Larval performance of three amphidromous shrimp species in the genus *Caridina* (Decapoda: Caridea: Atyidae) under different temperature and salinity conditions

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**Abstract.**—We examined the effects of temperature and salinity on larval survival, duration, and growth of three amphidromous atyid shrimps, *Caridina leucosticta*, *C. multidentata*, and *C. typus* to infer larval dispersal strategy in the sea. Larvae were reared under 25 combinations of five different temperature (20, 23, 26, 29, and 32°C) and salinity (4.25, 8.5, 17, 25.5, and 34 ppt) levels. Interspecific variability was detected in larval performance: *C. leucosticta* larvae were able to survive to moult into the juvenile stage at the lower salinity condition (8.5 ppt), *C. multidentata* larvae exhibited an ability to adapt to the wide range of salinity condition (17–34 ppt), and *C. typus* larvae adapted to the higher salinity condition (34 ppt) better than *C. leucosticta* larvae. Larval duration was less variable between species. Thus, salinity adaptation of larvae may play an important role in mediating the larval dispersal of the three *Caridina* species in the sea. Larval dispersal range may be most limited near the river mouth for *C. leucosticta*, and be moderate for *C. typus*, and *C. multidentata* larvae may be able to disperse broadly under the high salinity condition of the open sea.

**Key words:** Larval survival, larval growth, larval duration, larval dispersal strategy

**Electronic supplementary material.** The online version of this article contains supplementary material at https://www.jstage.jst.go.jp/article/crustacea/50/0/50_41/_article

**Introduction**

Freshwater shrimps in the family Atyidae are the most diversified decapod crustaceans (Decapoda: Caridea) that inhabit various freshwater bodies from fast-moving mountain streams to sluggish, oligohaline waters (De Grave *et al*., 2008; De Grave & Fransen, 2011). Atyid shrimps play important roles in stream food webs as primary consumers and food sources for predators (Pringle *et al*., 1993; Covich & McDowell, 1996; Covich *et al*., 1999; Crowl *et al*., 2001; Oeding *et al*., 2020).

Atyid shrimps exhibit one of two life history traits: landlocked or amphidromous (Shokita, 1979; Hayashi & Hamano, 1984; Bauer, 2013). Landlocked species complete their entire life-cycle within freshwater bodies, whereas larvae of amphidromous species require saline water for successful development (Shokita, 1979; Hayashi & Hamano, 1984; Nakahara *et al*., 2005). Newly hatched larvae (stage 1 zoeae) of amphidromous shrimps passively drift from freshwater environments to the sea, 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, 2007; Hamano *et al*., 2005; 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 (Hay-
mano & Hayashi, 1992; Hamano et al., 2005; Bauer, 2013; Yatsuya et al., 2013). Accordingly, amphidromous shrimp populations are connected through marine larval dispersal (Shokita, 1979; Bauer, 2013; Fujita et al., 2016).

Laboratory culture experiments have revealed that temperature and salinity are the most important environmental factors affecting larval survival, duration, and growth of decapod crustaceans (Anger, 2001, 2003). Both seawater temperature and salinity fluctuate temporally and spatially in natural environments. Therefore, information on larval performance under different temperature and salinity conditions is essential for a better understanding of population connectivity through marine larval dispersal in amphidromous shrimp species. However, the influence of temperature and salinity on the larval performance of amphidromous atyid shrimps remains largely unknown. To date, larval culture experiments at various salinity levels under particular temperatures have been conducted for several amphidromous atyid shrimp species (Hayashi & Hamano, 1984; Nakahara et al., 2005; Kawahara et al., 2019), and only a laboratory culture using larvae of *Caridina gracilirostris* De Man, 1892 has been performed under different temperature and salinity conditions (Heerbrandt & Lin, 2006).

