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# Feeding habits of stone flounder *Platichthys bicoloratus* larvae in Mutsu Bay, Japan

Feeding habits of stone flounder larvae

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**Abstract:** To clarify the feeding strategy of pelagic larvae of the stone flounder in Mutsu Bay, the dietary composition and prey size was investigated from February to April during 1989–1999. Diets were compared with the numerical and volumetric compositions and frequency of occurrence of each prey species. Mensuration formulae were applied to estimate individual prey volume in the diet, while the length of planktonic species was measured from net and water samples. Prey shapes were assumed as sphere, cylinder, ellipsoid, pyramid, two elliptical cones, or a combination of ellipsoid and cylinder. Prey-size range increased as the larvae grew. Pre-flexion larvae fed mainly on copepod nauplii. Flexion and post-flexion larvae ingested primarily appendicularians, with a suggestion that these larvae might depend on some parts of the microbial food web. Low frequencies of flexion and post-flexion larvae with empty guts (1.7 and 1.4%, respectively) might be derived from feeding on slow-swimming appendicularians. From a size comparison between “house”-like organ length and trunk length of the appendicularian *Oikopleura* sp., almost all house-like organs with trunks in the larval diet were non-expanded “house rudiments”, not expanded “houses”. Thus, stone flounder larvae may not chew the houses, but swallow the house rudiments with trunks.

**Key words:** appendicularia, copepod, flounder larva, Mutsu Bay, nauplius, *Oikopleura*, *Platichthys bicoloratus*, prey.

(Abstract: 200 words, Total: 5,753 words)

## INTRODUCTION

The stone flounder *Platichthys bicoloratus* is distributed in the coastal waters of temperate and subarctic zones in East Asia and is an important commercial fish species in Japan. Extensive studies on the early life history of this species have been reported, including egg development,<sup>1,2</sup> morphology of larvae and juveniles,<sup>1-4</sup> feeding habits,<sup>4-6</sup> starvation tolerance of larvae,<sup>7</sup> growth,<sup>5,8,9</sup> nursery areas,<sup>5,10,11</sup> tagging experiment of juveniles,<sup>12</sup> occurrence, transport and migration of larvae and juveniles<sup>13-17</sup> and predation by sand shrimp.<sup>18</sup> In Mutsu Bay, the peak-spawning period of stone flounder is December–January<sup>19</sup> and, subsequently, pelagic larvae occur from mid-December to late April (Takatsu T *et al.*, unpubl. data, 2006). The feeding habits of pelagic larvae are unknown, with the exception of a study in Wakasa Bay,<sup>4</sup> and geographical differences in larval diet are unclear. Flatfish larvae fundamentally change their morphology, with eye-migration, from late larval to juvenile stages. Empty-gut larvae frequently occur in these stages<sup>4,20</sup> and larvae may have high mortality rates during these critical stages.<sup>21</sup> To reveal the cause of stock fluctuations, a better understanding is needed of the feeding strategy during the metamorphosis stages. We have investigated the diet of pelagic larvae of the stone flounder, particularly during the flexion and post-flexion stages, in Mutsu Bay.

## MATERIALS AND METHODS

Between February and April in 1989–1999, pelagic larvae of stone flounder were collected in Mutsu Bay and its bay mouth (Fig. 1) from aboard the T/S *Ushio-maru* of Hokkaido University Faculty of Fisheries (128 tons) and a fishing boat (4.9 tons) (Table 1) as part of the fish larvae monitoring program. Larvae were collected by horizontal hauls with a ring net (0.8 m diameter and 0.33 mm mesh aperture), a larva net (1.3 m diameter, 0.62 mm mesh and 0.33 mm cod-end

mesh) and a non-closing beam-trawl net (2.0 m height  $\times$  2.5 m width square mouth and 13–3.1–0.33 mm mesh in 1989 and 13–3.1–0.72 mm in 1991, 1992 and 1998<sup>22</sup>). The ring net and the larva net were towed for 10 min at a speed of 1.0 m/s at 0, 5, 8, 10 and 20 m depths in March 1989 and 1999 (Table 1). The beam-trawl net was towed at a speed of 1.5 m/s for 20–22 min for oblique hauls or for 10–15 min for horizontal hauls at the target depths (12–51 m depth range). Towing manners of the beam-trawl net are detailed in Takatsu *et al.*<sup>23</sup> All sampling was performed in the daytime (08:41–16:24 hours). On board, fish larvae were preserved in a 5% buffered formalin-seawater solution in 1999, or a 5–10% buffered formalin-seawater solution for 24–36 h and, then, transferred to a 70% ethanol solution prior to 1999. Water temperature was measured with a VARIOSENS III (Impulsphysik) in 1989, a DBT (Met-Ocean) in 1991 and CTDs (Neil Brown in 1992 and Seabird in 1998 and 1999) at each sampling station.

In the laboratory, stone flounder larvae were counted and classified into four developmental stages; (i) yolk-sac, (ii) pre-flexion, (iii) flexion and (iv) post-flexion, corresponding to stages A-B, C-D, E-F and G-H, as defined by Minami.<sup>4</sup> Pre-flexion larvae were subdivided into stages C and D according to presence of a cartilaginous hypural bone. Eye-migration begins at late stage F. Body length (BL), upper jaw length (UJL) and mouth width (MW) of larvae were measured to the nearest 0.1 mm with an electric slide-caliper (Fig. 2). In this study, BL is the Fig.2 general term for notochord length (NL; snout tip to notochord tip in pre-flexion and flexion larvae) and standard length (SL; snout tip to the posterior edge of the hypurals in post-flexion larvae). Mouth depth (MD) was hypothesized as  $MD = \sqrt{2} \cdot UJL$ .<sup>24</sup> The scientific name of the stone flounder in this study conformed to Cooper and Chapleau.<sup>25</sup>

Digestive tracts, from the oesophagus to the anus, were removed and the contents were identified to the lowest practical taxa and counted. The contents were stained with methylene-blue solution (2 mg/100 mL of the environmental concentration) for detecting appendicularians (*Oikopleura* and *Fritillaria*). “House rudiments” of *Oikopleura* spp. and

“house rudiment-like organs” of *Fritillaria* spp. stained purple and blue-violet, respectively [Fig.3](#) (Fig. 3). *Oikopleura* and *Fritillaria* build and live in a complex mucoid balloon called a ‘house’, which is equipped with incurrent feeding filters.<sup>26-28</sup> As houses have fragile structures, they are not found in plankton net samples. The ‘house rudiment’ of *Oikopleura* is the non-expanded and initially secreted house, i.e. the animal discards the house and can expand the house rudiment over a short period of time.<sup>29</sup> *Fritillaria* spp. also live in houses and initially secrete ‘house rudiments’ that are attached on the trunk;<sup>27,28</sup> however, the morphology and productive process of ‘house rudiments’ is not clearly understood. Thus, the blue-violet organs attached to the trunk near the mouth of *Fritillaria* spp. (Fig. 3) are called “house rudiment-like organs” in this study. Tail morphology of both *Oikopleura* and *Fritillaria* spp. is important for identification to species (e.g. the distribution pattern of subchordal cells and structure of muscles; Fig. 2).<sup>30</sup> Nevertheless, tails are rarely found in the diet of stone flounder larvae (see Results), therefore, we identified appendicularians at the genus level, as either *Oikopleura* sp. or *Fritillaria* sp.

