水生動物 第2024巻
令和6年3月

Effects of temperature on reproduction of the atyid shrimp species *Caridina multidentata* and *Caridina typus* (Decapoda: Caridea: Atyidae) under laboratory conditions

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Abstract

We studied the effects of temperature on the reproduction of two atyid shrimp species of the genus *Caridina*, namely *C. multidentata* and *C. typus*, by culturing them at 20, 23 and 26 °C. Females of both species oviposited at all temperatures. Temperature did not affect the hatching rates in *C. typus*, but the hatching rates in *C. multidentata* were low at 26 °C. The number of newly hatched larvae from a single female was affected by temperature for both species and decreased with increasing temperatures. The reduction rate in the number of newly hatched larvae from 20 °C to 23 °C and 26 °C was 67 % and 88 % for *C. multidentata* and 27 % and 29 % for *C. typus*, respectively. Thus, high-temperature regimes negatively affected the reproductive output of these atyid shrimps.

Keywords: atyid shrimp; amphidromous shrimp; oviposition; larval hatching; temperature adaptation

Introduction

Caridina multidentata Stimpson 1860 and *Caridina typus* H. Milne-Edwards 1837 are freshwater shrimp of the family Atyidae (Decapoda: Caridea). *C. multidentata* and *C. typus* grow to 25–35 mm and 30–40 mm in body length, respectively (Suzuki and Naruse 2011). They are collected from natural habitats and sold commercially as ornamental shrimp.

To protect wild populations of *C. multidentata* and *C. typus*, artificial propagation techniques are needed to produce juveniles. These atyid shrimps are amphidromous; that is, oviposition and hatching occur in freshwater, but newly hatched larvae passively drift to the sea and develop into juveniles before migrating to adult habitats. Therefore, knowledge of the factors affecting the reproduction of adults in freshwater and larval survival and development in the sea is essential for the development of artificial propagation technologies.

Because decapod crustaceans are ectotherms, temperature strongly influences their biological processes (Anger 2001; Green et al. 2014; Ren et al. 2021). A laboratory study elucidated the appropriate temperature and salinity conditions for the larval survival and development of atyid shrimps, including *C. multidentata* and *C. typus* (Kondo et al. 2021). However, little is known about the influence of temperature on their reproductive traits such as oviposition, hatching and fecundity (i.e. number of offspring). In the present study, we cultured *C. multidentata* and *C. typus* under different temperature levels and examined their oviposition, hatching and number of newly hatched larvae.

Materials and Methods

Test shrimp

The wild *C. multidentata* collected from western Japan were purchased from an aquarium shop on 4 April 2017 and 14 May 2018 (information on the exact capture site was not available). The wild *C. typus* was captured on 19 August 2019 and 29 July and 24 August 2020 in the Banda River (34°58'N, 139°46'E), Chiba Prefecture, Japan.

Shrimp culture method

Shrimp culturing and experiments were conducted in a laboratory at the Tokyo University of Marine Science and Technology, Tokyo, Japan. The test shrimps were maintained in aerated aquaria (31 L volume) under natural photoperiod conditions. A small filter system (SUISAKU EIGHT M; Suisaku Corp., Tokyo, Japan) was placed at the bottom of the tank, which was covered with gravel. Aquatic plants *Egeria densa* were suspended in the aquaria. Aerated containers (860 mL volume) without aquatic plants were used when shrimps were cultured individually. The shrimps were fed daily with commercially formulated ornamental atyid shrimp food (COMET marsh shrimp; Itosui Corp., Tokyo, Japan), and one-third of the rearing water was replaced with fresh water twice a week.

Shrimp culture experiment

We tested three temperature levels for shrimp culturing (20, 23 and 26 °C), which correspond to the temperatures from spring to autumn during the reproductive season for atyid shrimps in the natural habitat of the Banda River (Yamada et al. 2024). Two culture experiments were conducted for each species. The temperature of the aquaria was controlled by heaters and an air conditioner in the laboratory.

In Experiment 1, sex was determined by the naked eye; that is, shrimps with a relatively wider carapace and longer pleopods were treated as female. Three and two test aquaria were used at each temperature level for *C. multidentata* and *C. typus*, respectively, and 30 shrimps (assumed sex ratio of 1:1) were cultured in each aquarium. Test *C. multidentata* were initially stocked in aquaria at 20 °C and then transferred directly from the stock aquaria to the test aquaria at the designated test temperatures. Test *C. typus* were initially stocked in aquaria at 23 °C and then the temperature was decreased or increased by 0.5 °C per day to the designated temperature levels for the 20 °C and 26 °C groups. Experiment 1 was conducted from 17 April to 16 October 2017 (182 days) for *C. multidentata* and from 24 August to 15 November 2019 (52 days) for *C. typus*.

