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House production by *Oikopleura dioica* (Tunicata, Appendicularia) under laboratory conditions

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We examined the generation time and the house renewal rate of Oikopleura dioica under various conditions. Animals were fed two flagellates, Isochrysis galbana and Tetraselmis sp., with concurrent determination of the carbon contents of body and house to estimate house production. The generation time was 6 days at 15°C, 4 days at 20°C and 3 days at 25°C at both 25 and 30 p.s.u. with a food concentration of 4×10^4 *cells* ml^{-1}*. The carbon content of newly secreted houses ranged from 0.5 to 0.8 μg, corresponding to 15.3 ± 4.8% of body carbon. The house renewal rates increased with increasing temperature and decreasing salinity. Food concentrations ranging from 100 to* 16×10^4 *cells* ml^{-1}*, body size and light condition had no effect on house renewal rate. Clogging of the inlet filter by adding the large diatom Ditylum sol caused an increase in house renewal rates. The total number and carbon content of houses during an animal's lifetime ranged from 46 to 53 houses and from 6.5 to 10.4 μg, respectively. Since daily house production calculated for the O. dioica population corresponded to 130–290% of its biomass and daily discarded house materials corresponded to 490–1100% of the biomass, this organism must play an important role as a producer of macroscopic aggregates.*

INTRODUCTION

Appendicularians, which are common in marine zooplankton communities, have been known for their extraordinarily unique mode of life. They collect small particles of food such as nano–pico phytoplankton, bacteria and even dissolved organic matter (Flood *et al.*, 1992) using a mucus structure called the 'house'. When a new house secreted by glandular epithelium on the animal's body is expanded, the old house is discarded. This replacement occurs quite often, sometimes several times or more per day. Discarded houses are used by various organisms as a source of food or surface habitat (Alldredge, 1972, 1976a,b; Ohtsuka and Kubo, 1991; Steinberg *et al.*, 1994; Steinberg, 1995; Mochioka and Iwamizu, 1996; Ohtsuka *et al.*, 1996) or contribute to the vertical transportation of organic matter to the deep sea (Silver and Alldredge, 1981).

Oikopleura dioica, which is distributed mainly in coastal waters (Fenaux, 1967) and is the most common and abundant appendicularian species in the inlet waters of Japan (Tokioka, 1955), has been examined most intensively because it is easily sampled and reared. Hence, much ecological information, including generation time, growth rate, filtering rate, fecundity and somatic production, has been accumulated. However, knowledge of house productivity for this species is limited. There has been only one report by Fenaux, which studied influences of temperature and food conditions on house renewal rate (Fenaux, 1985). Although house carbon content is one of the key parameters to estimate house production, we can find only one data set for the carbon content of the discarded house of *O. dioica* (Alldredge, 1979). Therefore, in the present study, we cultivated *O. dioica* under various conditions and measured generation times and house renewal rates. Carbon contents of the body and newly secreted, particle-free house were examined concurrently. Using these observations, we evaluated the number and carbon content of houses produced by an individual during its lifetime.

METHOD

Specimens of *O. dioica* were captured using a hand-towed plankton net with a large-volume cod-end at the Keihin

Canal in Tokyo Bay (Figure 1). Individuals were transferred to a 3 l glass beaker filled with seawater filtered through a membrane filter (0.45 μm pore size) and the salinity adjusted to 25 practical salinity units (p.s.u.), which was the ambient salinity at the collection site. This beaker was then mounted on a special apparatus to cultivate appendicularians (Sato *et al.*, 1999). Two flagellates, *Isochrysis galbana* (6 μm in cell length) and *Tetraselmis* sp. (13 μm in cell length), were cultured for food and fed. For use in our experiments, individuals of *O. dioica* were cultivated at 20°C and with a 12 h light:12 h dark cycle as a stock culture.

Determination of generation time

For experiments to determine the generation time, three different temperatures (15, 20 and 25°C) and two salinities (25 and 30 p.s.u.) were used. In all conditions, 2×10^4 cells ml^{-1} of *I. galbana* and 2×10^4 cells ml^{-1} of *Tetraselmis* sp. were fed. This was equivalent to 1200 μg C l^{-1} as particulate organic carbon (POC), determined by use of Yanagimoto MT-3 CHN analyzer. Mature males and females acclimatized to a target temperature were transferred to 50 ml flasks, and agitated to release gametes and fertilize eggs. The contents of flasks were poured into a beaker filled with adjusted sea water at the target condition and the beaker was mounted on the cultivating apparatus. Individuals were transferred to a fresh beaker every day by use of a wide-bore pipet. The generation time was determined as the period from fertilization until >50% of the surviving animals became fully mature. For these experiments, 10–30 individuals were sampled and fixed in 5% buffered formaldehyde–sea water solution every 12 h after fertilization. Immediately after fixation, the lengths of the trunk (including gonad) and tail were measured under a microscope.