Measurements for prey-size comparison and volume estimation were performed for each prey item in the diet using a binocular microscope with attached micrometer. Dimensions of all prey organisms were exclusive of appendages (Fig. 2). The act of being swallowed by a predator is not restricted by the largest prey length (three-dimensional length, e.g.  $L_p$  in copepods; Fig. 2), but usually by the second-largest length (SLL).<sup>31</sup> Therefore, SLLs shown in Figure 2 were used to compare prey sizes. Trunk depth ( $T_d$ ) for *Oikopleura* and trunk width ( $T_w$ ) for *Fritillaria* were treated as SLLs because these prey items were usually found in the larval digestive tracts as trunks with house rudiments or house rudiment-like organs, but without tails (see Results).

Volumes were used for dietary analysis, instead of prey weights, to avoid under- or over-estimation from differences in digestion speed or weight loss during fixation of prey organisms. Volumes of prey items were calculated from the geometric formulae in [Table 2](#) [Table 2](#) (modified Nishiyama and Hirano<sup>32</sup>). Each  $m'$  (species-specific ratio of prosome volume to the

whole body volume) for copepodites<sup>32</sup> was estimated from the mean of 20 individuals in zooplankton samples collected either with a plankton net (45 cm diameter and 0.33 mm mesh aperture) or in a Van Dorn bottle through a 40- $\mu$ m mesh sieve (Table 2). If an organism was flattened laterally or dorsoventrally, or collapsed in the diet, displacement lengths were calculated from linear regression formulae between prey lengths obtained from zooplankton samples (Table 3 and Fig. 2). Trunks of *Oikopleura* sp. in the diet were frequently collapsed because of digestion, but the house rudiment was less-digestible as enzymes to digest acidic polysaccharides are absent for fish larvae and most marine zooplankton<sup>27,33,34</sup>. In addition, viscera, composed of oesophagus, stomach, intestine and ovary, in the trunk of *Fritillaria* sp., are also less-digestible (see Results). Thus, trunk sizes of appendicularians were reconstructed from house rudiment length in *Oikopleura dioica* and stomach diameter in *Fritillaria borealis*. *O. dioica* and *F. borealis* were the most abundant species in Mutsu Bay during February–April (Takatsu T *et al.*, unpubl. data, 2006). Tail volumes of appendicularians were excluded to avoid over-estimation. If a particle was identifiable but immeasurable because of collapse or digestion (70 of a total of 2,034 prey items), mean prey lengths of the same prey type in the same digestive tracts were used for volume estimation. In the diet, unidentified prey organisms (4 unidentifiable copepod nauplii, 1 unidentifiable copepod copepodites, and 23 unidentifiable prey particles) and scarce prey (2 tintinnid *Ptychocylis* sp. and 1 *Oncaea* sp. copepodite) were ignored for volume estimation.

Table 3

Data on digestive tract content, according to the developmental stage of stone flounder larvae, were expressed as percentage occurrence frequency (F%: the percentage of larvae that consumed a particular type of prey) and number and volume percent (N% and V%: the percentage of each prey type to the total number and volume of prey items, respectively). Numbers of uncountable prey particles, caused by digestion, were excluded from estimation for N%; however, their occurrence was included in F%.

Diet overlap in N% between sampling areas is shown by a percentage similarity index (*PS*: same as the Bray–Curtis overlap index<sup>35</sup>) calculated in equation 1:

$$PS(\%)=100\{1-0.5\sum|(n_{iA}/\sum n_A)-(n_{iB}/\sum n_B)|\}$$

where  $n_{iA}$  and  $n_{iB}$  are number of individuals of prey item  $i$  in the diet of area  $A$  and  $B$ , respectively.

Because the gut fullness volume increased with larval growth, feeding intensities between sampling periods were compared by relative gut fullness (GF%). GF% was calculated per individual larva as the percentage of the prey volume to the saturation volume. Saturation volumes were substituted for larval BL in the regression formulae, which were estimated from the maximum volume of every 10 larvae in both flexion and post-flexion stages.

Mann–Whitney  $U$ -tests and Kruskal–Wallis tests were used to non-parametrically compare median values between two samples and three-or-more samples, respectively. Shirley–Williams tests and Steel–Dwass tests for non-parametric multiple comparisons were used between two adjacent ordinal three-or-more samples and between all pairs of less-ordinal three-or-more samples, respectively.<sup>36</sup> The difference in N% values between sampling areas was compared using the  $G$ -test.<sup>37</sup> Spearman's rank correlation coefficient ( $r_s$ ) was used to compare the relationship between GF% and environmental water temperature at depths where the larvae were collected by horizontal net tow. The relations between larval BL and MW, and BL and prey volume, were compared between larval developmental stages, using analysis of covariance (ANCOVA). Significance levels were set at 0.05.

## **RESULTS**

### **Relationship between larval body length and mouth width**



Mouth widths (MW) of stone flounder larvae were smaller than mouth depths (MD = 1.59 × mean MW). Because prey-capture of fish is restricted by the narrower length of mouth sizes (i.e. MW) and the ability of larvae to capture prey is closely correlated with MW,<sup>38</sup> the relationship between body length (BL) and MW is shown in Figure 4. MW increased with increasing BL and regression lines between BL and MW were not parallel between pre-flexion, flexion or post-flexion stages (ANCOVA:  $P=0.008$ ). The slope for flexion larvae with growth stagnation for notochord flexion (0.079) was greater than either pre-flexion or post-flexion larvae (0.065 and 0.048, respectively).