In Experiment 2, the performance of shrimp at the high temperature level (26 °C) was re-examined using test shrimps whose sex had been accurately determined. Prior to the experiment, shrimps were reared individually in containers at 23 °C, and when the shrimp moulted, sex was determined by observing the appendix masculina (presence = male; absence = female) on the endopod of the second pleopod of the exuviae remaining in the containers. Sexed individuals were kept in aquaria designated for each sex. Two test aquaria were used, and 10 males and 10 females were stocked in each aquarium at 23 °C. The temperature was increased by 0.5 °C per day to 26 °C. Experiment 2 was conducted from 21 June to 22 October 2018 (123 days, Aquarium 1) and from 15 July to 22 October 2018 (99 days, Aquarium 2) for *C. multidentata* and from 5 September to 20 November 2020 (76 days, both aquaria) for *C. typus*.

In both experiments, test shrimps were monitored daily for survival and oviposition, and the temperature of each aquarium was recorded. Ovigerous females were individually maintained in containers controlled at designated test temperatures. When the larvae hatched, the number of newly hatched larvae was counted, and the body length of each mother was measured to the nearest 0.5 mm from the posterior margin of the orbit to the dorsal posterior end of the telson by photographing on 1 mm square paper. Body length measurements were not conducted for two and three females of *C. multidentata* in the 20 °C and 23 °C groups, respectively, and for one female of *C. typus* in the 26 °C group due to a mistake. After the larvae hatched, the females were returned to the test aquaria. At the end of the experiments, the surviving shrimps were stored in a refrigerator at −20 °C and later sexed, and the body length of each shrimp was measured to the nearest 0.1 mm using a digital calliper. The means

Table 1. Survival rate and body length (BL) of two atyid shrimp species of the genus *Caridina* cultured at three temperature levels (20, 23 and 26 °C) at the end of the experiment. Survival rate = (number of surviving shrimps)/(number of initial test shrimps) × 100. Means ± standard deviations of daily culture temperature and BL are shown.

Species (experiment no.)	Temperature level (°C)	Aquarium no.	Daily culture temperature (°C)	Survival rate (%)	Male		Female	
					n	BL (mm)	n	BL (mm)
C. multidentata (1)	20	1	20.1 ± 0.2	80.0	10	33.2 ± 3.4	14	37.2 ± 3.4
		2	19.7 ± 0.1	83.3	5	31.4 ± 2.0	20	34.7 ± 3.3
		3	20.1 ± 0.1	73.3	9	31.1 ± 2.3	13	37.4 ± 3.5
	23	1	22.9 ± 0.2	73.3	7	33.1 ± 1.8	15	36.9 ± 3.1
		2	23.2 ± 0.1	90.0	11	32.6 ± 1.1	16	36.9 ± 2.9
		3	23.3 ± 0.1	83.3	8	32.9 ± 0.6	17	38.2 ± 2.2
	26	1	26.1 ± 0.2	40.0	3	32.9 ± 1.7	9	37.3 ± 2.5
		2	26.3 ± 0.1	20.0	0	-	6	39.2 ± 2.3
		3	26.0 ± 0.2	10.0	2	36.3 ± 0.5	1	41.9
C. multidentata (2)	26	1	26.0 ± 0.3	85.0	10	23.8 ± 1.5	7	27.0 ± 2.6
		2	26.0 ± 0.4	90.0	10	23.2 ± 1.9	8	27.4 ± 3.9
C. typus (1)	20	1	20.1 ± 0.4	100	19	19.7 ± 1.8	11	24.8 ± 2.5
		2	20.3 ± 0.3	100	19	19.2 ± 1.5	11	24.6 ± 1.6
	23	1	23.1 ± 0.3	86.7	18	18.9 ± 1.3	8	22.9 ± 1.7
		2	23.1 ± 0.4	93.3	17	19.3 ± 1.2	11	23.3 ± 1.0
	26	1	25.7 ± 0.8	83.3	20	18.3 ± 1.1	5	21.3 ± 1.1
		2	25.7 ± 0.9	83.3	19	18.7 ± 2.6	6	20.0 ± 2.6
C. typus (2)	26	1	25.9 ± 0.5	85.0	10	19.9 ± 0.8	7	19.3 ± 0.8
		2	26.0 ± 0.5	95.0	10	24.4 ± 2.0	9	25.3 ± 1.1

and standard deviations of the temperature records in each aquarium are shown in Table 1, and the temperatures were maintained at the designated levels.

