Effect of temperature on survival, intermolt period, and growth of juveniles of two mud crab species, *Scylla paramamosain* and *Scylla serrata* (Decapoda: Brachyura: Portunidae), under laboratory conditions

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Abstract

Temperature affects ectotherm's biological processes and plays a fundamental role in determining their geographical distribution. We elucidated the temperature adaptation of juveniles of two mud crab species in the genus *Scylla* De Haan, 1833 (in De Haan, 1833–1850): *Scylla paramamosain* Estampador, 1949 and *Scylla serrata* (Forskål, 1775), which occur mainly in temperate and subtropical/tropical areas in Japan, respectively. The first instar crabs (C1) were raised from three broods for each species and were individually cultured to molt to C2–C5 at different temperatures (15.2–30.5 °C). In *S. paramamosain*, C1 juveniles could molt to C2 at 15.4 °C and the survival rate was reduced at 29.8 °C through C4–C5. In *S. serrata*, C1 juveniles could not molt to C2 at 15.2 °C and the survival rate was not affected by temperature thereafter. The intermolt period was shortened at higher temperatures in both species. The carapace width of juveniles increased with increasing temperature but became smaller at 27.6–30.2 °C in *S. paramamosain*, whereas it appeared to reach a plateau at > 24 °C in *S. serrata*. The lower threshold temperature (95 % confidence interval) for the development of C1 juveniles was estimated as 13.65 °C (13.51–13.77 °C) for *S. paramamosain*, and 15.44 °C (15.12–15.72 °C) for *S. serrata*. Thus, juveniles of *S. paramamosain* and *S. serrata* adapted to lower and higher temperature conditions, respectively, reflecting their biogeographical distribution.

Keywords

Biogeography, geographical distribution, nursery culture, temperature adaptation, threshold temperature

Introduction

The thermal environment strongly influences biological processes, such as the behavior, survival, and growth of ectothermic animals, and it is an important factor that defines their distribution (Pörtner, 2001; Sunday *et al.*, 2011; Araújo *et al.*, 2013; Hoffmann *et al.*, 2013).

Mud crabs in the genus *Scylla* De Haan, 1833 (in De Haan, 1833–1850) (Decapoda: Brachyura: Portunidae) are important fishery resources in the Indo-Pacific, from warm temperate through subtropical to tropical regions (Keenan and Blackshaw, 1999; LeVay, 2001; Ogawa *et al.*, 2011; 2012). They consist of four species: *Scylla paramamosain* Estampador, 1949, *Scylla serrata* (Forskål, 1775), *Scylla olivacea* (Herbst, 1796), and *Scylla tranquebarica* (Fabricius, 1798) (Keenan and Blackshaw, 1999). In Asian and Oceanian countries, they are also treated as important aquaculture target species (Keenan and Blackshaw, 1999; Azra *et al.*, 2019). The main aquaculture target species are *S. paramamosain* and *S. serrata* (Allan and Fielder, 2004). The former is cultured mainly in China (Ye *et al.*, 2010) and Vietnam (Nghia *et al.*, 2007), and the latter mainly in the Philippines (Quinitio and Parado-Estepa, 2011) and Australia (Ruscoe *et al.*, 2004).

In Japan, three mud crab species, *S. paramamosain*, *S. serrata*, and *S. olivacea,* occur (Oshiro, 1988), and the two former species are important components of local fisheries in small brackish water bays and inlets (Obata *et al.*, 2006; Hamasaki *et al.*, 2011; Ogawa *et al.*, 2011; 2012). *Scylla paramamosain* is distributed on the Pacific coast of Honshu, a warm temperate area of Japan; *S. serrata* is mainly distributed in the Ryukyu Archipelago, a subtropical area of Japan that is more southern (Oshiro, 1988; Ogawa *et al.*, 2011; 2012). Therefore, it is hypothesized that *S. paramamosain* may adapt better to lower temperature environments than *S. serrata*. Hamasaki (2002; 2003) supported this hypothesis by determining the biological lower threshold temperature for egg development as 13.98 °C for *S. paramamosain* and 15.70 °C for *S. serrata*. Larvae of mud crabs grow into the juvenile stage through pelagic zoeal and megalopal stages (Keenan and Blackshaw, 1999; Hamasaki *et al.* 2011). After settlement, mud crab juveniles must survive and grow

through environmental adaptations. However, the temperature adaptation of the juvenile crabs of these species has not been clarified.

Some studies have examined the effects of temperature on the survival and development of juveniles of *S. paramamosain* (Gong *et al.*, 2015; Syafaat *et al.*, 2021) and *S. serrata* (Ruscoe *et al.*, 2004; Baylon, 2010) under laboratory conditions. However, the minimum temperature for culturing juveniles was higher in experiments for *S. serrata* (20 °C) than in those for *S. paramamosain* (14 °C). Consequently, further study under a similar culture temperature regime is required to clarify the temperature adaptation of these mud crab species. In the present study, we aimed to compare and evaluate the temperature adaptation of *S. paramamosain* and *S. serrata* through juvenile culture experiments.

Materials and Methods

Experimental animals

We conducted three culture experiments using juvenile crabs raised from three different broods for each species at the Yaeyama Field Station, Japan Fisheries Research and Education Agency, Ishigaki, Okinawa Prefecture, Japan.

Three wild mature females of *S. paramamosain* were caught in Urado Bay (33°31'N 133°33'E), Kochi Prefecture, Japan, in February 2020 (brood 1, 125 mm in carapace width (CW); brood 2, 131 mm CW; and brood 3, 127 mm CW). Those of *S. serrata* were collected in Iriomotejima Island (24°20'N 123°45'E), Okinawa Prefecture, Japan, in August 2018 (brood 1, 147 mm CW) or Okinawajima Island (26°20'N 127°51'E), Okinawa Prefecture, Japan, in October 2020 (brood 2, 156 mm CW; brood 3, 172 mm CW).

Females were reared individually in 200 L tanks with a flow-through water system under natural temperature and salinity conditions (around 34 ppt). They were fed frozen krill *Euphausia* sp. and short-necked clams *Ruditapes philippinarum* (A. Adams and Reeve, 1850). After the eggs were laid, we periodically observed their development and transferred each ovigerous female with mature eggs to a 500 L tank for larval hatching. Culture temperature (mean ±

standard deviation) during the egg incubation period was 22.4 ± 0.6 °C, 22.5 ± 0.7 °C, and 27.6 ± 0.6 °C for broods 1–3 of *S. paramamosain*, respectively, and 25.0 ± 0.5 °C, 21.8 ± 0.8 °C, and 23.3 ± 0.5 °C for broods 1–3 of *S. serrata*, respectively.

The newly hatched zoeae from each brood were stocked at a density of 20 individuals/L in a 500 L tank. They were fed rotifers *Brachionus plicatilis* Müller, 1786 species complex (S-morphotype) and *Artemia* nauplii. Larvae metamorphosed into megalopae 18–21 days after hatching. The 4–5-day-old megalopae from each brood were transferred into a 15 L container and fed *Artemia* nauplii. Rearing waters of zoeae and megalopae were not renewed during the culture period, and the water temperatures for culturing larvae were ~24–27°C. Larvae molted to the first instar crabs (C1) in late April 2020 (broods 1 and 2) and late July 2020 (brood 3) for *S. paramamosain* and in mid-December 2018 (brood 1), late March 2021 (brood 2), and late April 2021 (brood 3) for *S. serrata*.

Juvenile culture under different temperatures

The first instar crabs (C1) within a day of molting from the megalopae were used for the culture experiments under 4–7 different temperature levels within a range of 15–30 °C. Juveniles were cultured for the designated period in each species-brood until surviving animals had reached the designated instar crabs (second to fifth instar crabs, C2–C5), depending on the respective temperature levels. Culture temperature, culture period, and the instar at the end of the experiments are summarized for respective species-broods in Tab. 1. Low temperatures were not tested on older instar crabs because of a limited space for culturing juvenile crabs in the laboratory. The crab culture began on the same day in most treatments, but it exceeded 2 days in brood 3 of *S. paramamosain* and brood 1 of *S. serrata*, depending on their larval developmental processes.

The C1 juveniles were individually reared in 50 mL plastic tubes (Falcon™ Conical Centrifuge Tube,

Table 1. Culture temperature (mean ± standard deviation), culture period, and the instar at the end of culture experiments in two mud crab species, *Scylla paramamosain* and *S. serrata*.

