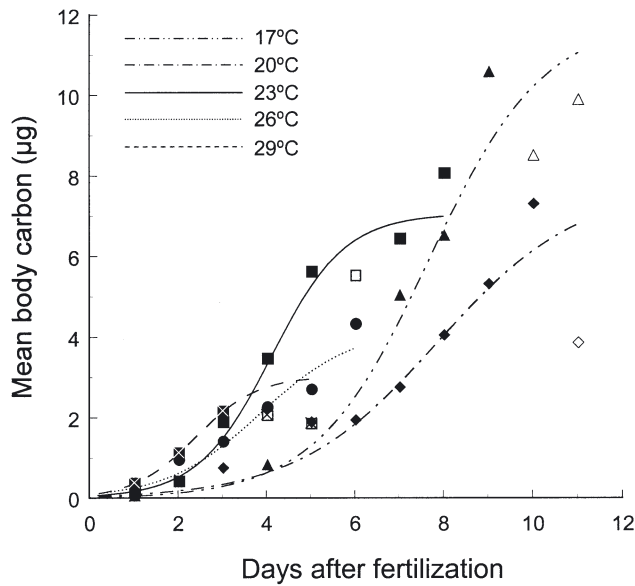


Fig. 1. *Oikopleura longicauda*. Growth as mean body carbon at 17°C (▲, △), 20°C (◆, ◇), 23°C (■, □), 26°C (●, ○) and 29°C (⊠, ⊞). Lines are logistic curves fit to growth at each temperature; open symbols represent data excluded for purpose of fitting the curves. Equations of logistic curves for 17, 20, 23, 26 and 29°C are: $C_t = 11.9/(1 + e^{5.97 - 0.778t})$, $7.77/(1 + e^{4.90 - 0.624t})$, $7.05/(1 + e^{4.94 - 1.20t})$, $4.23/(1 + e^{3.63 - 0.945t})$ and $3.05/(1 + e^{3.40 - 1.43t})$, respectively, where C_t = body carbon at t hours after fertilization. Chlorophyll *a* concentration in cultivation water = 0.5 to 0.7, 0.3 to 0.7, 0.4 to 1.4, 0.3 to 0.8 and 0.3 to 0.5 $\mu\text{g l}^{-1}$ for 17, 20, 23, 26 and 29°C respectively

tail length measurements. Therefore, the mean trunk length or C_B will be slightly underestimated in these cases. If the temporal changes of C_B are fit by logistic curves at this stage, the curves will deviate below the mean C_B . To fit the curve, we thus excluded the decreased C_B data (open symbols in Figs. 1 to 3). The resultant growth curves provided a good description of the temporal changes in C_B (Figs. 1 to 3) and could thus be used in Eq. (3). The total house number produced by an individual during its lifetime varied as a function of species and temperature, ranging from 88 to 149 for *Oikopleura longicauda*, 74 to 86 for *O. fusiformis* and 30 to 40 for *O. rufescens* (Table 5). Total C_{NH} and total C_{DH} ranged from 4.1 to 22.9 μg and 13.8 to 77.3 μg for *O. longicauda*, 7.7 to 8.8 μg and 25.0 to 28.8 μg for *O. fusiformis*, and 13.2 to 21.4 μg and 30.7 to 49.7 μg for *O. rufescens*, respectively. At temperatures exceeding 26°C, the total house number and total house carbon of *O. longicauda* decreased abruptly, possibly due to the shorter generation time and smaller body size at higher temperatures (Fig. 1). The total C_{NH} and total C_{DH} were 1.1 to 3.6 and 2.7 to 12 times higher than the C_B of mature individuals (Table 5).