Statistical analysis

Statistical analyses were performed in Experiment 1 using R statistical software (R4.3.2; R Core Team 2023) at a 5 % significance level. A binomial generalised linear model (GLM) (logit link) was employed to evaluate the effect of temperature levels (categorical explanatory variable) on test shrimp survival (two-vector response variable; number of surviving and dead shrimp). The number of ovigerous females found in each test aquarium was summed monthly. A Poisson generalised linear mixed-effects model (GLMM) (log link) was then used to assess differences in the number of ovigerous females (response variable) between months and temperature levels (categorical explanatory variables). Larval hatching success (response variable; present [1] or absent [0]) of ovigerous females was compared by temperature level (categorical explanatory variable) using binomial GLMM. The influence of body length (continuous explanatory variable) and temperature level (categorical explanatory variable) on the number of newly hatched larvae of females was evaluated using Poisson GLMM. Binomial GLM analyses were performed using the *brglm* function in the brglm package (Kosmidis and Firth 2021). GLMM analyses were conducted using the *glmer* function in the lme4 package (Bates et al. 2015). In the GLMM analyses, the identification number of the test aquaria was included in the random intercept effect.

Based on the results of the Poisson GLMM analyses, adjusted means with 95% confidence intervals for the number of newly hatched larvae were calculated for each temperature level for each species on a logarithmic scale using the *lsmeans* function (Lenth 2016), and these adjusted means were converted to antilogarithmic numbers.

Results

Survival

In Experiment 1, we assumed an equal sex ratio (1:1) at the start of the experiment; however, the sex

Table 2. Coefficient estimates with standard errors in binomial generalised linear models to evaluate the effects of temperature (20, 23 and 26 °C) (categorical explanatory variable) on survival (response variable) in two atyid shrimp species of the genus *Caridina*. Coefficient estimates of the categorical explanatory variables were calculated for 23 °C and 26 °C, representing the change in the response variable relative to the baseline category (20 °C).

Species	n	Coefficient	Estimate	SE	z value	p
C. multidentata	9	Intercept	1.2993	0.2569	5.058	< 0.0001
		23 °C	0.2082	0.3753	0.555	0.5790
		26 °C	-2.4726	0.3572	-6.923	< 0.0001
C. typus	6	Intercept	4.7960	1.4320	3.349	0.0008
		23 °C	-2.6690	1.4920	-1.789	0.0735
		26 °C	-3.2250	1.4720	-2.191	0.0285

ratio appeared to be female-biased for *C. multidentata* and male-biased for *C. typus* when surviving shrimps were sexed (Table 1). Survival rates of *C. multidentata* were significantly lower at 26 °C (10–40 %) ($p < 0.0001$), but not at 23 °C (73–90 %) ($p = 0.5790$), than at 20 °C (73–83 %) in Experiment 1 (Tables 1 and 2). In this experiment, high mortality occurred at the start of the culturing process in the 26 °C group, although the actual number of dead shrimps was not counted. However, relatively high survival rates (85–90 %) were observed at 26 °C for *C. multidentata* in Experiment 2 (Table 1). Survival rates of *C. typus* were significantly lower at 26 °C (83 %) ($p = 0.0285$), but not at 23 °C (87–93 %) ($p = 0.0735$), than at 20 °C (100 %) in Experiment 1 (Tables 1 and 2). Nevertheless, differences in survival rates between temperature groups of *C. typus* were small compared to those of *C. multidentata*, and high survival rates among *C. typus* were observed at 26 °C (85–95 %) in Experiment 2 (Table 1).

