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Note

New apparatuses for cultivation of appendicularians

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Appendicularians are pelagic tunicates known for their unique feeding behavior. These animals ingest small particles using a gelatinous structure called a "house", which is secreted around the animal from specialized epithelial cells (e.g., Flood 1978; King et al. 1980; Alldredge 1981; Flood et al. 1992). They are an important link between the tiny primary producers and the larger planktivorous predators (Alldredge & Madin 1982) and contribute to the vertical flux of organic matter due to the sinking of abandoned houses (Silver & Alldredge 1981; Taguchi 1982; Davoll & Youngbluth 1990). However, ecological and physiological studies of appendicularians have been limited to the past 30 years. Paffenhöfer (1970) developed a cultivating apparatus for copepods, which was devised to simulate environmental conditions by (a) reducing contact with surfaces (walls, bottom, water-air) by keeping food and feeders suspended, (b) preventing aggregations of both by creating, using the tumbling approach, gentle shear, and (c) reducing wall effects by using large volumes. By using this apparatus, he succeeded in rearing *Oikopleura dioica* and *Fritillaria borealis* in the laboratory for the first time (Paffenhofer 1973, 1976). The former species was reared through 19 consecutive generations. Fenaux & Gorsky (1979, 1983, 1985) improved this apparatus and reared several species of *Oikopleura* and *Fritillaria formica*. Nevertheless, we are still short of information on the generation time, egg production and house renewal rate for many species. To understand the ecology of lesser known appendicularians, we devised new instrumentation and successfully cultivated *Oikopleura dioica*, *O. longicauda*, *O. fusiformis*, *O. rufescens*, *Appendicularia sicula*, *Fritillaria formica*, *Megalocercus huxleyi* and *Stegosoma magnum*. Here, we first describe these new instruments in detail. Generation times of the appendicularian species reared are also reported.

Two types of apparatuses were devised. In Type 1, a metal frame holding a 3-liter glass beaker was directly set on the axis of a motor, which was fixed on a wooden stand at an angle of 20° (Fig. 1). This frame was adjusted to rotate at 3 revolutions min^{-1} by the use of speed reduction gear heads on the motor.

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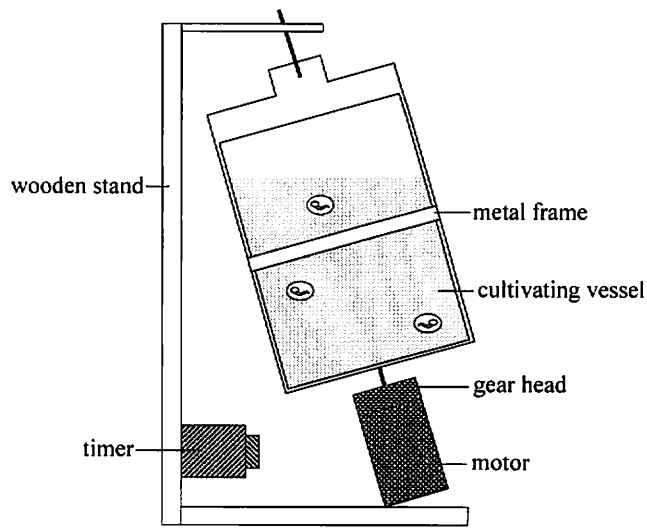

Fig. 1. Schematic diagram of type-1 apparatus.

A timer controlled the frame movement so as to repeat a 15s rotation and 15s repose alternately. This system works to gently agitate water in the beaker. Since the beaker rotates on a fixed axis, this apparatus allows easy observation and requires only a small space compared to conventional systems like Paffenhöfer's apparatus in which the cultivating vessel moves in a large circle.

Type 2 consisted of a motor fixed at an angle of 20° on a pipe (Fig. 2). A plastic arm with a pulley and clips to hold the cultivating vessel was attached to the motor axis. The same plastic arm was attached to a metal shaft mounted on the pipe using ball bearings. Two pulleys were connected to a belt so that the motor rotated both arms simultaneously. During operation, we mounted this system in a water bath and attached 3 or 5-liter plastic beakers filled with seawater. Water levels in the water bath and the beakers were adjusted to the same level to maintain the neutral buoyancy of the beakers, so as not to cause strain on the plastic arms. Water temperature in the beakers can be adjusted by regulating the temperature of the water bath.

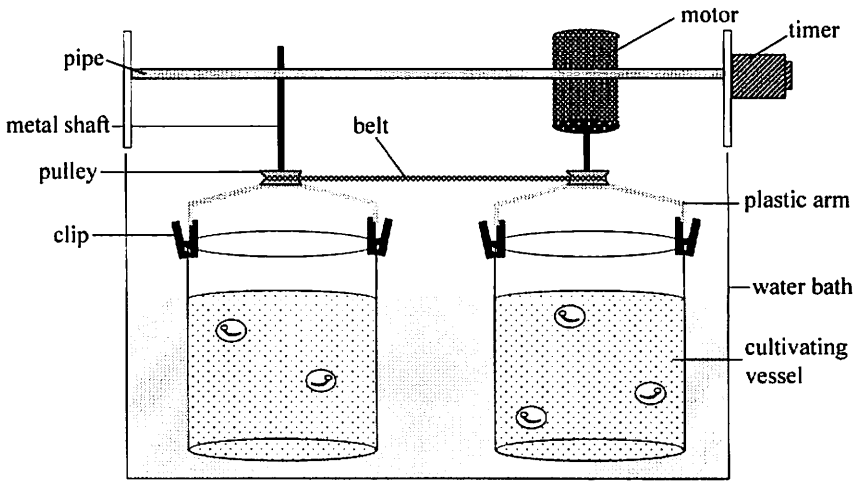

Fig. 2. Schematic diagram of type-2 apparatus.