	Scylla paramamosain				*Scylla serrata*			
Brood	Temperature (°C)	Start date	Period (days)	Instar	Brood	Temperature (°C)	Start date	Period (days)	Instar
	15.3 ± 0.4	22 Apr 2020	54	C2		19.0 ± 0.7	19 Dec 2018	34	C3
	19.3 ± 0.5	22 Apr 2020	52	C3		21.1 ± 0.6	19 Dec 2018	26	C3
	21.8 ± 0.2	22 Apr 2020	46	C4		23.1 ± 0.7	19 Dec 2018	31	C4
1	24.7 ± 0.2	22 Apr 2020	49	C5	1	24.5 ± 0.4	19 Dec 2018	26	C4
	25.8 ± 0.7	22 Apr 2020	44	C5		26.4 ± 1.3	19 Dec 2018	33	C5
	29.8 ± 0.5	22 Apr 2020	40	C5		28.1 ± 0.2	19 Dec 2018	27	C5
						30.4 ± 0.7	18 Dec 2018	27	C5
	15.3 ± 0.4	23 Apr 2020	52	C2		15.2 ± 0.3	23 Mar 2021	24	C1
	19.3 ± 0.4	23 Apr 2020	49	C3		19.6 ± 0.9	23 Mar 2021	38	C3
2	21.8 ± 0.2	23 Apr 2020	49	C4	2	21.9 ± 0.3	23 Mar 2021	39	C4
	24.7 ± 0.2	23 Apr 2020	45	C5		24.8 ± 0.1	23 Mar 2021	23	C4
	25.9 ± 0.8	23 Apr 2020	47	C5		28.1 ± 0.3	23 Mar 2021	30	C5
	29.8 ± 0.5	23 Apr 2020	39	C5		28.5 ± 1.9	23 Mar 2021	28	C5
	19.0 ± 0.6	29 Jul 2020	35	C3		19.1 ± 0.8	25 Apr 2021	30	C3
	21.0 ± 0.3	29 Jul 2020	31	C3		22.4 ± 0.2	25 Apr 2021	28	C4
3	23.3 ± 0.5	28 Jul 2020	25	C4	3	25.4 ± 0.4	25 Apr 2021	28	C5
	27.7 ± 0.2	28 Jul 2020	32	C4		30.5 ± 0.4	25 Apr 2021	24	C5
	30.3 ± 0.7	28 Jul 2020	25	C4					

C1–5, second to fifth instar crabs

Corning Japan KK, Tokyo, Japan) containing 40 mL UV-sterilized seawater (salinity, ~34 ppt). The experimental tube was maintained in temperature-controlled incubation chambers with a photoperiod cycle of 12 h light and 12 h dark (MT1-201, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). Ten crabs were used for each temperature level in all experiments.

Daily observations were made on the occurrence of molting and whether the crabs were alive or dead. After observations, all the culture water was replaced, and *Artemia* nauplii was given as food at densities of 5 individuals/mL for C1, 12 individuals/mL for C2, and 15 individuals/mL for C3–C5. Carapace width (the widest portion of the carapace) of cultured crabs was measured using digital calipers, and measurements were taken during the intermolt period at each instar.

Data analysis

Statistical analyses were performed using R statistical software (R4.1.1; R Core Team, 2021) at a 5 % significance level. To evaluate the effect of temperature on the survival of the juveniles of each species while considering brood differences, we used a bias-reduced generalized linear model (BRGLM) with a binomial distribution, that is, a logistic model. In the BRGLM analysis, binary survival (1) or death (0) until the respective instar crabs was a response variable, and temperature and brood identity were continuous and categorical explanatory variables, respectively. The coefficients of a logistic equation (with standard errors; z–values with probabilities) were estimated using the *brglm* function (logit link) implemented in the brglm package (Kosmidis and Firth, 2021). The survival analysis was not performed for C4 of *S. serrata* because all crabs survived at all temperature levels.

We evaluated the influence of temperature (T) and brood (B) (continuous and categorical explanatory variables, respectively) on the intermolt period (number of days) and growth (carapace width) of each instar crab in each species (response variables) using a generalized linear model (GLM) with a Poisson distribution and a linear model (LM), respectively. In these analyses, two models were applied considering the nonlinear effect of temperature: model 1, $y \sim T + T^2 + B$ and model 2, $y \sim T + B$. The coefficients of the models were estimated, and their statistical

significance was evaluated using the *glm* function for Poisson-GLM (log link) analyses and the *lm* function for LM analyses. The model with a lower Akaike information criterion (Akaike, 1973) value was selected and shown to be the best for each instar crab in each species. Differences in the intermolt period and carapace width between the broods of each species were tested with Tukey's method using the *glht* function in the multcomp package (Hothorn *et al.*, 2008).

To compare the low-temperature adaptation of juveniles of the two species, the relationship between temperature (T) and intermolt period (D) was evaluated using the heat summation theory equation (Hamasaki, 2003): $D = a/(T - b)$. The parameters *a* and *b* are the so-called "thermal constant" and "lower threshold temperature (LTT)", respectively, for biological development. The thermal constant summarizes the effective temperature for development (> threshold temperature) up to a selected biological endpoint. We used the data set of temperature and the intermolt period of C1 juveniles to determine the heat summation theory equation because they were cultured at the widest temperature range among respective instar crabs in both species. The parameters were estimated using a nonlinear ordinary least-squares method and evaluated with *t*-tests using the *nls* function. The 95 % confidence interval (CI) for each parameter was estimated with the *confint* function.

RESULTS

Survival

The survival rate of juveniles was not significantly different among broods in both species (Tab. 2). In *S. paramamosain*, temperature significantly affected the survival of juveniles and their ability to reach C2, C4, and C5 (Tab. 2). Survival rates decreased at the lowest temperature level (15.3 °C) when juveniles molted to C2 although they still showed high values (80 %) (Fig. 1). Then, survival rates were maintained above 90 % at 19.0–30.3 °C. However, they largely declined with increasing temperature from 24.7 to 29.8 °C through C4–C5, with final survival rates of 100 % and 30–40 % at 24.7 and 29.8 °C, respectively. In *S. serrata*, temperature significantly affected the

Table 2. Coefficient estimates with standard errors (SE) for the explanatory variables in the generalized linear model with a binomial distribution to evaluate the juvenile survival (alive or not) until the respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in two mud crab species *Scylla paramamosain* and *S. serrata*. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1). The statistical analysis was not performed for C4 of *S. serrata* because all crabs survived at all temperature levels. Crabs from brood no. 3 of *S. paramamosain* were cultured until molting to C4.

Instar	Coefficient	*S. paramamosain*					*S. serrata*			
		N	Estimate	SE	z value	*P*	N	Estimate	SE	z value	*P*
C2	Intercept	170	-3.3116	2.0216	-1.638	0.1014	170	-23.1229	8.5585	-2.702	0.0069
	T		0.2854	0.1065	2.680	0.0074		1.3785	0.4404	3.130	0.0018
	B-2		0.6761	0.8407	0.804	0.4213		-0.8579	1.9626	-0.437	0.6620
	B-3		0.4154	1.0221	0.406	0.6844		-1.9861	1.6649	-1.193	0.2329
C3	Intercept	150	3.7569	2.3165	1.622	0.1050	150	-12.0152	7.7443	-1.551	0.1208
	T		-0.0486	0.0907	-0.536	0.5920		0.7220	0.3489	2.069	0.0385
	B-2		0.8860	0.9856	0.899	0.3690		-0.1669	1.7407	-0.096	0.9236
	B-3		-0.0132	0.7744	-0.017	0.9860		-0.9602	1.7048	-0.563	0.5733
C4	Intercept	110	18.9302	5.9271	3.194	0.0014					
	T		-0.6169	0.2055	-3.002	0.0027					
	B-2		0.7883	0.9115	0.865	0.3871					
	B-3		0.7806	0.8595	0.908	0.3638					
C5	Intercept	60	17.8407	4.4009	4.054	0.0001	70	-18.2917	15.6493	-1.169	0.2420
	T		-0.6244	0.1581	-3.950	0.0001		0.7462	0.5779	1.291	0.1970
	B-2		0.2519	0.6977	0.361	0.7180		0.8937	1.7525	0.510	0.6100
	B-3							2.3064	1.8213	1.266	0.2050

C2–5, second to fifth instar crabs; N, number of crabs

survival of juveniles and their ability to reach C2 and C3 (Tab. 2). Survival rates decreased at lower temperature levels (15.2–19.6 °C) through C2–C3, and juveniles could not molt to C2 at the lowest temperature level (15.2 °C) (Fig. 2). Then, survival rates showed relatively high values (> 80 %) through C4–C5 at test temperatures (21.9–30.5 °C).

