Dietary effects of phytoplankton and zooplankton on larval survival, duration and growth of four Caridina species (Decapoda: Caridea: Atyidae) under laboratory conditions

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Abstract.—We conducted laboratory experiments to evaluate the dietary effects of phytoplankton and zooplankton on larval survival, duration and growth of four amphidromous atyid shrimps, Caridina leucosticta, C. multidentata, C. serratirostris, and C. typus. Larvae were reared by being fed commercially preserved or cultured phytoplankton Tetraselmis sp. and cultured zooplankton rotifers. Larvae survived to juveniles by being fed cultured phytoplankton and rotifers; however, C. serratirostris larvae died due to being trapped to the surface tension of the water during moultting. Rotifers significantly improved larval survival and development, particularly in C. multidentata and C. typus. C. leucosticta larvae moulted to juveniles with high survival rates by being fed only cultured phytoplankton, suggesting that they may develop in eutrophic brackish environments with substantial phytoplankton biomass. C. multidentata larvae had prolonged larval durations under limited nutritional conditions, suggesting their ability to broadly disperse in the sea. C. typus had a longer larval duration than C. leucosticta. Thus, Caridina species might exhibit different larval dispersal strategies in the sea. Larval duration varied, but body size did not vary among the species under the different feeding conditions, indicating that larvae moulted to juveniles after reaching the threshold size of each species needed to migrate to their adult freshwater habitat.

Keywords: Amphidromous shrimp, larval export strategy, larval dispersal, feeding condition

Electronic supplementary material. The online version of this article contains supplementary material at https://www.jstage.jst.go.jp/article/crustacea/49/0/49_225/_article

Introduction

Freshwater shrimp in the family Atyidae occur worldwide, except Antarctica; they are the most diversified decapod crustaceans (Decapoda: Caridea) and are found in various freshwater bodies from fast-moving mountain streams to sluggish, oligohaline waters (De Grave et al., 2008; De Grave & Fransen, 2011). Atyid shrimp play important roles in stream food webs as primary consumers (Pringle et al., 1993; Croll et al., 2001; March & Pringle, 2003; Oeding et al., 2020). Some atyid species are fished for local food (Holthuis, 1980; De Grave et al., 2008), and they are also harvested wild and cultured for the aquarium trade (Heerbrandt & Lin, 2006; De Grave et al., 2008, 2015). At present, almost one-third of freshwater caridean shrimp species are threatened or near-threatened due to human activity (De Grave et al., 2015).

Most atyid shrimp species exhibit an amphidromous life cycle (Shokita, 1979; Hayashi & Hamano, 1984; Bauer, 2013). Adults inhabit
and reproduce in freshwater environments, but their larvae require saline water for successful development (Shokita, 1979; Hayashi & Hamano, 1984; Nakahara et al., 2005). Newly hatched larvae (stage 1 zoeae) passively drift from freshwater environments to the sea (Ideguchi et al., 2000, 2007; Hamano et al., 2005), and they develop for a relatively long period with complex zoeal stages in the brackish waters of estuaries and coastal bays or in the open sea (Hayashi & Hamano, 1984; Ideguchi et al., 2000; Bauer, 2013; Yatsuya et al., 2013). After recruitment to the mouth of a coastal river or stream, juveniles migrate up to the adult freshwater habitat (Hamano & Hayashi, 1992; Hamano et al., 2005; Bauer, 2013; Yatsuya et al., 2013). Accordingly, information on the environmental factors affecting larval survival, duration and growth is essential for a better understanding of the ecological processes of amphidromous atyid shrimp in the sea, including larval survival and dispersal, population connectivity, recruitment dynamics and geographical distribution.

