Prolonged maturation time in female *Caprella acanthogaster* (Crustacea, Amphipoda) at low temperature under laboratory conditions

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Abstract.—Caprellids are epifaunal crustaceans, widely distributed in marine environments. The life histories of many temperate caprellids have been described, but the growth pattern of a boreal species has not previously been observed. To fill this gap, a boreal species, *Caprella acanthogaster*, was reared at 5°C under laboratory conditions and observed it from hatching to maturation and oviposition. Males were recognizable from instar IV and females from instar V. Female maturation was observed at instar VIII (115 days) and instar IX (126 days), whereas males did not mature morphologically in this study. The females matured at instar IX and oviposited subsequent to molting. These periods from hatching to maturation and oviposition are the longest known among previously reared female caprellids. Caprellids are known to mature rapidly in temperate regions. However, female *C. acanthogaster* required 4 months to mature at 5°C while males need an even longer but unknown period. These observations at a low temperature fill a gap in the current information on caprellid life histories.

Introduction

Caprellids are small epifaunal crustaceans living on algae, bryozoans and hydrozoans and are widely distributed around the world (McCain & Steinberg, 1970). They are secondary producers and prey for fish, playing a role in the transfer of energy in coastal ecosystems (Cain, 1980; 1983; 1989; Norderhaug & Christie, 2011; Best & Stachowicz, 2012). As the population densities of caprellids generally show large seasonal fluctuations (e.g., Mukai, 1971; Ashton *et al.*, 2010; Zakhama–Sraieb *et al.*, 2011), it is essential to understand the population dynamics of these animals to

understand the dynamics of total production in epifaunal communities. Population dynamics are internally controlled by life history traits such as growth and reproductive patterns. After the successful rearing of *Caprella danilevskii* Czerniavski, 1868 through its life history by Takeuchi & Hirano (1991), several studies have described the life histories of caprellid species under laboratory conditions with rearing temperature of 20°C. These studies showed that growth to maturity is rapid over approximately one month and that a brief reproductive interval of 1–2 weeks occurs after maturation (Gardella, 1962; Sakaguchi, 1989; Takeuchi & Hirano, 1992a, b; Cook *et al.*, 2007; Boos, 2009; Hosono 2009, 2011; Baeza–Rojano *et al.*, 2011). This rapid growth and high reproductive potential adequately explains the rapid population growth of caprellids over short periods frequently reported in field studies (e.g., Imada & Kikuchi, 1984; Aoki, 1988; Takeuchi *et al.*, 1990).

The life history traits of caprellids revealed by laboratory experiments contribute to the understanding of population dynamics in the field. However, such laboratory experiments have been conducted primarily in temperate species, and no previous studies have examined the life history of a boreal species at low temperatures. In a high latitude area, Costello & Myers (1989) found ovigerous females of *C. acanthifera* Leach 1814 in limited periods from April to June and October to November in Lough Hyne, Ireland (51°N30', 10°18'W; temperature range: 8–18°C). Heptner (1963) found that reproduction in *C. septentrionalis* Krøyer, 1838 occurred only in early summer in the White Sea (66°34'N, 33°08'E; –1.1–12). These observations imply that the growth

and maturation of caprellids could be slow in low-temperature regions compared with temperate regions. To fill the gap in the available information about growth patterns at low temperatures in caprellids, we reared a boreal species, *C. acanthogaster* Mayer, 1980, at 5°C and described its growth and maturation pattern from hatching to maturation and oviposition under laboratory conditions.

Materials and Methods

Three ovigerous females were collected on 15 January 2002 in Usujiri, on the Pacific coast of northern Japan (41° 57' N, 141° 58' E). These females were individually transferred to 40-ml polystyrene containers (4 cm diameter, 7 cm height) with seawater and a small piece, approximately 5 cm, of filamentous red algae (*Neorhodomela larix*) as substrate. The water temperature of the rearing container was kept at 5°C throughout the experiment. The details of the rearing system are presented by Hosono (2009). The photoperiod was maintained at 12 h light: 12 h dark. The females were fed daily with commercial goldfish flakes (Tetrafin, developed by Tetra Japan Inc.). When juveniles emerged from the female brood pouch, the parent was removed from the rearing container. The emerged young were classified as juveniles of instar I and 0 days according to Takeuchi & Hirano (1991). The juveniles were reared in groups of 10–20 individuals and were transferred to individual rearing when the presence of sexual characters was confirmed. Observations and water exchange were conducted under a binocular microscope (Olympus SZX 12) every day. Moulting was identified with increment of the number of flagellar articles in antennal and size increment. When molting was confirmed, a picture was taken with a digital camera attached to the microscope to measure body length using image analysis software (NIH Image 1.56, National Institutes of Health, USA), and then appearance of sexual character was checked. The sum of the axial length from the anterior of the head to the posterior tip of pereonite