The objective of this study was to evaluate the effects of temperature and salinity on larval survival, duration, and growth of three amphidromous atyid shrimp species in the genus *Caridina* H. Milne-Edwards, 1837. Specifically, we examined *C. leucosticta* Stimpson, 1860, *C. multidentata* Stimpson, 1860, and *C. typus* H. Milne-Edwards, 1837, all of which are commonly occurring species in western and southern Japan (Shokita, 1979; Hamano & Hayashi, 1992; Suzuki et al., 1993; Usami et al., 2008; Saito et al., 2012). Our results highlight the variability in the larval performance of *Caridina* shrimps under different temperature and salinity conditions, and ecological implications are 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 2017 to 2019. Wild mature female and male shrimps were captured using scoop nets at the following locations from May to June: *C. leucosticta* in the Heda Okawa River (34°58′N, 138°47′E), Shizuoka Prefecture; *C. multidentata* and *C. typus* in the Banda River (34°58′N, 139°46′E), Chiba Prefecture, Japan.

Shrimps were cultured in aerated aquaria (23 l volume) (30–40 shrimps per tank, unknown sex ratio) under natural photoperiod conditions at approximately 23°C, according to the method detailed in our previous study (Hamasaki et al., 2020a). Larval culture experiment was conducted once for *C. leucosticta* and *C. multidentata* using newly hatched larvae (stage 1 zoeae) from single females cultured in 2018, and twice for *C. typus* using those from single females cultured in 2017 and 2019.

Stage 1 zoeae from each species-brood were sampled, fixed with 5% neutral formalin for one day, and then preserved in 70% ethanol. The carapace length of 10 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 analysing 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 1.

#### Experimental temperature and salinity conditions

We evaluated the larval performance of the three *Caridina* species under the 25 combina-
lations of five different temperature (20, 23, 26, 29, and 32°C) and salinity (4.25, 8.5, 17, 25.5, and 34 ppt) levels. Temperature levels were selected according to the seawater temperature profiles (∼20–31°C) around the Japanese coastal areas (Japan Meteorological Agency; https://www.data.jma.go.jp/gmd/kaiyou/data/db/kaikyo/jun/sst_HQ.html) during the reproductive season of the Caridina shrimps (Shokita, 1979; Hamano & Hayashi, 1992; Ideguchi et al., 2000; Hamano et al., 2005; Yamahira et al., 2007; Yatsuya et al., 2013). Salinity levels were set assuming the salinity profiles from the river mouth to the open sea where Caridina larvae may develop (Ideguchi et al., 2000; Yatsuya et al., 2013; Urakawa et al., 2015).

Larvae were cultured using temperature-controlled incubation chambers with a 14 h light and 10 h dark photoperiod cycle (MT1–201, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). Larval rearing water with different salinities was prepared using dechlorinated tap water and artificial seawater salts (Sealife, Marinetech Co. Ltd., Tokyo, Japan). Rearing temperatures were recorded every 30 min during larval culture periods using data loggers (Thermochron SL, KN Laboratories Co. Ltd., Osaka, Japan). The mean ± standard deviation values of larval rearing temperatures for each species-brood are summarized in Table 1. Larval rearing temperatures of each species were referred to as 20, 23, 26, 29, and 32°C for convenience.

### Table 1. Hatching date and carapace length (CL) of stage 1 zoeae of three atyid shrimps in the genus *Caridina*, and actual temperature measurements in designated temperature groups for rearing the larvae at 20, 23, 26, 29, and 32°C. Values in upper and lower lines indicate the means and standard deviations for each species-brood. Number of larvae measured for the CL was 10 in each species-brood.