Laboratory culture experiments have tested the various biotic and abiotic environmental factors affecting larval survival, duration and growth in decapod crustacean species (Anger, 2001; Zeng et al., 2020). Excluding larvae with obligate lecithotrophy, feeding conditions such as prey type and density are an important biotic factor largely influencing the larval performance of decapod crustaceans (Anger, 2001; Zeng et al., 2020). Atyid shrimp larvae can survive to the juvenile stage (sensu Møller et al., 2020) by being fed phytoplankton and/or detritus (Hayashi & Hamano, 1984; Nakahara et al., 2005; Heerbrandt & Lin, 2006). Nakahara et al. (2005) tested three different marine phytoplankton species,Chaetoceros gracilis, Nannochloropsis oculata and Tetraselmis tetratele, for culturing larvae of two amphidromous shrimps, Caridina leucosticta Stimpson, 1860 and Caridina typus H. Milne-Edwards, 1837, and they detected higher larval survival to the juvenile stage when Tetraselmis was fed to the larvae than when the other phytoplankton species were fed to the larvae. They further revealed the optimal feeding density of Tetraselmis for culturing these larvae. Additionally, Nakahara et al. (2005) documented that later stage larvae of C. leucosticta could ingest euryhaline zooplankton, specifically the rotifer Brachionus plicatilis, in a preliminary larval rearing trial. However, the dietary effect of zooplankton on the larval performance of atyid shrimp has not been thoroughly examined.

Our objective in the present study was to evaluate the dietary effects of phytoplankton Tetraselmis sp. and zooplankton rotifers on larval survival, duration and growth of four atyid shrimp species of the genus Caridina H. Milne-Edwards, 1837, namely C. leucosticta, C. multidentata Stimpson, 1860, C. serratirostris De Man, 1892, and C. typus, all of which are commonly occurring in Japan (Shokita, 1979; Hamano & Hayashi, 1992; Suzuki et al., 1993; Usami et al., 2008; Saito et al., 2012). Our results highlighted the variability in the larval performance of atyid shrimp species under different feeding conditions, and ecological implications were discussed in terms of larval dispersal strategy.

Materials and Methods

Larval source

Culture experiments were conducted in a laboratory at Tokyo University of Marine Science and Technology, Tokyo, Japan, from 2016 to 2018. Wild mature females and males of the test shrimp were captured using scoop nets at the following locations from April to May: C. leucosticta in the Heda Okawa River (34°58′N, 138°47′E), Shizuoka Prefecture, and C. serratirostris and C. typus in the Banda River (34°58′N, 139°46′E), Chiba Prefecture, Japan. The wild C. multidentata collected from western Japan were purchased from an aquarium trader (information on the exact capture location was not available).
not available).

Females were cultured with males in aerated aquaria (23 l) under natural photoperiod conditions (30–40 shrimps per tank, unknown sex ratio). Using a heater and an air conditioner in the laboratory, the aquarium temperature was controlled at approximately 23°C, which is equivalent to the summer temperature during the reproductive season of atyid shrimp in the natural habitat of the Banda River. Artifical diets for ornamental shrimp or fish were given daily to the animals, and half of the rearing water was exchanged with new water every three days. Ovigerous females were transferred and individually stocked in 1-l beakers. Ovigerous females were fed an artificial diet each day, and 200 ml of rearing water was exchanged daily with new water.

The larval hatching period extended from May to September, and newly hatched larvae (stage 1 zoeae) from two brooding females of each species were used for the larval culture experiments. Stage 1 zoeae from each brood were sampled, fixed with 5% neutral formalin for one day and then preserved in 70% ethanol; however, we accidentally released all of the stage 1 zoeae of *C. serratirostris* brood 1, and *C. typus* brood 1 hatched in 2016 before collecting the specimens. The carapace length of 10–15 specimens of each species-brood was measured from the posterior margin of the sessile eyes to the posterior end of the carapace (Nakahara *et al.*, 2007) using a microscope equipped with a digital camera and image analyzing system (Nikon Digital Sight and NIS-Elements software, Nikon Corp., Tokyo, Japan). The larval hatching date and carapace length of stage 1 zoeae of each species-brood are summarized in Table S1 in the electronic supplementary material.

**Phytoplankton and zooplankton foods**

We used euryhaline phytoplankton *Tetraselmis* sp. (cell diameter, ~10–12 μm) and euryhaline zooplankton, the rotifer *Brachionus pli-

*catilis* species complex (small-morphotype; body size, ~0.1–0.2 mm in loria length), which are effective foods in larviculture of various aquatic organisms (Muller-Feuga, 2000; Anger, 2001; Hagiwara *et al.*, 2007).