7 along the longitudinal mid-line of each pereonite was used as the body length.

The sex of caprellids can be identified based on the presence of oostegites at pereonites 3–4 in the female and based on the abdominal appendages in the male. The abdominal appendages at a younger instar are difficult to confirm because a caprellid attaches to its algal substrate. To observe the presence of abdominal appendages, a newly moulted juvenile was transferred onto a small piece of cotton from its algal substrate in a petri dish filled with seawater, and then the cotton with the juvenile was transferred on glass slide. The juvenile was laid down on its back on the slide, and checked through a slit-like space of the cotton from the ventral side of the juvenile under a binocular microscope. Presence of female oostegites can be easily checked in the condition that a caprellid attaches to its substrate. Maturity in females was categorized into three stages based on the formation of oostegites, using categories similar to those in Takeuchi & Hirano (1991). Immature: hemispherical rudiments of oostegites appear at the base of the gills; penultimate: oostegites are lamelliform; mature: oostegites bear setae along their edges and form a brood pouch.

The growth of the body was quantified with the regression curves in Takeuchi & Hirano (1991): $L = \alpha_1 x^{\beta_2} + \gamma_1$ in the male and $L = \alpha_2 / \{1 + \beta_2 \exp(\gamma_2 x)\}$ in the female, where L is the body length, x is the instar and $\alpha_{1,2}$, $\beta_{1,2}$ and $\gamma_{1,2}$ are parameters determined by sex. Data on juveniles from instars I to VI were pooled for males and females in the estimation of the parameters of the equations. The cumulative days to each instar were compared between sexes using a Mann–Whitney U -test to examine the difference in molting patterns between sexes. The relationship between instars and days to each instar was described with an exponential growth curve according to Hosono (2011): $D = \exp(\alpha_3 x + \beta_3) + \gamma_3$, where x is the instar, D is the cumulative days to the instar and α_3 , β_3 and γ_3 are parameters to be estimated. All estimates of the parameters of nonlinear regression curves were performed with R statistical software, version 2.14 (R Development Core Team, 2011). The fit of all

non-linear regression curves were described by the following index similar to R-squared (hereafter referred as R^2): $(1-d)/\sum_1^n (y_i - \bar{y})^2$, where, d is deviance of a regression model, n is number of data used in curve estimation, y_i indicates each data of length or days, and $\bar{y}$ is mean of y_i .

Results

No sexual traits appeared until instar III. Individuals with male abdominal appendages of a secondary sexual characteristic began to appear at instar IV ($38.5 \text{ days} \pm 2.3 \text{ SD}$), and all males could be identified at instar V (Table 1, Fig. 1). Immature females appeared at instar V ($54.8 \text{ days} \pm 13.1 \text{ SD}$). Lamelliform oostegites in the penultimate stage in females were observed from instar VII to IX. The molt to the mature stage in the females was confirmed at instar VIII (115 days) and instar IX (126 days) (Table 1, Fig. 1). In the case of maturation at instar IX (126 days), the female oviposited following her maturation. One female was still in the penultimate stage at instar IX, indicating that the female would mature on and after instar X. During the rearing experiment, the latest molt was observed at 145 days at instar IX in a male and at 150 days at instar VIII in a female.

There was no significant difference between sexes in molt timing in most instars (Fig. 1; Mann-Whitney U -test, $p > 0.05$) except for the earlier molting to instar VII in the male than in the female ($p < 0.001$). Although we could not test the significance of differences in molt timing between sexes at instar IX because of the small sample size, the timing in the two sexes overlapped. Therefore, data on both sexes were combined to estimate the relationship between the instar and the cumulative days to each instar. The relationship was estimated as follows: $D = \exp(0.10x + 4.47) - 96.12$ ($R^2=0.95$, Fig. 1).