Determination of carbon contents of body and house

Body carbon weights (BC) of various sized individuals were measured, and the relationship between trunk length and BC was examined. To make the animals break out of their houses and paralyze their tails, individuals were transferred to 3% KCl solution. After measurement of trunk length, 1–30 individuals of similar size were transferred onto pre-ashed (480°C for 3 h), 24 mm Whatman GF/C glass fiber filters and dried at 60°C for 24 h. All samples were stored in a desiccator for future measurement with the CHN analyzer. While the animal is still in its old house, it has already secreted a new one, which is carried as a rudiment in collapsed form on the trunk (Alldredge, 1976b). Thus, BC determined at this stage would include house rudiments. Newly secreted, particle-free houses were collected to determine the house carbon content (HC). The animal, which was larger than >700 μm in trunk lengthing was forced to abandon its old house by prodding the house and starting to expand a new one. While expanding the house, the animal was transferred to membrane-filtered (0.2 μm pore size) sea water to prevent

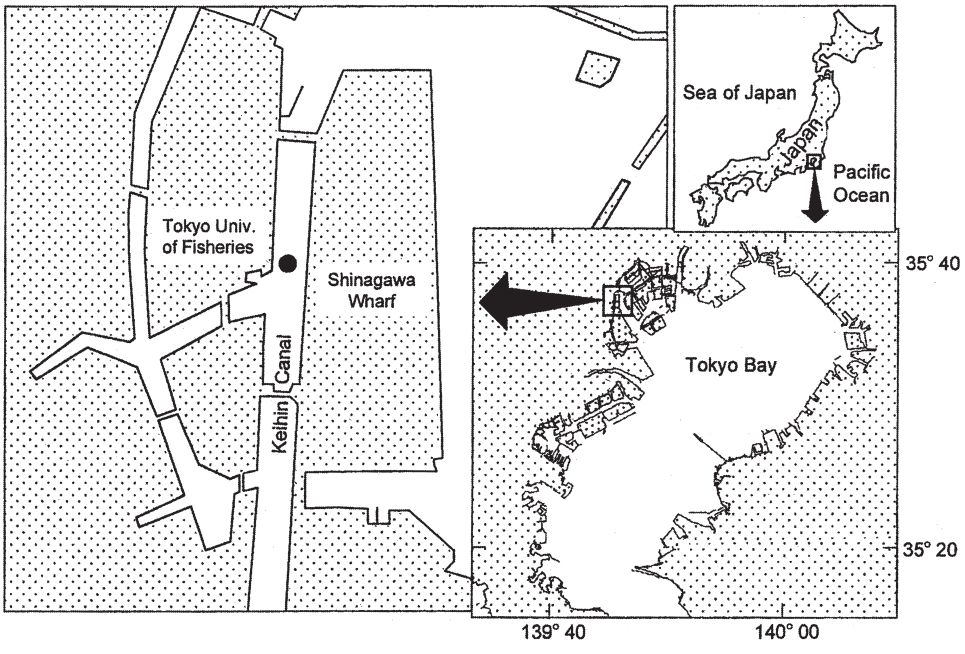

Fig. 1. Map showing the location of the sampling site (●) in Tokyo Bay.

particles from adhering to the house. Because newly secreted, particle-free houses are completely transparent and difficult to handle, each animal was then transferred to membrane-filtered seawater, to which we added powder of pre-ashed GF/C filter. The powder was drawn into the house and made it visible. Animals and houses were separated by transferring them to 3% KCl solution. About 10–20 of these animals and houses were separately pipetted onto pre-ashed 24 mm GF/C filters and dried. The carbon contents of these samples were determined using the method noted above.

Determination of house renewal rates

We determined the house renewal rates under various conditions, as shown in Table I. The animals were deposited individually in 250 ml glass jars filled with 200 ml of sea water adjusted to each condition (or 25 ml glass jars filled with 20 ml of sea water for individuals <400 μm in trunk length). These animals were cultivated at the target condition for 12 h in advance. Glass jars containing individuals were slanted at an angle of 20° on a table of 50 cm diameter, rotating at 2 r.p.m. with an alternating cycle of 15 s rotation and 15 s stationary in order to agitate water in the jars (Sato *et al.*, 1999). All individuals were transferred to new glass jars every 4 h to minimize the reduction of food concentration as a result of the animal's feeding, and discarded houses in the old jars were counted. After feeding for 12 h, all individuals were fixed and trunk lengths were measured. In the experiments conducted at 25 p.s.u. and 4×10^4 cells ml^{-1}, various-sized individuals were employed to examine the relationship between body size and house renewal rate, while in other experiments, individuals >500 μm in trunk length were employed. We also added a diatom, *Ditylum sol* (70 μm in cell width and 40 μm in cell length), in sea water to examine the influence of clogging of the inlet filter, which spans the water intake of the house and traps large particles before they enter the house [100×30 μm mesh size for large individuals of *O. dioica*; (Fenaux, 1986)]. Experiments were conducted under illumination of 2–6 μE m^{-2} s^{-1}, except for one under dark conditions to investigate the possibility of a diurnal rhythm in house renewal. In this latter experiment, we did not transfer animals to other jars during the experiment, in order always to keep them in the dark. Animals were not transferred in the illuminated control either.

Table I: Experimental conditions for determination of house renewal rate of O. dioica

Salinity (p.s.u.)	Temperature (°C)	Food concentration (cells ml^{-1})	Light condition	*Ditylum sol* concentration (cells ml^{-1})
25	15	4×10^4	L	
	20	1×10^2	L	
		1×10^3	L	
		2×10^3	L	
		4×10^3	L	
		1×10^4	L	
		2×10^4	L	
		4×10^4	L	
		8×10^4	L	
		16×10^4	L	
		4×10^4	L	50
		4×10^4	L	100
		4×10^4	L	200
	25	4×10^4	L	
		4×10^4	D	
30	15	4×10^4	L	
	20	4×10^4	L	
	25	4×10^4	L	
35	20	4×10^4	L	

Food concentrations are expressed as the total number of *I. galbana* and *Tetraselmis* sp. per unit volume of water. Each food organism was added equally in cell number. L, illuminated at 2–6 μE m^{-2} s^{-1}; D, dark.