Intermolt period

Model 1 was selected as the best for describing the relationship between temperature and the intermolt period at C1–C3 of *S. paramamosain* and C1–C2 of *S. serrata* and model 2 for other instar crabs. However, the temperature did not significantly affect the intermolt period at C4 of *S. serrata* (Tab. 3). The intermolt period decreased exponentially (model 1) or linearly (model 2) with increasing temperatures (Figs. 3, 4). Inter-brood variability was evident in the intermolt periods of the instar crabs, *S. paramamosain* (C1–C2) and *S. serrata* (C2–C3). However, the trends of temperature effects on the intermolt period were similar among broods in both species (Figs. 3, 4).

Growth

The C1 juveniles could be assigned unbiasedly to respective test temperature groups because the carapace width of C1 juveniles did not differ significantly among test temperatures in both species (Tab. 4 and Figs. 5, 6). When the carapace width of C1 juveniles was compared among broods, it was significantly different among broods in *S. serrata* but not in *S. paramamosain* (Tab. 4 and Figs. 5, 6). Model 1 was selected as the best for describing the relationship between temperature and carapace width at C2–C4 of *S. paramamosain* and C2–C3 of *S. serrata*, and model 2 for other instar crabs. However, the temperature did not significantly affect the carapace width at C5 of both species (Tab. 4). The relationship between temperature and carapace width was expressed by an upward convex curve by model 1 in both species (Figs. 5, 6), and the carapace width tended to reach peaks at around 26 °C in *S. paramamosain* (Fig. 5). However, it did not largely decrease even at higher temperatures (> 30 °C) in *S. serrata* (Fig. 6). Inter-brood variability was evident in carapace width at
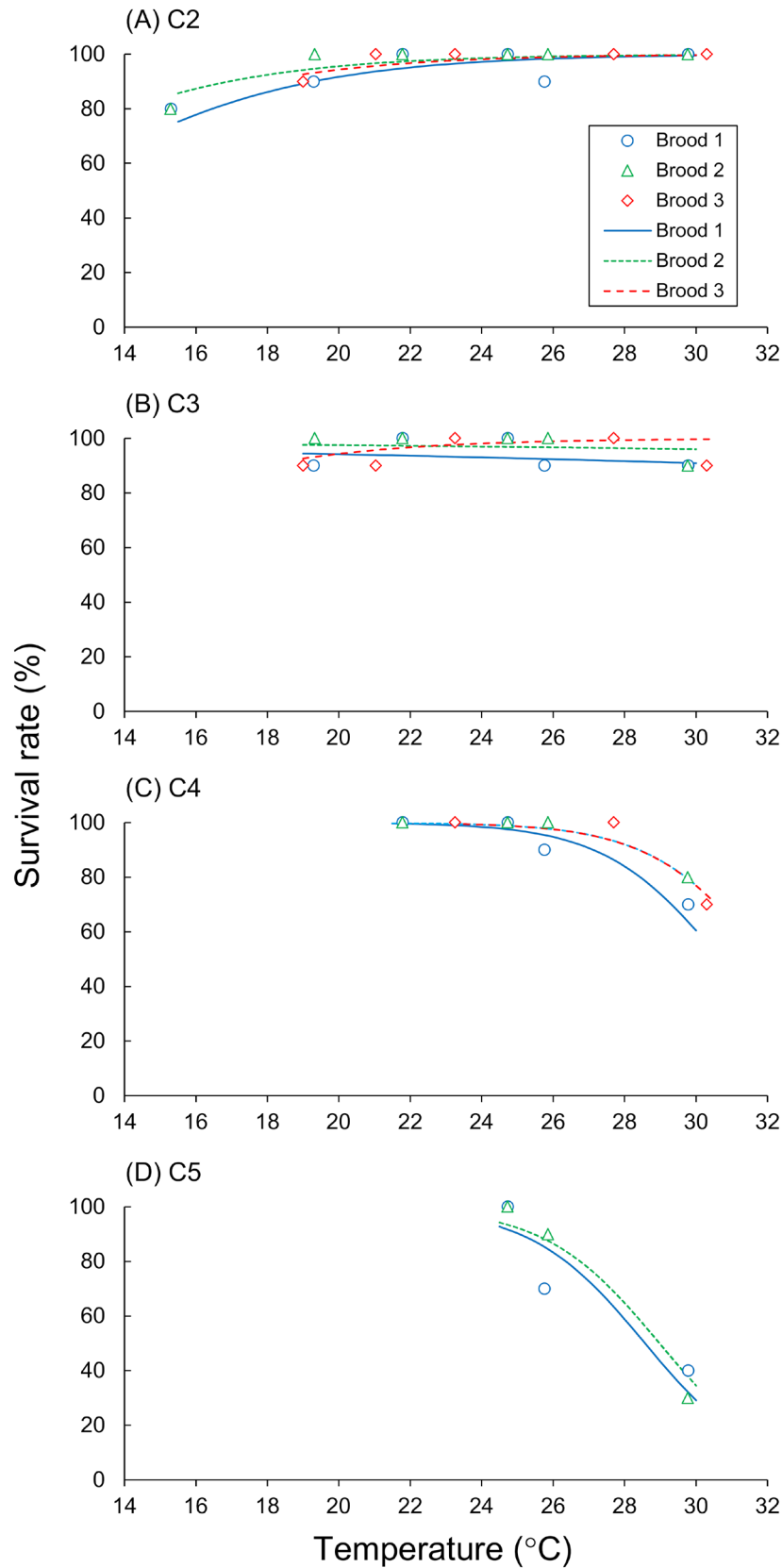
Figure 1. Survival rates of *Scylla paramamosain* juveniles under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Tab. 2. Significant differences were not found among broods at all instar crabs.
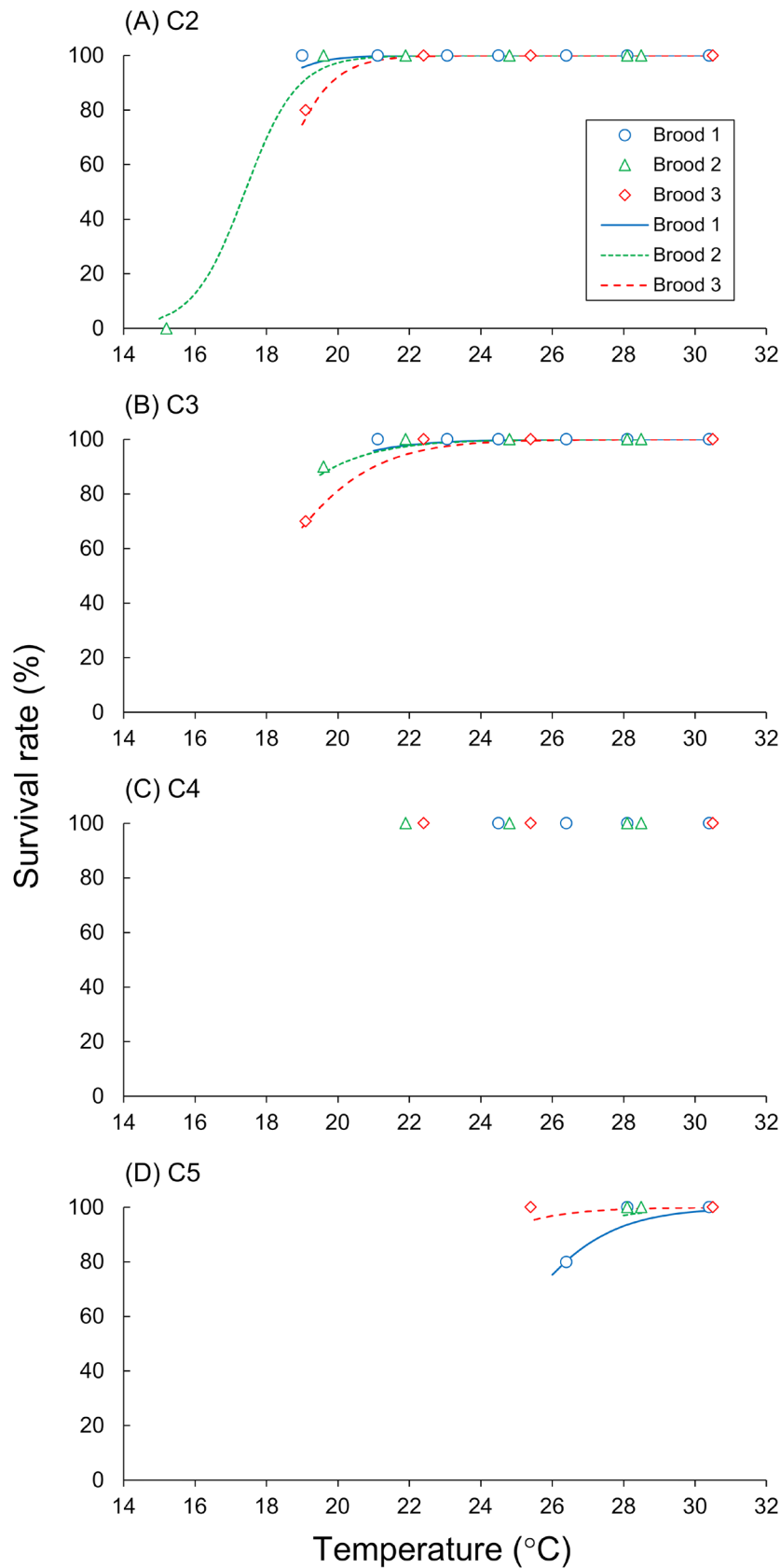
Figure 2. Survival rates of *Scylla serrata* juveniles under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Tab. 2. Significant differences were not found among broods at all instar crabs.