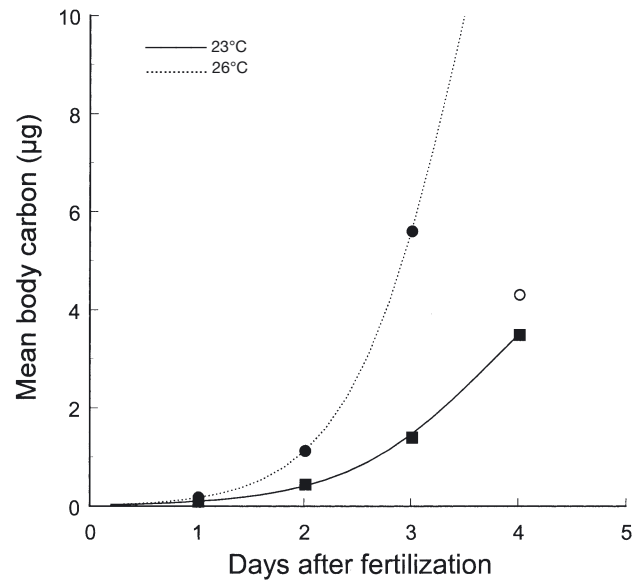


Fig. 2. *Oikopleura fusiformis*. Growth as mean body carbon at 23°C (■, □) and 26°C (●, ○); open symbols as in Fig. 1. (At 23°C, tunicates survived to Day 5, but were not sampled after Day 4.) Equations of logistic curves for 23 and 26°C are: $C_t = 5.96/(1 + e^{5.58 - 1.48t})$ and $21.0/(1 + e^{6.59 - 1.86t})$, respectively. Chlorophyll *a* concentrations in cultivation water were 0.4 to 1.2 and 0.3 to 1.2 $\mu\text{g l}^{-1}$ for 23 and 26°C, respectively

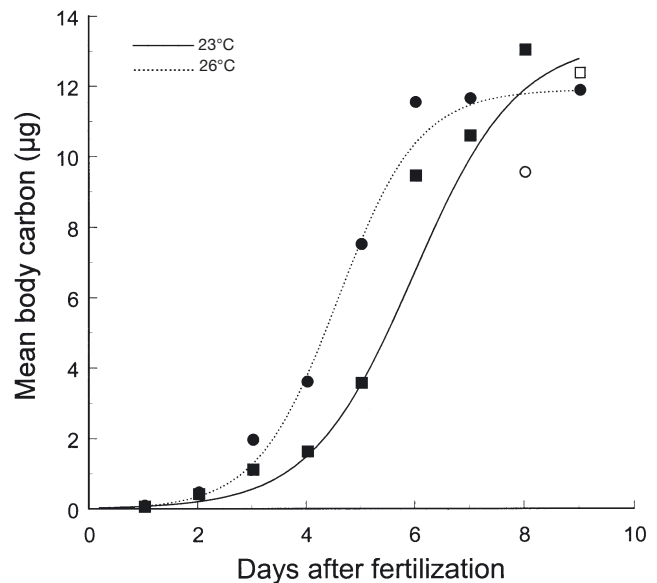


Fig. 3. *Oikopleura rufescens*. Growth as mean body carbon at 23°C (■, □) and 26°C (●, ○); open symbols as in Fig. 1. Equations of logistic curves for 23 and 26°C are: $C_t = 13.3/(1 + e^{6.29 - 1.05t})$ and $11.9/(1 + e^{6.13 - 1.34t})$, respectively. Chlorophyll *a* concentrations in cultivation water were 0.4 to 2.7 and 0.6 to 1.1 $\mu\text{g l}^{-1}$ for 23 and 26°C, respectively

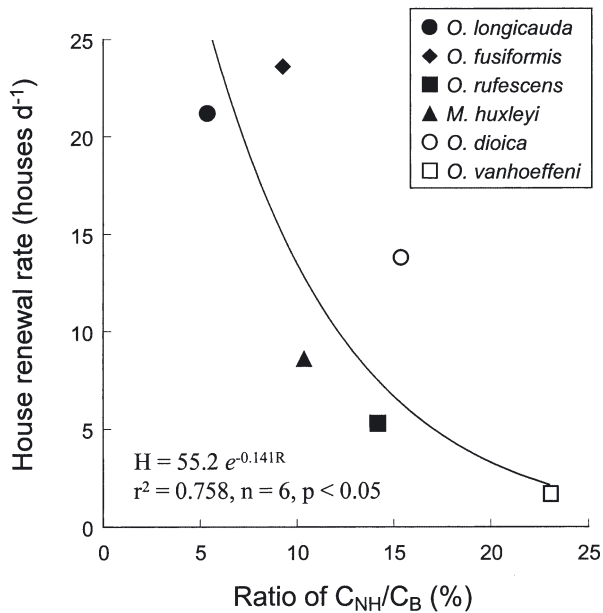


Fig. 4. *Oikopleura* spp. and *Megalocercus huxleyi*. Relationship between ratios of C_{NH}/C_B (R , C content of new house/C content of body) and house renewal rates (H) at -0.6 to $5.8^{\circ}C$ for *O. vanhoeffeni* and at $23^{\circ}C$ for the other 5 species. For *O. vanhoeffeni* temperature and house renewal rates are from Riehl (1992, in Flood & Deibel 1998) and C_{NH}/C_B ratio from Deibel (1986); for *O. dioica* data are from Sato et al. (2001); for remaining species, data are from present study

