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. Paffenhofer (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. justiformis, 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. 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 Paffenhofer’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.
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).

*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.
of the appendicularian species varied between 3 to 8 days (Table 1). Generation times were only 5 to 6 days. The characteristic feature of the short generation time of appendicularians can be attributed to their high growth rates (Hopcroft & Roff 1995; Uye & Ichino 1995). Even in the case of the large species *M. huxleyi* and *S. magnum*, which reached lengths of 11.4 mm and 9 mm in tail length, respectively, the generation times were only 5 to 6 days.

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


