Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

An improved cultivation device for appendicularians with notes on the biology of *Fritillaria* sp. collected in Sagami Bay, Japan

Riki Sato*

Misaki Marine Biological Station, School of Science, the University of Tokyo, 1024 Koajiro, Misaki, Miura, Kanagawa, Japan

Research Article

*Present address: Fisheries Resources Institute, Japan Fisheries Research and Education Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa, Japan.

Cite this article: Sato R (2023). An improved cultivation device for appendicularians with notes on the biology of *Fritillaria* sp. collected in Sagami Bay, Japan. *Journal of the Marine Biological Association of the United Kingdom* **103**, e68, 1–6. https://doi.org/10.1017/S0025315423000541

Received: 30 March 2023
Revised: 29 June 2023
Accepted: 14 July 2023

Keywords:
Appendicularians; cultivation; device; *Fritillaria*; mucus structure

Corresponding author:
Riki Sato; Email: sato_riki39@fra.go.jp

Abstract

To cultivate zooplankton species with highly fragile mucoid structures, a new type of cultivation device was developed, which uses a novel method to gently circulate the water in the culture vessel. Using this device, a species of the fritillarid appendicularia (*Fritillaria* sp.) collected in Sagami Bay was successfully cultivated. This form resembles *Fritillaria haplostoma* or *Fritillaria formica tuberculata*, but some taxonomical characteristics differed from those of the latter forms. A novel device comprising a polycarbonate bucket with a motor-driven rotating cylinder inserted into the water in the bucket was developed and maintained *Fritillaria* sp. over ten filial generations in the laboratory. The animals reached trunk lengths of 1026 ± 85 μm (mean ± SD) four days after fertilization at 23°C. The instantaneous growth rates were calculated as 1.11–1.72 at 20–26°C, representing a 3.0–5.6-fold daily increase in body weight. The house constructed by this species is extraordinarily fragile, typically has a barrel-like or cylindrical shape, and accommodates its entire body and food-concentrating filter. At 23°C, the species produced houses at a rate of 28 ± 2.8 houses d^{-1}. The new device is useful for the continuous cultivation of the fragile form of fritillarid appendicularia, and even for various other zooplankton. It also shows that *Fritillaria* sp. could be a significant secondary producer and transporter of organic matter in marine ecosystems due to its high growth and house production rates.

Introduction

Appendicularians are pelagic tunicates that form an important component of marine planktonic communities worldwide; they are notable for their construction of elaborate mucus structures (or 'houses'), which they use to collect small food particles. Paffenhöfer (1973) first reported the successful cultivation of a neritic appendicularian species, *Oikopleura dioica* Fol, 1872, using a unique apparatus comprising a culture vessel mounted on a motor-driven frame, which was rotated in a circular orbit to gently agitate the water and suspend animals and their food in the water (Paffenhöfer, 1970). Subsequently, several additional methods and devices have been developed and applied to the cultivation of appendicularians. These include a rotating paddle (Fenaux and Gorsky, 1985), water injection with a rotating paddle (Galt, 1987), a temperature-gradient plankton kreisel (Gorsky *et al.*, 1986), a diffusion tube (Patry *et al.*, 2020), and improved rotating culture vessel (Sato *et al.*, 1999). Because the rotating paddle device has a simple structure and is easy to construct, it has become indispensable in maintaining *O. dioica* cultures in the laboratory (Chioda *et al.*, 2002; Nishida, 2008; Bouquet *et al.*, 2009; Martí-Solans *et al.*, 2015; Masunaga *et al.*, 2020). Consequently, significantly more ecological, physiological, and genetic information has been accumulated for this species of the Oikopleuridae family compared to other families of Appendicularia (Kowalevskiidae and Fritillariidae).

Fritillarid species are common in appendicularian assemblages and sometimes occur in high densities (Tokioka, 1955; Shiga, 1985; Presta *et al.*, 2015), however, laboratory investigations of their life cycles are limited. Paffenhöfer (1976) determined the generation time of *Fritillaria borealis* Lohmann, 1896 by conducting rearing experiments with his unique cultivation apparatus. Sato *et al.* (1999) modified Paffenhöfer's instrumentation and cultivated *Fritillaria formica digitata* Lohmann in Lohmann & Bückmann, 1926 (Sato, 2000) and *Appendicularia sicula* Fol, 1874 (Sato *et al.*, 1999) through several life cycles to examine their generation time. Recently, Henriet *et al.* (2022) reported a morphogenetic study of *Fritillaria borealis typica* Lohmann, 1896 cultured in the laboratory for several months using the rotating paddle method. Although these studies revealed some new information on the ecology and physiology of fritillarids, information on their biology remains limited.