| Species    | Hatching date | CL (mm)       | Culture temperature groups (°C) | 20 | 23 | 26 | 29 | 32 |
|------------|---------------|---------------|---------------------------------|----|----|----|----|----|
| *C. leucosticta* | August 14, 2018 | 0.238         | 20.1                            | 23.1| 26.3| 29.3| 33.0|    |
|             |               | 0.015         | 0.2                             | 0.3 | 0.2 | 0.3 | 0.4 |    |
| *C. multidentata* | June 25, 2018  | 0.380         | 20.1                            | 22.9| 26.4| 29.2| 32.3|    |
|             |               | 0.052         | 0.2                             | 0.3 | 0.2 | 0.2 | 0.3 |    |
| *C. typus* | August 23, 2017 | 0.276         | 20.3                            | 22.6| 26.1| 29.6| 32.8|    |
|             |               | 0.018         | 0.5                             | 0.3 | 0.3 | 0.3 | 0.3 |    |
|             | August 15, 2019 | 0.293         | 20.2                            | 23.5| 26.5| 29.5| 32.7|    |
|             |               | 0.015         | 0.3                             | 0.3 | 0.2 | 0.2 | 0.3 |    |

Larvae were housed individually in the wells of six-well cell culture plates, which contained 8 ml of artificial saline water in each well. Larvae were fed euryhaline phytoplankton *Tetraselmis* sp. at $1 \times 10^5$ cells ml$^{-1}$ and euryhaline zooplankton, the rotifer *Brachionus plicatilis* species complex (small-morphotype) at 20 individuals ml$^{-1}$, which are effective foods for culturing larvae of atyid shrimps (Hamasaki et al., 2020a, 2020b). *Tetraselmis* sp. and rotifers were cultured according to the methods detailed in our previous studies (Hamasaki et al., Crustacean Research 50).
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. 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 behaviour as well as the external morphology. Larvae were considered as having reached the juvenile stage when they were able to steadily settle on the bottom of a rearing container using their endopods of the pereiopods and swim in the normal manner using pleopods for propulsion, while showing the morphological characteristics that equalled or advanced those described for the first juvenile stage of *C. multidentata* (Hayashi & Hamano, 1984) and other *Caridina* species (Nakahara et al., 2007). The final survival rate of larvae was defined for each temperature-salinity combination in each species as: (number of larvae that moulted into the juvenile stage) / (number of initial larvae) × 100.

The number of zoeal stages was reported for the three *Caridina* species as follows: *C. leucosticta*, 7–9, mainly 7; *C. multidentata*, 9; and *C. typus*, 9 (Hayashi & Hamano, 1984; Nakahara et al., 2007). However, the number of moults to the juvenile stage highly varied within the species, and fragile exuviae of small larvae were easily lost or overlooked during the culture operations in our previous culture experiments using atyid shrimp larvae (Hamasaki et al., 2020a, 2020b). Therefore, the moulting events were not considered as larval performance in the present study.

Surviving juveniles of each species 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). One specimen of *C. leucosticta* reared at 26°C-17 ppt and one of *C. typus* reared at 23°C-25.5 ppt were not measured because of damage to their carapaces.

**Statistical analysis**

Statistical analyses were performed for each species using R statistical software (R4.0.2; R Core Team, 2020) at a 5% significance level. Data for *C. typus* larvae cultured in 2017 and 2019 were combined for subsequent analysis.

We used a generalized linear model (GLM) with a binomial distribution to evaluate the influences of temperature and salinity on larval survival to moult into the juvenile stage, i.e. the binary survival (1) or death (0) (response variable). Effects of temperature and salinity on larval duration, i.e. the number of days required to moult into the juvenile stage and larval growth, i.e. the carapace length of juveniles (response variables) were also evaluated using the Poisson-GLM and a general linear model (LM), respectively.

In the GLM and LM analyses, we applied four models of which continuous explanatory variables were comprised of mean temperature (*T*) and salinity (*S*) for culturing larvae, or their interaction term (*T* × *S*) and/or quadratic terms (*T*² and *S*²), considering their interactions and nonlinear effects: model 1, \( y \sim T + S + T^2 + S^2 + T \times S \); model 2, \( y \sim T + S + T^2 + S^2 \); model 3, \( y \sim T + S + T \times S \); and model 4, \( y \sim T + S \). The model with the lowest Bayesian Information Criterion (BIC) value (Schwarz, 1978) was selected as the most suitable to describe the effects of temperature and salinity on larval performance in each species. The coefficients of the models were estimated, and their statistical significance was evaluated using the *glm* function for binomial (logit link) and Poisson (log link) GLM analyses and the *lm* function for LM analyses.