Fig.4

### Diet of pelagic stone flounder larvae

Table 4 shows the diet composition of stone flounder larvae by development stage in the daytime, regardless of sampling period. Larvae with empty digestive tracts were scarce in flexion and post-flexion larvae (1.7 and 1.4%, respectively). Pre-flexion larvae, at the first feeding stage (stage C), fed mainly on copepod nauplii (F%=50, N%=50, V%=92). Rotiferan *Trichocerca* sp. and crustacean eggs were found in diets. Pre-flexion larvae at stage D fed mainly on copepod nauplii (F%=77, N%=54, V%=55.6) and appendicularians (F%=46, N%=20, V%=39.7), but fed on a variety of food items. *Centropages* nauplii, *Pseudocalanus* nauplii and *Oikopleura* sp. occupied a relatively higher V% (35.6%, 10.5% and 35.7%, respectively). Flexion larvae (stages E and F) and post-flexion larvae (stages G and H) fed mainly on appendicularians, especially *Oikopleura* sp. (flexion: F%=88, N%=60.5, V%=91.9; post-flexion: F%=96, N%=81.9, V%=94.3). Although bivalve larvae, cladocerans and copepodites occurred in the diet in stage D and later stages, they were rarely found (F%≤17).

Table 4

Diet composition of flexion and post-flexion larvae were compared between sampling

periods (Fig. 5). In five of six sampling periods, *Oikopleura* sp. were predominant in N% and V% values (44–100 and 67–100%); however, *Fritillaria* sp. showed higher N% and V% for flexion larvae (46 and 32%) and N% of post-flexion larvae (42%) in March 1999 than other periods. Bivalve larvae had relatively high N% and V% values (29 and 28%) in post-flexion larvae in March 1998. Cladoceran *Evadne nordmanni* showed relatively high N% and V% (28 and 10%) in post-flexion larvae in April 1991.

Fig.5

Diet overlap in N% between sampling areas and N% values of major prey items by area are shown in Table 5. Data from flexion and post-flexion larvae, collected in March 1989, April 1991, February 1992 and March 1998, were used because relatively larger numbers of larvae were obtained (42–97 individuals). There were significant differences in N% between areas (*G*-test;  $P=0.002$  in March 1989 and  $P<0.001$  in the other three sampling periods). Similarity indices between areas showed 55–94%. N% values of *Oikopleura* sp. were larger (42–98 %) than other prey items throughout the area in these four periods.

Table 5

House rudiments attached to the trunks of *Oikopleura* sp. and house rudiment-like organs attached to the trunks of *Fritillaria* sp. were stained purple and blue-violet, respectively (Fig. 3). They were clearly detectable from other prey particles stained blue in the diet. In a few cases, an inflated house with trunk was found in the diet; however, there was no case of house rudiment length ( $Hrl>Tl$ ) in the diet (Fig. 2). House rudiments with trunks and whole tails accounted for 1.4%, with trunks and torn-off tails 1.6% and with trunks 77% of the total number of individuals of *Oikopleura* sp. in the diet (Table 6). The remaining 20% were only house rudiments, without trunks or tails. Thirteen of 20 individuals of *Oikopleura* sp. with trunks and whole tails were found in the anterior part of the digestive tract (foregut). A trunk of *Fritillaria* sp. with a viscus, house rudiment-like organ and torn-off tail occurred in the diet of one stone flounder larva collected in March 1998 (0.8%; Table 6). Viscera with house rudiment-like organs, but without trunk epithelium or tail, were frequently found and occupied 78% of the total number of

Table 6

individuals of *Fritillaria* sp. in the diet.

### Prey size distribution in diet

Median and ranges of SLLs were 110 and 44–220  $\mu\text{m}$ , 170 and 96–410  $\mu\text{m}$ , and 320 and 59–1200  $\mu\text{m}$  for copepod nauplii, copepodites, and *Oikopleura* sp., respectively (Fig. 6). Median SLLs of *Oikopleura* sp., *E. nordmanni* and bivalve larvae in digestive tracts increased with the developmental stage of stone flounder larvae.

Fig.6

Median ratio of SLL to MW (SLL/MW) and the 10–90 percentile range of the ratios for all prey items were 32 and 12–48%, respectively. Median SLL/MW for *Oikopleura* sp. (45%) was significantly larger than for copepod nauplii (39%) or copepodites (36%; Steel–Dwass test, both  $P < 0.001$ , but  $P > 0.20$  for nauplii vs. copepodites).

### Gut fullness

Total prey volume in the larval diet increased as the larvae grew, especially in the pre-flexion stage (Fig. 7). Because two regression lines of flexion and post-flexion larvae, between BL and maximum prey volume from every 10 larvae, were not significantly different (ANCOVA: slope:  $P = 0.95$ , intercept:  $P = 0.64$ ), a combined regression line for flexion and post-flexion larvae was used to estimate relative gut fullness (GF%). No significant correlation was found between GF% of individual larvae and environmental water temperature where the flexion and post-flexion larvae were collected ( $n = 211$ ,  $P = 0.25$ ; minimum median maximum temperatures 4.2, 5.6, and 8.2  $^{\circ}\text{C}$ ). Median GF% differed significantly between sampling periods (Kruskal–Wallis test,  $P < 0.001$ ). By the Steel–Dwass test, GF% values in March 1998 and 1999 (21 and 22%, respectively) were significantly lower than in March 1989, April 1991 and February 1992 (51,

Fig.7

Table 7

52 and 44%, respectively;  $0.05 \geq P > 0.01$  or  $P < 0.001$ ; Table 7). In February 1991, median GF%, based on 15 individual flexion and post-flexion larvae, showed relatively higher values (57%); however, it did not differ significantly from others.

## DISCUSSION

“House rudiments” of *Oikopleura* spp., “house rudiment-like organs” of *Fritillaria* spp. and other zooplankton are frequently transparent in the digestive tract contents of fish larvae and plankton net samples. However, in this study, they were distinguishable by color after the addition of methylene blue solution (Fig. 3). With appendicularians, the addition of methylene blue solution prevents under-counting of samples.

In Mutsu Bay, the range of prey size for stone flounder larvae expanded as the larvae grew (Fig. 6). Pre-flexion larvae (stages C and D) fed mainly on copepod nauplii, while flexion and post-flexion larvae fed mainly on appendicularians (Table 4). Although there were geographical differences in diet composition in flexion and post-flexion stages, diet overlaps were relatively high (55–94 %) and appendicularians were largely dominant (Fig. 5 and Table 5). In Wakasa Bay (Fig. 1), pre-flexion larvae of stone flounder also fed on copepod nauplii (68–81 % in F%).<sup>4</sup> Copepodites (mainly *Centropages* sp.) occurred most frequently in the diet of flexion and post-flexion larvae in Wakasa Bay (35–78 % in F%), however, *Oikopleura* spp. did not (1.1–12.5 %).<sup>4</sup> Thus, there was a geographical difference in diet between these two bays. The ecological role of appendicularians in food webs has been discussed previously,<sup>42,46–48</sup> and larvae of flatfish frequently feed on appendicularians.<sup>20,39–45</sup> Appendicularians feed efficiently within complex mucus filter “houses” on a wide size spectrum of naturally occurring particles (about  $<0.2 \mu\text{m}$  to  $100 \mu\text{m}$ ), including relatively large particles (e.g., ciliates, dinoflagellates and diatoms) but also dissolved or small sized particulate organic matter (e.g., submicron colloids,