Lifetime production and carbon content of houses

The mean trunk lengths measured every 12 h after fertilization were converted to BC using a trunk length–BC regression. For mature individuals in the samples, trunk length was converted from tail length using tail–trunk length regressions at each condition, because the gonads of fully matured individuals ruptured on fixation and trunk length could not be measured. Shiga reported that the relationship between trunk length (TR) and tail length (TA) of *Oikopleura labradoriensis* was expressed as the power function: $TA = \alpha TR^{\beta}$ (α and β are constants), which was divided into two parts at a distinct inflection point (Shiga, 1976). Thus, we looked for the inflection point by eye on the regression of tail length to trunk length, and separated data into two groups at the inflection point. BC increments with time at each condition were fitted by the logistic curves, expressed as:

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

where C_t is the BC at t h after fertilization, and b (intrinsic rate of natural increase per 12 h), C_∞ (maximum BC theoretically reached) and a are specific constants (Nose *et al.*, 1988). A period from fertilization to unfolding n-th houses (t_n, h) was calculated from:

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

where t_1 is the period from fertilization to unfolding of the first house and t_h is the house renewal interval, which is equal to the reciprocal of the house renewal rate. Extrapolating generation time into t_n of equation (2), the total number of houses produced by an individual during its lifetime can be calculated. Given the total house number m, the total HC (THC) produced by an individual during its lifetime can be calculated using the following equation, combining (1) and (2):

$$THC = \sum_{n=1}^{m} C_n \times r \quad (3)$$

where r is a HC/BC ratio. Because collecting the large quantity of newly secreted, particle-free houses of smaller individuals for carbon analysis was difficult, we have assumed that the HC/BC ratio is constant through the lifetime of the animal.

RESULTS

Generation time

Under both salinities, the generation time was 6 days at 15°C, 4 days at 20°C and 3 days at 25°C. Some individuals released gametes 5, 3 and 2 days after fertilization, respectively.

Carbon contents of body and house

The relationship between trunk length (TR; μm) and BC (μg) was expressed as:

$$BC = 2.62 \times 10^{-8}\,TR^{2.83}\ (r = 0.948,\ n = 59)$$

This relationship is similar to those reported for the same species (King *et al.*, 1980; Gorsky *et al.*, 1988).

The range of HC of four samples was 0.5–0.8 μg per house, with a mean (± SD) of 0.6 ± 0.1 μg. These values corresponded to 10.8–25.9% of BC, with a mean of 18.4 ± 6.7%, although BC here did not include carbon of the house rudiment because individuals in this experiment had discarded old and new houses consecutively. For estimation of total HC produced by an animal in its lifetime, we use the BC with the house rudiment in equations (1) and (3). This means that the HC/BC ratio extrapolated in equation (3) should be as HC/(BC with the house rudiment). We recalculated the HC/BC ratio assuming a carbon content of house rudiment equivalent to HC, i.e. HC/(BC + HC), and obtained a HC/BC ratio of 15.3 ± 4.8%.

House renewal rate

The relationships between house renewal rates (HR; houses h^{-1}) and temperature (T) or salinity (S) were expressed as:

$$HR = 0.0395\,T - 0.208$$
(at 25 p.s.u., $r = 0.850$, $n = 281$, $P < 0.001$)

$$HR = 0.0369\,T - 0.210$$
(at 30 p.s.u., $r = 0.878$, $n = 57$, $P < 0.001$)

$$HR = -0.0110\,S + 0.843$$
(at 20°C, $r = -0.441$, $n = 214$, $P < 0.001$)

The house renewal rates at a food concentration of 4×10^4 cells ml^{-1} increased with increasing temperature and decreasing salinity (Figure 2).

The house renewal rates were observed to be constant over all animal sizes since trunk lengths and house renewal rates were not significantly correlated (Figure 3).

Under dark conditions, the mean house renewal rate (± SD) was 0.80 ± 0.08 houses h^{-1} ($n = 12$), while in the illuminated control, the mean house renewal rate was 0.80 ± 0.10 houses h^{-1} ($n = 18$). These values were not significantly different (t-test, $P > 0.9$).

We observed a slight tendency toward increased mean house renewal rates with increasing food concentration

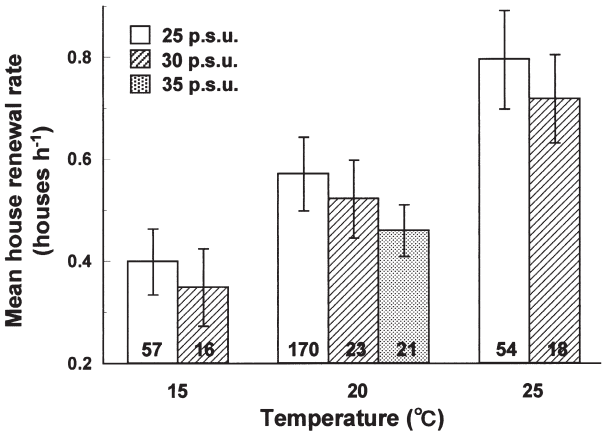

Fig. 2. Mean house renewal rate of *O. dioica* at each temperature and salinity. Numbers of individuals examined are given in boxes. Vertical bars denote the SD.