We generated the response-surface contour plots of larval survival rate and duration using the *counter* function based on the calculations derived by substituting the designated tempera-
ture (18–35°C, 0.5°C interval) and salinity (4–36 ppt, 1 ppt interval) combinations into the selected model equations of respective species (model 2). For interspecific comparisons of larval survival on an identical scale, response-surface contour plots were generated based on the relative survival rates against the highest survival rate (derived from model 2) in each species. Response-surface contour plots were not generated for the carapace length of juveniles because model 4 (containing only linear terms) was selected for all species and the effects of temperature and salinity on larval growth could be easily inferred from the coefficients of the linear terms. For interspecific comparisons of temperature and salinity adaptations, the temperature and salinity ranges showing the relative survival rates ≥ 50% and ≥ 90%, and the optimum temperature and salinity combination exhibiting the highest survival rate and larval duration under these conditions were estimated for each species based on the calculations for generating the response-surface contour plots.

■ Results

Larval survival

The relationship between temperature and final survival rate of larvae at designated salinity levels and that between salinity and final survival rate of larvae at designated temperature levels tended to be represented by a convex curve in each species (Fig. 1). Among the four models applied to evaluate the effects of temperature and salinity on larval survival, model 2 \( (\upsilon - T + S + T^2 + S^2) \) was selected as the best for all species and all the coefficients of linear and quadratic terms were statistically significant (Table 2).

Larvae of *C. leucosticta* survived to the juvenile stage at 20–29°C and 8.5–34 ppt with higher survival rates at 23–26°C and 17–25.5 ppt (Fig. 1A). Larvae of *C. multidentata* survived to the juvenile stage at 20–32°C and 17–34 ppt with higher survival rates at 23–29°C and 17–25.5 ppt, and they also showed high survival rates at 34 ppt under 26–29°C conditions (Fig. 1B). Although overall survival rates tended to be lower in larvae of *C. typus* than in those of other species (Fig. 1), larval survival response to temperature and salinity was found to be similar to *C. multidentata*: *C. typus* larvae survived to the juvenile stage at 20–29°C and 17–34 ppt with higher survival rates at 23–26°C and 17–25.5 or 34 ppt (Fig. 1C).

Larvae of *C. leucosticta* were thus able to survive to moult into the juvenile stage at the
Table 2. Coefficient estimates with standard errors (SE) for the explanatory variables in the generalized linear model (GLM) or general linear model (LM) to best describe the effects of temperature (T) and salinity (S) on larval survival (alive or not) and duration (days) to reach the juvenile stage, and carapace length (mm) of juveniles of three atyid shrimps in the genus *Caridina*. Four models were applied in the binomial and Poisson GLM analyses for larval survival and duration, respectively, and those in the LM analysis for the carapace length: model 1, \(y \sim T + S + T^2 + S^2 + T \times S\); model 2, \(y \sim T + S + T^2 + S^2\); model 3, \(y \sim T + S + T \times S\); and model 4, \(y \sim T + S\). The model with the lowest Bayesian Information Criterion (BIC) value was selected as the best for each larval performance in each species. Model 2 was selected for larval survival and duration and model 4 was selected for carapace length in all species. See supplementary Tables S1, S2, and S3 for the coefficient estimates and BIC values of all models for *C. leucosticta*, *C. multidentata*, and *C. typus* respectively.