The body growth patterns of both sexes were similar until instar VIII (Fig. 2; Mann-Whitney U -test, $p > 0.05$). Although we could not perform statistical tests because of the small sample size in instar IX, the body lengths of the sexes at this instar were markedly different (mean body length: 12.0 and 10.1 mm in male and female,

Fig. 1. Days to each instar in *Caprella acanthogaster* at 5°C. Vertical bars indicate standard deviations. Symbols of male and female are shifted to avoid overlapping of error bars.

Table 1. Number of animals reared in each developmental stage

Instar	Juvenile	Male	Female		
			Immature	Penultimate	Mature
I	30	—	—	—	—
II	30	—	—	—	—
III	30	—	—	—	—
IV	21	8	—	—	—
V	4	13	10	—	—
VI		13	12	—	—
VII		13	8	4	—
VIII		7		11	1
IX		4		1	2

Fig. 2. Body growth pattern of *Caprella acanthogaster* at 5°C. Symbols are same as in Fig. 1

Fig. 3. Relationship between maturation times and temperature based on the previously published data. Black symbols represent the referenced data, and a white symbol represents the data from this study. Each number near plots corresponds to the reference numbers in Table 2.

respectively). The body lengths of the matured females were 8.5 mm (instar VIII) and 10.1 mm (instar IX). The body growth pattern in each sex was quantified as follows: $L = 0.23x^{1.69} + 1.15$ ($R^2=0.94$) in the male and $L = 12.16 / \{1 + 12.76\exp(-0.43x)\}$ ($R^2=0.97$) in the female (Fig. 2).

Discussion

Female *Caprella acanthogaster* required approximately 4 months to mature. In caprellids, growth patterns from hatching to maturation have primarily been obtained from laboratory studies at a temperature of 20°C (Table 2). These studies reported a brief maturation time for females of approximately 1 month, e.g., 29.0 days in *C. equilibra* Say, 1818; and 26.3 days in *C. dilatata* Kroyer, 1843 reported by Baeza-Rojano & Guerra-García (2013). At lower temperature, the maturation time of *C. mucica* at 5°C is predicted to be 140.7 days based on the growth curve for instars I–III in Hosono (2011). The predicted period is similar to our results in female *C. acanthogaster*. Based on the published results of 17 rearing experiments in 12 caprellids, an inverse relationship was estimated between temperature and maturation

rates in caprellids (Fig. 3; $y = 348.9(x - 1.91)^{-0.87}$). This inverse relationship in caprellids indicates that the maturation rate decreases considerably with decreasing temperatures.

Information of prolonged maturation time and low growth rate observed in female *C. acanthogaster* at 5°C enables us to interpret the reproductive dynamics of the species in low temperature season in the field. Hosono & Sakurai (2006) investigated the population structure of the species at the same location of the sampling area in this study and suggested that recruitment would be restricted during this period because of the occurrence of mature females and no juveniles from February to April of the low-temperature season (mean temperature: 3.4°C). It is known that oviposition interval of *C. mutica* that is often found in the habitat of *C. acanthogaster*, closely correlates with moulting interval, becoming longer at lower temperatures (mean moulting interval: 33.4, 20.4, 11.7, 9.7 days at 5, 10, 15, 20°C, respectively; Hosono, 2009). The oviposition interval of *C. acanthogaster* can be estimated as 22.6 days in the case of the moulting from instar IX to instar X at 5°C. If *C. acanthogaster* have a similar response in reproductive rate to decreasing temperature, the rate would be reduced in the period of February to April. Additionally abnormal development of brooding eggs at 5°C in *C. mutica* was frequently observed. These negative factors to reproductive activity at low temperature are consistent with the restricted recruitment observed in the natural population in low temperature season.