We also developed a device for transferring large appendicularians such as *M. huxleyi* and *S. magnum* from one vessel to another. This consisted of a transparent acrylic pipe (12–31 mm internal diameter) and a lid, covered with a silicon sponge rubber sheet, that was attached to a guiding bar (Fig. 3a). For transferal, the mouth of the acrylic pipe was set in the water so as to put the animal between the mouth of the pipe and the lid (Fig. 3b). Then the animal was gently sucked into the pipe by use of a nipple connected to the upper end of the pipe, and the bar was pulled to close the pipe lid (Fig. 3c). The device containing the animal was then placed in the water of another vessel and the animal gently released through the reverse procedure to that mentioned above. This device can be made cheaper and more easily than the device reported by Acuña et al. (1994).

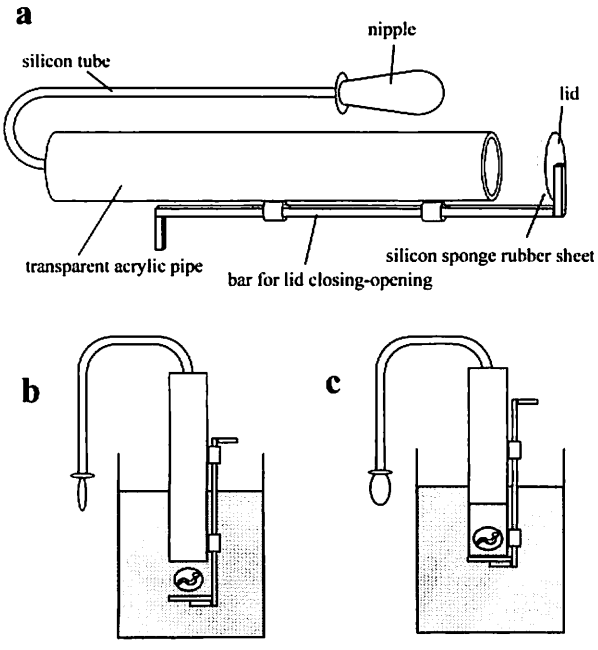

Fig. 3. Transfer device. **a.** Whole view. **b.** Before suction of animal into device. **c.** After suction of animal and closing mouth of device.

Oikopleura dioica specimens were captured using a hand-towed plankton net with a large volume cod-end at Keihin Canal in Tokyo Bay. Seawater for cultivation was filtered through a membrane filter (0.45-μm pore size) and adjusted to 25 PSU (ambient salinity at the collection site). Cultured flagellates, *Isochrysis galbana* (5.8 μm in cell length) and *Tetraselmis* sp. (12.7 μm in cell length), were fed at 3×10^4 cells ml^{-1} and 5×10^3 cells ml^{-1}, respectively. A glass beaker filled with seawater and a dozen of the captured oikopleurid individuals was mounted on the metal frame of the type-1 apparatus and rotation commenced. These individuals, within their houses, were transferred into fresh seawater every 2 to 3 d by the use of wide-bore pipettes. Most cultivations were carried out in the laboratory at 20°C and with a 12L : 12D light cycle. Under these conditions, *O. dioica* matured and released their gametes 4 to 5 d after fertilization (Table 1). In this apparatus, *O. dioica* was successfully cultivated to the 54th filial generation.

Other species were collected in surface waters 100 to 300 m off the Banda Marine Laboratory of the Tokyo University of Fisheries, Tateyama, Chiba Prefecture. *Oikopleura longicauda*, *O. fusiformis*, *O. rufescens*, *M. huxleyi* and *S. magnum* were captured individually with their houses in 300 ml transparent plastic vessels by diving. *Appendicularia sicula* and *F. formica* were gently sorted out in the laboratory from seawater collected by a diver using a 20-liter polyethylene tank. Each species was separately transferred into beakers filled with ambient seawater filtered through a 30-μm mesh. These beakers were mounted on the plastic arms of the type-2 apparatus. The animals, within their houses, were transferred into freshly filtered seawater every 0.5 to 2 d depending on their density and size. This was done through the use of wide-bore pipettes for small individuals or the above-mentioned transfer device for large individuals of large species. Cultivations were conducted at 20, 23 and 26°C under a 12L : 12D light cycle. *Oikopleura longicauda* grew well when fed on 3 species of cultured algae

Table 1. Generation times of appendicularian species cultivated in the laboratory during the present study. ND: no data.