| Species         | Coefficient | Larval survival | | Larval duration | | Carapace length | |
|-----------------|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 |             | Estimate (SE) | SE | z value | P      | Estimate (SE) | SE | z value | P      | Estimate (SE) | SE | t value | P      |
| *C. leucosticta*| Intercept   | -77.6950 (13.4566) | -5.774 | <0.0001 | 16.9972 (1.4984) | 11.343 | <0.0001 | 1.0633 (0.0463) | 22.985 | <0.0001 |
|                 | T           | 5.5994 (1.0435) | 5.366 | <0.0001 | -1.0279 (0.1854) | -8.482 | <0.0001 | -0.0094 (0.0018) | -5.254 | <0.0001 |
|                 | S           | 1.0804 (0.1317) | 8.204 | <0.0001 | 0.0012 (0.0009) | 0.066 | 0.9470 | -0.0019 (0.0009) | -2.250 | 0.0271 |
|                 | T^2         | -0.1118 (0.0208) | -5.365 | <0.0001 | 0.0184 (0.0025) | 7.447 | <0.0001 | 0.0001 (0.0005) | 0.280 | 0.7790 |
|                 | S^2         | -0.0266 (0.0032) | -8.192 | <0.0001 | 0.0001 (0.0005) | 0.280 | 0.7790 | 0.0001 (0.0005) | 0.280 | 0.7790 |
| *C. multidentata*| Intercept   | -73.2582 (11.7709) | -6.224 | <0.0001 | 14.9307 (0.9279) | 16.090 | <0.0001 | 1.4985 (0.0454) | 33.037 | <0.0001 |
|                 | T           | 4.5397 (0.8351) | 5.436 | <0.0001 | -0.7368 (0.0676) | -10.897 | <0.0001 | -0.0091 (0.0016) | -5.827 | <0.0001 |
|                 | S           | 1.5566 (0.2153) | 7.228 | <0.0001 | -0.1182 (0.0273) | -4.330 | <0.0001 | -0.0029 (0.0009) | -3.107 | 0.0023 |
|                 | T^2         | -0.0867 (0.0159) | -5.442 | <0.0001 | 0.0126 (0.0013) | 9.556 | <0.0001 | 0.0001 (0.0005) | 0.280 | 0.7790 |
|                 | S^2         | -0.0327 (0.0045) | -7.296 | <0.0001 | 0.0023 (0.0005) | 4.247 | <0.0001 | 0.0001 (0.0005) | 0.280 | 0.7790 |
| *C. typus*      | Intercept   | -69.8081 (10.6700) | -6.542 | <0.0001 | 20.9492 (1.7929) | 11.685 | <0.0001 | 1.0221 (0.1023) | 9.990 | <0.0001 |
|                 | T           | 4.6462 (0.7981) | 5.821 | <0.0001 | -1.2096 (0.1327) | -9.117 | <0.0001 | -0.0048 (0.0035) | -1.397 | 0.1670 |
|                 | S           | 1.0282 (0.1897) | 5.420 | <0.0001 | -0.0774 (0.0340) | -2.280 | 0.0226 | 0.0013 (0.0016) | 0.826 | 0.4120 |
|                 | T^2         | -0.0927 (0.0158) | -5.855 | <0.0001 | 0.0216 (0.0027) | 8.067 | <0.0001 | 0.0001 (0.0007) | 1.767 | 0.0773 |
|                 | S^2         | -0.0206 (0.0038) | -5.443 | <0.0001 | 0.0012 (0.0007) | 1.767 | 0.0773 |
lower salinity condition (8.5 ppt) whereas those of *C. multidentata* survived to the juvenile stage at the higher temperature level (32°C). Increased survival ability in the lower salinity conditions by *C. leucosticta* larvae could also be seen in the survival curves in relation to days after hatching of the larvae reared at 4.25 ppt. The number of days until all larvae died was greater, specifically 11–37 days in *C. leucosticta* compared to 5–13 days in *C. multidentata* and 7–12 days in *C. typus* (see supplementary Figs. S1–S3).

