博士学位論文

ノコギリガザミ類の稚ガニ期における共食いと温度適応に関する研究

2022 年度

（2023 年 3 月）

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第１章　序論

1. 1 ノコギリガザミ類について

ノコギリガザミ類は十脚目 Decapoda、ワタリガニ科 Portunidae、ノコギリガザミ属 *Scylla* に属する大型のカニ類であり、インド・太平洋の熱帯から亜熱帯域の主に内湾や汽水域に生息する。美味で市場価値が高いことから、インド・太平洋沿岸諸国における水産上重要な大型甲殻類である（Keenan & Blackshaw, 1999; Le Vay, 2001; Ogawa *et al*., 2011; Ogawa *et al*., 2012; 古川ら，2014）。本属は 4 種、すなわち、トゲノコギリガザミ *Scylla paramamosain* Estampador, 1949、アミメノコギリガザミ *S. serrata* (Forskål, 1775)、アカテノコギリガザミ *S. olivacea* (Herbst, 1796)、ミナミノコギリガザミ *S. tranquebarica* (Fabricius, 1798) から構成され（Keenan *et al*., 1998; 阪地 ・ 伏屋，2015）、本邦にはミナミノコギリガザミを除く 3 種が分布する（大城，1988）。成体は主に汽水域や内湾域に生息し、交尾したメスは沖合に移動して産卵し（Le Vay, 2001）、孵化したゾエアは約 2 週間で 4 回の脱皮を経てメガロパに変態した後、1 週間程度で稚ガニに変態する（Keenan & Blackshaw, 1999）。

1. 2 世界のノコギリガザミ類を対象とした産業

ノコギリガザミ類は天然漁獲に加え、マングローブ域などで大規模な養殖が行われている（Keenan & Blackshaw, 1999; Shelley, 2008）。近年、世界の養殖生産量の増加が著しく、トゲノコギリガザミでは 2010 年の 112,408 トンから 2019 年には 160,616 トンに、アミメノコギリガザミでは 2010 年に 36,993 トンから 2019 年には 132,957 トンに増加した（FAO，2021）。一方、天然漁獲はアミメノコギリガザミで 2010 年の 35,338 トンから 2020 年の 51,139 トンに微増するにとどまっている（FAO，2021）（図 1.1）。

養殖の多くは天然種苗を用いており（Allan & Fielder, 2003; Keenan & Blackshaw, 1999; Le Vay, 2001; Marichamy & Rajapackiam, 2001）、生息地の破壊、養殖用種苗や人工種苗生産向けの成熟雌の過剰漁獲による天然個体群への影響が懸念されており（Le Vay, 2001; Walton *et al*., 2006）、持続可能な養殖産業構築のために人工種苗の供給が望まれてきた。ノコギリガザミ類の種苗生産は難しく（Allan & Fielder, 2003; Le Vay *et al*.,

2008)、卵菌症（加治 *et al.*, 1991; 浜崎・畑井，1993a，1993b)、幼生期の疾病（Hamasaki *et al.*, 2011)、幼生の過剰発育による大量死（Hamasaki *et al.*, 2002; 高野ら，2004）など様々な問題が発生したが、精力的な種苗生産技術開発の結果、一部の国では人工種苗による養殖も始まっている（Allan & Fielder, 2003)。

ノコギリガザミ類の養殖産業が抱えるもう一つの課題として、共食いによる減耗が挙げられる。ノコギリガザミ類を含む多くのカニ類の中間育成工程やその後の養殖工程において、共食いによる減耗は生残率を低下させる深刻な問題であり（Allan & Fielder, 2003; Quinitio & Parado-Estepa, 2011; Williams & Primavera, 2001)、持続可能なノコギリガザミ類養殖の構築を目指すうえで、共食い防除策の開発は喫緊の課題である。