Table 3. Coefficient estimates with standard errors (SE) for the explanatory variables in the generalized linear model with a Poisson distribution to evaluate the intermolt periods (number of days) of respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in two mud crab species *Scylla paramamosain* and *S. serrata*. Two models were applied: model 1, $y \sim T + T^2 + B$ and model 2, $y \sim T + B$. The model with a lower Akaike information criterion (AIC) value was selected and shown to be the best for each instar in each species. See Appendix, Tabs. A1 and A2, for the coefficient estimates and AIC values of all models for *S. paramamosain* and *S. serrata,* respectively. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1). Crabs from brood no. 3 of *S. paramamosain* were cultured until molting to C4.

Instar	Coefficient	S. paramamosain					S. serrata			
		N	Estimate	SE	z value	P	N	Estimate	SE	z value	P
	Intercept		11.7114	0.4980	23.516	< 0.0001		11.1156	1.4312	7.766	< 0.0001
	T		-0.6955	0.0480	-14.483	< 0.0001		-0.6403	0.1213	-5.279	< 0.0001
C1	T²	164	0.0120	0.0011	10.876	< 0.0001	158	0.0105	0.0025	4.176	< 0.0001
	B-2		-0.0291	0.0502	-0.580	0.5617		0.1235	0.0670	1.843	0.0653
	B-3		-0.2209	0.0671	-3.294	0.0010		-0.0324	0.0749	-0.433	0.6647
	Intercept		14.1412	1.2720	11.118	< 0.0001		9.7669	1.3495	7.237	< 0.0001
	T		-0.8699	0.1077	-8.080	< 0.0001		-0.5087	0.1144	-4.449	< 0.0001
C2	T²	144	0.0154	0.0022	6.885	< 0.0001	156	0.0077	0.0024	3.252	0.0012
	B-2		-0.0370	0.0622	-0.596	0.5514		0.1355	0.0620	2.185	0.0289
	B-3		-0.1511	0.0641	-2.359	0.0183		-0.1056	0.0718	-1.472	0.1411
	Intercept		14.1041	2.5929	5.439	< 0.0001		4.4759	0.3065	14.603	< 0.0001
	T		-0.8541	0.2028	-4.212	< 0.0001		-0.0918	0.0117	-7.862	< 0.0001
C3	T²	101	0.0154	0.0039	3.938	< 0.0001	120				
	B-2		-0.0670	0.0671	-0.999	0.3180		0.1033	0.0725	1.425	0.1542
	B-3		-0.0636	0.0775	-0.821	0.4120		-0.1475	0.0849	-1.738	0.0823
	Intercept		5.0630	0.6360	7.961	< 0.0001		2.7240	0.6702	4.064	< 0.0001
C4	T	43	-0.0874	0.0246	-3.554	0.0004	68	-0.0169	0.0235	-0.719	0.4720
	B-2		-0.1258	0.0775	-1.623	0.1047		0.0718	0.0934	0.769	0.4420
	B-3							-0.1008	0.0988	-1.019	0.3080

C1–4, first to fourth instar crabs; N, number of crabs

C2–C4 of *S. paramamosain* and all instar crabs of *S. serrata*. However, the trends of temperature effects on juvenile growth were similar among broods in both species (Figs. 5, 6).

Low temperature adaptation

The analyses of the heat summation theory equation estimated the LTT (95 % CI) for the development of C1 juveniles as 13.65 °C (13.51– 13.77 °C) for *S. paramamosain* and 15.44 °C (15.12– 15.72 °C) for *S. serrata* (Tab. 5).

DISCUSSION

The present study demonstrated interspecific variation in the temperature adaptation of two mud crab species: *S. paramamosain* and *S. serrata*. The C1 juveniles were able to molt to C2 with 80 % survival rates at 15.4 °C, and survival rates were maintained above 90 % at all test temperatures (19.0–30.3 °C) until C3 in *S. paramamosain* (Fig. 1). On the other hand, C1 juveniles were not able to survive to C2 at 15.2 °C, and survival rates decreased to 70 % until C3 at 19.1 °C in *S. serrata* (Fig. 2). Survival rates of *S. paramamosain* juveniles declined to 30–40 % until C5 at 29.8 °C (Fig. 1), whereas all *S. serrata* juveniles survived to C5 even at 30.5 °C (Fig. 2). Additionally, the carapace width of juveniles increased with increasing temperature but became smaller at 27.6–30.2 °C in *S. paramamosain* (Fig. 5), whereas it appeared to reach a plateau at > 24 °C in *S. serrata* (Fig. 6). Thus, our results highlight that juveniles of *S. paramamosain* and *S. serrata* adapt to lower and higher temperature conditions, respectively.

The intermolt period of juveniles exponentially or linearly decreased with increasing temperature