prochlorophytes, cyanobacteria, bacterioplankton and nanoflagellates).<sup>27,46,47</sup> Relatively large particles are also consumed by other herbivores including some copepods (grazing food chain); however, dissolved or small sized particulate organic matter is not preyed.<sup>46,47</sup> Appendicularians short-circuit the microbial food web and transfer energy from very small particles to larval fish.<sup>42,46–48</sup> Thus, stone flounder larvae in Mutsu Bay depend on some parts of the microbial food web through the feeding on appendicularians. Larvae with empty digestive tracts occurred frequently in Wakasa Bay (stages E,F,G, and H at 33, 20, 7.7, and 33%, respectively),<sup>4</sup> but rarely in Mutsu Bay (flexion: 1.7%; post-flexion: 1.4%). The low frequencies of empty-gut individuals in Mutsu Bay, compared to Wakasa Bay, are possibly the results of feeding success on a higher abundance of appendicularians. However, there is no comparable data on zooplankton abundance in these two bays, thus, we cannot discuss the difference in prey selectivity.

In March 1998 and 1999, *Fritillaria* sp. were relatively abundant in the diet composition of stone flounder larvae compared to *Oikopleura* sp. (Fig. 5). In addition, relative gut fullness was lower in March 1998 and 1999 than other periods in 1989–1991 (Table 7). These results may reflect the size difference between *Oikopleura* sp. and *Fritillaria* sp. (Fig. 6). Moreover, *Oikopleura* sp. showed a broad size range and accounted for a majority of the diet during the broad metamorphosis stages, i.e. in flexion and post-flexion stages. Accordingly, *Oikopleura* sp. may play an important food source for stone flounder larvae.

Although the mean house diameter of 10 species of *Oikopleura* is 9.4-fold larger than the trunk length of the animal (range: 3.8–22.7-fold; Table 6.2 in Flood and Deibel<sup>27</sup>), there was no case of a “house”-like-organ length greater than trunk length in the diet of stone flounder larvae. Accordingly, “house”-like organs with trunks of *Oikopleura* sp. in the diet must be non-expanded “house rudiments”, not expanded “houses”. With peculiar, large, fang-like teeth,<sup>49</sup> leptocephalus larvae of anguilloid fishes can feed on the larger houses of *Oikopleura* spp.; however, stone flounder larvae do not have these characteristic teeth. Thus, stone flounder larvae

may not chew the house, but swallow the house rudiment with the trunk.

Median size ratio of prey to larval mouth (SLL/MW) for *Oikopleura* sp. was larger than for copepod nauplii and copepodites. This phenomenon shows that appendicularians are weak swimmers<sup>26</sup> and have low escape speed from predation. However, appendicularians rarely abandon the house in response to predation.<sup>26</sup> Stone flounder larvae, in many cases, fed on naked *Oikopleura* sp. in the very short period when they discard the house. *O. dioica* frequently produces new houses (4–16 houses/day depending on food and temperature conditions<sup>29</sup>), and appendicularians expand new houses in 1.5–5 min.<sup>26</sup> Although mature individuals of *Oikopleura longicauda* discards the house to spawn,<sup>50</sup> stone flounder larvae could also feed on small individuals (perhaps immature individuals; Fig. 6) and larval-feeding opportunities might not depend on the spawning behavior of *Oikopleura*.

House rudiments or house rudiment-like organs of appendicularians occurred more frequently than trunks in the diet of stone flounder larvae (Table 6) because enzymes to digest acidic polysaccharides are absent in fish larvae and most marine zooplankton.<sup>27,33,34</sup> As tails of appendicularians occurred rarely in the stone flounder diet, while those of *Oikopleura* sp. were found mainly in the foregut, larvae might easily digest the tails. It is also possible that larvae primarily attack the trunk and not the tail. However, knowledge of the feeding behavior of stone flounder larvae on appendicularians, either *in situ* or in the laboratory, is lacking and should be the subject of future research.

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**Fig. 1.** (Left) Location of Mutsu Bay, and (right) sampling stations with isobaths, showing sampling stations where stone flounder larvae were collected (●) and not collected (○). Mutsu Bay was divided into three regions, the bay mouth (M), West Bay (W) and East Bay (E).

**Fig. 2.** (A) Measurements of stone flounder larvae, (B) *Oikopleura* sp. in a “house”, and (C) various zooplankton as prey for larvae. Notochord length (NL) for pre-flexion and flexion larvae, and standard length (SL) for post-flexion larvae were used; these lengths were generalized as body length (BL) in this study. Abbreviations for prey lengths were as follows; *D*: body depth; *Dp* and *Du*: prosome and urosome depths; *EDi*: egg diameter; *Hrl*: house rudiment length; *L*: body length; *Lp* and *Lu*: prosome and urosome lengths; *Sd*, *Sl* and *Sw*: shell depth, length and width; *Si*: stomach diameter; *TailL*: tail length; *Td*, *Tl* and *Tw*: trunk depth, length and width; *ThD* and *ThDi*: thecal depth and diameter; *W*: body width; *Wp* and *Wu*: prosome and urosome widths. Abbreviations with an underline show the second largest length (SLL) of three-dimensional lengths, provided that *Dp* in *Paracalanus parvus* and *Pseudocalanus newmani* were used instead of *Wp*, because  $Dp > Wp$  (see Table 3).

**Fig. 3.** Photographs of appendicularians. (A) *Oikopleura dioica* (left) and *Fritillaria borealis* (right) in their environment, (B) trunk, house rudiment and part of tail of *Oikopleura* sp. in diet of stone flounder larvae, (C) house rudiment of *Oikopleura* sp. in the diet, (D) house rudiment-like organ of *Fritillaria* sp. in the diet. All specimens were stained with methylene blue solution.

**Fig. 4.** Relationship between BL and MW of larval stone flounder by developmental stage. There was a significant difference between slopes of the regression lines by least-square method (ANCOVA:  $P=0.008$ ). Regression equations (in mm) are: pre-flexion larvae, stages C-D (○),  $MW=0.065 \times BL - 0.047$  ( $r^2=0.78$ ,  $n=18$ ,  $P<0.001$ ); flexion larvae, stages E-F (■),  $MW=0.079 \times BL - 0.10$  ( $r^2=0.55$ ,  $n=116$ ,  $P<0.001$ ); and post-flexion larvae, stages G-H (□),

$MW=0.048 \times BL - 0.20$  ( $r^2=0.26$ ,  $n=154$ ,  $P<0.001$ ).