Accordingly, the larval survival response to temperature and salinity conditions varied in different *Caridina* species, and the responsesurface contour plots well illustrated the interspecific variability in larval survival (Fig. 2). The temperature and salinity ranges showing the relative survival rates ≥ 50% (1) and ≥ 90% (2), and the optimum temperature and salinity combination exhibiting the highest survival rate (3) were estimated as follows: *C. leucosticta*, (1) 19.5–30.5°C and 9–31 ppt, (2) 21.5–28.5°C and 13–27 ppt, and (3) 25°C and 20 ppt; *C. multidentata*, (1) 19–33.5°C and 12–35 ppt, (2) 21–31.5°C and 15–32 ppt, and (3) 26°C and 24 ppt; and *C. typus*, (1) 21–29°C and 16–34 ppt, (2) 23–27°C and 21–29 ppt, and (3) 25°C and 25 ppt.

**Larval duration**

Larval duration tended to decrease with increasing temperature in all species, and species-specific changes of larval duration in relation to salinity levels were observed (Fig. 3). Model 2 \( y = T + S + T^2 + S^2 \) was selected as the best to describe the effects of temperature and salinity on larval duration for all species (Table 2), and all coefficient estimates of the linear and quadratic terms of temperature were statistically significant.

In *C. leucosticta*, larval duration tended to increase with increasing salinity (Fig. 3A), but the coefficients of linear and quadratic terms of salinity were not statistically significant (Table...
In *C. multidentata*, the relationship between larval duration and salinity tended to be represented by a convex curve (Fig. 3B), and the coefficients of linear and quadratic terms of salinity were statistically significant (Table 2). In *C. typus*, larval duration tended to decrease with increasing salinity (Fig. 3C), and the coefficient of the linear term of salinity was statistically significant (Table 2).

Interspecific variability was thus observed in the relationships between salinity and larval duration.

**Fig. 3.** Number of days required to moult into the juvenile stage (larval duration) of *Caridina leucosticta* (A), *C. multidentata* (B), and *C. typus* (C). Larvae were reared under the 25 combinations of five different temperature (20, 23, 26, 29, and 32°C) and salinity (4.25, 8.5, 17, 25.5, and 34 ppt) levels. No larvae survived to the juvenile stage at 4.25 ppt. Bars and vertical lines indicate mean and standard deviation values, respectively. Numbers of individuals are shown above the bars. See Table 1 for the actual rearing temperature (mean and standard deviation values) for each species.

**Fig. 4.** Response-surface contour plots of larval duration at different temperature and salinity combinations in *Caridina leucosticta* (A), *C. multidentata* (B), and *C. typus* (C). Contour plots were generated using the larval duration calculated from the model 2 equation that best described the effects of temperature and salinity on larval duration to moult into the juvenile stage in each species. See Table 2 for the equations of respective species. Viable environments for larvae are represented by the three different grey shade areas, indicating the temperature and salinity combinations at relative larval survival rates ≥10%, ≥ 50%, and ≥ 90%, respectively. The black circles indicate the point estimate of temperature and salinity combination at the highest larval survival rate. See Fig. 2 for the surface-response contour plots of larval survival rates.
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duration, but larval duration varied less between salinity levels under the identical temperature level in all species (Fig. 3). Larval duration under the temperature and salinity ranges showing the relative survival rates ≥ 50% (1) and ≥ 90% (2), and the optimum temperature and salinity combination exhibiting the highest survival rate (3) were estimated based on the response-surface contour plots (Fig. 4) as follows: *C. leucosticta*, (1) 15–56 days, (2) 15–33 days, and (3) 18 days; *C. multidentata*, (1) 15–55 days, (2) 14–35 days, and (3) 16 days; and *C. typus*, (1) 16–51 days, (2) 17–29 days, and (3) 20 days.

**Larval growth**

Although the carapace length of juveniles varied less between the different temperature and salinity levels (Fig. 5), model 4 ($y = 7 + S$) was selected as the most suitable to describe the effects of temperature and salinity on larval growth in all species (Table 2). Significant negative effects of temperature and salinity on larval growth were detected in *C. leucosticta* and *C. multidentata*, but not in *C. typus* (Table 2).