In considering the cultivation of zooplankton species with highly fragile mucoid structures, the author speculated that any major disturbance or thrusting movement of the water by a rotating paddle blade or water injection at the inlet openings should be limited as much as possible because such hydraulic pressure could distort or twist mucilaginous constructions. The methods of Paffenhöfer (1970) and Sato *et al.* (1999), in which culture contents are circulated due to shearing between the water and the inside wall of the culture vessel, are suitably gentle and limit the destruction of the fragile structures. However, the former requires a large space and

specific installation sites because the culture vessel and mounting flame move in a circle. This device may also be top-heavy at sites such as shipboard laboratories. Although Sato *et al.* (1999) later mitigated these disadvantages by having the culture vessel rotate on a fixed axis in the same position, much labour and time are required to construct this apparatus, and due to the placement of the vessel on a rotating shaft, it is not suitable for larger culture vessels, as the weight can lead to breakage of the device.

Therefore, the author tried to improve on the available cultivation devices, using a novel method that circulates the water smoothly and gently without any oscillation and strong hydraulic pressure. At the time of this study, a species of the family Fritillariidae (*Fritillaria* sp.) was collected at a site near our laboratory and reared using the improved device. This study reports this new cultivation device as well as the growth, house morphology, and house renewal rate of this species.

Materials and methods

The device used in this study consisted of two units: a rotating polypropylene cylinder driven by a motor mounted on a metal frame pedestal, and a polycarbonate bucket that formed the culture vessel (Figure 1). The cylinder (12 cm in diameter × 13 cm in height) comprised a lidded 2 L polypropylene bottle, the bottom of which was removed using a heavy-duty cutter knife (Figure 1A). This was fastened directly onto the axis of a speed-controllable motor with a gear head (US206-401 and 2GN90 K, Oriental Motor Co. Ltd., Tokyo, Japan) (Figure 1B, C). A metal frame pedestal was made using a store-brought angle bar and a metal plate for assembling a steel rack or a metal gadget (Figure 1B). A bucket containing 9 L of seawater was set at an angle of 20° to facilitate lateral and vertical water movement. The cylinder-motor unit was attached to the top of the bucket, such that the cylinder was partially submerged in the seawater (Figure 1D), and rotated at 3–5 rpm.

The animals used in the experiment were collected from surface waters in Sagami Bay, off Misaki Marine Biological Station, School of Science, the University of Tokyo, Miura, Kanagawa Prefecture, Japan (35°16'N; 139°60'E). Surface water was sampled by submerging a 20 L polyethylene tank. In the laboratory, this was gently poured into a beaker illuminated from behind. The animals were sorted and transferred into the polycarbonate bucket filled with ambient seawater filtered through a 30-μm mesh using a wide-bore pipette. The transferred individuals were then maintained with setting the cylinder-motor unit to expand their houses and resume feeding. Individuals were transferred into fresh mesh-filtered ambient seawater every 0.5–2 days, depending on the density and size of the animals. The number of transferred individuals varied from dozens of premature individuals up to 200 individuals with trunk lengths of 200–300 μm.

At maturity, the oocyte-filled posterior region of the trunk could be seen with the naked eye. About 10 of these individuals were transferred to a 50 ml flask and agitated to release gametes and fertilize eggs. The contents of the flask were poured into the bucket of the cultivation device, and cultivation experiments were then conducted under a 12 L:12 D light cycle at three different temperatures (20, 23, and 26°C) with the cultivation device placed in an incubator. Every 24 h after fertilization, 5–11 individuals were sampled and fixed in a 5% buffered formaldehyde seawater solution, and their trunk lengths (the distance between the upper lip of the mouth and the posterior edge of the trunk) and tail lengths were immediately measured under a microscope. Some specimens were captured with a digital camera (Panasonic DMC-GH2) fitted on the microscope to facilitate morphological observation of the animal's body. The growth rate was calculated by examining the change in body weight during the cultivation period. The trunk length was converted into the ash-free dry weight using a conversion equation obtained for *Fritillaria haplostoma* Fol, 1872 (Hopcroft *et al.*, 1998). The trunk lengths of some mature individuals could not be measured because their gonads had ruptured and released eggs at the sampling time. Therefore, the relationship between tail length and trunk length was determined to obtain the trunk length of the ruptured animals. The instantaneous growth rate (g) was calculated as follows:

$$g = \frac{\ln W_m - \ln W_1}{t - 1}$$

where W_m and W_1 are the mean ash-free dry weights of individuals on the final cultivation day and one day after fertilization, respectively, and t is the generation time in days.

A single individual was also maintained in the culture vessel at 23°C for 12 h, and the discarded houses were collected every 1–2 h and counted to determine the house renewal rate. For morphological observations of the house, cultured alga (*Tetraselmis* sp.) was added to the water. The algae adhered to the outer membrane of the house and thus diminished house transparency and facilitated the observation of its shape and structure. The houses occupied by animals in the culture vessel were recorded using a digital camera (Canon Power Shot G16) in close-up and video mode. Discarded houses were also transferred into a Petri dish and photographed with the digital camera.

Results

The cultivated *Fritillaria* individuals (Figure 2) resembled *Fritillaria formica tuberculata* Lohmann in Lohmann & Bückmann, 1926 or *F. haplostoma*, considering the strongly bent compact trunk and dorsal oikoplastic layer located upstream of the stomach (Figure 2B) for the former, or the narrow tail musculature (Figure 2C) and large upper lip (Figure 2F) for the latter. However, they did not have the protruding lips with lateral lobes of the former or elongated cylindrical testis of the latter (Bückmann and Kapp, 1975; Fenaux, 1993). Therefore, these individuals are provisionally regarded here as *Fritillaria* sp.