**Fig. 5.** Composition by number (upper) and volume (lower) of digestive tract contents of stone flounder larvae in the flexion (left) and post-flexion stage (right) by sampling period in Mutsu Bay. Numerals above bars show numbers of larvae examined.

**Fig. 6.** Prey-size (SLL in Fig. 2) differences in larval stone flounder diet by developmental stage. Different letters indicate significant differences at  $P<0.05$  in the same prey item by the Mann–Whitney  $U$ -test between two samples and by the Shirley–Williams test between two adjacent ordinal three samples. Superscripts indicate sample sizes.

**Fig. 7.** Relationship between BL of stone flounder larva (mm) and prey volume in the digestive tract ( $\text{mm}^3$ ) in Mutsu Bay. The regression line of flexion and post-flexion larvae combined was shown, and the formula was as follows:  $\log_{10}(\text{prey volume} + 0.0001) = 0.162 \times BL - 1.98$  ( $r^2=0.90$ ,  $n=26$ ,  $P<0.001$ ).

## 陸奥湾におけるイシガレイ仔魚の摂餌生態

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イシガレイ浮遊仔魚の摂餌戦略を解明するために，1989–1999年2–4月に陸奥湾で食物組成と餌サイズを調べた。餌サイズ範囲は仔魚の成長と共に拡大した。脊索屈曲前仔魚は主にかいあし類ノープリウスを，屈曲中仔魚と屈曲後仔魚は主に尾虫類を捕食し，低い空消化管率（1.7%と1.4%）は恐らく遊泳速度の遅い尾虫類を効率よく摂餌することによってもたらされるものと考えられた。仔魚は尾虫類 *Oikopleura* sp.の「包巢」を噛み砕くのではなく，躯幹を伴う「包巢原基」を丸呑みして捕食するものと判断された。

(248字)

**Table 1.** Sample list of stone flounder larvae for dietary analysis in Mutsu Bay

Date	Sampling Gear and Towing Method	Numbers of sampling stations /samples	Number of larvae used				Body length (mm)		
			Preflexion (stage C)	Preflexion (stage D)	Flexion (stages E-F)	Postflexion (stages G-H)	Median	Min.	Max.
1-6 Mar. 1989 <sup>*</sup>	Beam-trawl, horizontal	18/23	1	6	24	58	10.3	3.4	15.3
	Larva net, horizontal	2/4	0	0	5	10	11.8	5.6	13.3
26-27 Feb. 1991 <sup>*</sup>	Beam-trawl, horizontal	6/8	1	1	12	2	7.5	2.8	11.9
	Beam-trawl, oblique	1/1	0	2	1	1	5.1	4.3	10.9
9-11 Apr. 1991 <sup>*</sup>	Beam-trawl, horizontal	14/14	0	1	20	31	10.2	4.5	13.8
25-26 Feb. 1992 <sup>*</sup>	Beam-trawl, horizontal	9/11	1	0	23	13	9.8	3.2	15.8
5-6 Mar. 1998 <sup>*</sup>	Beam-trawl, oblique	2/2	0	1	24	18	8.7	6.7	12.2
27 Mar. 1999 <sup>**</sup>	Ring net, horizontal	2/4	3	2	8	8	7.8	3.5	12.0

<sup>\*</sup>: T/S *Ushio-maru*, <sup>\*\*</sup>: a fishing boat.

**Table 2.** Prey shapes and volume formulae used in this study

Prey type	Shape	Formula	Source
Coscinodiscineae	cylinder	$V = \pi \cdot (ThDi/2)^2 \cdot ThD$	this study
BIVALVIA larvae	two elliptic cones	$V = 2 \cdot 1/3 \cdot \pi \cdot (Sl/2) \cdot (Sw/2) \cdot (Sd/2)$	this study
POLYCHAETA larvae	cylinder	$V = \pi \cdot L \cdot (W/2) \cdot (D/2)$	this study
<i>Trichocerca</i> sp.	ellipsoid	$V = 4/3 \cdot \pi \cdot (L/2) \cdot (W/2)^2$	this study
Cladocera	pyramid	$V = 1/3 \cdot L \cdot W \cdot D$	this study
Copepoda nauplii	ellipsoid	$V = 4/3 \cdot \pi \cdot (L/2) \cdot (W/2)^2$	modified Nishiyama and Hirano <sup>32</sup>
Copepoda copepodites	ellipsoid + cylinder	$V = Vp/m'$ $= [4/3 \cdot \pi \cdot (Lp/2) \cdot (Wp/2) \cdot (Dp/2)]/m'$ $m' = Vp/(Vp + Vu)$ $= Vp/[Vp + \pi \cdot Lu \cdot (Wu/2) \cdot (Du/2)]$	modified Nishiyama and Hirano <sup>32</sup>
Crustacean eggs	sphere	$V = 4/3 \cdot \pi \cdot (EDi/2)^3$	Nishiyama and Hirano <sup>32</sup>
Appendicularia (trunk)	ellipsoid	$V = 4/3 \cdot \pi \cdot (Tl/2) \cdot (Td/2) \cdot (Tw/2)$	this study

\* *Paracalanus parvus*:  $m' = 0.977$ , *Pseudocalanus newmani*:  $m' = 0.966$ , *Centropages abdominalis*:  $m' = 0.949$ , *Acartia omorii*:  $m' = 0.967$ , *Oithona similis*:  $m' = 0.944$ .

Each  $m'$  for copepodite is species specific ratio of prosome volume to the whole body volume. Dimensions of prey are shown in Figure 2.