Figure 3. Intermolt periods of *Scylla paramamosain* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Tab. 3. Differences in the intermolt period between broods at each instar (P < 0.05) are indicated by different lowercase letters in the tables following the brood numbers.
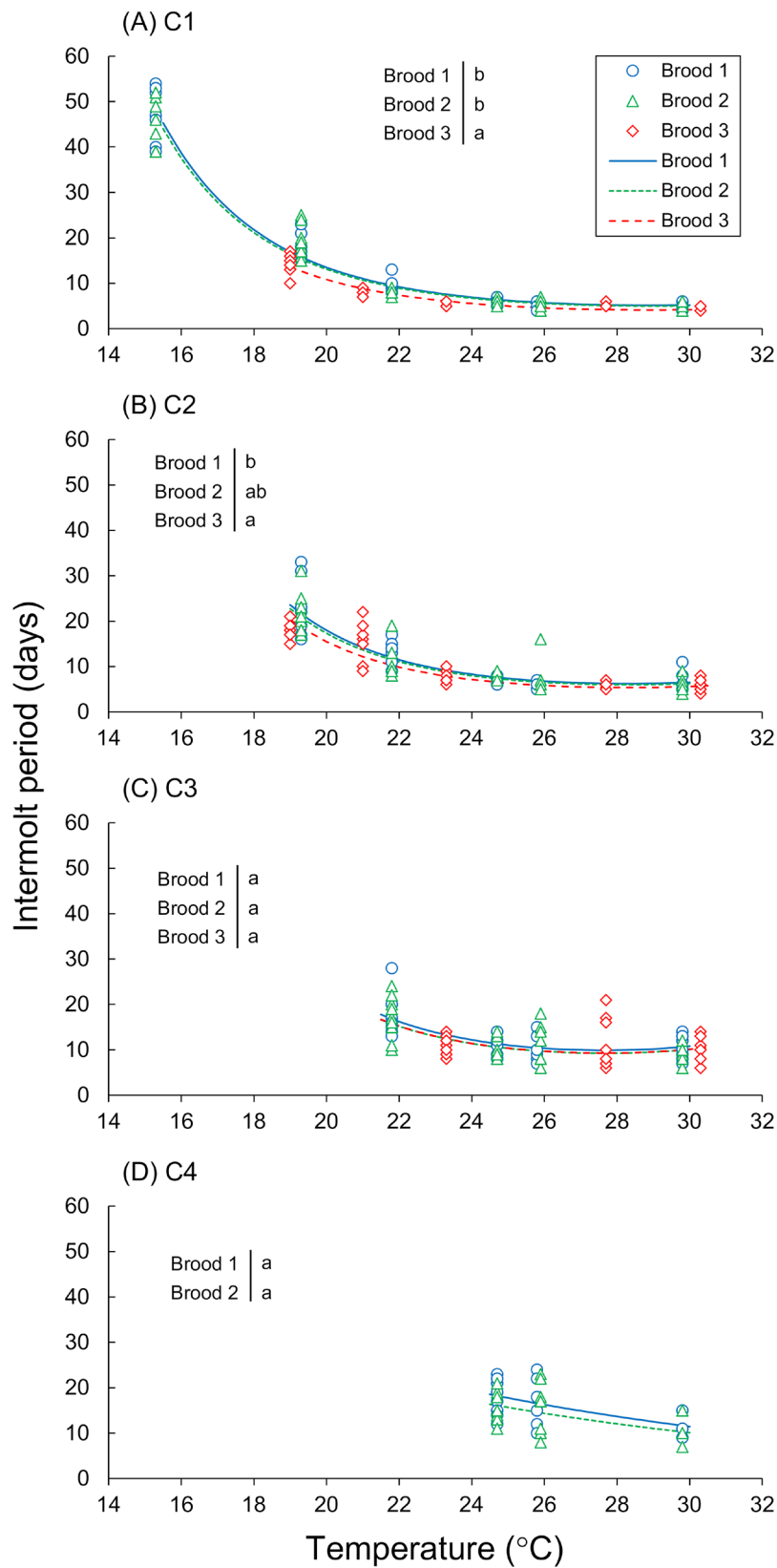
Figure 4. Intermolt periods of *Scylla serrata* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Tab. 3. Differences in the intermolt period between broods at each instar (P < 0.05) are indicated by different lowercase letters in the tables following the brood numbers.
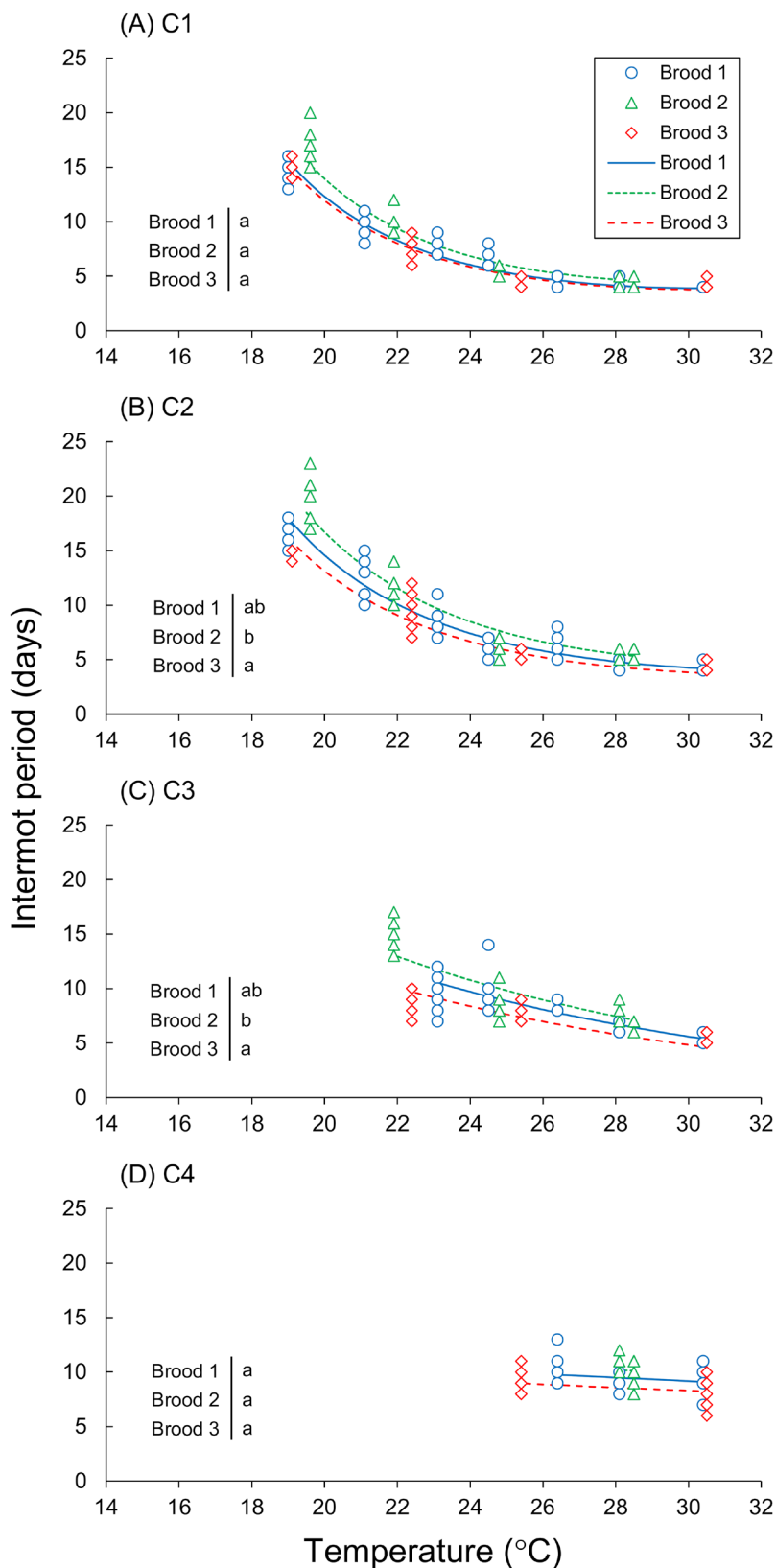
Table 4. Coefficient estimates with standard errors (SE) for the explanatory variables in the general linear model to evaluate the carapace widths of respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in two mud crab species *Scylla paramamosain* and *S. serrata*. Two models were applied: model 1, $y \sim T + T^2 + B$ and model 2, $y \sim T + B$. The model with a lower Akaike information criterion (AIC) value was selected and shown to be the best for each instar in each species. See Appendix, Tabs. A3 and A4, for the coefficient estimates and AIC values of all models for *S. paramamosain* and *S. serrata*, respectively. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1). Crabs from brood no. 3 of *S. paramamosain* were cultured until molting to C4.

Instar	Coefficient	N	*S. paramamosain* Estimate	SE	z value	P	N	*S. serrata* Estimate	SE	z value	P
	Intercept		3.4829	0.0627	55.539	< 0.0001		3.7647	0.0373	100.840	< 0.0001
	T		0.0005	0.0026	0.186	0.8520		-0.0018	0.0015	-1.240	0.2170
C1	T^2	170					170				
	B-2		0.0465	0.0282	1.649	0.1010		-0.1730	0.0143	-12.122	< 0.0001
	B-3		0.0435	0.0298	1.458	0.1470		-0.1068	0.0159	-6.737	< 0.0001
	Intercept		-0.1561	0.4767	-0.327	0.7438		1.0344	0.7065	1.464	0.1450
	T		0.3871	0.0419	9.233	< 0.0001		0.3312	0.0581	5.698	< 0.0001
C2	T^2	164	-0.0074	0.0009	-8.227	< 0.0001	158	-0.0061	0.0012	-5.175	< 0.0001
	B-2		0.0676	0.0450	1.505	0.1344		-0.5110	0.0334	-15.286	< 0.0001
	B-3		-0.1491	0.0473	-3.152	0.0019		-0.3753	0.0365	-10.272	< 0.0001
	Intercept		-6.9159	1.6019	-4.317	< 0.0001		-2.6792	1.1255	-2.380	0.0185
	T		1.0327	0.1321	7.821	< 0.0001		0.7298	0.0925	7.893	< 0.0001
C3	T^2	144	-0.0201	0.0027	-7.489	< 0.0001	156	-0.0131	0.0019	-7.032	< 0.0001
	B-2		0.0969	0.0798	1.215	0.2260		-0.8105	0.0530	-15.301	< 0.0001
	B-3		-0.1036	0.0814	-1.273	0.2050		-0.6027	0.0580	-10.399	< 0.0001
	Intercept		-12.1186	5.6778	-2.134	0.0354		8.0636	0.3502	23.028	< 0.0001
	T		1.5130	0.4405	3.435	0.0009		0.0539	0.0131	4.123	0.0001
C4	T^2	101	-0.0291	0.0085	-3.434	0.0009	120				
	B-2		0.4286	0.1462	2.932	0.0042		-1.4096	0.0765	-18.417	< 0.0001
	B-3		0.1465	0.1639	0.894	0.3735		-0.5169	0.0967	-5.345	< 0.0001
	Intercept		11.1068	2.3906	4.646	< 0.0001		10.3924	0.9338	11.130	< 0.0001
C5	T	43	-0.0735	0.0914	-0.804	0.4260	68	0.0439	0.0327	1.343	0.1840
	B-2		0.2996	0.3241	0.924	0.3610		-2.0146	0.1367	-14.740	< 0.0001
	B-3							-0.9462	0.1375	-6.881	< 0.0001

C1–5, first to fifth instar crabs; N, number of crabs

(Figs. 3, 4), a phenomenon that has been observed in many decapod crustacean species (Anger, 2001). Based on the data set of temperature and the intermolt period at C1, the lower threshold temperature (LTT) (95 % CI) for juvenile development was estimated as 13.65 °C (13.51–13.77 °C) for *S. paramamosain* and 15.44 °C (15.12–15.72 °C) for *S. serrata* (Tab. 5). The LTT for egg development has been estimated to be 13.98 °C for *S. paramamosain* (Hamasaki, 2002) and 15.70 °C for *S. serrata* (Hamasaki, 2003). The LTT for zoeal development to the megalopal stage has also been estimated as 15.08 °C for *S. serrata* (Hamasaki, 2003). Consequently, LTT estimates for egg and zoeal

developments matched those for juvenile development in *S. paramamosain* and *S. serrata*, suggesting that the low-temperature adaptations may be similar regardless of the life history stage in these mud crab species.