Fritillaria sp. produced an extraordinary mucilaginous and fragile house that was easily distorted or broken, even by moderate water turbulence or gentle prodding. However, the rotating cylinder of the novel cultivation device never damaged or distorted their houses, and the animals were maintained in good condition through more than ten filial generations.

The animals matured and released their gametes 4–5 days, 3–4 days, and 3 days after fertilization at 20, 23, and 26°C, respectively. The mean tail length reached 2137 ± 177 μm (± SD, $n = 7$) on the final day at 23°C (Figure 3) and was not statistically different from those of 11 individuals at 20°C and eight individuals at 26°C (Kolmogorov–Smirnov test for consistency with a normal distribution, $0.16 < D < 0.19$, $0.40 < P < 0.81$; Bartlett test for the equality of the error variances, $\chi^2 = 2.44$, $P = 0.30$; one-way ANOVA, $F_{2,23} = 3.42$, $P = 0.64$). The relationships between tail length (Ta, μm) and trunk length (Tr, μm) were plotted at each temperature and found to overlap (Figure 4), and thus all data were combined to fit one regression equation in a form according to Shiga (1976):

$$\mathrm{Tr} = 0.607 \times \mathrm{Ta}^{0.967} \ (n = 68,\ r^2 = 0.974)$$

By applying this equation, the mean trunk length was estimated as 1026 ± 85 μm on the final day at 23°C. Instantaneous growth rates were calculated as 1.11, 1.34, and 1.72 at 20, 23, and 26°C, respectively, representing a 3.0–5.6-fold daily increase in body weight.

https://doi.org/10.1017/S0025315423000541 Published online by Cambridge University Press

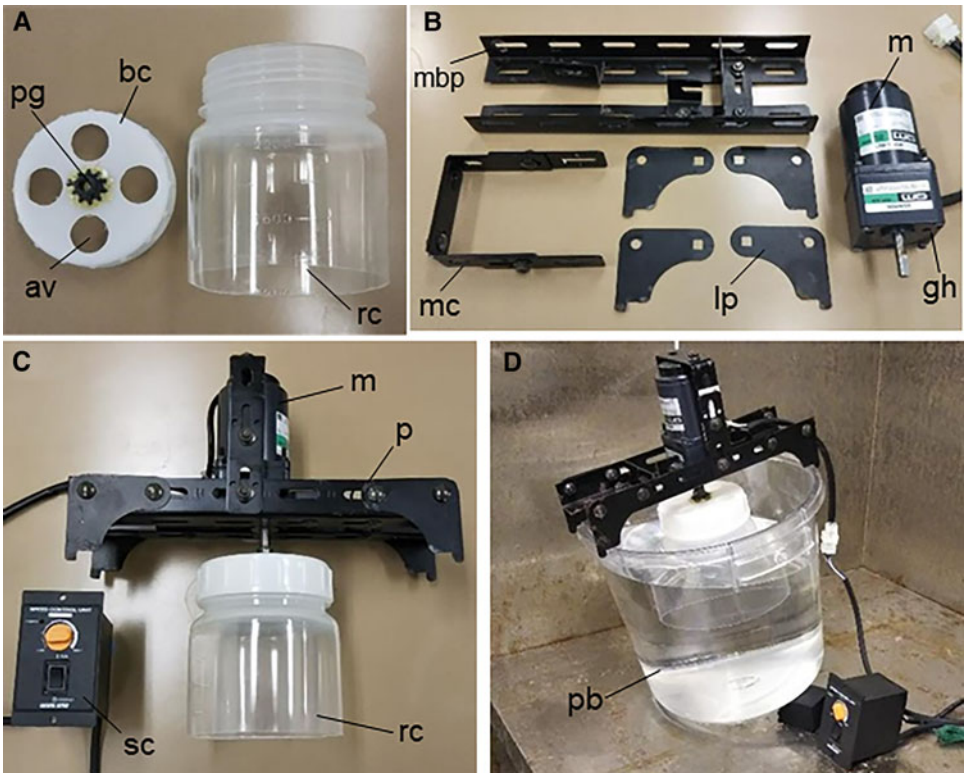

Figure 1. Cultivation device: (A) cylinder and bottle cap. A pinion gear to hold the motor axis was glued onto the bottle cap with epoxy adhesive. Small holes were drilled around the pinion gear so that epoxy adhesive could extend to both sides of the cap. Four large holes in the lid form an air vent during experiments; (B) disassembled metal pedestal. The leg plates were cut to fit the edge of the bucket; (C) assembled cylinder-motor unit; (D) experimental setup with an angled polycarbonate bucket containing seawater. bc, bottle cap; pg, pinion gear; av, air vent; rc, rotating cylinder; mbp, main body of pedestal; m, motor; mc, motor clamp; lp, leg plate; gh, gear head; p, pedestal; sc, speed controller; pb, polycarbonate bucket.