**Discussion**

The present study highlights the interspecific variability in the larval performance under a range of temperature and salinity conditions for amphidromous atyid shrimp, *C. leucosticta*, *C. multidentata*, and *C. typus* (Figs. 1 and 2). *C. leucosticta* larvae best adapted to the lower salinity conditions, *C. multidentata* larvae exhibited an ability to adapt to the wide range of temperature and salinity environments, and *C. typus* larvae adapted to the higher salinity condition better than *C. leucosticta* larvae (Fig. 2).

A few studies have examined the effects of salinity on larval survival of *C. leucosticta*, *C. multidentata*, and *C. typus* (Hayashi & Hamano, 1984; Nakahara et al., 2005). Hayashi & Hamano (1984) reared *C. multidentata* larvae by feeding detritus made of an artificial diet for freshwater fish and rice bran under five different salinity levels (0, 8.5, 17, 25.5, and 34 ppt) at 25°C. They reported survival rates to the juvenile stage as 0, 0, 66.7, 11.1, and 11.1% at the respective salinity levels. Nakahara et al. (2005) reared *C. multidentata* larvae by feeding *Tetraselmis tetrathele* under five different salinity levels (0, 8.5, 17, 25.5, and 34 ppt) at 25–27°C. They reported mean survival rates to the juvenile stage as 0, 0, 94.4, 3.3, and 14.4% for *C. leucosticta* and 0, 0,
22.3, 37.8, and 26.7% for *C. typus*, respectively. Larval survival responses to different salinity conditions in *C. leucosticta* and *C. typus* examined by Nakahara *et al.* (2005) were equivalent to those in the present study. Larvae of *C. multidentata* reared by Hayashi & Hamano (1984), however, showed lower survival rates at higher salinity levels compared with those in the present study. The appropriate feeding conditions for culturing *Caridina* larvae (Hamasaki *et al.*, 2020a) employed in the present study might have improved the larval performance of *C. multidentata*. To the best of our knowledge, only a laboratory culture has been conducted for *C. gracilirostris* under several temperature (24, 27, and 30°C) and salinity (10, 15, and 20 ppt) combinations and the optimum conditions for larval survival has been determined as 27°C and 15 ppt (Heerbrandt & Lin, 2006) similar to those for *C. leucosticta*.

Larvae of amphidromous shrimp develop in the brackish waters of estuaries and coastal bays or in the open sea (Shokita, 1979; Hayashi & Hamano, 1984; Ideguchi *et al.*, 2000, 2007; Hamano *et al.*, 2005; Bauer, 2013; Yatsuya *et al.*, 2013). It has been suggested that amphidromous shrimp species requiring lower salinities for larval development exhibit restricted larval dispersal, and vice versa (Shokita, 1979; Fujita *et al.*, 2016). Considering the different larval survival responses to salinities demonstrated for the three *Caridina* species in the present study, larval dispersal range may be most limited in *C. leucosticta* and moderate in *C. typus*, whereas *C. multidentata* larvae may be able to disperse broadly under the high salinity condition of the open sea.

Hamasaki *et al.* (2020a) reared larvae of *C. leucosticta, C. multidentata, and C. typus* under different feeding conditions using *Tetraselmis* and/or rotifers. They reported that *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) because the larvae of *C. leucosticta* showed higher survival rates than those of other species even when they were fed *Tetraselmis* alone. Thus, the salinity adaptation (demonstrated in the present study) as well as feeding habits (Hamasaki *et al.*, 2020a) of *C. leucosticta* larvae suggest limited larval dispersal in the brackish waters of estuaries and coastal bays near the river mouth.