DISCUSSION

In a previous study (Lohmann 1909), house renewal rate by *Oikopleura rufescens* was 6 houses d^{-1} , similar the rate measured in our study. However, house renewal rates reported for *Kowalevskia tenuis* (Fol 1872) and *O. longicauda* (Taguchi 1982) were considerably different from our rates. Fol (1872) observed that *K. tenuis* built 1 house every 2 h when kept in a sufficiently large vessel. Taguchi (1982) obtained a house renewal rate of 5.3 houses d^{-1} for *O. longicauda* by using a complex calculation incorporating the abundance of discarded houses, tunicate abundance, losses due to sedimentation, and losses due to predation. Although we cannot find conclusive reasons for the considerable difference between these studies, we believe that our results reflect more natural values of house renewal rates since we reared these species in fairly good condition up to spawning for *K. tenuis* and through more than 10 filial generations for *O. longicauda*.

The C_{DH} of each species obtained here was lower than that reported by Alldredge (1976b) or Taguchi (1982). These differences may be due to differences in the size of the houses collected and the quality and quantity of particles trapped on or in the houses.

Relationship between house renewal rate and house carbon content

House renewal rates varied between species. House renewal rate of *Oikopleura rufescens* was lower than in other species, except for *O. cophocerca* (Table 1). *O. rufescens* builds a small, highly gelatinous and firm house (Alldredge 1976b, 1977). The firmness of this house may result from a high C_{NH}/C_B ratio and small house size. Among the 4 species examined, *O. rufescens* had the highest C_{NH}/C_B ratio (Table 2). For *O. cophocerca*, low house renewal rate may result in a high C_{NH}/C_B ratio. This presumption is supported by our observation that the houses of *O. cophocerca* were highly gelatinous and very firm.

In contrast, *Oikopleura longicauda* produced many houses per day with a low C_{NH}/C_B ratio. This species is known to have no inlet filters. Except for large species such as *O. vanhoeffeni*, *O. villafrancae* or *Megalocercus huxleyi*, the mesh width of inlet filters is 13 to $35 \mu m$ (Alldredge 1977, Fenaux 1986, Flood & Deibel 1998). *O. longicauda* thus could draw particles $>35 \mu m$ into its house, a particle size that is precluded by inlet filters in other species. According to Flood & Deibel (1998), $100 \mu m$ plastic beads were readily drawn into the house of *O. longicauda*, even by small individuals. This means that the food-concentrating filter would clog quickly with larger particles, and the organisms would have to build new houses more frequently. To produce many houses per day with a low C_{NH}/C_B ratio may be advantageous for *O. longicauda*, as it allows more efficient filtration of food and energy investment for house synthesis relative to a high house renewal rate.

Oikopleura fusiformis builds a mucilaginous and fragile house (Alldredge 1976b, 1977), which seems to be so sticky that it can easily accumulate particles. In our experiments, of the total particles drawn into the house, the proportion that accumulated internally without being ingested was larger for *O. fusiformis* than for *O. longicauda* and *O. rufescens* (R. Sato et al. unpubl. data). Thus, this species would need to build new houses frequently, explaining its high house renewal rates. However, the C_{NH}/C_B ratio of *O. fusiformis* was comparable to that of *Megalocercus huxleyi*, and house renewal rates of the former were about 3 times higher than that of the latter, resulting in a much higher carbon investment for house synthesis per unit time. Because carbon invested in house materials is supplied from ingested food, the difference in house productivity between the 2 species may derive from the difference in capacity of food collection or ingestion.