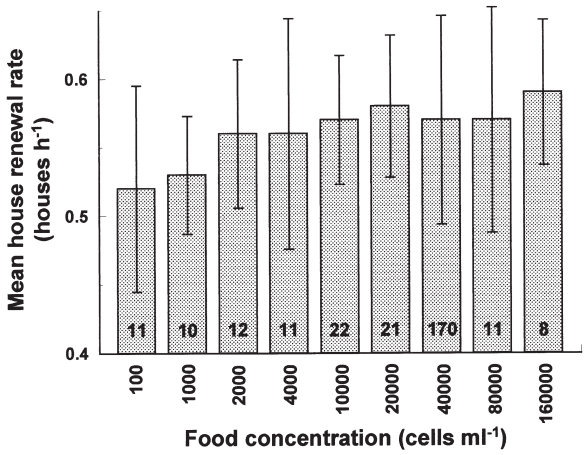

Fig. 4. Mean house renewal rate of *O. dioica* at various food concentrations at 20°C and 25 p.s.u. Numbers of individuals examined are given in the columns. Vertical bars denote the SD.

(Figure 4). However, there was no significant difference among mean house renewal rates at various food concentrations, as determined by analysis of variance ($F = 1.21$, $P > 0.2$). The minimum value of 0.52 ± 0.08 at 100 cells ml^{-1} was only 88% of the maximum value of 0.59 ± 0.05 at 16×10^4 cells ml^{-1}, in spite of more than a three order of magnitude increase in food concentration.

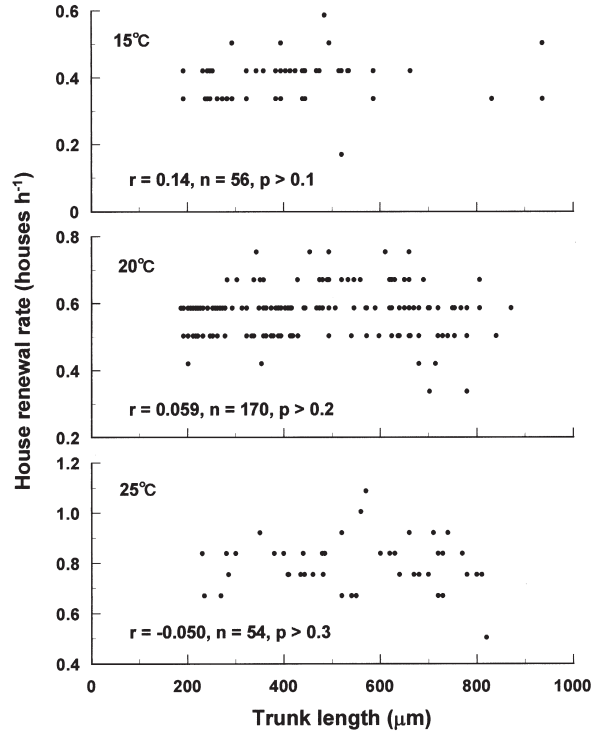

Fig. 3. Relationships between trunk length and house renewal rate of *O. dioica* at various temperatures at 25 p.s.u.

We also observed that clogging of the inlet filter by *D. sol* added to sea water caused an increase in house renewal rate at ≥100 cells ml^{-1} (Figure 5). There was a significant difference among mean house renewal rates as a result of analysis of variance ($F = 2.94$, $P < 0.05$).

Lifetime production and carbon content of houses

We observed that individuals required 13 h at 15°C, 10 h at 20°C and 7 h at 25°C until they unfolded their first house after fertilization at both 25 and 30 p.s.u. Given the mean generation time of 144 h at 15°C, 96 h at 20°C and 72 h at 25°C, we can assume that 50 ± 6.2–53 ± 8.4 houses at 25 p.s.u. or 46 ± 6.6–48 ± 5.6 houses at 30 p.s.u. could be produced by an individual (Table II).

Under our laboratory conditions, inflection points for

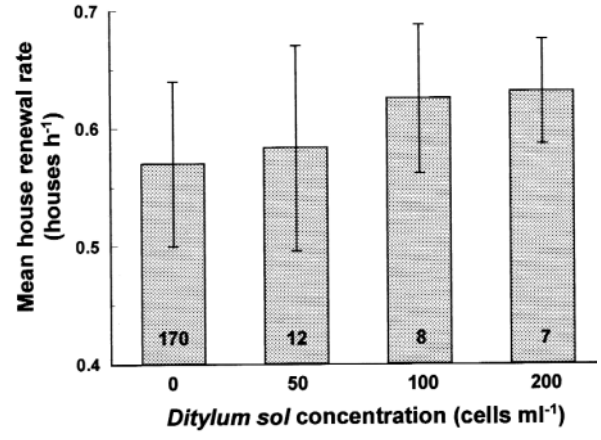

Fig. 5. Mean house renewal rate of *O. dioica* with *D. sol* at 20°C and 25 p.s.u. Numbers of individuals examined are given in the columns. Vertical bars denote the SD.