Oviposition

Ovigerous females were found in all aquaria of both species, but not in one aquarium of *C. multidentata* at 26 °C in Experiment 1 (Table 3). Ovigerous females of *C. multidentata* were found from April (mainly May) to September (Fig. 1a, b), and those of *C. typus* were found from August to October (Fig. 1c, d). In Experiment 1, the number of ovigerous females of both species was significantly higher at 23 °C (*C. multidentata*, $p = 0.0415$; *C. typus*, $p = 0.0461$), but not at 26 °C (*C. multidentata*, $p = 0.8421$; *C. typus*, $p = 0.1862$), than at 20 °C (Table 4). The number of ovigerous females of both species was higher than the number of surviving females in some aquaria. This was particularly evident for *C. multidentata* in

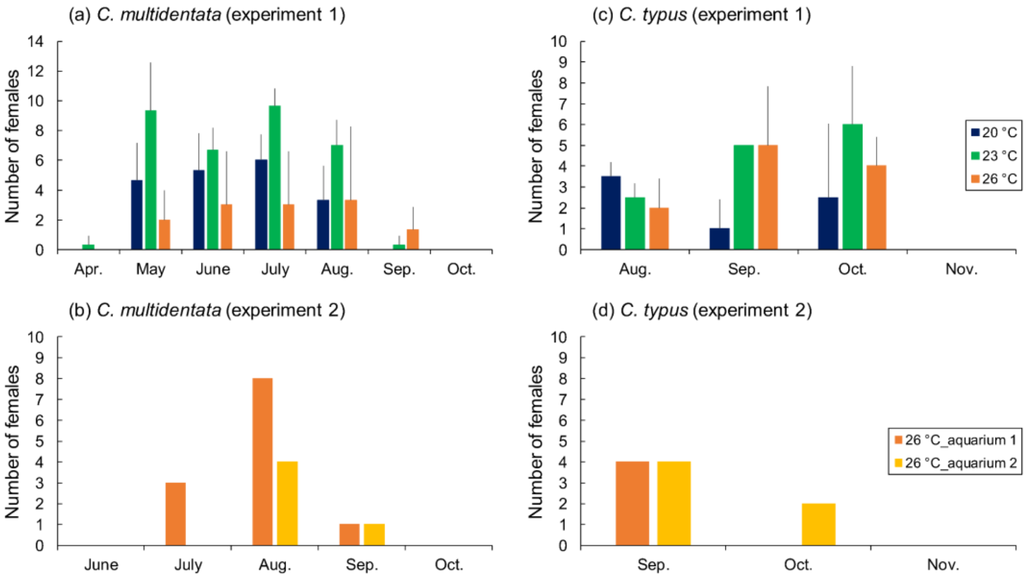

Fig. 1. Monthly number of ovigerous females of two atyid shrimp species in the genus *Caridina*, namely *C. multidentata* (a, b) and *C. typus* (c, d), cultured at 20, 23 and 26 °C. Mean values of three and two aquaria are shown for Experiment 1, and each aquarium value is shown for Experiment 2. Vertical lines indicate the standard deviation.

Table 3. Oviposition and hatching of two atyid shrimp species of the genus *Caridina* cultured at three temperature levels (20, 23 and 26 °C). Hatching rate = (number of females that hatched eggs)/(number of ovigerous females − number of dead ovigerous females) × 100. Means ± standard deviations (ranges) of the egg incubation period and larval hatching period of individual females are shown.

Species (experiment no.)	Temperature level (°C)	Aquarium no.	Number of ovigerous females*	Number of females that hatched eggs	Hatching rate (%)	Egg incubation period (days)	Hatching period (days)
C. multidentata (1)	20	1	20 (1)	15	78.9	24.7 ± 5.1 (16–36)	2.3 ± 1.8 (1–7)
		2	14 (1)	9	69.2	22.8 ± 4.2 (15–30)	1.9 ± 0.9 (1–3)
		3	24 (1)	14	60.9	24.1 ± 4.1 (16–32)	2.4 ± 1.4 (1–5)
	23	1	29	21	72.4	17.3 ± 3.5 (13–26)	3.4 ± 2.0 (1–8)
		2	28	14	50.0	16.4 ± 4.1 (12–26)	2.3 ± 1.9 (1–7)
		3	43 (1)	24	57.1	19.7 ± 5.1 (14–31)	2.7 ± 2.1 (1–7)
	26	1	30 (1)	8	27.6	14.6 ± 5.3 (9–25)	1.8 ± 1.2 (1–4)
		2	0	-	-	-	-
		3	8	6	75.0	13.2 ± 4.9 (10–22)	2.8 ± 1.5 (1–4)
C. multidentata (2)	26	1	12	2	16.7	23.5 ± 4.9 (20–27)	1.0 ± 0.0 (1)
		2	5	0	0	-	-
C. typus (1)	20	1	6	5	83.3	22.2 ± 5.5 (17–31)	3.4 ± 1.7 (2–6)
		2	8	7	87.5	21.9 ± 5.8 (15–34)	2.1 ± 1.7 (1–5)
	23	1	11	10	90.9	14.3 ± 4.7 (6–24)	1.7 ± 1.3 (1–5)
		2	16	13	81.3	15.7 ± 4.4 (10–24)	1.4 ± 1.1 (1–5)
	26	1	11 (1)	8	80.0	15.4 ± 4.4 (11–23)	1.6 ± 1.4 (1–5)
		2	11	11	100	10.0 ± 1.5 (8–13)	1.7 ± 1.2 (1–4)
C. typus (2)	26	1	4 (1)	3	100	11.6 ± 2.1 (10–14)	1.0 ± 0.0 (1)
		2	6	6	100	13.7 ± 3.6 (10–19)	1.3 ± 0.5 (1–2)