**Table 3.** Regression formulae of prey shapes used in this study

Prey type	Formula
BIVALVIA larvae	$W_s = 0.553L_s$ ( $n=23, r^2=0.93$ ) $D_s = 0.852L_s$ ( $n=60, r^2=0.97$ )
POLYCHAETA larvae (Spionidae)	$W = 0.354L$ ( $n=20, r^2=0.70$ ) $D = 0.286L$ ( $n=20, r^2=0.80$ )
<i>Evadne nordmanni</i>	$L = 1.19D + 0.330$ ( $n=218, r^2=0.59$ ) $W = 0.798D - 0.0404$ ( $n=205, r^2=0.48$ )
<i>Podon leuckarti</i>	$L = 0.835D$ ( $n=60, r^2=0.51$ ) $W = 0.466D$ ( $n=60, r^2=0.41$ )
Copepoda nauplii	
<i>Paracalanus parvus</i>	$W = 0.374L + 0.0182$ ( $n=343, r^2=0.87$ )
<i>Pseudocalanus newmani</i>	$W = 0.455L + 0.0130$ ( $n=76, r^2=0.87$ )
<i>Centropages abdominalis</i>	$W = 0.432L + 0.0102$ ( $n=128, r^2=0.93$ )
<i>Acartia omorii</i>	$W = 0.537L$ ( $n=80, r^2=0.95$ )
<i>Oithona similis</i>	$W = 0.473L + 0.0062$ ( $n=1329, r^2=0.91$ )
<i>Corycaeus</i> sp.	$W = 0.544L$ ( $n=40, r^2=0.87$ )
<i>Oncaea</i> sp.1 (long 1st antennae)	$W = 0.401L + 0.0112$ ( $n=33, r^2=0.92$ )
<i>Oncaea</i> sp.2 (short 1st antennae)	$W = 0.299L + 0.0313$ ( $n=23, r^2=0.82$ )
Copepoda copepodites	
<i>Paracalanus parvus</i>	$W_p = 0.392L_p$ ( $n=291, r^2=0.71$ ) $D_p = 0.415L_p$ ( $n=272, r^2=0.64$ )
<i>Pseudocalanus newmani</i>	$W_p = 0.402L_p$ ( $n=98, r^2=0.77$ ) $D_p = 0.403L_p$ ( $n=91, r^2=0.64$ )
<i>Centropages abdominalis</i>	$W_p = 0.370L_p$ ( $n=47, r^2=0.77$ ) $D_p = 0.360L_p$ ( $n=44, r^2=0.62$ )
<i>Oithona similis</i>	$W_p = 0.412L_p$ ( $n=78, r^2=0.62$ ) $D_p = 0.321L_p$ ( $n=20, r^2=0.55$ )
Appendicularia	
<i>Oikopleura dioica</i>	$Tl = 1.18Hrl$ ( $n=37, r^2=0.92$ ) $Td = 0.628Hrl$ ( $n=37, r^2=0.83$ ) $Tw = 0.576Hrl$ ( $n=37, r^2=0.87$ ) $Td = 0.141TailL$ ( $n=51, r^2=0.79$ )
<i>Fritillaria borealis</i>	$Tl = 2.60Tw + 0.180$ ( $n=112, r^2=0.69$ ) $Td = 0.516Tw + 0.042$ ( $n=114, r^2=0.65$ ) $Tl = 7.61Si$ ( $n=106, r^2=0.79$ ) $Td = 1.56Si$ ( $n=108, r^2=0.71$ ) $Tw = 2.02Si$ ( $n=108, r^2=0.55$ ) $Tw = 0.144TailL$ ( $n=109, r^2=0.49$ )

Zooplankton samples collected with either a plankton net or a Van Dorn bottle were used for the measurements, and each prey length in mm is shown in Figure 2. All probabilities of regression formulae were  $P < 0.001$ .

**Table 4.** Percent frequency of occurrence (F%), percent by number (N%), and percent by volume (V%) of food items in digestive tracts of stone flounder larvae in daytime

Prey type	Preflexion (stage C)			Preflexion (stage D)			Flexion (stages E-F)			Postflexion (stages G-H)		
	F%	N%	V%	F%	N%	V%	F%	N%	V%	F%	N%	V%
Coscinodiscineae	0	0	0	8	5	<0.1	3	0.7	0.1	1	0.2	<0.1
<i>Ptychocylis</i> sp.	0	0	-	8	3	-	0	0	-	0	0	-
POLYCHAETA larvae	0	0	0	0	0	0	1	0.1	<0.1	4	0.5	<0.1
BIVALVIA larvae	0	0	0	15	3	0.6	4	1.9	0.4	2	2.9	1.2
<i>Trichocerca</i> sp.	17	10	6	8	3	0.3	1	0.1	<0.1	0	0	0
Cladocera (total)	0	0	0	8	2	<0.1	6	3.5	1.9	12	8.9	3.9
<i>Evadne nordmanni</i>	0	0	0	8	2	<0.1	5	3.3	1.9	9	6.5	3.0
<i>Podon leuckarti</i>	0	0	0	0	0	0	1	0.1	<0.1	6	2.4	0.8
Copepod nauplii (total)	50	50	92	77	54	55.6	18	8.0	1.0	1	0.1	<0.1
<i>Paracalanus</i>	17	10	2	38	11	4.7	1	0.1	<0.1	0	0	0
<i>Pseudocalanus</i>	17	10	61	15	7	10.5	9	2.6	0.6	1	0.1	<0.1
<i>Centropages</i>	17	10	9	23	23	35.6	5	1.3	0.2	0	0	0
<i>Acartia</i>	0	0	0	0	0	0	1	0.1	<0.1	0	0	0
<i>Oithona</i>	33	20	20	31	8	4.7	6	2.8	0.2	0	0	0
<i>Oncaea</i>	0	0	0	0	0	0	3	0.7	<0.1	0	0	0
Unidentified nauplii	0	0	-	23	5	-	2	0.3	-	0	0	-
Copepod copepodites (total)	0	0	0	8	2	3.5	17	4.5	2.0	6	0.6	0.3
<i>Paracalanus parvus</i>	0	0	0	0	0	0	6	1.3	0.8	1	0.1	0.1
<i>Pseudocalanus newmani</i>	0	0	0	0	0	0	3	0.4	0.6	1	0.2	0.1
<i>Centropages abdominalis</i>	0	0	0	0	0	0	3	0.4	0.1	1	0.2	0.2
<i>Oithona similis</i>	0	0	0	8	2	3.5	10	2.2	0.4	1	0.2	<0.1
<i>Oncaea</i> sp.	0	0	-	0	0	-	0	0	-	1	0.1	-
Unidentified copepodites	0	0	-	0	0	-	1	0.1	-	0	0	-
Crustacean eggs	17	10	3	8	3	0.2	12	2.8	0.3	4	0.7	<0.1
Appendicularia (total)	0	0	0	46	20	39.7	97	76.9	94.5	98	85.5	94.5
<i>Oikopleura</i> sp.	0	0	0	38	15	35.7	88	60.5	91.9	96	81.9	94.3
<i>Fritillaria</i> sp.	0	0	0	15	5	4.0	29	16.4	2.6	11	3.6	0.3
Unidentified	33	30	-	23	5	-	7	1.5	-	6	0.7	-
Mean and SD number of food organisms*	1.7	±	1.37	4.8	±	3.11	5.9	±	3.79	9.3	±	8.28
Mean and SD volume of food organisms (mm <sup>3</sup> )*	5.2·10 <sup>-4</sup>	±	1.05·10 <sup>-3</sup>	5.7·10 <sup>-3</sup>	±	5.98·10 <sup>-3</sup>	0.10	±	0.0978	0.40	±	0.394
Number of fish examined	6			13			117			141		
Number of empty digestive tracts	1			0			2			2		
Median BL (mm) of fish examined	3.4			4.6			7.9			11.5		
Range of BL (min.-max.; mm)	2.8	-	3.8	3.6	-	6.7	4.5	-	11.2	8.1	-	15.8

\* SD: standard deviation.