The influence of temperature on the survival and development of juveniles of *S. paramamosain* and *S. serrata* has been studied as a basis for developing nursery culture techniques in aquaculture. Gong *et al.* (2015) reared C1 juveniles of *S. paramamosain* from China until molting to C2 at 14, 20, 26, 32, and 39 °C and reported that juveniles could molt at 20, 26, and 32 °C with survival rates of 87, 95, and 91 %, respectively. Syafaat *et al.* (2021) reared C1

Figure 5. Carapace widths of *Scylla paramamosain* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Tab. 4. Differences in carapace width between broods at each instar (P < 0.05) are indicated by different lowercase letters in the tables following the brood numbers.
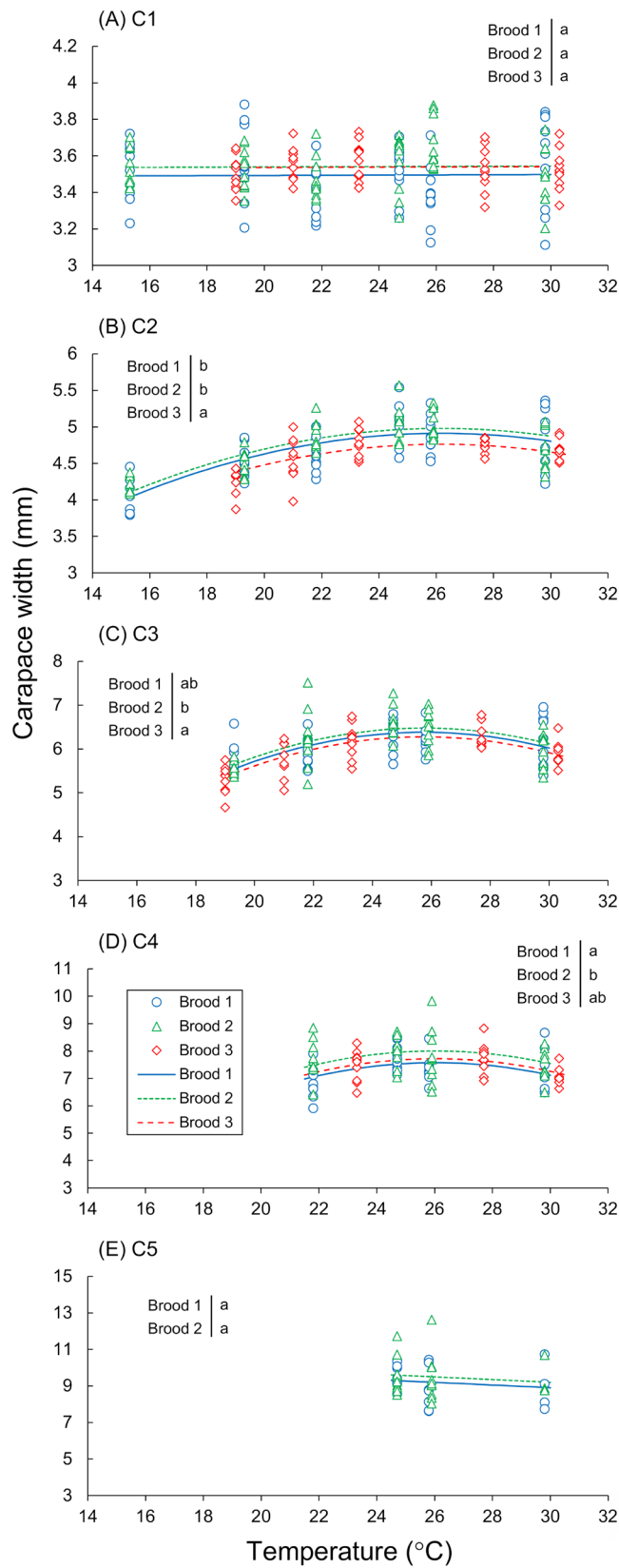
Figure 6. Carapace widths of *Scylla serrata* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Tab. 4. Differences in carapace width between broods at each instar (P < 0.05) are indicated by different lowercase letters in the tables following the brood numbers.
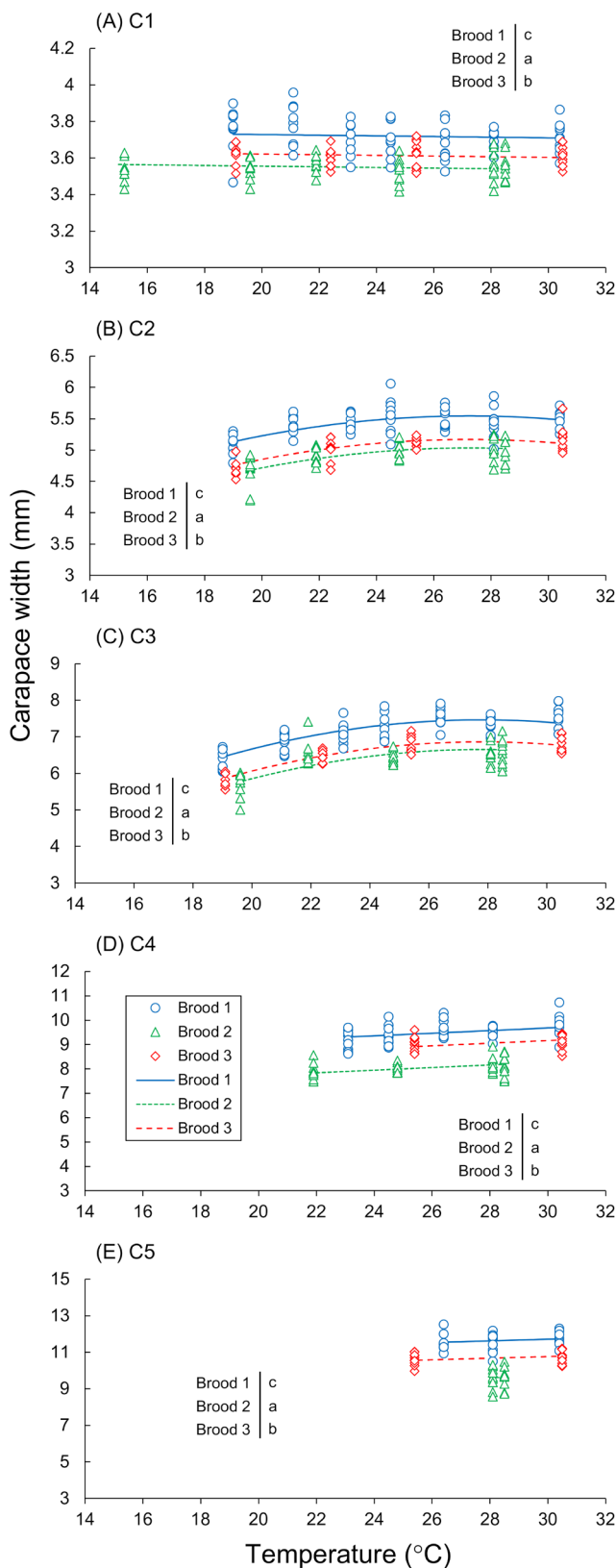
Table 5. Coefficient estimates with standard errors (SE) and 95 % confidence intervals (CI) of the heat summation theory equation $[D = a/(T − b)]$ describing the relationship between mean temperature (T) and the intermolt period (D, number of days) of the first instar crab in the two mud crab species *Scylla paramamosain* and *S. serrata*.