There was a relationship between the C_{NH}/C_B ratio and the house renewal rate at $23^{\circ}C$ for 6 species (Fig. 4). For *Oikopleura dioica*, house renewal rate at

23°C and 35 psu was calculated using relationships between house renewal rate and temperature and between house renewal rate and salinity (Sato et al. 2001). For *O. vanhoeffeni*, a large cold-water species, the house renewal rate of 1.7 ± 0.78 houses d^{-1} measured at temperatures between -0.6 and $+5.8^\circ\text{C}$ (Riehl 1992 in Flood & Deibel 1998), and the $C_{\text{NH}}/C_{\text{B}}$ ratio of 23% (Deibel 1986) are tentatively used in Fig. 4. The house renewal rate (H ; houses d^{-1}) decreased exponentially with increasing $C_{\text{NH}}/C_{\text{B}}$ ratio (R ; %) (Fig. 4), expressed as

$$H = 55.2 e^{-0.141R} \quad (8)$$

$(r^2 = 0.758, n = 6, p < 0.05)$

This suggests that the house renewal rates counter-balance $C_{\text{NH}}/C_{\text{B}}$ ratios, i.e. the carbon investment for each house. Species with lower carbon investment for each house have higher house renewal rates and vice versa.

Importance of appendicularians as producers of macroscopic aggregates

Clarke & Roff (1990) and Hopcroft & Roff (1998) estimated the house production (= new house production) by appendicularian communities in Jamaica, and suggested that annual house production could be about 10 to 40% of the annual production of the entire copepod community. In another study, Tomita et al. (1999) estimated the house production by *Oikopleura longicauda* populations in Toyama Bay, Japan, and showed that the annual house production of this species was much higher than the annual production by the euphausiid *Euphausia pacifica*, the most abundant zooplankton at the study site (Hirakawa et al. 1992). House production in Jamaica was calculated by applying rate data for *O. dioica* and *O. rufescens* to appendicularian communities that included various species (Clarke & Roff 1990, Hopcroft & Roff 1998), and in the Toyama Bay study, rate data for *O. dioica* were applied to *O. longicauda* populations. Because the house renewal rates and the $C_{\text{NH}}/C_{\text{B}}$ or $C_{\text{DH}}/C_{\text{B}}$ ratios vary considerably by species (Tables 1 to 3), the approach in these studies will have caused some errors. Nevertheless, these studies do indicate that house production by appendicularians comprises a considerable fraction of secondary production in marine ecosystems.

High house productivity by appendicularians is also shown by our results. The house renewal rates of *Oikopleura dioica*, a coastal species (Tokioka 1955, Fenaux et al. 1998), are regulated by temperature and salinity (Sato et al. 2001). In this study, the house renewal rates of many species were also affected by temperature (Table 1). For the species we examined (which are

oceanic species: Tokioka 1955), variation in salinity may not be an important modifier of house renewal rates *in situ*. House production can be safely estimated from the abundance of the appendicularians and temperature in the field. For example, peak concentration of *O. longicauda*, often the dominant appendicularian species in warm oceanic waters (Tokioka 1940, 1960, Bückmann & Kapp 1975, Fenaux et al. 1998), was 83 ind. m^{-3} in Suruga Bay, central Japan (Itoh 1990). Since water temperature was about 23°C at this time (Fig. 2 in Itoh 1990), the maximum number of discarded houses in Suruga Bay can be estimated as $1760 \text{ m}^{-3} \text{ d}^{-1}$ using the house renewal rate of $21.2 \text{ houses d}^{-1}$ for each individual (Table 1). Taguchi (1982) found that the abundance of discarded houses of *O. longicauda* in Kaneohe Bay, Hawaii, reached $1.3 \times 10^5 \text{ houses m}^{-3}$. Discarded houses of *O. longicauda* could thus be an important source of macroscopic aggregates in the water column, depending on the abundance of these tunicates. Since *O. fusiformis* and *O. rufescens* are also distributed widely in warm oceanic waters (Tokioka 1940, 1960, Bückmann & Kapp 1975, Fenaux et al. 1998), their discarded houses may also be a common component of macroscopic aggregates.

The daily new house production by *Oikopleura longicauda*, *O. fusiformis*, *O. rufescens* and *Megalocercus huxleyi* corresponded to between 50 and 250% of their biomass, and daily discarded house production corresponded to between 110 and 820% of the biomass (Table 4). These high rates indicate that appendicularians produce a large number of macroscopic aggregates in the form of houses. Furthermore, *O. longicauda*, *O. rufescens* and *O. fusiformis* invest 4 to 23 μg of carbon in new house materials and release 14 to 77 μg of carbon as discarded houses during their generation time of 3 to 10 d (Table 5). This is between 1.1 and 12 times the amount of carbon in a mature individual. This means that, on a carbon basis, house production by appendicularians can be much larger than somatic production. High productivity of macroscopic aggregates by appendicularians may be widely occurring events because of their ubiquitous distribution in the oceans.