Two kinds of *Tetraselmis* sp. were used: one was commercial, as a condensed paste of *Tetraselmis* sp. stored at 4°C (preserved *Tetraselmis*) (*Tetraselmis* 3600®, Reed Mariculture Inc., Campbell, CA, USA), and the other was live *Tetraselmis* sp. cultured in our laboratory (cultured *Tetraselmis*). We selected preserved *Tetraselmis* as a potential food for culturing atyid larvae because of its easy availability. In our laboratory, *Tetraselmis* sp. were cultured in glass containers with seawater (salinity, 34 ppt; 24–26°C) supplemented with fertilizers for algal culture (KW21, Daiichi Seimo Co. Ltd., Kumamoto, Japan) under constant illumination conditions. *Tetraselmis* sp. are green unicellular algae with four distinct flagella. The algal cells of cultured *Tetraselmis* can move around freely in seawater, whereas those of preserved *Tetraselmis* were intact but nonviable.

The rotifers were cultured in plastic containers with seawater (salinity, 24 ppt; 23–24°C) supplied with the commercial, condensed freshwater phytoplankton *Chlorella vulgaris* (Super Chlorella V12, Chlorella Industry, Tokyo, Japan).

**Larval culture experiments**

Larvae were cultured under five different feeding conditions: 1) preserved *Tetraselmis* (PT), 2) cultured *Tetraselmis* (CT), 3) rotifers (R), 4) preserved *Tetraselmis* and rotifers (PTR), and 5) cultured *Tetraselmis* and rotifers (CTR). Thirty newly hatched larvae (stage 1 zoeae) were used for each feeding condition in each species brood, and larval culture experiments were conducted twice using larvae hatched from two different broods of each species. Some (1 or 2) zoeal larvae of *C. typus* brood 1 were lost accidentally during the culture operations, and these larvae were excluded.
Larvae were housed individually in the wells of six-well cell culture plates, which contained 8 ml artificial saline water in each well. *Tetraselmis* species were fed to larvae at $1 \times 10^5$ cells ml$^{-1}$ because Nakahara et al. (2005) revealed it as the best feeding density for larval survival of *C. leucosticta* and *C. typus*. Rotifers were given to larvae at 20 individuals ml$^{-1}$. The salinity of the larval rearing water was set at 17 ppt using artificial seawater salts (Sealife, Marinetech Co. Ltd., Tokyo, Japan) because saline water of $\sim$17 ppt could sustain larval survival to the juvenile stage in *C. leucosticta*, *C. multidentata*, and *C. typus* (Hayashi & Hamano, 1984; Nakahara et al., 2005). Using temperature-controlled incubation chambers with a photoperiod cycle of 14 h of light and 10 h of dark, the larval rearing temperature was set at 26°C, which is equivalent to the summer sea-surface temperature on the Pacific coasts of Japan during the reproductive season of atyid shrimp.

Each morning, larvae were transferred to clean culture wells with fresh saline water and food using a glass pipette, and the numbers of live and dead larvae were recorded under a stereomicroscope. The presence of an exuvia was also checked for each larva. Larval rearing was terminated when all surviving larvae had moulted to the juvenile stage. We observed later-stage larvae under the stereomicroscope and determined whether they moulted to the juvenile stage based on their external morphology described for the first juvenile stage of *C. multidentata* (Hayashi & Hamano, 1984) and other *Caridina* species (Nakahara et al., 2007). Additionally, larval behaviour as well as the external morphology was considered for determining the settlement stage for the larvae of *C. typus* brood 2; larvae were reared until they were able to steadily settle on the bottom of a rearing container using the endopods of the peraeopods and swim in the normal manner using pleopods for propulsion.

Surviving juveniles of each species-brood were fixed and preserved similar to the stage 1 zoea specimens, and the carapace length was measured from the posterior margin of the orbit to the posterior end of the carapace (Nakahara et al., 2007).