Effects of an artificial rearing condition on the growth pattern of *C. acanthogaster* may need to be evaluated. As for developmental traits of sexual characteristics in the species, our observation that male morphological traits appear earlier than female morphological traits is similar to that reported for natural populations (Hosono & Sakurai, 2006). Male morphological traits begin to appear in the 2–3 mm size class and in the female at the 4–5 mm size class, as is known from the natural population structure (Fig. 4 in Hosono & Sakurai, 2006). As for the maturation size of female, Hosono & Sakurai (2006) also reported that the female size at maturation (the size at which 50% of all females are mature)

increased with decreasing temperatures. The results of that study showed that mature and immature females co-occurred over a broad size range of 9–14 mm in April (mean temperature 10°C) (Fig. 5 in Hosono & Sakurai, 2006). This maturation pattern reported in the field is consistent with our observations that females matured at body lengths of 8.5 and 10.1 mm and that one female was still immature at body length of 10.2 mm. These developmental patterns correspond to that in the natural population, and indicate the present rearing experiments have good reproducibility, at least, as for developmental pattern in female. On the other hand, males did not appear to reach mature stages in this study. The mature males of *C. acanthogaster* have extremely elongated pereonites 1 and 2 compared with the other pereonites (Takeuchi, 1995, Hosono & Munehara, 2001). In the males observed in the experiment, the ratio of the length of pereonite 2 to the body length at the later instars remained shorter (0.16, 0.17 and 0.17 at instars VII, VIII and IX, respectively) than the corresponding ratio for pereonite 3 (0.17, 0.18, 0.18), indicating that the males did not reach mature stages during the experimental period.

Survival rates to mature stages in this

study were relatively low in both sexes. It is unclear whether the low survival rate results from the direct effect of low temperature and/or cumulative effects of handling for elongated rearing period. Examination of abdominal appendages during early instars could have had a negative influence on the survival rate in this study. Further examination on the low survival rate and quantification of growth pattern in adult stage are needed.

In the present study, we found a long period of 4 months to maturity based on the observations from hatching to maturation of female *C. acanthogaster* at 5°C. This maturation time at a low temperature is considerably longer than that previously reported in temperate regions. Many caprellids are known from high latitudes as well (Vassilenko, 1974). To understand differences in life history traits or in temperature responses among all caprellids, experiments under low-temperature conditions and studies of the life histories of species occurring at high latitudes are particularly needed.

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I would like to extend my sincerest

Table 2. Maturation time of female caprellids reported in previously published literature. "Ref. No." corresponds to the number plotted in Figure 3

Ref. No.	Species	Temperature (°C)	Days to maturation instar (Days)	References
1	<i>Caprella acanthogaster</i> Mayer, 1980	5	120.5	This study
2	<i>C. brevirostris</i> Mayer 1903	20	27.5	Takeuchi and Hirano (1991)
3	<i>C. cristibrachium</i> Mayer, 1903	20	35.0	Hosono (2006)
4	<i>C. danilevskii</i> Czerniavski, 1868	20	20.0	Takeuchi and Hirano (1991)
5	<i>C. dilatata</i> Kroyer, 1843	20	26.3	Baeza-Rojano and Guerra-García (2013)
6	<i>C. equilibra</i> Say, 1818	20	29.0	Baeza-Rojano and Guerra-García (2013)
7	<i>C. generosa</i> Arimoto, 1977	20	19.5	Takeuchi and Hirano (1991)
8	<i>C. grandimana</i> Mayer, 1882	17	38.4	Baeza-Rojano <i>et al.</i> (2011)
9	<i>C. laeviuscula</i> Mayer, 1903	12	44.2	Gardella (1962)
10	<i>C. mutica</i> Schurin, 1935	5	140.7	InstarX estimated based on the growth curve in Hosono (2011)
11	<i>C. mutica</i>	10	54.0	Instar VII in Boos (2009)
12	<i>C. mutica</i>	10	56.9	InstarX in Hosono (2011)
13	<i>C. mutica</i>	15	41.3	InstarX in Hosono (2011)
14	<i>C. mutica</i>	16	38.1	Instar VII in Boos (2009)
15	<i>C. mutica</i>	20	31.6	InstarX in Hosono (2011)
16	<i>C. okadai</i> Arimoto, 1930	20	20.6	Takeuchi and Hirano (1992b)
17	<i>C. scaura</i> Templeton, 1836	20	32.0	Sakaguchi (1989)

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