**Table 5.** Diet overlap by percentage similarity index (Bray-Curtis index) among sampling areas and numerical percentages (N%) of major prey items in each area.

Mar. 1989	Similarity index			N%			
	Bay mouth	West	East	<i>Oikopleura</i> sp.	<i>Fritillaria</i> sp.	<i>Evadne</i> <i>nordmanni</i>	BIVALVIA larvae
Bay mouth				97%	0%	0%	0%
West	94%			94%	5.5%	0%	0%
East	89%	90%		87%	2.6%	0.4%	1.2%

Apr. 1991	Similarity index			N%			
	Bay mouth	West	East	<i>Oikopleura</i> sp.	<i>Fritillaria</i> sp.	<i>Evadne</i> <i>nordmanni</i>	BIVALVIA larvae
Bay mouth				98%	0%	0%	0%
West	64%			63%	12%	13%	0.8%
East	55%	76%		55%	0.8%	33%	0%

Feb. 1992	Similarity index			N%			
	Bay mouth	West	East	<i>Oikopleura</i> sp.	<i>Fritillaria</i> sp.	<i>Evadne</i> <i>nordmanni</i>	BIVALVIA larvae
Bay mouth				-	-	-	-
West	-			88%	0%	0%	0%
East	-	89%		98%	0%	0.9%	0%

Mar. 1998	Similarity index			N%			
	Bay mouth	West	East	<i>Oikopleura</i> sp.	<i>Fritillaria</i> sp.	<i>Evadne</i> <i>nordmanni</i>	BIVALVIA larvae
Bay mouth				53%	38%	0%	0%
West	-			-	-	-	-
East	65%	-		42%	14%	0.7%	26%

N% data of flexion and post-flexion larvae were combined; -: no data.

**Table 6.** States of appendicularian particles in the diet of stone flounder larvae in the daytime

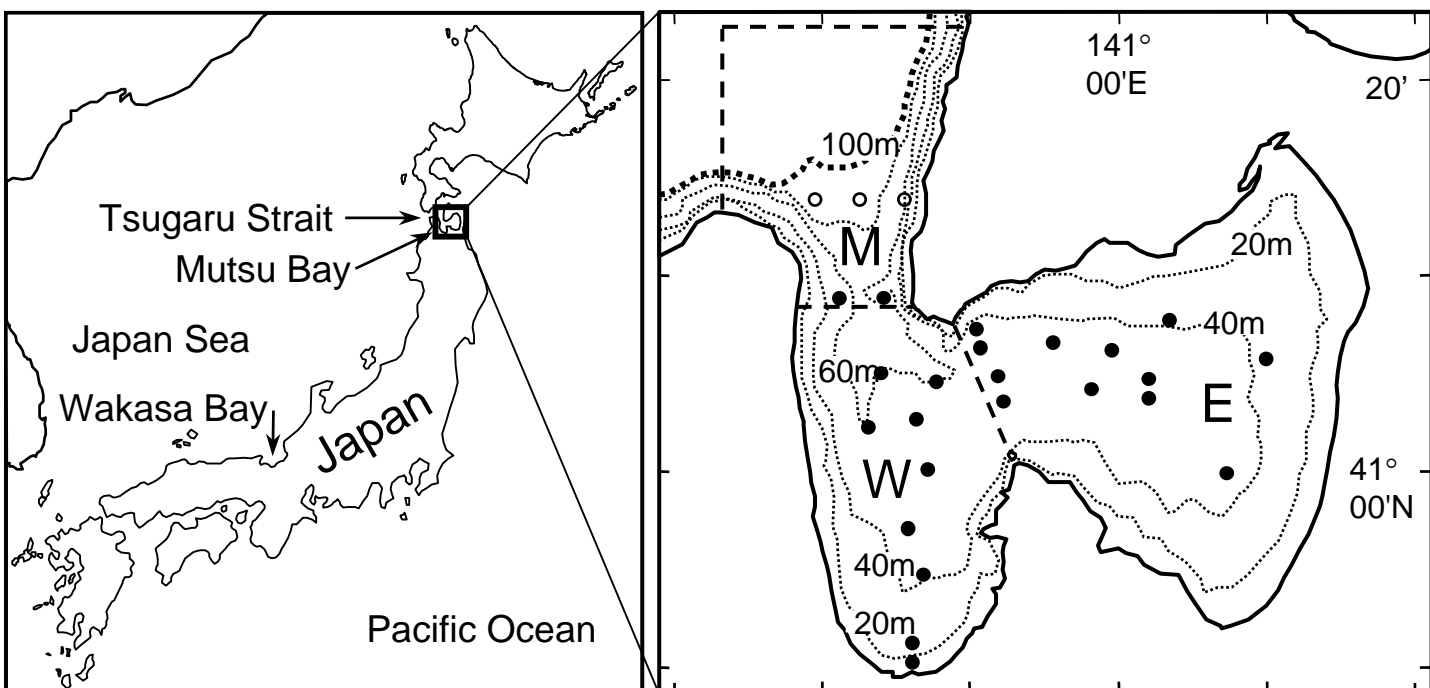
Prey type	State	Nr. of inds. and particles	%
<i>Oikopleura</i> sp.	House rudiment+trunk+whole tail	20	1.4
	House rudiment+trunk+torn-off tail	24	1.6
	House rudiment+trunk	1,130	77.3
	House rudiment	288	19.7
	Broken house rudiment*	(31)*	-
<i>Fritillaria</i> sp.	Viscus+trunk+house rudiment like organ+torn-off tail	1	0.8
	Viscus+trunk+house rudiment like organ	5	3.8
	Viscus+trunk	23	17.6
	Viscus+house rudiment like organ	102	77.9
	House rudiment like organ*	(7)*	-
	Broken viscus or trunk*	(25)*	-

\*: particles were not measurable and counted as numbers of particles. -: no data.