*Number of ovigerous females died is shown in parentheses.

Table 4. Coefficient estimates with standard errors in Poisson generalised linear mixed-effects models to evaluate the differences in the number of ovigerous females (response variable) between months and temperature levels (20, 23 and 26 °C) (categorical explanatory variables) in two atyid shrimp species of the genus *Caridina*. Coefficient estimates of the categorical explanatory variables were calculated for May to September and 23 °C and 26 °C, representing the change in the response variable relative to the baseline category (April and 20 °C).

Species	n	Coefficient	Estimate	SE	z value	p
C. multidentata	48	Intercept	-2.3381	1.0135	-2.307	0.0211
		May	3.8713	1.0066	3.846	0.0001
		June	3.8068	1.0073	3.779	0.0002
		July	4.0254	1.0051	4.005	0.0001
		August	3.7136	1.0084	3.683	0.0002
		September	1.6094	1.0914	1.475	0.1403
		23 °C	0.5464	0.2680	2.039	0.0415
		26 °C	-0.0637	0.3196	-0.199	0.8421
C. typus	18	Intercept	0.5754	0.3436	1.675	0.0940
		September	0.3185	0.3286	0.969	0.3324
		October	0.4463	0.3202	1.394	0.1633
		23 °C	0.6568	0.3293	1.994	0.0461
		26 °C	0.4520	0.3419	1.322	0.1862

Aquarium 3 (43 ovigerous females/17 surviving females) at 23 °C and Aquaria 1 (30/9) and 3 (8/1) at 26 °C in Experiment 1 (Tables 1 and 3), suggesting multiple oviposition by a single female. This was supported by the number of ovigerous females (12) of *C. multidentata* in Experiment 2 with an initial stocking of 10 females.

Hatching

Hatching rates in ovigerous females of *C. multidentata* were significantly lower at 26 °C (28–75 %) (p = 0.0085), but not at 23 °C (50–72 %) (p = 0.2633), than at 20 °C (61–79 %) in Experiment 1 (Tables 3 and 5). For *C. multidentata*, low hatching rates at 26 °C (0–17 %) were also observed in Experiment 2 (Table 3). For *C. typus*, hatching rates at 23 and 26 °C (80–100 %) were not significantly different from those at 20 °C (83–88 %) in Experiment 1 (p = 0.6660–0.9640), and hatching was observed in all ovigerous females in Experiment 2 (Tables 3 and 5). In both species, larval hatching took one to several days for a single female, and the mean number of days from oviposition to first hatching, that is, the mean egg incubation period, tended to decrease with increasing temperature levels, but the variation in egg incubation periods was large even within each temperature level (15–36 days, 12–31 days and 9–27 days at 20, 23 and 26 °C for *C. multidentata*, respectively; 15–34 days, 6–24 days and 8–23 days at 20, 23 and 26 °C for *C. typus*, respectively) (Table 3).

Table 5. Coefficient estimates with standard errors in binomial generalised linear mixed-effects models to evaluate the effects of temperature (20, 23 and 26 °C) (categorical explanatory variable) on hatching (response variable) in two atyid shrimp species of the genus *Caridina*. Coefficient estimates of the categorical explanatory variables were calculated for 23 °C and 26 °C, representing the change in the response variable relative to the baseline category (20 °C).