The carbon content of a newly secreted house corresponds to 23% of C_{B} for *Oikopleura vanhoeffeni* (Deibel 1986), 15.3% of C_{B} for *O. dioica* (Sato et al. 2001) and 10 to 20% of ash-free dry weight for *O. rufescens* (Alldredge 1976c). These data and our $C_{\text{NH}}/C_{\text{B}}$ values in this paper indicate that the houses contained a high proportion of the total carbon produced by the appendicularians. Moreover, discarded houses (Table 3) contained much more carbon than newly secreted ones (Table 2) because of adhering particles. Contrary to our results, however, Alldredge (1976b) reported that

the carbon content of newly secreted, particle-free houses of *O. rufescens* was higher than that of field-collected discarded houses. As reported by Sato et al. (2001), houses collected in the field may have been discarded for some length of time before collection, allowing decomposition of house materials and reduction of carbon content to be advanced by the activity of microorganisms attached to the houses. Thus, it is likely that the carbon content of houses will continuously increase while they are occupied and decrease after they are discarded. Although newly secreted, particle-free houses contain very low or undetectable amounts of nitrogen (Alldredge 1976b, Riehl 1992 in Flood & Deibel 1998), accumulation of phytoplankton, detritus and fecal materials in the house also enriches them with nitrogen (Alldredge 1976b). Discarded houses are therefore potentially valuable to other organisms. Various organisms such as bacteria (Alldredge & Youngbluth 1985, Davoll & Silver 1986, Davoll & Youngbluth 1990), ciliates (Caron et al. 1986, Davoll & Silver 1986), planktonic crustaceans (Alldredge 1972, 1976a,b, Ohtsuka & Kubo 1991, Ohtsuka et al. 1993, 1996, Steinberg & Silver 1994, Steinberg 1995) or anguilloid larvae (Mochioka & Iwamizu 1996) are known to utilize discarded appendicularian houses.

Daily new house and discarded house production by *Oikopleura dioica* corresponds to 130–290% and 490–1100% of the animal biomass, respectively (Sato et al. 2001). In the present study, daily house production by *O. fusiformis* was comparable to that of *O. dioica*, but that by *O. longicauda*, *O. rufescens* and *Megalocercus huxleyi* was lower. The high abundance of *O. dioica*, sometimes exceeding 1×10^4 ind. m^{-3} (Seki 1973, Paffenhöfer 1976, Anakubo & Murano 1991, Uye & Ichino 1995, Nakamura et al. 1997, Nakamura 1998), is only occasionally recorded for oceanic larvacean species (Alldredge 1982, Hopcroft & Roff 1998). The abundance and biomass of appendicularians thus appear to be generally lower in oceanic than coastal waters, and therefore house productivity in oceanic waters will generally be lower than that in coastal waters. Ohtsuka et al. (1993), however, observed the attachment of copepods to appendicularian houses in oceanic waters dominated by oceanic species, but not in coastal waters dominated by *O. dioica*. This suggests that the discarded houses are more important as a food source for copepods (and perhaps also for other organisms) in oligotrophic oceanic waters than in eutrophic coastal waters. Appendicularian assemblages in oceanic waters may be more important in aggregate food webs even though their total production is smaller.

Discarded houses sink at a rate of 26 to 189 $m d^{-1}$ (Silver & Alldredge 1981, Taguchi 1982, Gorsky et al. 1984, Hansen et al. 1996), except for the discarded

houses of the giant appendicularian *Bathochordaeus* sp., which sink at a rate of $818 \pm 199 m d^{-1}$ (Hamner & Robison 1992). The species examined here are found primarily in the upper 100 m of the water column (Tomita 1998), so sinking houses can potentially contribute to the transport of organic matter synthesized in the euphotic zone to deeper, less productive waters. However, as stated earlier, particulate organic matter contained in the discarded houses decreases over time and therefore would decrease while sinking through the water column. Therefore, the amount of organic matter actually transported to deeper water by the sinking of discarded houses will be smaller than the discarded house production estimated in this study.

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