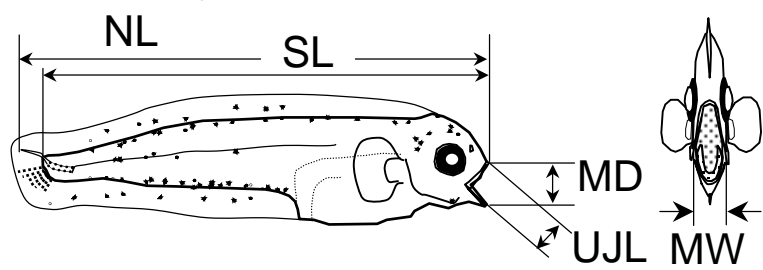
**Table 7.** Result of non-parametric multiple comparison in gut fullness between samples by the Steel-Dwass test

	Mar. 1989	Feb. 1991	Apr. 1991	Feb. 1992	Mar. 1998	Mar. 1999	<i>n</i>	Median GF%
Mar. 1989							95	51%
Feb. 1991	insig.						15	57%
Apr. 1991	insig.	insig.					51	52%
Feb. 1992	insig.	insig.	insig.				36	44%
Mar. 1998	***	insig.	***	***			42	21%
Mar. 1999	*	insig.	*	*	insig.		16	22%

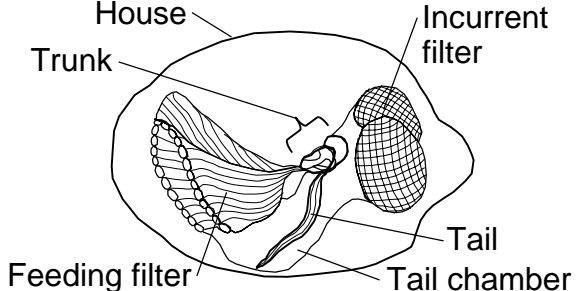
insig.:  $P > 0.05$ ; \*:  $0.05 \geq P > 0.01$ ; \*\*:  $0.01 \geq P \geq 0.001$ ; \*\*\*:  $P < 0.001$ .



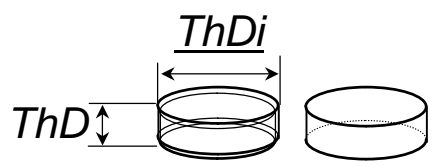
**A** *Platichthys bicoloratus* larva



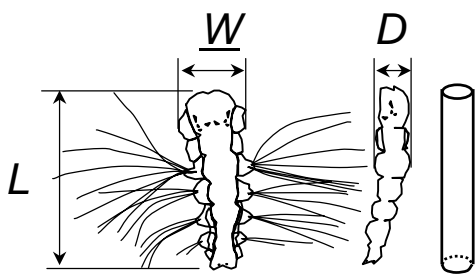
**B** *Oikopleura* sp. in a "house"



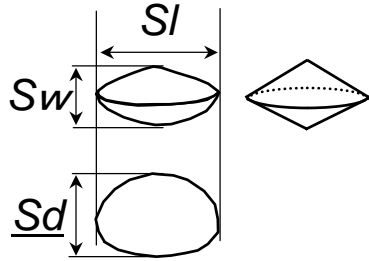
**C** Coscinodiscineae



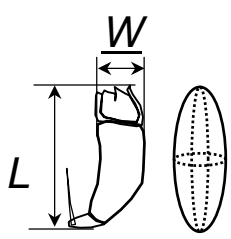
POLYCHAETA larva



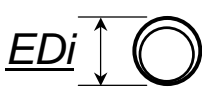
BIVALVIA larva



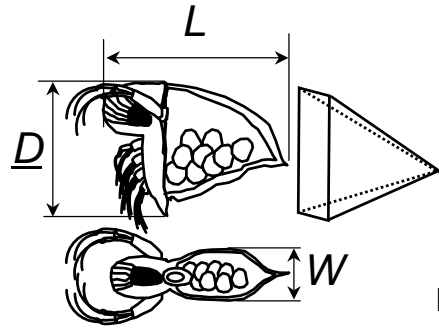
*Trichocerca* sp.



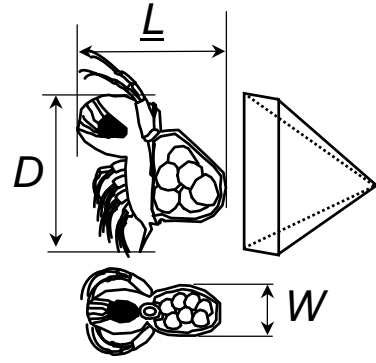
Crustacean egg



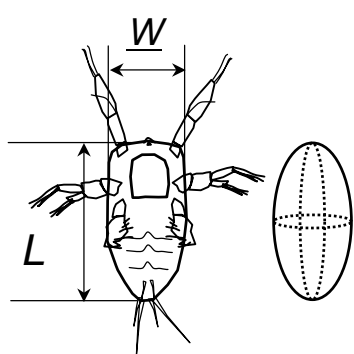
*Evadne nordmanni*



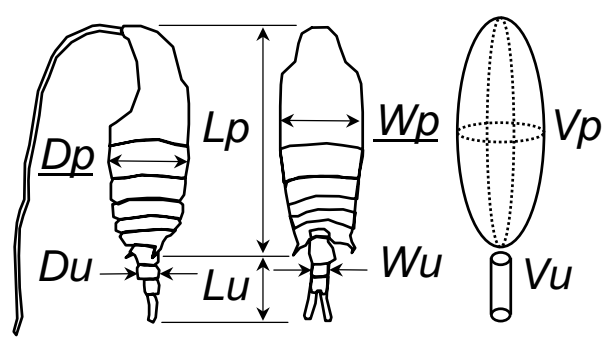
*Podon leuckarti*



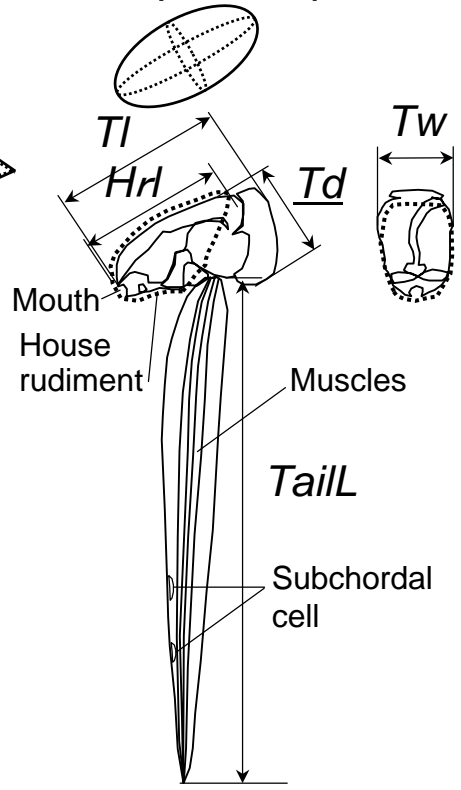
Copepod nauplius



Copepod copepodite



*Oikopleura* sp.



*Fritillaria* sp.