Table II: Total number and carbon content of houses produced by individual O. dioica during their lifetime at each temperature and salinity

Salinity (p.s.u.)	Temperature (°C)	Generation time (h)	Mature individual body C (mean ± SD; μg)	Total house number (mean ± SD; houses ind.$^{-1}$)	Total house C (μg ind.$^{-1}$)	Total house C/mature individual body C
25	15	144	3.3 ± 0.95	53 ± 8.4	6.5	2.0
	20	96	3.2 ± 0.98	50 ± 6.2	7.5	2.3
	25	72	2.6 ± 0.70	53 ± 6.3	7.4	2.8
30	15	144	5.0 ± 2.4	47 ± 9.9	10.4	2.1
	20	96	5.2 ± 1.9	46 ± 6.6	9.1	1.8
	25	72	2.5 ± 0.64	48 ± 5.6	7.4	3.0

O. dioica appeared to be ~1500 μm in tail length (Figure 6). At 20°C and 25 p.s.u., and at 25°C and 30 p.s.u., however, constants β of power equations fit to two groups of data were not significantly different at $P < 0.05$. For these conditions, only one power equation was thus fit for all data.

As shown in Figure 7, logistic curves represented BC increments well and were available to use for equation (3). The total HC produced by an individual in its lifetime ranged between 6.5 and 10.4 μg, corresponding to 1.8–3.0 times the BC of a mature individual (Table II).

DISCUSSION

It had long been believed that clogging of the filters triggered house abandonment (Lohmann, 1909; Alldredge, 1976b). Fenaux, however, demonstrated that animals abandoned and expanded their houses even when they were maintained in artificial sea water without particles to clog the filters (Fenaux, 1985). House renewal appears to depend on the house rudiment secretion rate, which may be controlled by physiological conditions (Flood and Deibel, 1998).

The house renewal rate increased linearly with increasing temperature, as Fenaux reported (Fenaux, 1985). According to our new results, it also increased with decreasing salinity (Figure 2). Although particles drawn into the house adhered to and accumulated densely in the food-concentrating filter at higher food concentrations, the house renewal rate increased by only 10% with increasing food concentration from 100 to 16×10^4 cells ml^{-1} (3–4800 μg C l^{-1}). Furthermore, it was rather constant at 2×10^3–8×10^4 cells ml^{-1} (60–2400 μg C l^{-1}) (Figure 4). Since these food concentrations may be within the range of those naturally encountered by *O. dioica*, food concentration does not appear to be a main factor in regulating house renewal rates in comparison with temperature and salinity. Acuña and Kiefer (Acuña and Kiefer, 2000) also reported little effect of food concentration on house renewal rates of *O. dioica*, although Fenaux (Fenaux, 1985) showed that the house renewal rate increased when algae were added to sea water. The decrease in house renewal rate at $<1 \times 10^3$ cells ml^{-1} may rather be due to shortage of food.

Body size and light condition had no effect on house renewal rate (Figure 3), indicating that animals produce houses periodically through their lifetime, if the environmental conditions are constant. To prevent clogging of the inlet filter, appendicularians of the Oikopleuridae momentarily suspend tail beating and reverse the flow of water with their ciliated spiracles, so that the water flowing out through the inlet filter sweeps away the large particles trapped there (Alldredge, 1976b). However, at