The house was typically barrel-like or cylindrical (Figure 5); however, some had a conical shape (Figure 5E), even when occupied. Houses of large individuals had longitudinal lengths of 7–10 mm. The house enveloped the entire animal body and food-concentrating filter and featured a funnel-like dent on one side, leading to a single water inlet just behind the occupying animal (Figure 5C, F). The elastic food-concentrating filter was alternatingly inflated with tail beating and deflated with tail arrest. The outer membrane of the house surrounding the animal and food-concentrating filter did not respond to the tail's movement (Supplementary video S1). The animals did not exhibit vigorous swimming with the house during feeding cycles, as was reported for *Fritillaria pellucida* (Busch, 1851) (Bone *et al*., 1979) and *F. borealis* (Flood, 2003). The house renewal rate was estimated as 28 ± 2.8 houses d^{-1} at 23°C ($n = 6$).

Discussion

Tokioka (1956) compared the taxonomic characters of ten forms of *Fritillaria* and proposed that they should be placed into sibling species forming a *Fritillaria haplostoma*-complex. *Fritillaria* sp. resembles *F. haplostoma*, especially in the trunk shape of small specimens from the lagoon waters of the Palao Islands (Tokioka, 1955). The testis of *Fritillaria* sp. was roundish (Figure 2B, E), differing from the elongated cylindrical testis of *F. haplostoma*, although that of large individuals of the latter caught in the oceanic waters of the Palao Islands by Tokioka (1955) showed roundish objects resembling the testis in the posterior part of the trunk. The taxonomy of a specific group of the genus *Fritillaria* is unclear; thus, further studies, including taxonomical scrutiny of *Fritillaria* sp., *F. haplostoma,* and related species, are needed. Considering these facts, the laboratory cultivation of various forms of fritillarids will be beneficial to taxonomical differentiation by providing specific ecological and physiological information and materials for the genetic analysis of each species.

Cultivation device

The cultivation device with the rotating cylinder worked effectively during the rearing of this appendicularian species as it did not damage or distort the extremely delicate houses or induce animals to discard their houses. Because the culture vessel itself remained stationary, the device could be set up in an incubator or shipboard laboratory and the design could be applied to larger culture vessels, as needed. The large culture vessels will help to reduce the wall effect and contact with the water–air surface, to maintain a larger number of animals or species with a larger body or house, and to prevent rapid depletion of food with an adequate animal concentration in the vessel. We also cultivated other planktonic animals (R. Sato, pers. obs.). For example, the fritillarid species *F. pellucida* was cultivated through three filial generations, the doliolid *Dolioletta gegenbauri* (Uljanin, 1884) was cultivated through two full life cycles with alternation of asexual and sexual generations, and a thecosome pteropod species (probably genus *Creseis*) was maintained for one week up to spawning, exhibiting normal floating in the water by spreading its feeding net. These results indicate that this device could be effective for cultivating various zooplankton.

The rotating paddle method performed effectively in the cultivation of *F. borealis typica* (Henriet *et al*., 2022). This species produces a compact house relative to the animal's body size and swims with the deflated house after pumping water into the food-concentrating filter during its feeding cycle (Flood, 2003). This indicates that the house of this form may be durable against any thrusting movement of the water caused by the rotating

https://doi.org/10.1017/S0025315423000541 Published online by Cambridge University Press