Species	N	Coefficient	Estimate	SE	95% CI		t value	P
S. paramamosain	164	a	77.4811	2.7298	72.2493	82.8884	28.38	< 0.0001
		b	13.6452	0.0667	13.5112	13.7709	204.46	< 0.0001
S. serrata	158	a	56.1539	1.9054	52.6309	59.9455	29.47	< 0.0001
		b	15.439	0.1564	15.1242	15.7226	98.73	< 0.0001

N, number of crabs.

juveniles of *S. paramamosain* from Malaysia at constant temperatures of 24, 28, and 32 °C and ambient temperatures of 27–30 °C for 45 days and reported respective survival rates as 87, 97, 80, and 93 %. They also reported that the growth rate was highest at 28 °C, followed by 32 °C and ambient temperature (27–30 °C), and lowest at 24 °C. Syafaat *et al.* (2021) further evaluated the critical thermal minimum of C1 juveniles of *S. paramamosain*, which was defined as the temperature when crabs were on their backs and could not recover an upright posture, and they recorded it as 17–18 °C. Thus, the lower temperature (14 °C) at which C1 juveniles of Chinese *S. paramamosain* by Gong *et al.* (2015) could not molt coincided with the LTT (13.65 °C) of Japanese *S. paramamosain* in our experiments. However, Malaysian *S. paramamosain* by Syafaat *et al.* (2021) appeared to exhibit a higher temperature adaptation compared with Japanese *S. paramamosain* probably because of the higher habitat temperatures in tropical Malaysia.

As for *S. serrata*, Ruscoe *et al.* (2004) reared C2 juveniles raised in Australia at 20, 25, 30, and 35 °C for 18 days. They reported a lower survival rate (36 %) at 20 °C than other temperatures (94–98 %) as well as minimum and maximum growth rates at 20 and 30 °C, respectively. Baylon (2010) reared C1 juveniles raised in the Philippines until molting to C2 at 20, 26, and 32 °C and recorded slightly lower survival rates (~83 %) at 20 °C compared with other temperatures (> 90 %: survival rates derived from figure 1 by Baylon (2010)). Thus, the performance of *S. serrata* juveniles under different temperatures appeared to be similar between previous and present studies conducted in subtropical or tropical areas, and our results could clarify the lower critical temperature for juvenile development in *S. serrata*.

Besides temperature, salinity is an important environmental factor affecting the physiological process of decapod crustaceans (Anger, 2001). Gong *et al.* (2015) reported slightly lower survival rates (75 %) of *S. paramamosain* juveniles from C1 to C2 at 5 ppt compared with those (96–97 %) at 10–40 ppt under 26 °C. Ruscoe *et al.* (2004) and Baylon (2010) conducted juvenile culture experiments for *S. serrata* under the different salinity and temperature combinations. They reported that juveniles could not survive at 0 ppt but exhibited similar performance at a broad range of salinity (5–45 ppt). Thus, juveniles of *S. paramamosain* and *S. serrata* are euryhaline, and the temperature adaptation of these species inferred by the present study may be general in natural environments with a wide range of salinity.

Our results supported the hypothesis that *S. paramamosain* may adapt better to lower temperature environments than *S. serrata*, based on the biogeography of these species in Japan. Ogawa *et al.* (2011) examined the species compositions of mud crabs in commercial catch from brackish inlets on Iriomotejima Island (24°20'N 123°45'E), Ryukyu Archipelago, during the period from September 2001 to August 2005. They reported that two species, *S. serrata* and *S. olivacea*, were identified in the area, with *S. serrata* being the dominant species (> 95 % of the catch). Moreover, Ogawa *et al.* (2012) examined the species compositions of mud crabs in commercial catches from Urado Bay (33°31'N 133°33'E) during the period from October 2008 to October 2009. They reported that three mud crab species were identified in the area, with *S. paramamosain* being the dominant species (74 % of the catch), followed by *S. serrata* (23 %) and *S. olivacea* (3 %). In Urado Bay, *S. serrata* and *S. olivacea* accounted for only several percent of

the total catch during the late 1980s (Sugimoto and Hasegawa, 1989; 1990). The Kuroshio Current plays an important role in transferring the larvae of southern marine organisms from warmer to cooler latitudes in the northwestern Pacific region (Iida *et al.*, 2010; Soeparno *et al.*, 2012; He *et al.*, 2015; Chang et *al.*, 2018). *Scylla serrata* larvae hatched in the Ryukyu Archipelago may disperse to the temperate bays and inlets via the Kuroshio Current, and the survival rate of juveniles settled there might have improved under global warming conditions.

The present study highlighted the temperature adaptation of early juveniles of two mud crab species, *S. paramamosain* and *S. serrata*. Further studies will be required to elucidate the temperature adaptation of these species throughout an entire life cycle to understand and predict future changes in the species compositions and the northern limit of their distribution under a global warming scenario.

Acknowledgements

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APPENDIX

Table A1. Coefficient estimates with standard errors (SE) for the explanatory variables in the generalized linear model with a Poisson distribution to evaluate the intermolt periods (number of days) of respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in *Scylla paramamosain*. Two models were applied: model 1, $y \sim T + T^2 + B$ and model 2, $y \sim T + B$. The Akaike information criterion (AIC) was calculated for each model to select the best model with a lower AIC value. The bold AIC value was a lower between those of models 1 and 2. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1). Crabs from brood no. 3 were cultured until molting to C4.

Instar	N	Coefficient	Model 1				Model 2			
			Estimate	SE	z value	*P*	Estimate	SE	z value	*P*
C1	164	Intercept	11.7114	0.4980	23.516	< 0.0001	6.4983	0.1154	56.318	< 0.0001
		T	-0.6955	0.0480	-14.483	< 0.0001	-0.1819	0.0057	-32.181	< 0.0001
		T^2	0.0120	0.0011	10.876	< 0.0001				
		B-2	-0.0291	0.0502	-0.580	0.5617	-0.0333	0.0502	-0.664	0.5070
		B-3	-0.2209	0.0671	-3.294	0.0010	-0.3430	0.0651	-5.265	< 0.0001
		(AIC)	**718.7**				828.3			
C2	144	Intercept	14.1412	1.2720	11.118	< 0.0001	5.5099	0.1808	30.478	< 0.0001
		T	-0.8699	0.1077	-8.080	< 0.0001	-0.1331	-0.0077	17.286	< 0.0001
		T^2	0.0154	0.0022	6.885	< 0.0001				
		B-2	-0.0370	0.0622	-0.596	0.5514	-0.0363	0.0621	-0.584	0.5590
		B-3	-0.1511	0.0641	-2.359	0.0183	-0.0994	0.0636	-1.563	0.1180
		(AIC)	**678.1**				722.8			
C3	101	Intercept	14.1041	2.5929	5.439	< 0.0001	3.9478	0.2710	14.568	< 0.0001
		T	-0.8541	0.2028	-4.212	< 0.0001	-0.0570	0.0108	-5.286	< 0.0001
		T^2	0.0154	0.0039	3.938	< 0.0001				
		B-2	-0.0670	0.0671	-0.999	0.3180	-0.0675	0.0671	-1.006	0.3140
		B-3	-0.0636	0.0775	-0.821	0.4120	-0.0627	0.0771	-0.813	0.4160
		(AIC)	**533.9**				547.3			
C4	43	Intercept	-2.2059	12.9929	-0.170	0.8652	5.0630	0.6360	7.961	< 0.0001
		T	0.4506	0.9608	0.469	0.6391	-0.0874	0.0246	-3.554	0.0004
		T^2	-0.0099	0.0177	-0.560	0.5754				
		B-2	-0.1300	0.0779	-1.669	0.0951	-0.1258	0.0775	-1.623	0.1047
		(AIC)	248.0				**246.4**			

C1–4, first to fourth instar crabs; *N*, number of crabs

Table A2. Coefficient estimates with standard errors (SE) for the explanatory variables in the generalized linear model with a Poisson distribution to evaluate the intermolt periods (number of days) of respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in *Scylla serrata*. Two models were applied: model 1, $y \sim T + T^2 + B$ and model 2, $y \sim T + B$. The Akaike information criterion (AIC) was calculated for each model to select the best model with a lower AIC value. The bold AIC value was a lower between those of models 1 and 2. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1).