Species	n	Coefficient	Estimate	SE	z value	p
C. multidentata	191	Intercept	0.8097	0.3046	2.658	0.0079
		23 °C	-0.4179	0.3736	-1.119	0.2633
		26 °C	-1.2810	0.4871	-2.630	0.0085
C. typus	62	Intercept	1.7918	0.7638	2.346	0.0190
		23 °C	-0.0426	0.9364	-0.045	0.9640
		26 °C	0.4595	1.0658	0.431	0.6660

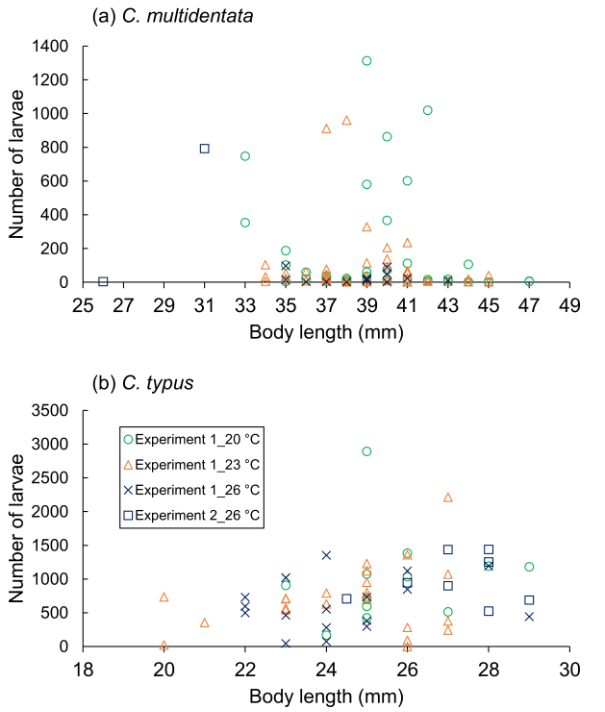

Fig. 2. The relationship between female body length and number of newly hatched larvae of two atyid shrimp species in the genus *Caridina*, namely *C. multidentata* (a) and *C. typus* (b), cultured at 20, 23 and 26 °C in Experiments 1 and 2.

Number of newly hatched larvae

The number of newly hatched larvae from a single female was highly variable, ranging from 1 to 1313 in *C. multidentata* and 1 to 2894 in *C. typus* (Fig. 2), and was negatively and positively correlated with female body length in *C. multidentata* ($p < 0.0001$) and *C. typus* ($p < 0.0001$), respectively (Table 6). The number of newly hatched larvae was significantly lower at 23 and 26 °C than at 20°C for both species (*C. multidentata*, $p \leq 0.0001$–0.0017; *C. typus*, $p =$ 0.0051–0.0093) (Table 6 and Fig. 3). The adjusted means of the number of newly hatched larvae at 23 and 26 °C decreased to 33 % and 12 % and 73 % and 71 % of that at 20 °C for *C. multidentata* and *C. typus*, respectively.

Table 6. Coefficient estimates with standard errors in Poisson generalised linear mixed-effects models to evaluate the effects of female body length (BL) (continuous explanatory variable) and temperature (20, 23 and 26 °C) (categorical explanatory variable) on the number of newly hatched larvae (response variable) in two atyid shrimp species of the genus *Caridina*. Coefficient estimates of the categorical explanatory variables were calculated for 23 °C and 26 °C, representing the change in the response variable relative to the baseline category (20 °C).

Species	n	Coefficient	Estimate	SE	z value	p
C. multidentata	106	Intercept	8.1602	0.2777	29.383	< 0.0001
		BL	-0.0750	0.0030	-24.902	< 0.0001
		23 °C	-1.1218	0.3569	-3.143	0.0017
		26 °C	-2.1558	0.4027	-5.354	< 0.0001
C. typus	53	Intercept	5.1077	0.1140	44.822	< 0.0001
		BL	0.0706	0.0028	25.455	< 0.0001
		23 °C	-0.3255	0.1252	-2.600	0.0093
		26 °C	-0.3509	0.1253	-2.801	0.0051

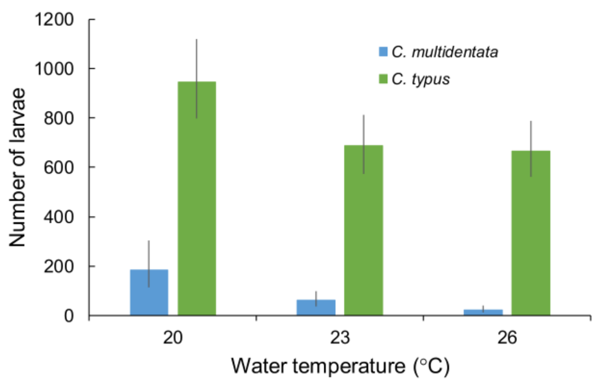

Fig. 3. Adjusted mean values with 95% confidence intervals (vertical bars) of the number of newly hatched larvae of two atyid shrimp species in the genus *Caridina*, namely *C. multidentata* and *C. typus*, cultured at 20, 23 and 26 °C in Experiment 1.