**Statistical analysis**

Statistical analyses were performed using R statistical software (R3.6.2; R Core Team, 2019) at a 5% significance level. A survival analysis was conducted to compare the survival curves among the different feeding conditions for each species with a log-rank test stratified by brood identity using the `survdiff` function implemented in the survival package (Therneau, 2020). A generalized linear mixed-effects model (GLMM) with a Poisson distribution was conducted to compare the number of days required to moult to the juvenile stage, i.e., larval duration (response variable) among the different feeding conditions (categorical explanatory variable) for each species, taking into account the inter-brood variability (Zuur et al., 2009). The larval growth, i.e., the carapace length of juveniles (response variable), was also compared among the different feeding conditions (categorical explanatory variable) for each species using a linear mixed-effects model (LMM). The statistical significance of the explanatory variable was evaluated using the `glmer` function (log link) (Bate et al., 2015) and the `Anova` function (type II Wald chi-square test) (Fox & Weisberg, 2011) in the GLMM analysis and the `lme` function and `anova` function (marginal type F-test) (Pinheiro et al., 2020) in the LMM analysis. In both analyses, the identity of the brood was included as a random intercept effect.

The difference in the number of moults during the larval stage among the different feeding conditions was not statistically tested because some exuviae of the larvae appeared to be missed during the culture operations (see the Results section).
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Results

Larval survival and duration

Similar larval performance was observed between the different broods of each species (Fig. 1). Survival analyses detected significant differences in the larval survival curves among the different feeding conditions for all species (C. leucosticta, $\chi^2 = 298.1$; C. multidentata, $\chi^2 = 237.0$; C. serratirostris, $\chi^2 = 35.00$; and C. typus, $\chi^2 = 253.5$; df = 4 and $P < 0.0001$ for all species) (Fig. 1).

In C. leucosticta, when the larvae were provided with the preserved Tetraselmis and/or rotifers under the PT, R and PTR conditions, only 10.0–30.0% of the larvae moulted 1–1.6 times on average, and many larvae died by approximately 13 days after hatching (Fig. 1A, B; see supplementary Table S2 for survival and moulting data of the larvae that died before
moult ing to the juvenile stage). The survival of *C. leucosticta* larvae was significantly improved when they were fed cultured *Tetraselmis* and rotifers under the CT and CTR conditions, as the survival rates to the juvenile stage reached 70.0–83.3% in the CT group and 83.3–93.3% in the CTR group (Fig. 1A, B; see supplementary Table S3 for survival, duration and growth data of larvae that survived to the juvenile stage). Survival rates slightly decreased from 17–23 days after hatching in larvae of the CT group compared with those of the CTR group, and larval durations to moult to the juvenile stage were significantly longer in the CT group (mean, 21.9–26.1 days) than in the CTR group (17.5–21.0 days) ($\chi^2 = 25.70, df = 1, P < 0.0001$) (Figs. 1A, B and 2A; Table S3).

In *C. multidentata*, under the PT, R and PTR conditions, 20.0–66.7% of the larvae moulted 1–1.3 times on average, but almost all died by approximately 16 days after hatching (Fig. 1C, D; Table S2). In the CTR group, *C. multidentata* larvae moulted to juveniles 21.4–24.7 days after hatching on average with high survival rates of 73.3–83.3% (Fig. 1C, D; Table S3). In the CT group, larvae showed high survival rates at over 90% until 14–17 days after hatching; then, larval mortality steadily increased, and 10.0–13.3% of the larvae moulted to the juvenile stage while showing significantly longer larval durations (mean, 31.7–59.0 days) than those of the CTR group (21.4–24.7 days) ($\chi^2 = 126.3, df = 1, P < 0.0001$) (Figs. 1C, D and 2A; Table S3). In the CT group, prolonged larval survival was observed in two cases: one was a larva that died 75 days after hatching in brood 1, and the other was a larva in brood 2 that moulted to the juvenile stage 95 days after hatching (Fig. 1C, D; Tables S2 and S3).

In *C. serratirostris*, the larvae never moulted, and all died by approximately 14 days after hatching under the PT, R and PTR conditions; however, 50.0–73.3% of the larvae moulted 1.3–1.8 times on average, but no larvae survived to the juvenile stage even under the CT and CTR conditions (Fig. 1E, F; Table S2). Larvae with exuvia during the moulting process were frequently observed to be attracted to the surface tension of the water in the rearing wells, and these larvae eventually died.