Instar	N	Coefficient	Model 1				Model 2			
			Estimate	SE	z value	*P*	Estimate	SE	z value	*P*
C1	158	Intercept	11.1156	1.4312	7.766	< 0.0001	5.2113	0.1992	26.167	< 0.0001
		T	-0.6403	0.1213	-5.279	< 0.0001	-0.1362	0.0085	-16.064	< 0.0001
		T^2	0.0105	0.0025	4.176	< 0.0001				
		B-2	0.1235	0.0670	1.843	0.0653	0.1096	0.0668	1.639	0.1010
		B-3	-0.0324	0.0749	-0.433	0.6647	-0.0109	0.0746	-0.146	0.8840
		(AIC)	**618.4**				633.6			
C2	156	Intercept	9.7669	1.3495	7.237	< 0.0001	5.4282	0.1869	29.046	< 0.0001
		T	-0.5087	0.1144	-4.449	< 0.0001	-0.1383	0.0080	-17.353	< 0.0001
		T^2	0.0077	0.0024	3.252	0.0012				
		B-2	0.1355	0.0620	2.185	0.0289	0.1241	0.0619	2.006	0.0448
		B-3	-0.1056	0.0718	-1.472	0.1411	-0.0940	0.0717	-1.311	0.1898
		(AIC)	**641.7**				650.1			
C3	120	Intercept	8.8986	3.2271	2.757	0.0058	4.4759	0.3065	14.603	< 0.0001
		T	-0.4356	0.2500	-1.742	0.0814	-0.0918	0.0117	-7.862	< 0.0001
		T^2	0.0066	0.0048	1.377	0.1684				
		B-2	0.0896	0.0734	1.221	0.2220	0.1033	0.0725	1.425	0.1542
		B-3	-0.1746	0.0872	-2.002	0.0453	-0.1475	0.0849	-1.738	0.0823
		(AIC)	500.4				**500.3**			
C4	68	Intercept	14.3761	21.2851	0.675	0.4990	2.7240	0.6702	4.064	< 0.0001
		T	-0.8475	1.5166	-0.559	0.5760	-0.0169	0.0235	-0.719	0.4720
		T^2	0.0148	0.0269	0.548	0.5840				
		B-2	0.1105	0.1177	0.939	0.3480	0.0718	0.0934	0.769	0.4420
		B-3	-0.1584	0.1441	-1.099	0.2720	-0.1008	0.0988	-1.019	0.3080
		(AIC)	296.8				**295.1**			

C1–4, first to fourth instar crabs; *N*, number of crabs

Table A3. Coefficient estimates with standard errors (SE) for the explanatory variables in the general linear model to evaluate the carapace widths of respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in *Scylla paramamosain*. Two models were applied: model 1, y ~ T + T^2 + B and model 2, y ~ T + B. The Akaike information criterion (AIC) was calculated for each model to select the best model with a lower AIC value. The bold AIC value was a lower between those of models 1 and 2. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1). Crabs from brood no. 3 were cultured until molting to C4.

Instar	N	Coefficient	Model 1				Model 2			
			Estimate	SE	t value	*P*	Estimate	SE	t value	*P*
C1	170	Intercept	3.4940	0.2885	12.109	< 0.0001	3.4829	0.0627	55.539	< 0.0001
		T	-0.0005	0.0256	-0.019	0.9850	0.0005	0.0026	0.186	0.8520
		T^2	0.00002	0.0006	0.038	0.9690				
		B-2	0.0465	0.0283	1.643	0.1020	0.0465	0.0282	1.649	0.1010
		B-3	0.0435	0.0300	1.453	0.1480	0.0435	0.0298	1.458	0.1470
		(AIC)	-144.7				**-146.6**			
C2	164	Intercept	-0.1561	0.4767	-0.327	0.7438	3.6727	0.1227	29.932	< 0.0001
		T	0.3871	0.0419	9.233	< 0.0001	0.0440	0.0050	8.708	< 0.0001
		T^2	-0.0074	0.0009	-8.227	< 0.0001				
		B-2	0.0676	0.0450	1.505	0.1344	0.0677	0.0535	1.265	0.2079
		B-3	-0.1491	0.0473	-3.152	0.0019	-0.1336	0.0563	-2.375	0.0187
		(AIC)	**5.7**				61.8			
C3	144	Intercept	-6.9159	1.6019	-4.317	< 0.0001	4.9676	0.2597	19.130	< 0.0001
		T	1.0327	0.1321	7.821	< 0.0001	0.0459	0.0103	4.458	< 0.0001
		T^2	-0.0201	0.0027	-7.489	< 0.0001				
		B-2	0.0969	0.0798	1.215	0.2260	0.1001	0.0941	1.063	0.2896
		B-3	-0.1036	0.0814	-1.273	0.2050	-0.1900	0.0951	-1.997	0.0477
		(AIC)	**146.3**				193.1			
C4	101	Intercept	-12.1186	5.6778	-2.134	0.0354	7.2792	0.6078	11.977	< 0.0001
		T	1.5130	0.4405	3.435	0.0009	0.0022	0.0238	0.092	0.9269
		T^2	-0.0291	0.0085	-3.434	0.0009				
		B-2	0.4286	0.1462	2.932	0.0042	0.4302	0.1541	2.792	0.0063
		B-3	0.1465	0.1639	0.894	0.3735	0.1350	0.1728	0.781	0.4366
		(AIC)	**199.6**				209.3			
C5	43	Intercept	62.7017	54.7034	1.146	0.2590	11.1068	2.3906	4.646	< 0.0001
		T	-3.8829	4.0361	-0.962	0.3420	-0.0735	0.0914	-0.804	0.4260
		T^2	0.0698	0.0740	0.944	0.3510				
		B-2	0.3303	0.3262	1.013	0.3170	0.2996	0.3241	0.924	0.3610
		(AIC)	133.1				**132.0**			

C1–5, first to fifth instar crabs; *N*, number of crabs

Table A4. Coefficient estimates with standard errors (SE) for the explanatory variables in the general linear model to evaluate the carapace widths of respective instar crabs (response variable) under different temperature (T) and brood (B, nos. 1–3) conditions (explanatory variables) in *Scylla serrata*. Two models were applied: model 1, $y \sim T + T^2 + B$ and model 2, $y \sim T + B$. The Akaike information criterion (AIC) was calculated for each model to select the best model with a lower AIC value. The bold AIC value was a lower between those of models 1 and 2. The coefficient estimate for brood was the output for brood nos. 2 and 3, representing changes in the response variable relative to the baseline category (brood no. 1).

Instar	N	Coefficient	Model 1				Model 2			
			Estimate	SE	t value	P	Estimate	SE	t value	P
C1	170	Intercept	3.7390	0.1799	20.787	< 0.0001	3.7647	0.0373	100.840	< 0.0001
		T	0.0004	0.0154	0.025	0.9800	-0.0018	0.0015	-1.240	0.2170
		T²	0.0000	0.0003	-0.144	0.8850				
		B-2	-0.1726	0.0145	-11.870	< 0.0001	-0.1730	0.0143	-12.122	< 0.0001
		B-3	-0.1066	0.0159	-6.690	< 0.0001	-0.1068	0.0159	-6.737	< 0.0001
		(AIC)	-368.6				**-370.6**			
C2	158	Intercept	1.0344	0.7065	1.464	0.1450	4.6559	0.1052	44.276	< 0.0001
		T	0.3312	0.0581	5.698	< 0.0001	0.0310	0.0042	7.465	< 0.0001
		T²	-0.0061	0.0012	-5.175	< 0.0001				
		B-2	-0.5110	0.0334	-15.286	< 0.0001	-0.5012	0.0361	-13.900	< 0.0001
		B-3	-0.3753	0.0365	-10.272	< 0.0001	-0.3960	0.0392	-10.092	< 0.0001
		(AIC)	**-86.2**				-62.7			
C3	156	Intercept	-2.6792	1.1255	-2.380	0.0185	5.1602	0.1782	28.962	< 0.0001
		T	0.7298	0.0925	7.893	< 0.0001	0.0810	0.0071	11.492	< 0.0001
		T²	-0.0131	0.0019	-7.032	< 0.0001				
		B-2	-0.8105	0.0530	-15.301	< 0.0001	-0.7858	0.0607	-12.945	< 0.0001
		B-3	-0.6027	0.0580	-10.399	< 0.0001	-0.6422	0.0662	-9.695	< 0.0001
		(AIC)	**56.6**				98.8			
C4	120	Intercept	5.7355	3.4703	1.653	0.1010	8.0636	0.3502	23.028	< 0.0001
		T	0.2320	0.2645	0.877	0.3820	0.0539	0.0131	4.123	0.0001
		T²	-0.0034	0.0050	-0.674	0.5020				
		B-2	-1.4067	0.0768	-18.305	< 0.0001	-1.4096	0.0765	-18.417	< 0.0001
		B-3	-0.5093	0.0976	-5.219	< 0.0001	-0.5169	0.0967	-5.345	< 0.0001
		(AIC)	93.9				**92.3**			
C5	68	Intercept	42.1151	30.0139	1.403	0.1650	10.3924	0.9338	11.130	< 0.0001
		T	-2.2180	2.1393	-1.037	0.3040	0.0439	0.0327	1.343	0.1840
		T²	0.0402	0.0380	1.057	0.2940				
		B-2	-1.9092	0.1691	-11.293	< 0.0001	-2.0146	0.1367	-14.740	< 0.0001
		B-3	-1.1011	0.2008	-5.484	< 0.0001	-0.9462	0.1375	-6.881	< 0.0001
		(AIC)	96.0				**95.2**			

C1–5, first to fifth instar crabs; *N*, number of crabs