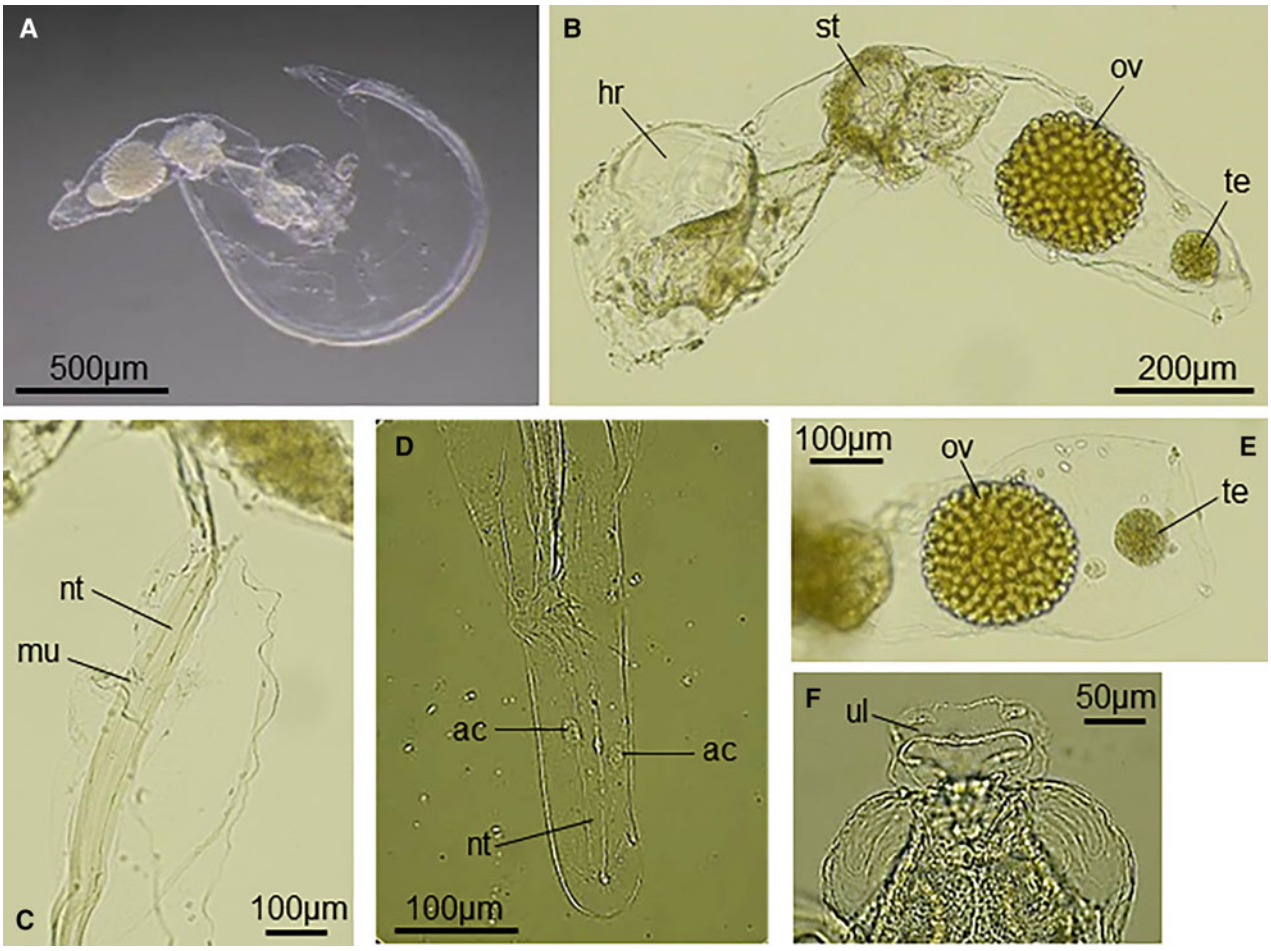

Figure 2. *Fritillaria* sp.: (A) right-side view of the whole animal; (B) left-side view of the trunk with the tail detached; (C) proximal portion of the tail showing narrow musculature. The tail fin was partially torn; (D) distal part of the tail showing the paired amphichordal cells on each side of the notochord tip; (E) dorsal view of the posterior trunk; (F) dorsal view of the mouth. hr, house rudiment; st, stomach; ov, ovary; te, testis; nt, notochord; mu, musculature; ac, amphichordal cell; ul, upper lip.

blade, resulting in the successful culture of the species with this device. It is not clear whether the continuous cultivation of *Fritillaria* sp. with its extremely fragile mucoid house is possible using other techniques such as the rotating paddle method or those mentioned in the introduction. However, the rotating cylinder device used in this study is perfectly suitable for cultivating this fragile fritillarid continuously. The abundance of different cultivation methods and devices will benefit the development of aquatic biology since the successful cultivation of a particular species is crucial to understanding the function of that species in the ecosystem, as this is difficult to observe directly in the natural environment (Paffenhöfer and Harris, 1979).

Growth and house of *Fritillaria* sp

The generation time of *Fritillaria* sp. was only several days, which is consistent with those of other appendicularian species such as *O. dioica* (Fenaux, 1976; Paffenhöfer, 1976) or some oceanic

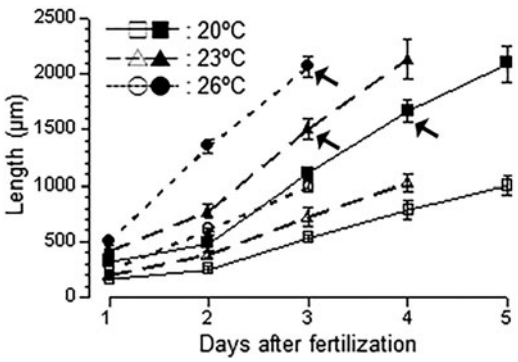

Figure 3. Changes in trunk length (open symbols) and tail length (solid symbols) of *Fritillaria* sp. over time after fertilization at 20°C (squares and solid lines), 23°C (triangles and dashed lines), and 26°C (circles and dotted lines). Trunk lengths include calculated values from tail length (see text and Figure 4) after mature individuals were first observed (indicated by arrows). Each point represents mean ± standard deviation.