1．3 本邦におけるノコギリガザミ類の状況

本邦には、トゲノコギリガザミ、アミメノコギリガザミ、アカテノコギリガザミの 3 種が生息しており、前 2 種が主要な漁獲対象となっている（Hamasaki *et al.*, 2011; Obata *et al.*, 2006; Ogawa *et al.*, 2011, 2012)。両種の分布には地理的な差異がみられ、トゲノコギリガザミは静岡県の浜名湖や高知県の浦戸湾など本州の太平洋沿岸の温帯域を主な生息域とするのに対し（Hamasaki & Kitada, 2008; Hamasaki *et al.*, 2011; 伊藤，2000)、アミメノコギリガザミは南西諸島を中心とした亜熱帯域に主に分布する（Ogawa *et al.*, 2011, 2012; 大城・今井，2003)。国内における漁獲量は（図 1.2)、トゲノコギリガザミは浜名湖にて年間 6 トン前後（静岡県水産技術研究所浜名湖分場，2022)、浦戸湾では 1 トン前後とされている（高知県水産試験場，2017)。アミメノコギリガザミは、沖縄県においてタイワンガザミを含むガザミ類として統計がとられており、近年では年間 3–5 トン前後が水揚げされている（沖縄県漁業協同組合連合会，2022)。太田ら（2017）によると、近年の沖縄県内における資源水準は低位に分類され、長期・短期傾向のいずれも減少とされている。

両種は栽培漁業の対象種として、日本栽培漁業協会を中心にトゲノコギリガザミは 1979 年から、アミメノコギリガザミは 1985 年から技術開発が開始され（日本栽培漁業協会，2003）、親ガニの養成（Hamasaki, 2002, 2003)、種苗生産（Suprayudi *et al.*, 2002; Hamasaki *et al.*, 2002)、中間育成（伊藤，1997a)、放流（伊藤，1997b; 伊藤・上村，1993; Imai *et al.*, 2002）などについて取り組まれてきた。日本栽培漁業協会による種苗放流は両種とも 1988 年から開始され（日本栽培漁業協会，2003)、トゲノコギリ

ガザミでは2010年の986,000尾（水産庁・独立行政法人水産総合研究センター, 2012）、アミメノコギリガザミは 2002 年の 469,000 尾（水産庁ら，2004）をもって終了した（図 1.3）。近年では静岡県と高知県で数万尾程度の小規模な放流が行われている（水産研究・教育機構，2020; 水産庁増殖推進部ら，2021）　。

本邦はノコギリガザミ類の生息地の北限に位置する。水温は外温動物の成長、生存、行動や生理に大きく影響することから（Araujo *et al*., 2013; Portner, 2001; Sunday *et al*., 2011）、今後の温暖化に伴って分布の拡大や資源構成の変化が起こる可能性がある。国内の将来的な分布状況を予測する上で、ノコギリガザミ類の温度適応を解明することが求められる。

1．4　本論文の構成

本論文は、トゲノコギリガザミとアミメノコギリガザミの共食い防除技術開発の基礎的知見収集及び温度適応に関する実験について筆者が取り組んだ研究成果を取りまとめたものであり、第 1 章の序論に続く第 2 章～第 4 章と第 5 章の総合考察より構成される。

第 2 章ではトゲノコギリガザミとアミメノコギリガザミの稚ガニの体サイズ差が共食いと胸脚欠損に及ぼす影響について、サイズの異なる 2 尾のカニを 24 時間同一容器で飼育することによって明らかにした。第 3 章では、トゲノコギリガザミとアミメノコギリガザミの稚ガニの飼育水温が生残と成長に与える影響について、種苗生産した稚ガニを水温別に個別飼育を行って調べた。第 4 章では、トゲノコギリガザミとアミメノコギリガザミの稚ガニの高温・低温耐性について飼育実験を行い、半数致死水温と半数歩行停止水温を明らかにした。第 5 章では総合考察として、第 2 章から第 4 章の結果に基づき、トゲノコギリガザミとアミメノコギリガザミの共食い防除技術の高度化に向けた課題について整理するとともに、温暖化環境下における本邦のノコギリガザミ類の資源構成の変化について考察を行った。なお、第 2 章から第 4 章の内容は下記学術論文として公表済みである。