In *C. typus*, under the PT, R and PTR conditions, brood 1 larvae never moulted, and all died by approximately 10 days after hatching. In addition, 6.7–33.3% of brood 2 larvae moulted 1–1.1 times on average, but all died by approximately 13 days after hatching (Fig. 1G, H; Table S2). The CT and CTR conditions sustained larval survival to the juvenile stage; the difference in the survival rates between these conditions grew from 12–17 days after hatching, and the final survival rates were higher in the CTR group (46.4–76.7%) than in
the CT group (13.3–13.8%) (Fig. 1G, H; Table S3). Larval durations were significantly shorter in the CTR group (mean, 25.2–28.6 days) than in the CT group (27.3–40.0 days) ($\chi^2 = 10.64, df = 1, P = 0.0011$) (Figs. 1G, H and 2A; Table S3).

Larval growth and moulting

A significant difference was not found in the carapace length of the juveniles based on the feeding conditions for all species ($C. leucosticta$, $F_{1, 96} = 1.386, P = 0.2420$; $C. multidentata$, $F_{1, 51} = 0.915, P = 0.3433$; and $C. typus$, $F_{1, 41} = 0.489, P = 0.4884$) (Fig. 2B). The number of moults to the juvenile stage varied from 4–15 in $C. leucosticta$, 5–19 in $C. multidentata$, and 6–17 in $C. typus$ (Table S3), and the minimum moulting frequencies were lower than the number of zoeal stages reported for these species ($C. leucosticta$, 7–9, mainly 7; $C. multidentata$, 9; $C. typus$. 9) (Hayashi & Hamano, 1984; Nakahara et al., 2007), suggesting that some exuviae were missed during the culture operations. Nevertheless, the mean number of moults tended to be larger in larvae of the CT group than those of the CTR group for each species (broods 1–2) as follows: $C. leucosticta$, 9.5–11.2 and 7.6–8.7 times; $C. multidentata$, 9.3–12.8 and 7.3–7.8 times; and $C. typus$, 8.5–13.8 and 8.3–11.3 times in the CT and CTR groups, respectively (Table S3).

Discussion

Our larval culture experiments demonstrated that feeding conditions significantly affected the larval survival and duration for the four Caridina species (Figs. 1 and 2). Feeding cultured Tetraselmis and cultured Tetraselmis with rotifers to the larvae could sustain their survival and development to the juvenile stage in $C. leucosticta$, $C. multidentata$ and $C. typus$, and rotifer supplementation largely improved larval survival and accelerated larval developmental velocity. Intraspecific variability in the number of larval stages has been determined for decapod crustaceans and is especially common in caridean shrimps, and the causes of the variability have been attributed to genetic and maternal factors and to environmental stress, such as unfavourable salinities and temperatures and limited nutritional conditions (Knowlton, 1974; Gore, 1985; Anger, 2001; Zeng et al., 2004; Quinn, 2016). In the present study, the number of moults to the juvenile stage highly varied within and between feeding conditions (Table S3), and the number of moults tended to be smaller in larvae under good nutritional conditions supplemented with rotifers than under the other nutritional conditions, although the exuviae might have been missed for some larvae during the culture operations. In $C. typus$ with 9 zoal stages (Nakahara et al., 2007), the mean number of moults was larger in brood 2 (11.3–13.8 times) than brood 1 (8.3–8.5 times) because brood 2 larvae were cultured for relatively longer periods to exhibit steady settlement behaviour on the bottom of the rearing containers (Fig. 1G, H).

The larvae of $C. serratirostris$ could still moult when fed with cultured Tetraselmis and cultured Tetraselmis with rotifers (Table S2), but no survived to the juvenile stage (Fig. 1E, F) because the larvae died while being trapped by the surface tension of the rearing water during the moulting process. Nakahara et al. (2005) conducted a group culture of $C. serratirostris$ larvae by stocking 30 stage 1 zoeae in each of two glass beakers containing 200 ml of saline water without aeration (salinity, 25 ppt; 25–27°C) by supplying cultured Tetraselmis tetrathele at $1 \times 10^5$ cells ml$^{-1}$. They reported that larvae moulted to the juvenile stage 34–46 days after hatching at a mean survival rate of 53%. The larvae of the other three species were sometimes observed to be attracted to the water surface in the present study, but they could return to the water column in the rearing wells. Larval attraction to the surface tension of the water during the moulting process may be a
specific phenomenon in shallow water in small rearing containers, but the reason why C. serratirostris larvae are vulnerable to the surface tension of water remains unknown. To elucidate the effects of feeding conditions on the larval performance of C. serratirostris, relatively large containers with deeper water would be recommended for culturing the larvae.