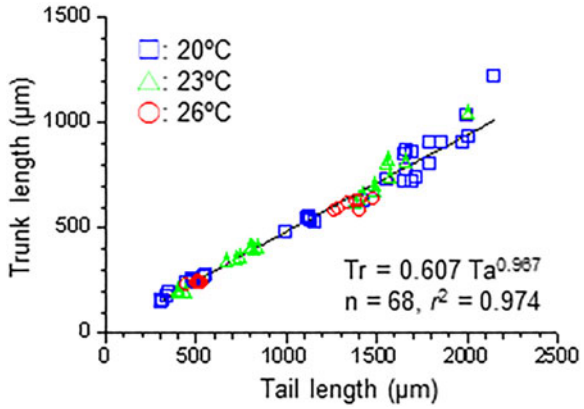

Figure 4. Relationships between tail length (Ta) and trunk length (Tr) of *Fritillaria* sp. at 20°C (blue squares, $n = 33$), 23°C (green triangles, $n = 22$), and 26°C (red circles, $n = 13$). For regression, all data were combined and fitted to one power function, as shown in the graph. The line denotes the regression line for all data.

https://doi.org/10.1017/S0025315423000541 Published online by Cambridge University Press

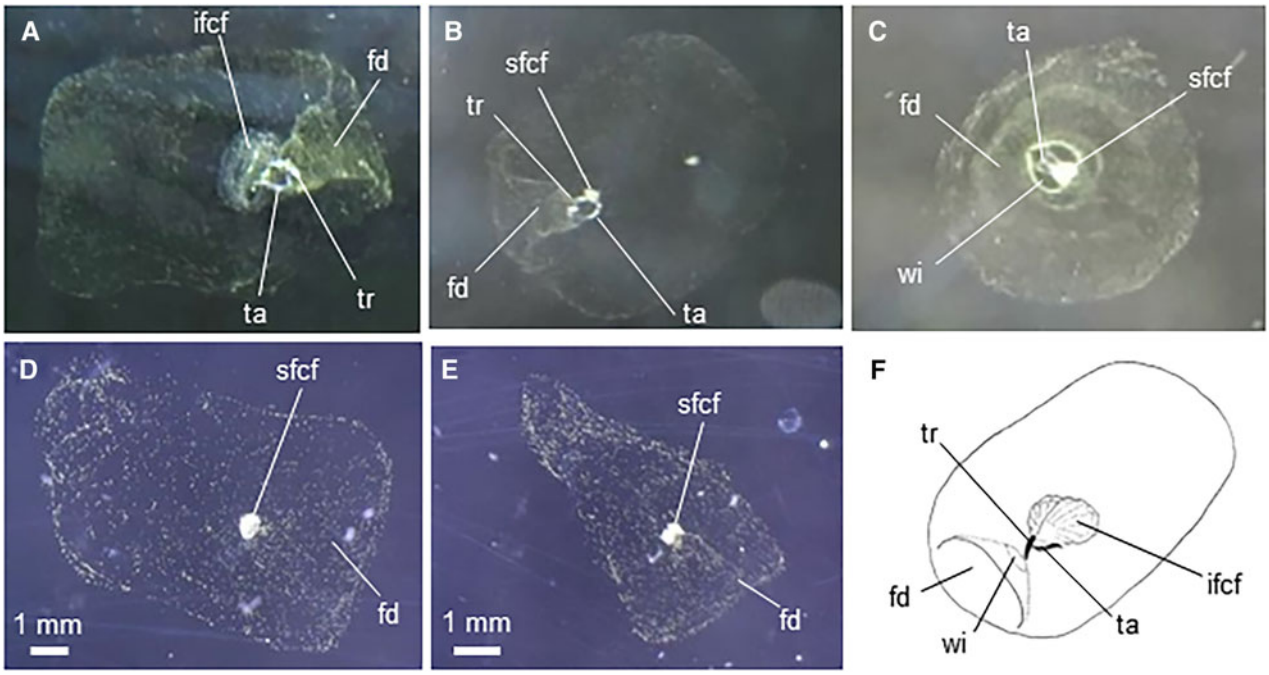

Figure 5. House of *Fritillaria* sp.: (A) video frame of an occupied house with the animal undulating its tail, left-side view; (B) video frame of an occupied house with the animal resting its tail, right-side view; (C) video frame of an occupied house, view from the water-inlet side; (D, E) photographs of houses just after being abandoned; (F) schematic of an occupied house with the animal inflating food-concentrating filter. tr, trunk; ta, tail; ifcf, inflated food-concentrating filter; sfcf, shrunken food-concentrating filter; fd, funnel-like dent; wi, water inlet.

warm-water forms (Sato *et al.*, 1999). A short generation time of 6–7 days was reported even for the cold-water species *F. borealis typica* at a low temperature of 12°C (Henriet *et al.*, 2022). The instantaneous growth rates calculated here were also within the range of those obtained for fritillarid species incubated in microcosms at 28°C *in situ* (Hopcroft *et al.*, 1998). These appendicularian growth rates are higher than those of copepods (Huntley and Lopez, 1992). Due to the short life cycle and high growth rate, appendicularian somatic productivity can sometimes be comparable with or higher than that of dominant crustacean zooplankton (Nakamura, 1998; Tomita *et al.*, 1999). Therefore, *Fritillaria* sp. could play the role of a significant secondary producer, along with other appendicularian species.