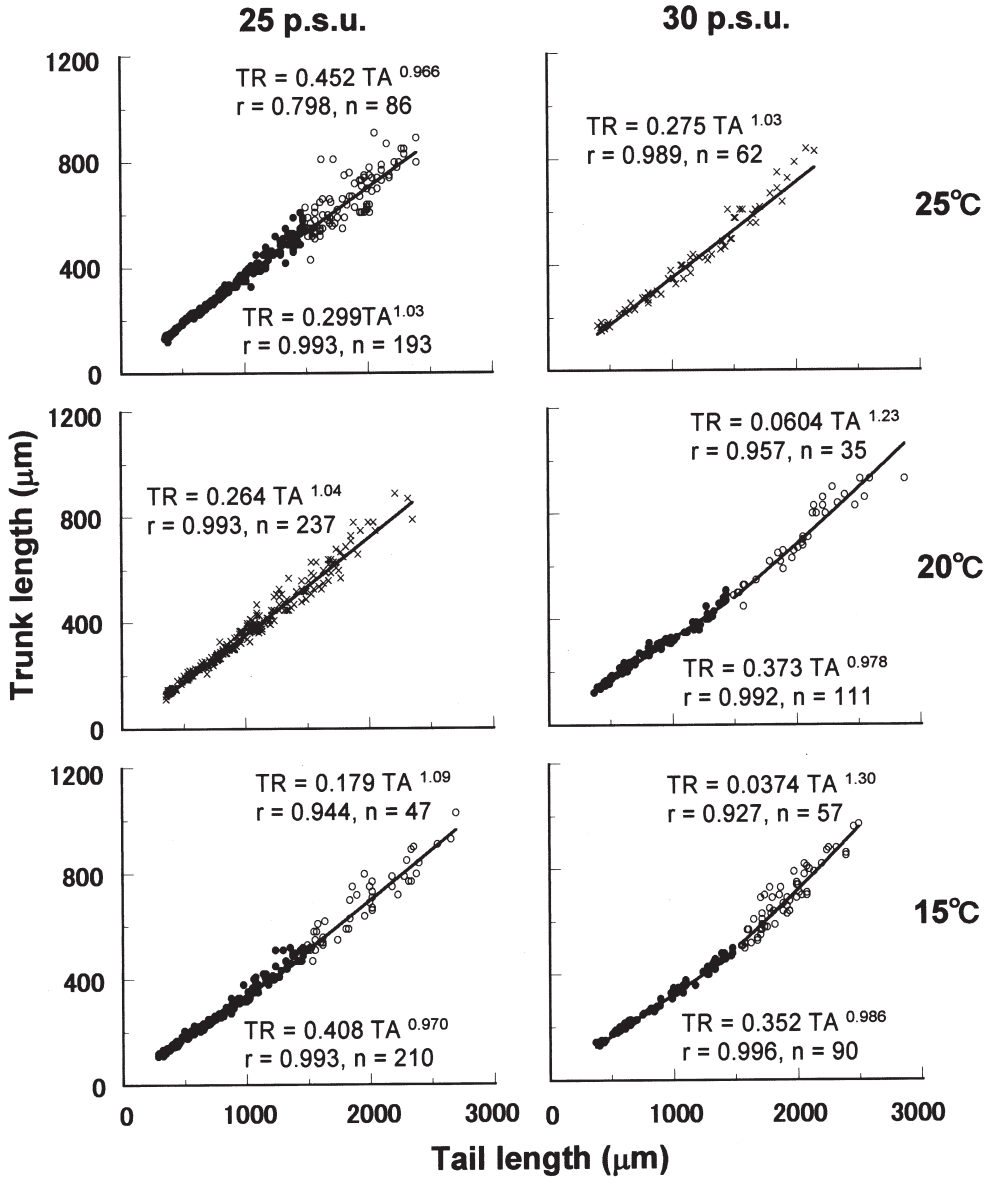

Fig. 6. Relationships between tail length (TA) and trunk length (TR) of *O. dioica* at each temperature and salinity. Data were fit to the power function $TR = \alpha TA^{\beta}$. When data were separated into two different size categories, i.e. >1500 μm (○) and <1500 μm (●) in tail length, and the null hypothesis (H_0) that $\beta_1 = \beta_{10}$ was rejected at $P < 0.05$, data of each category were fit to power functions separately. When H_0: $\beta_1 = \beta_{10}$ was not rejected at $P < 0.05$, all data are shown by crosses and fit to one power function.

D. sol concentrations >100 cells ml^{-1}, the inlet filter was clogged and the house renewal rate increased (Figure 5). In Tokyo Bay, large or chain (or mass) forming phytoplankton such as *Skeletonema costatum*, *Chaetoceros debile*, *Thalassiosira* spp., *Rhizosolenia* spp. or *Ceratium furca* sometimes occur, reaching hundreds or thousands of cells per milliliter (Nomura and Yoshida, 1997). In such an extreme environment, large particles may accelerate the increase in house renewal rate and the growth of individuals or populations of *O. dioica* may deteriorate, since increased house renewal rates due to clogging of the inlet filter will result in greater investment of materials for house synthesis relative to food intake.

Hansen *et al.* reported that the abundance of houses discarded by an *O. dioica* population was ~300 houses m^{-3} with an animal abundance of 160 individuals (ind.) m^{-3} in East Sound, Orcas Island, WA (Hansen *et al.*, 1996). We can deduce that a much larger number of houses must be produced and discarded episodically by *O. dioica* populations, since individual *O. dioica* produced 8.4–19 houses

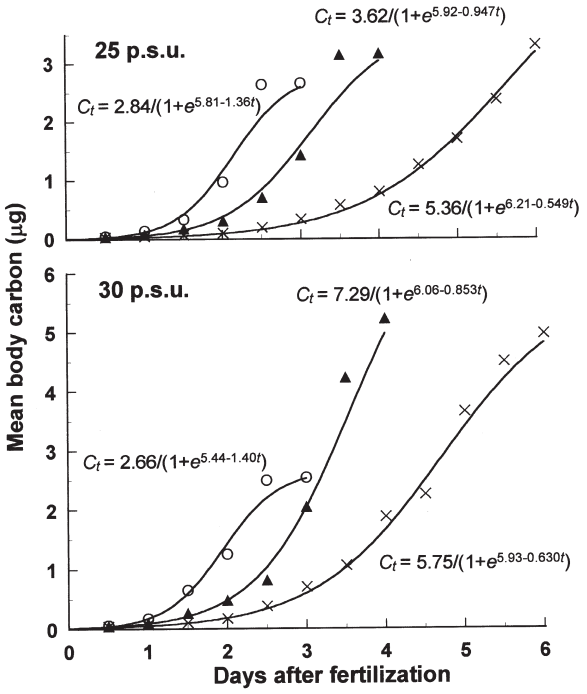

Fig. 7. Logistic curves fit to growth of *O. dioica* expressed by mean body carbon at each temperature and salinity. ×, 15°C; ▲, 20°C; ○, 25°C. The equation of the logistic curve for each condition is given in the figure. *t*, days after fertilization.

in a day under our experimental conditions and a high abundance of this species exceeding 1×10^4 ind. m^{-3} in the field has frequently been observed (Seki, 1973; Anakubo and Murano, 1991; Uye and Ichino, 1995; Nakamura *et al.*, 1997). Assuming a highly eutrophic environment, for example, where ingestible POC is 1200 μg l^{-1}, *O. dioica* abundance is 1×10^4 ind. m^{-3} and salinity is 30 p.s.u., we can calculate the number of daily discarded houses ranging from 8.4×10^4 (15°C) to 1.7×10^5 houses m^{-3} (25°C). This is equivalent to the value reported by Taguchi, who found that the abundance of discarded houses of *Oikopleura longicauda* in Kaneohe Bay, Hawaii, reached 1.3×10^5 houses m^{-3} (Taguchi, 1982).