In our larval culture experiments, among the two kinds of phytoplankton, only cultured Tetraselmis sustained larval survival and development to the juvenile stage in C. leucosticta, C. multidentata, and C. typus, even though early larvae appeared to ingest both preserved and cultured Tetraselmis since green microalgal substances were observed in the digestive tracts of the larvae under the stereomicroscope. Fatty acid content and composition in live food have been determined to largely affect larval survival, growth and development of aquatic organisms, including decapod crustaceans (Montaini et al., 1995; Anger, 2001; Tocher, 2010). Montaini et al. (1995) reported that the fatty acid profile of the condensed cells of Tetraselmis suecica preserved at 4°C remained unchanged over a storage time of 90 days. Nakahara et al. (2007) reported that the early larvae of Caridina species ingested phytoplankton via suspension feeding behaviour. If the nutritional values of the preserved and cultured Tetraselmis used in the present study were similar, then the efficiency of suspension feeding of cultured Tetraselmis by larvae might be enhanced by the voluntary movement of their live algal cells in larval rearing water, but the suspension feeding efficiency for preserved Tetraselmis might substantially decrease because of their nonviable cells, leading to nutritional deficiency in the larvae supplied with the preserved Tetraselmis.

Rotifers alone did not sustain larval survival and development, suggesting that at least early larvae could not ingest rotifers. Hayashi & Hamano (1984) reported that rotifers with green algae (mainly Chlorella) did not maintain the larvae of C. multidentata under healthy conditions. In the present study, supplementing rotifers with cultured Tetraselmis significantly improved larval survival and development from 12–23 days after hatching compared with supplementing cultured Tetraselmis alone (Fig. 1). These results indicate that larvae might be able to ingest rotifers well from the middle to late larval stages, as preliminarily observed by Nakahara et al. (2005). Additionally, Nakahara et al. (2007) documented that larvae of Caridina species begin to capture and ingest a lump of food organisms using developed endopods of the thoracic appendages. Ingesting larger rotifers (~0.1–0.2 mm) rather than smaller Tetraselmis sp. (~10–12 μm) should improve the feeding efficiency of larvae, resulting in a higher survival rate and faster developmental velocity of larvae (Figs. 1 and 2). Some stage 1 zoeae supplemented with rotifers alone could moult in some species-broods (Table S2). It has been reported that C. multidentata larvae survive to juveniles by feeding on detritus made of an artificial diet for freshwater fish and rice bran (Hayashi & Hamano, 1984). Therefore, these stage 1 zoeae might take detritus, such as organic materials carried in the rearing water with rotifers and/or the faeces of rotifers. This might be supported by the occurrence of a few longer surviving larvae under the feeding condition with preserved Tetraselmis supplemented with rotifers in C. leucosticta brood 1 (Fig. 1A) and C. multidentata brood 2 (Fig. 1D). Rotifers can be cultured and reproduced by feeding Tetraselmis sp. (Hagiwara et al., 2007).

The moulting rates of the C. multidentata larvae were higher than those of the other species under the limited feeding conditions with preserved Tetraselmis and/or rotifers (Table S2). This result might be because some newly hatched larvae of C. multidentata could moult once, but those of C. leucosticta, C. serratirostris, and C. typus never moulted under the starved conditions in saline water (salinity, 17
ppt; 26°C) (Hamasaki et al., unpublished data). Additionally, Hayashi & Hamano (1984) reported that *C. multidentata* larvae began to take food as stage 3 zoeae in saline water (salinity, ~17 ppt; 25°C). Thus, early zoeae of *C. multidentata* are potentially lecithotrophic, and those of other *Caridina* species are planktrotrophic. In general, *C. leucosticta*, *C. serratoriostris*, and *C. typus* are abundant in lower reaches with riverbank vegetation, whereas *C. multidentata* inhabits middle and upper reaches with boulders (Saito et al., 2012). It has been suggested that the lecithotrophy stage of newly hatched larvae of decapod crustaceans is adapted to an insufficient, unpredictable, or seasonally short production of food (Shokita, 1979; Anger, 2001, 2006; Anger & Hayd, 2010). Potential lecithotrophy of the early larvae of *C. multidentata* might be an adaptation to the downstream trip in the freshwater environment with limited food conditions.