According to Flood (2003), the compact house of *F. borealis* does not fully enclose the animal. By contrast, the *Fritillaria* sp. house reached lengths nearly ten times longer than the animal's trunk, and its outer membrane completely covered the animal. These morphological differences between their houses may reflect each species' behaviour and functional response. Contractility of the entire compact house of *F. borealis* without a non-deformable outer membrane may enable the animal to swim with the deflated house during its feeding cycle. Contrarily, *Fritillaria* sp. did not exhibit vigorous swimming with the house during feeding cycles as the particularly fragile large house could be damaged by such behaviour. Images of the house of *Fritillaria rex* Hopcroft and Robison, 2005, a large mesopelagic species, were recorded using a remotely operated vehicle and show the structure enveloping the animal and the food-concentrating filter, expressed as 'a diffuse outer sphere' (Hopcroft and Robison, 2005). This structure seems to correspond to the outer membrane of the *Fritillaria* sp. house. The house of *F. borealis* does not have the diffuse outer structure around the animal and the food-concentrating filter (Flood, 2003). Thus, such a structure may be characteristic of the house of a distinct group of fritillarid forms. A single water inlet observed in the house of *Fritillaria* sp., which is different from the house of oikopleurid species with paired openings, was also described in *F. borealis* (Flood, 2003), but it is not obvious in the *in situ* images of *F. rex* (Hopcroft and Robison, 2005). This conformity suggests that the single water inlet may be one of the characteristics of the *Fritillaria* house. However, we need to examine other fritillarid species since Flood (2003) proposed that the general house morphology of *F. borealis* belonging to the subgenus *Eurycercus* should not be applied to the entire genus, including the other subgenus *Acrocercus*, which is characterized by a distal part of the tail ending in a simple process which is not notched (Fenaux, 1993; Shiga, 1997) like that of *Fritillaria* sp. (Figure 2D).

The house renewal rate of *Fritillaria* sp. is one of the highest reported (Flood and Deibel, 1998; Sato *et al.*, 2003). Sato *et al.* (2003) suggested that those species producing houses with lower carbon content have higher house renewal rates. This indicates that the house of *Fritillaria* sp. may contain a lower amount of carbon, which may account for the severe fragility of the house. The larger size of the house relative to the animal could also contribute to its extreme fragility due to the reduced density of the materials per unit volume of the house. Despite the possible low carbon content of the house, this form may significantly contribute to the cycling and transport of organic matter in the marine environment as an active producer of macroscopic aggregates owing to the high house renewal rate.

In general, many fritillarid appendicularians have smaller bodies than the oikopleurids, except for large mesopelagic species (Hopcroft and Robison, 2005). The small size of the fritillarids may mean that they have a lower contribution to and impact on marine ecosystems when compared with oikopleurids. Additionally, the former has received less research attention, and there is a shortage of ecological and physiological information relating to them. The results of this study emphasize that the fritillarid appendicularians may be a more important producer and transporter of organic matter in marine ecosystems than what is currently proposed due to their high growth and house production rates.

https://doi.org/10.1017/S0025315423000541 Published online by Cambridge University Press

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315423000541.

Data. Raw data are available from the author upon reasonable request.

Acknowledgements. I thank the staff of Misaki Marine Biological Station, School of Science, the University of Tokyo, for their co-operation. I am grateful to Dr K. Hidaka, Japan Fisheries Research and Education Agency, for critically reading the manuscript.

Author's contributions. The author performed the entirety of this research.

Financial support. This work was supported by the Research Institute of Marine Invertebrates.

Competing interest. None.

References

Bone Q, Gorski G and Pulsford AL (1979) On the structure and behaviour of *Fritillaria* (Tunicata: Larvacea). *Journal of the Marine Biological Association of the United Kingdom* **59**, 399–411.

Bouquet JM, Spriet E, Troedsson C, Otterå H, Chourrout D and Thompson EM (2009) Culture optimization for the emergent zooplanktonic model organism *Oikopleura dioica*. *Journal of Plankton Research* **31**, 359–370.

Bückmann A and Kapp H (1975) Taxonomic characters used for the distinction of species of Appendicularia. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* **72**, 201–247.

Chioda M, Eskeland R and Thompson EM (2002) Histone gene complement, variant expression, and mRNA processing in a Urochordate *Oikopleura dioica* that undergoes extensive polyploidization. *Molecular Biology and Evolution* **19**, 2247–2260.

Fenaux R (1976) Cycle vital d'un appendiculaire *Oikopleura dioica* Fol, 1872, Description et chronologie. *Annales de l'Institut Océanographique, Paris* **52**, 89–101.

Fenaux R (1993) The classification of Appendicularia (Tunicata): history and current state. *Mémoires de l'Institut Océanographique, Monaco* **17**, 1–123.

Fenaux R and Gorsky G (1985) Nouvelle technique d'elevage des appendiculaires. *Rapports et Procès-verbaux des Réunions – Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* **29**, 291–292.

Flood PR (2003) House formation and feeding behaviour of *Fritillaria borealis* (Appendicularia: Tunicata). *Marine Biology* **143**, 467–475.

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

Galt CP (1987) Phylum Chordata, subphylum Urochordata, class Larvacea (Appendicularia). In Strathmann MF (ed.), *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. Seattle, WA: University of Washington Press, pp. 640–646.