Species	Temperature (°C)	Food	Food concentration[c] (μgCl $^{-1}$)	Generation time (days)	Filial generation number
Oikopleura dioica	20	Cultured phytoplankton[a]	800	4–5	54
Oikopleura longicauda	23	Cultured phytoplankton[b]	ND	4–5	14
Oikopleura fusiformis	20	Natural particles	ND	6	3
Oikopleura rufescens	23	Natural particles	100–130	7–8	3
Megalocercus huxleyi	23	Natural particles	140–190	5	1
Stegosoma magnum	26	Natural particles	ND	5–6	1
Appendicularia sicula	23	Natural particles	120–190	3–4	4
Fritillaria formica	23	Natural particles	90–110	3	8

[a] *Isochrysis galbana* and *Tetraselmis* sp.
[b] *Isochrysis galbana*, *Tetraselmis* sp. and unidentified flagellate.
[c] Food concentration was determined with a CHN analyzer (Yanagimoto MT-3).

(*Isochrysis galbana*, *Tetraselmis* sp. and an unidentified flagellate of 2-μm cell diameter) in membrane-filtered (0.45-μm pore size) seawater.

Under these laboratory conditions, generation times for all of the appendicularian species varied between 3 to 8 d (Table 1). These results are consistent with previous studies which showed that appendicularians have a considerably shorter generation time than copepods (Paffenhöfer 1976; Fenaux 1977; Esnal et al. 1985; Hopcroft & Roff 1995). Even in the case of the large species *M. huxleyi* and *S. magnum*, which reached 11.4 mm and 9 mm in tail length, respectively, the generation times were only 5 to 6 d. The characteristic feature of the short generation time of appendicularians can be attributed to their high growth rates (Hopcroft & Roff 1995; Uye & Ichino 1995; Nakamura et al. 1997).

This study showed that the new apparatuses were effective in the successful rearing of appendicularians. These apparatuses will thus contribute to the further understanding of appendicularian biology, and can also be used in the cultivation of other zooplankton species.

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Literature Cited

Acuña, J. L., D. Deibel & Sooley 1994. A simple device to transfer large and delicate planktonic organisms. *Limnol. Oceanogr.* **39**: 2001–2003.

Alldredge, A. L. 1981. The impact of appendicularian grazing on natural food concentrations in situ. *Limnol. Oceanogr.* **26**: 247–257.

Alldredge, A. L. & L. P. Madin 1982. Pelagic tunicates: unique herbivores in the marine plankton. *Bio Sci.* **32**: 655–663.

Davoll, P. J. & M. J. Youngbluth 1990. Heterotrophic activity on appendicularian (Tunicata: Appendicularia) houses in mesopelagic regions and their potential contribution to particle flux. *Deep-Sea Res.* **37**: 285–294.

Esnal, G. B., C. Sankarankutty & R. J. Castro 1985. Diurnal and seasonal fluctuations of *Oikopleura dioica* FOL, 1872 (Tunicata, Appendicularia) in the mouth of the river Potengi (North Brazil). *Physis* **43A**(105): 65–71.

Fenaux, R. 1977. Life history of the appendicularians (genus *Oikopleura*). *Proc. Symp. Warm Water Zoopl. Spl. Publ.* UNESCO/NIO. 497–510.

Fenaux, R. & G. Gorsky 1979. Tecqniques d'élevage des appendiculaires. *Ann. Inst. océanogr., Paris* **55**: 195–200.

Fenaux, R. & G. Gorsky 1983. Cycle vital et croissance de l'appendiculaire *Oikopleura longicauda* (VOGT), 1854. *Ann. Inst. océanogr., Paris* **59**: 107–116.

Fenaux, R. & G. Gorsky 1985. Nouvelle technique d'élevage des appendiculaires. *Rapp. Comm. Int. Mer Médit.* **29**: 291–292.

Flood, P. R. 1978. Filter characteristics of Oikopleurid's houses. *Bull. Mar. Sci.* **34**: 173–175.

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

Hopcroft, R. R. & J. C. Roff 1995. Zooplankton growth rates: extraordinary production by the larvacean *Oikopleura dioica* in tropical waters. *J. Plankton Res.* **17**: 205–220.

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

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

Paffenhöfer, G.-A. 1970. Cultivation of *Calanus helgolandicus* under controlled conditions. *Helgoländer Wiss. Meeresunters.* **20**: 346–359.

Paffenhöfer, G.-A. 1973. The cultivation of an appendicularian through numerous generations. *Mar. Biol.* **22**: 183–185.

Paffenhöfer, G.-A. 1976. On the biology of appendicularia of the southeastern North Sea, p. 437–455. In *10th European Symposium on Marine Biology* (eds. Persoone, G. & E. Jaspers). Universa Press, Belgium.

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

Taguchi, S. 1982. Seasonal study of fecal pellets and discarded houses of Appendicularia in a subtropical inlet, Kaneohe bay, Hawaii. *Estuar. Coast. Shelf Sci.* **14**: 545–555.

Uye, S. & S. Ichino 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.