Although the survival and development of larvae of *C. leucosticta*, *C. multidentata* and *C. typus* were significantly improved by feeding them cultured *Tetraselmis* supplemented with rotifers, interspecific variation was observed in the larval performance under the different feeding conditions. The supplementation effects of rotifers on larval survival were quite large in *C. multidentata* and *C. typus* compared with those in *C. leucosticta*, and the larvae of *C. leucosticta* showed higher survival rates even when they were fed cultured *Tetraselmis* alone (Fig. 1). Thus, *C. leucosticta* larvae may have a high ability to survive to the juvenile stage in the eutrophic environment in the brackish waters of estuaries and coastal bays, where primary production is high and phytoplankton biomass is much higher than microzooplankton biomass (Uye et al., 1999; Ara & Hiromi, 2009). In our larval culture experiments, the larvae of *C. multidentata* could prolong the larval duration when they were fed cultured *Tetraselmis* alone, and the larval duration of *C. typus* brood 1 tended to be longer than that of *C. leucosticta* (Figs. 1 and 2). Consequently, larval performance under the different feeding conditions may suggest that *C. leucosticta* larvae develop within estuaries and coastal bays, *C. multidentata* has the ability to disperse broadly in the sea, and *C. typus* exhibits broader larval dispersal than *C. leucosticta*.

Fujita et al. (2016) conducted phylogeographic studies for *C. leucosticta*, *C. multidentata* and *C. typus* to compare their genetic population structures and to predict their marine larval dispersal patterns based on the sequences of the mitochondrial DNA cytochrome c oxidase subunit I gene and the control region. They suggested that *C. leucosticta* larvae develop near river mouths because this species had a genetically heterogeneous population structure, whereas larvae of *C. multidentata* and *C. typus* disperse in the oceanic environment because these species exhibited homogeneous population structures. Based on the haplotype network patterns, genetic diversity and population size, Fujita et al. (2016) suggested that larval dispersal ability is high in *C. multidentata*, followed by that in *C. typus*, and is low in *C. leucosticta*. Thus, marine larval dispersal patterns of the three *Caridina* species inferred from the larval culture experiments and comparative phylogeographic studies were well matched.

The number of days required to moult to the juvenile stage highly varied under the different feeding conditions, but the feeding conditions did not significantly affect the larval growth of *C. leucosticta*, *C. multidentata* and *C. typus* (Fig. 2). After completing a pelagic larval life stage and recruiting to the mouth of a coastal river or stream, atyid juveniles go through an additional stage, i.e., they must migrate up to the adult freshwater habitat (Hamano & Hayashi, 1992; Hamano et al., 2005; Bauer, 2013; Yatsuya et al., 2013). Juveniles may require a species-specific physical status to accomplish migration to the adult habitat. Therefore, the body size of juveniles did not vary under dif-
different nutritional conditions, and larvae required relatively long durations to reach the threshold size at the juvenile stage of each species under limited nutritional conditions. The body sizes of *C. multidentata* juveniles were larger than those of the other species (Fig. 2B). *C. multidentata* mainly inhabits the middle and upper reaches (Hamano & Hayashi, 1992; Saito *et al.*, 2012), and the larger body size of the juveniles might be advantageous for completing the long trip to reach the adult habitat while climbing over vertical walls (Hamano & Hayashi, 1992).

The present study highlighted the interspecific variability in the larval performance under the different feeding conditions for amphidromous atyid shrimp species, *C. leucosticta*, *C. multidentata*, and *C. typus*. To further understand the early life history traits of atyid shrimp, larval culture techniques should be improved for *C. serratirostris*, and an investigation of dietary effects of phytoplankton and zooplankton on larval performance will be required for other atyid shrimp species.

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