The ratio of daily house production to appendicularian biomass can be calculated by multiplying the HC/BC ratio and the house renewal rate. Hopcroft and Roff estimated that the daily house production of an appendicularian community dominated by *O. longicauda* in Kingston Harbour, Jamaica (salinity 34–36‰; temperature 27–30°C), was equivalent to 150–300% of appendicularian biomass (Hopcroft and Roff, 1998). Using 15.3% for the HC/BC ratio, daily house production of the *O. dioica* population ranged from 130% (30 p.s.u., 15°C) to 290% (25 p.s.u., 25°C) of its biomass. Considering that a variety of particles including fecal pellets are accumulated in the house due to feeding and defecation (Hansen *et al.*, 1996), the discarded houses must contain a greater amount of carbon, although Alldredge reported that the carbon contents of newly secreted, particle-free houses of *Oikopleura rufescens* were higher than those of discarded ones (Alldredge, 1976c). These houses collected in the field may have been passed a certain period of time after they were discarded, so that decomposition of house materials and reduction of carbon contents could be advanced by microorganisms attached to the houses. Thus, we presume that the newly discarded houses contain a greater amount of carbon than the newly secreted, particle-free ones. The carbon content of 2.3 μg per discarded house of *O. dioica* could be calculated from the data listed in the tables of Alldredge (Alldredge, 1979). This value for the discarded house is 3.8 times the carbon content of a newly secreted, particle-free house obtained in this study. This result yields daily house materials discarded by an *O. dioica* population ranging from 490 to 1100% of its biomass.

Crustacean zooplankton discard organic carbon as a molt through their growth. For example, the molt of the copepod *Calanus pacificus* contains 3.8% of its BC (Vidal, 1980) and a similar value (4%) is found in the euphausiid *Euphausia pacifica* (Jerde and Lasker, 1966). According to Lasker, annually discarded molts of *E. pacifica* were equivalent to seven times its biomass based on dry weight, or 2.8 times its biomass based on carbon weight calculated from percent chemical composition data (Lasker, 1966). On the other hand, *O. dioica* invests carbon corresponding to 15.3% of BC in a house, or 1.8–3.0 times greater than that in the mature individuals in houses completely produced during several days (Table II). Taking such a high carbon content of the house and house productivity into account, *O. dioica* is considered to be very different from crustaceans as a producer of macroscopic aggregates and a transporter of organic carbon.

Discarding a large amount of organic matter as a house, larvaceans must occupy an important position in the marine ecosystems. We have no doubt that various organisms utilize discarded houses of oceanic species (Alldredge, 1972, 1976a,b; Ohtsuka and Kubo, 1991; Steinberg *et al.*, 1994; Steinberg, 1995; Mochioka and Iwamizu, 1996; Ohtsuka *et al.*, 1996). However, Ohtsuka *et al.* reported that no attachment of copepods to appendicularian houses was observed in inshore waters dominated by *O. dioica*, in contrast to the offshore area dominated by oceanic species (Ohtsuka *et al.*, 1993). Their result indicates that the way houses are utilized may differ between offshore and inshore regions. We still need information about the utilization of *O. dioica* and its houses by other organisms in coastal marine ecosystems.

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REFERENCES

Acuña, J. L. and Kiefer, M. (2000) Functional response of the appendicularian *Oikopleura dioica*. *Limnol. Oceanogr.*, **45**, 608–618.

Alldredge, A. L. (1972) Abandoned larvacean houses: a unique food source in the pelagic environment. *Science*, **177**, 885–887.

Alldredge, A. L. (1976a) Field behavior and adaptive strategies of appendicularians (Chordata: Tunicata). *Mar. Biol.*, **38**, 29–39.

Alldredge, A. L. (1976b) Appendicularians. *Sci. Am.*, **235**, 95–102.

Alldredge, A. L. (1976c) Discarded appendicularian houses as sources of food, surface habitats, and particulate organic matter in planktonic environments. *Limnol. Oceanogr.*, **21**, 14–23.

Alldredge, A. L. (1979) The chemical composition of macroscopic aggregates in two neritic seas. *Limnol. Oceanogr.*, **24**, 855–866.

Anakubo, T. and Murano, M. (1991) Seasonal variation of zooplankton in Tokyo Bay. *J. Tokyo Univ. Fish.*, **78**, 145–165.

Fenaux, R. (1967) *Les Appendiculaires des Mers d'Europe et du Bassin Méditerranéen*. Maison et Cie, Paris.

Fenaux, R. (1985) Rhythm of secretion of oikopleurid's houses. *Bull. Mar. Sci.*, **37**, 498–503.

Fenaux, R. (1986) The house of *Oikopleura dioica* (Tunicata, Appendicularia): structure and functions. *Zoomorphology*, **106**, 224–231.

Flood, P. R. and Deibel, D. (1998) The appendicularian house. In Bone, Q. (ed.), *The Biology of Pelagic Tunicates*. Oxford University Press, Oxford, pp. 105–124.