Direct observation of larval dispersal in the sea is difficult. Therefore, genetic population structures of decapod crustaceans have been studied for evaluating the population connectivity through larval dispersal in the sea, based on the assumption that if larval dispersal is high, the species will have a genetically homogenous population structure (e.g. Weber *et al.*, 2000; Domingues *et al.*, 2010; Laurenzano *et al.*, 2013; Fujita *et al.*, 2016; Hamasaki *et al.*, 2017). The marine larval dispersal patterns of the three *Caridina* species inferred from the larval culture experiments conducted by the present study and Hamasaki *et al.* (2020a) are supported by the comparative phylogeographic studies performed by Fujita *et al.* (2016). Fujita *et al.* (2016) collected *C. leucosticta, C. multidentata,* and *C. typus* specimens throughout their distribution range in Japan and sequenced the mitochondrial DNA cytochrome c oxidase subunit I gene and the control region. Considering 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 of *C. typus*, and is low in *C. leucosticta*.

The reproductive season of *Caridina* species extends from spring to late summer or early autumn in their distributional areas in Japan (Shokita, 1979; Hamano & Hayashi, 1992; Ideguchi *et al.*, 2000; Hamano *et al.*, 2005; Yamahira *et al.*, 2007; Yatsuya *et al.*, 2013). Seawater areas with appropriate temperatures for the larval survival of the three *Caridina* species (>
~21°C) exist broadly during the reproductive season around the Ryukyu Archipelago in southern Japan and the Pacific and the Sea of Japan in western Japan, the primary habitats of Caridina species (Japan Meteorological Agency; https://www.data.jma.go.jp/gmd/kaiyou/data/db/kaikyo/jun/sst_HQ.html).

Pelagic larval duration is an indicator of dispersal potential in marine organisms (Anger, 2001; Shanks, 2009). The present study revealed that temperature largely influenced larval durations of the three Caridina species, but salinity less affected them (Fig. 3) as is generally known for decapod crustaceans (Anger, 2001, 2003). Larval duration under the temperature and salinity combinations exhibiting the relative survival rates ≥ 50% and ≥ 90% was estimated as 15–56 days and 15–33 days for C. leucosticta, 15–55 days and 14–35 days for C. multidentata, and 16–51 days and 17–29 days for C. typus, respectively (Fig. 4). Thus, interspecific variability in the larval duration was small, suggesting that interspecific variability in the larval survival response to salinities is responsible for the larval dispersal range of the Caridina species in the sea. It should be noted, however, that the larvae of Caridina species, particularly C. multidentata, could prolong the larval duration to moult into the juvenile stage under limited nutritional conditions (Hamasaki et al., 2020a).

Larval growth was negatively influenced by temperature and salinity in C. leucosticta and C. multidentata, but not in C. typus (Fig. 5; Table 2). Overall, however, the carapace length of juveniles varied less under the different temperature and salinity conditions in each species. 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, it is considered that Caridina juveniles did not exhibit the intraspecific variation in body size.

Our results suggest that larval adaptations to salinities may mediate the larval dispersal range in the sea, and that seawater areas with appropriate temperatures for larval survival and development expand broadly during the reproductive season of the three Caridina species in Japan. Sea-surface temperature, however, has risen in the whole of Japan due to global warming (Japan Meteorological Agency; http://www.data.jma.go.jp/cpdinfo/index_temp.html). The expanding seawater areas with temperatures in excess of 30°C in summer may negatively affect larval survival and development, particularly for C. leucosticta and C. typus. It should be noted, however, that the highest setting temperature (32°C) in the present study might near the upper lethal temperature of larvae of the Caridina species, and larvae of C. leucosticta and C. typus might have died under the rearing temperatures (32.7–33.0°C) just above the lethal temperature, whereas larvae of C. multidentata could have survived to the juvenile stage under the rearing temperature (32.3°C) just below the lethal temperature. Further studies will be required to elucidate the upper thermal adaptation of larvae of the three Caridina species for evaluating the population extinction risk through changes in larval performance due to global warming effects.

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