Discussion

For *C. multidentata*, survival rates were lower at 26 °C (10–40 %) than at 20 and 23 °C (73–90 %) in Experiment 1, but survival rates were high even at 26 °C (85–90 %) in Experiment 2 (Table 1). For *C. typus*, survival rates tended to decrease with increases in temperature, but the differences between temperature levels were small (83–100 %) in Experiment 1, and the survival rates were high even at 26 °C (85–95 %) in Experiment 2 (Table 1). In Experiment 1 with *C. multidentata*, the test shrimps were transferred directly from the stock aquaria at 20 °C to the test aquaria at 20, 23 and 26 °C, and the high early mortality occurred in the 26 °C aquaria. In other experiments, *C. multidentata* and *C. typus* were stocked in aquaria at 23 °C, and then the temperature decreased or increased by 0.5 °C per day, for which no early mortality was observed. Therefore, the abrupt increase in temperature from 20 °C to 26 °C may have been responsible for the low survival of *C. multidentata* at 26 °C in Experiment 1.

Ovigerous females of *C. multidentata* were found mainly from May to September, and those of *C. typus* were found from August to October (Fig. 1). The reproductive season extends from May to September for *C. multidentata* (Takeda 1972; Hamano and Hayashi 1992; Yamada et al. 2024) and from June to October for *C. typus* (Imai and Onuki 2022; Maruyama and Okamoto 2022; Yamada et al. 2024) in streams and rivers with temperature profiles commonly recorded in western Japan. In Experiment 1, *C. multidentata* was cultured from April to October and *C. typus* from August to November at three constant temperature levels. However, the end of the reproductive season was similar between captive and natural conditions. The photoperiod has been known to affect the reproduction of decapod crustaceans (e.g. Crocos and Kerr 1986; Matsuda et al. 2002; Kim et al. 2010), including *C. multidentata* (Takeda 1972). Takeda (1972) reported that *C. multidentata* oviposited during the winter season under culture conditions controlled at 20 °C and a photoperiod longer than 13 h. In the present study, the test shrimp were cultured under the natural photoperiod cycle, and a reduced photoperiod shorter than 13 h in September for *C. multidentata* and 11 h in October for *C. typus* may have been an environmental cue for the end of breeding season.

The proportion of ovigerous females (i.e. number of ovigerous females/number of test females) between temperature groups was not compared because the sex ratio at the start of the culturing process and the mortality profile of the test shrimps were not determined in Experiment 1 for both species. However, the number of ovigerous females was higher at 23 °C than at 20 °C (Tables 3 and 4), where aquaria had similar numbers of surviving females at the end of the culture experiment (Table 1). In the present study, multiple oviposition by a single female was suggested to occur in *C. multidentata* and *C. typus* because the number of ovigerous females was higher than the number of surviving females in some aquaria, particularly among *C. multidentata* at 23 and 26 °C in Experiment 1, which featured a long culture period (182 days) (Tables 1 and 3). High-temperature regimes may have accelerated the gonadal maturation cycle, as is known for decapods (e.g. Crocos and Kerr 1986; Matsuda et al. 2002; Kim et al. 2010). Multiple oviposition by a single female was observed in *C. multidentata* by Takeda (1972), who reared a male–female pair (10 pairs in total) under natural temperature and photoperiod conditions and reported that a single female oviposited 8–11 times during a breeding season.

The egg incubation period of *C. multidentata* and *C. typus* tended to decrease with increases in temperature (Table 3), as is known for decapods (e.g. Wear 1974), but the variation in egg incubation periods within the same temperature regimes was large (e.g. 6–24 days at 23 °C for *C. typus*). This was probably due to the missed oviposition dates of females in aquaria; that is, ovigerous females were found some days after oviposition. In addition, in some females of both species, larval hatching from a single female took several days. In previous studies, egg incubation periods for *C. multidentata* were reported to be 13–23 days at around 20 °C, 20–22 days at 22–23 °C, 16–19 days at around 25 °C, 9–11 days at > 25 °C (Takeda 1972) and about 27–30 days at 19–20 °C (Shokita 1979). Furthermore, Hamano and Hayashi (1992) documented that when ovigerous females of *C. multidentata* were reared from oviposition at 20 °C, some larvae began to hatch after 21–29 days, and synchronous hatching of many larvae occurred after 26–56 days. Thus, the incubation periods of *C. multidentata* eggs appear to vary, even at similar temperatures.