Flood, P. R., Deibel, D. and Morris, C. C. (1992) Filtration of colloidal melanin from sea water by planktonic tunicates. *Nature*, **355**, 630–632.

Gorsky, G., Dallot, S., Sardou, J., Fenaux, R., Carre, C. and Palazzoli, I. (1988) C and N composition of some Mediterranean zooplankton and micronecton species. *J. Exp. Mar. Biol. Ecol.*, **124**, 133–144.

Hansen, J. L. S., Kiørboe, T. and Alldredge, A. L. (1996) Marine snow derived from abandoned larvacean houses: sinking rates, particle content and mechanisms of aggregate formation. *Mar. Ecol. Prog. Ser.*, **141**. 205–215.

Hopcroft, R. R. and Roff, J. C. (1998) Production of tropical larvaceans in Kingston Harbour, Jamaica: are we ignoring an important secondary producer? *J. Plankton Res.*, **20**, 557–569.

Jerde, C. W. and Lasker, R. (1966) Molting of euphausiid shrimps: shipboard observations. *Limnol. Oceanogr.*, **11**, 120–124.

King, K. R., Hollibaugh, J. T. and Azam, F. (1980) Predator–prey interactions between the larvacean *Oikopleura dioica* and bacterioplankton in enclosed water columns. *Mar. Biol.*, **56**, 49–57.

Lasker, R. (1966) Feeding, growth, respiration, and carbon utilization of a euphausiid crustacean. *J. Fish. Res. Board Can.*, **23**, 1291–1317.

Lohmann, H. (1909) Die Gehäuse und Gallertblasen der Appendicularien und ihre Bedeutung für die Erforschung des Lebens im Meer. *Verh. Dtsch. Zool. Ges.*, **19**, 200–239.

Mochioka, N. and Iwamizu, M. (1996) Diet of anguilloid larvae: leptocephali feed selectively on larvacean houses and fecal pellets. *Mar. Biol.*, **125**, 447–452.

Nakamura, Y., Suzuki, K., Suzuki, S. and Hiromi, J. (1997) Production of *Oikopleura dioica* (Appendicularia) following a picoplankton 'bloom' in a eutrophic coastal area. *J. Plankton Res.*, **19**, 113–124.

Nomura, H. and Yoshida, M. (1997) Recent occurrence of phytoplankton in the hyper-eutrophicated inlet, Tokyo Bay, central Japan. *La Mer*, **35**, 107–121.

Nose, Y., Ishii, T. and Shimizu, M. (1988) *Suisan Shigengaku*. Tokyo University Press, Tokyo.

Ohtsuka, S. and Kubo, N. (1991) Larvaceans and their houses as important food for some pelagic copepods. *Bull. Plankton Soc. Jpn*, **Spec. Vol.**, 535–551.

Ohtsuka, S., Kubo, N., Okada, M. and Gushima, K. (1993) Attachment and feeding of pelagic copepods on larvacean houses. *J. Oceanogr.*, **49**, 115–120.

Ohtsuka, S., Böttger-Schnack, R., Okada, M. and Onbé, T. (1996) *In situ* feeding habits of Oncaea (Copepoda: Poecilostomatoida) from the upper 250 m of the central Red Sea, with special reference to consumption of appendicularian houses. *Bull. Plankton Soc. Jpn*, **43**, 89–105.

Sato, R., Yu, J., Tanaka, Y. and Ishimaru, T. (1999) New apparatuses for cultivation of appendicularians. *Plankton Biol. Ecol.*, **46**, 162–164.

Seki, H. (1973) Red tide of *Oikopleura* in Saanich Inlet. *La Mer*, **11**, 17–22.

Shiga, N. (1976) Maturity stages and relative growth of *Oikopleura labradoriensis* LOHMANN (Tunicata, Appendicularia). *Bull. Plankton Soc. Jpn*, **23**, 31–45.

Silver, M. W. and Alldredge, A. L. (1981) Bathypelagic marine snow: deep-sea algal and detrital community. *J. Mar. Res.*, **39**, 501–530.

Steinberg, D. K. (1995) Diet of copepods (*Scopalatum vorax*) associated with mesopelagic detritus (giant larvacean houses) in Monterey Bay, California. *Mar. Biol.*, **122**, 571–584.

Steinberg, D. K., Silver, M. W., Pilskaln, C. H., Coale, S. L. and Paduan, J. B. (1994) Midwater zooplankton communities on pelagic detritus (giant larvacean houses) in Monterey Bay, California. *Limnol. Oceanogr.*, **39**, 1606–1620.

Taguchi, S. (1982) Seasonal study of fecal pellets and discarded houses of Appendicularia in a subtropical inlet, Kaneohe Bay, Hawaii. *Estuarine Coastal Shelf Sci.*, **14**, 545–555.

Tokioka, T. (1955) General consideration on Japanese appendicularian. *Publ. Seto Mar. Biol. Lab.*, **4**, 93–103.

Uye, S. and Ichino, S. (1995) Seasonal variations in abundance, size composition, biomass and production rate of *Oikopleura dioica* (FOL) (Tunicata: Appendicularia) in a temperate eutrophic inlet. *J. Exp. Mar. Biol. Ecol.*, **189**, 1–11.

Vidal, J. (1980) Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of *Calanus pacificus* and *Pseudocalanus* sp. *Mar. Biol.*, **56**, 135–146.

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