Gorsky G, Fenaux R and Palazzoli I (1986) Une methode de maintien en suspension des organismes zooplanctoniques fragiles. *Rapports et Procès-verbaux des Réunions - Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* **30.2.204**, 21–22.

Henriet S, Aasjord A and Chourrout D (2022) Laboratory study of *Fritillaria* lifecycle reveals key morphogenetic events leading to genus-specific anatomy. *Frontiers in Zoology* **19**, 26.

Hopcroft RR and Robison BH (2005) New mesopelagic larvaceans in the genus *Fritillaria* from Monterey Bay, California. *Journal of the Marine Biological Association of the United Kingdom* **85**, 665–678.

Hopcroft RR, Roff JC and Bouman HA (1998) Zooplankton growth rates: the larvaceans *Appendicularia*, *Fritillaria* and *Oikopleura* in tropical waters. *Journal of Plankton Research* **20**, 539–555.

Huntley ME and Lopez MD (1992) Temperature-dependent production of marine copepods: a global synthesis. *The American Naturalist* **140**, 201–242.

Martí-Solans J, Ferrández-Roldán A, Godoy-Marín H, Badia-Ramentol J, Torres-Aguila NP, Rodríguez-Marí A, Bouquet JM, Chourrout D, Thompson EM, Albalat R and Cañestro C (2015) *Oikopleura dioica* culturing made easy: a low-cost facility for an emerging animal model in EvoDevo. *Genesis (New York, N.Y.: 2000)* **53**, 183–193.

Masunaga A, Liu AW, Tan Y, Scott A and Luscombe NM (2020) Streamlined sampling and cultivation of the pelagic cosmopolitan larvacean, *Oikopleura dioica*. *Journal of Visualized Experiments* **160**, e61279.

Nakamura Y (1998) Blooms of tunicates *Oikopleura* spp. and *Dolioletta gegenbauri* in the Seto Inland Sea, Japan, during summer. *Hydrobiologia* **385**, 183–192.

Nishida H (2008) Development of the appendicularian *Oikopleura dioica*: culture, genome, and cell lineages. *Development, Growth & Differentiation* **50**, S239–S256.

Paffenhöfer GA (1970) Cultivation of *Calanus helgolandicus* under controlled conditions. *Helgoländer wissenschaftliche Meeresuntersuchungen* **20**, 346–359.

Paffenhöfer GA (1973) The cultivation of an appendicularian through numerous generations. *Marine Biology* **22**, 183–185.

Paffenhöfer GA (1976) On the biology of Appendicularia of the southern North Sea. In Persoone G and Jaspers E (eds), *Proceedings of the 10th European Symposium on Marine Biology, Ostend, Belgium*, 17–23 September 1975. Wetteren: Universa Press, Vol. **2**, pp. 437–455.

Paffenhöfer GA and Harris RP (1979) Laboratory culture of marine holozooplankton and its contribution to studies of marine planktonic food webs. *Advances in Marine Biology* **16**, 211–308.

Patry WL, Bubel M, Hansen C and Knowles T (2020) Diffusion tubes: a method for the mass culture of ctenophores and other pelagic marine invertebrates. *PeerJ* **8**, e8938.

Presta ML, Hoffmeyer MS and Capitanio FL (2015) Population structure and maturity stages of *Fritillaria borealis* (Appendicularia, Tunicata): seasonal cycle in Ushuaia Bay (Beagle Channel). *Brazilian Journal of Oceanography* **63**, 279–288.

Sato R (2000) *Ecological study of appendicularians* (PhD thesis). Tokyo University of Fisheries, Tokyo, Japan. (in Japanese).

Sato R, Tanaka Y and Ishimaru T (2003) Species-specific house productivity of appendicularians. *Marine Ecology Progress Series* **259**, 163–172.

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

Shiga N (1976) Maturity stages and relative growth of *Oikopleura labradoriensis* LOHMANN (Tunicata, Appendicularia). *Bulletin of the Plankton Society of Japan* **23**, 81–95.

Shiga N (1985) Seasonal and vertical distributions of Appendicularia in Volcano Bay, Hokkaido, Japan. *Bulletin of Marine Science* **37**, 425–439.

Shiga N (1997) Class appendiculata. In Chihara M and Murano M (eds), *An Illustrated Guide to Marine Plankton in Japan*. Tokyo: Tokai University Press, pp. 1393–1414. (in Japanese).

Tokioka T (1955) General consideration on Japanese appendicularian fauna. *Publications of the Seto Marine Biological Laboratory* **4**, 251–261.

Tokioka T (1956) *Fritillaria arafoera* n. sp., a form of sibling species: *Fritillaria haplostoma*-complex (Appendicularia: Chordata). *Pacific Science* **10**, 403–406.

Tomita M, Ikeda T and Shiga N (1999) Production of *Oikopleura longicauda* (Tunicata: Appendicularia) in Toyama Bay, southern Japan Sea. *Journal of Plankton Research* **21**, 2421–2430.