Hatching rates of ovigerous females were low at 26 °C (0–75 %; mean: 29.8 %) compared to 20 and 23 °C (50–79 %; mean: 64.8 %) in *C. multidentata*, whereas they did not differ between temperature levels (80–100 %; mean: 90.4 %) in *C. typus* (Table 3). In ovigerous females that did not produce a larva, the eggs were completely lost from their pleopods. It is known that females lose their eggs due to various biotic and abiotic factors, such as failure of the eggs to adhere to the pleopod setae during oviposition, unfertilised and arrested egg development, mechanical egg loss, and egg mortality due to inappropriate temperature and salinity conditions, predation and parasitism (Kuris 1991).

In Takeda's (1972) culture experiments with *C. multidentata*, a male–female pair was reared in a 15 L aerated bucket (rearing water exchange rate: 100 % every three days), and larval hatching occurred in all broods of all ovigerous females during a breeding season, even at high-temperature conditions of 26–28 °C. In the present study, rearing water quality may have deteriorated in the small containers (860 mL) with a low water exchange rate (33 % twice a week), and the degree of rearing water deterioration may have increased at 26 °C. The sensitivity to water deterioration may be higher in *C. multidentata* than in *C. typus*. Therefore, the present study may have recorded a high proportion of females that completely lost their eggs, especially at 26 °C. This hypothesis should be tested in future studies by rearing *C. multidentata* in containers with different volumes and water exchange rates.

The number of eggs attached to the female pleopods is known to be positively correlated with female body length in *C. multidentata* and *C. typus* (Shokita 1979). In the present study, the number of newly hatched larvae was positively correlated with female body length in *C. typus*, but a negative correlation between these variables was observed in *C. multidentata* (Table 6 and Fig. 2), probably influenced by egg loss. The number of newly hatched larvae decreased with increases in temperature, and the rate of decrease with temperatures that increased from 20 °C to 23 °C and 26 °C was higher in *C. multidentata* (67 % and 88 %) than in *C. typus* (27 % and 29 %) (Fig. 3). As ectotherms, atyid shrimps may experience increases in metabolism with increases in temperature, which may reduce the energy expenditure required for reproductive output. This physiological response in high-temperature regimes may be stronger in *C. multidentata* because these test specimens seemed to be better adapted to low-temperature regimes than *C. typus* specimens. The lethal temperature for *C. multidentata* adults was lower than that for *C. typus* adults (Maruyama and Okamoto 2022). *C. typus* was not collected, but *C. multidentata* was collected from streams and rivers during the overwintering period (Yamada et al. 2024). Reduced reproductive output coupled with higher egg loss rates at higher temperatures may have been responsible for the temperature-dependent reduction in the number of newly hatched *C. multidentata* larvae.

Takeda (1972) documented that the number of eggs increased from 2440 to 5170 dependent on the body length of 25–31 mm in wild *C. multidentata*. Shokita (1979) reported the mean number of eggs attached to the pleopods and mean female body length to be 2607 and 35.1 mm for wild *C. multidentata* and 2188 and 26.4 mm for wild *C. typus*. Thus, the number of newly hatched larvae was much lower than the number of eggs attached to the pleopods in both atyids, especially in *C. multidentata* (Figs. 2 and 3). The egg loss rate may have been higher as a result of the captive status of the *C. multidentata* and *C. typus*.

Our experiments highlighted the negative effects of high temperatures on the reproductive performance of *C. multidentata* and *C. typus* in captivity. To improve the broodstock management techniques for these species, further studies are needed to investigate the effects of water quality (e.g. ammonia in the water) (Zhao et al. 2020; Li et al. 2022) on the incubation success of female eggs and to elucidate the egg loss rate by counting the number of eggs attached to the pleopods.

Acknowledgments

We thank the members of the laboratory for helping with laboratory work. We are grateful to two anonymous reviewers for their valuable comments and suggestions. This study was supported by JSPS KAKENHI grant number JP17K00578 to KH.

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Received: 5 February 2024 | Accepted: 1 March 2024 | Published: 4 March 2024