- Sanda, T., Shimizu, T., Dan, S., & Hamasaki, K., 2021. Effect of body size on cannibalism in juvenile mud crab *Scylla serrata* (Decapoda: Brachyura: Portunidae) under laboratory conditions. Crustacean Research 50, 87-93. （第 2 章 1 節）

- Sanda, T., Iwasaki, T., Dan, S., & Hamasaki, K., 2023. Effect of body size on cannibalism in juvenile mud crab *Scylla paramamosain* (Decapoda: Brachyura: Portunidae) under laboratory conditions. Aquatic Animals 2023, AA2023-2（第 2 章 2 節）
- Sanda, T., Shimizu, T., Iwasaki, T., Dan, S., & Hamasaki, K., 2022. 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, Nauplius 30, e2022012. （第 3 章）
- Sanda, T., Shimizu, T., Dan, S., & Hamasaki, K., 2022. Temperature tolerance of juveniles of two mud crab species, *Scylla paramamosain* and *Scylla serrata* (Decapoda: Brachyura: Portunidae). Crustacean Research 51, 129-135.（第 4 章）

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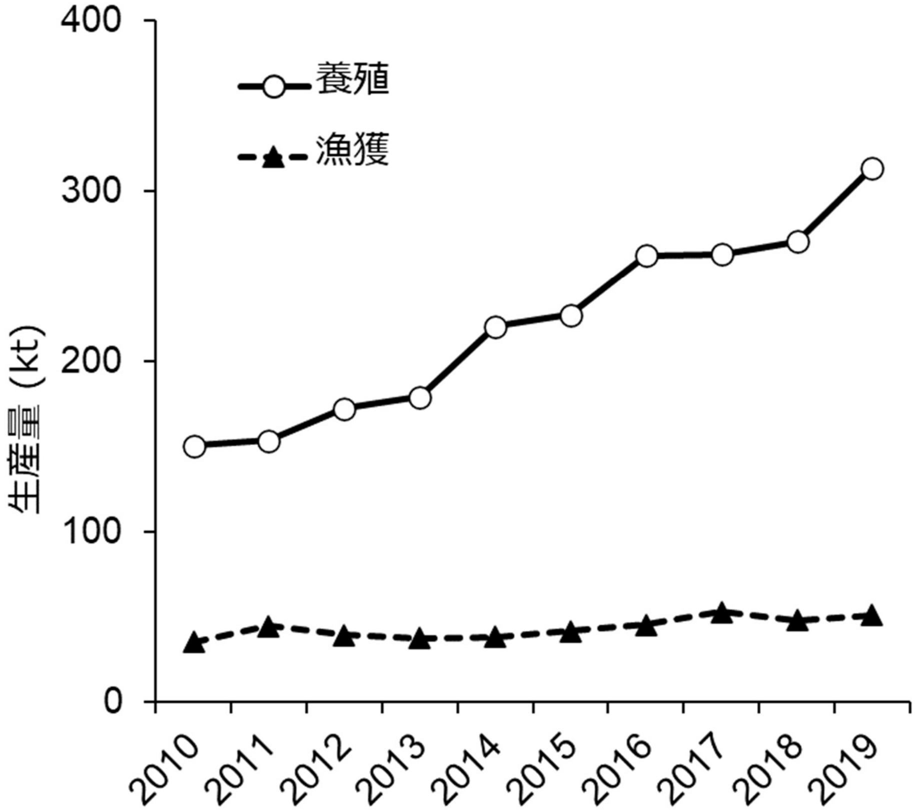

図 1.1. 世界のノコギリガザミ類の漁獲量と養殖生産量の推移

漁獲量はアミメノコギリガザミのみ、養殖生産量はアミメノコギリガザミ、トゲノコギリガザミ、アカテノコギリガザミの合算（FAO yearbook. Fishery and Aquaculture Statistics 2019 より作成）

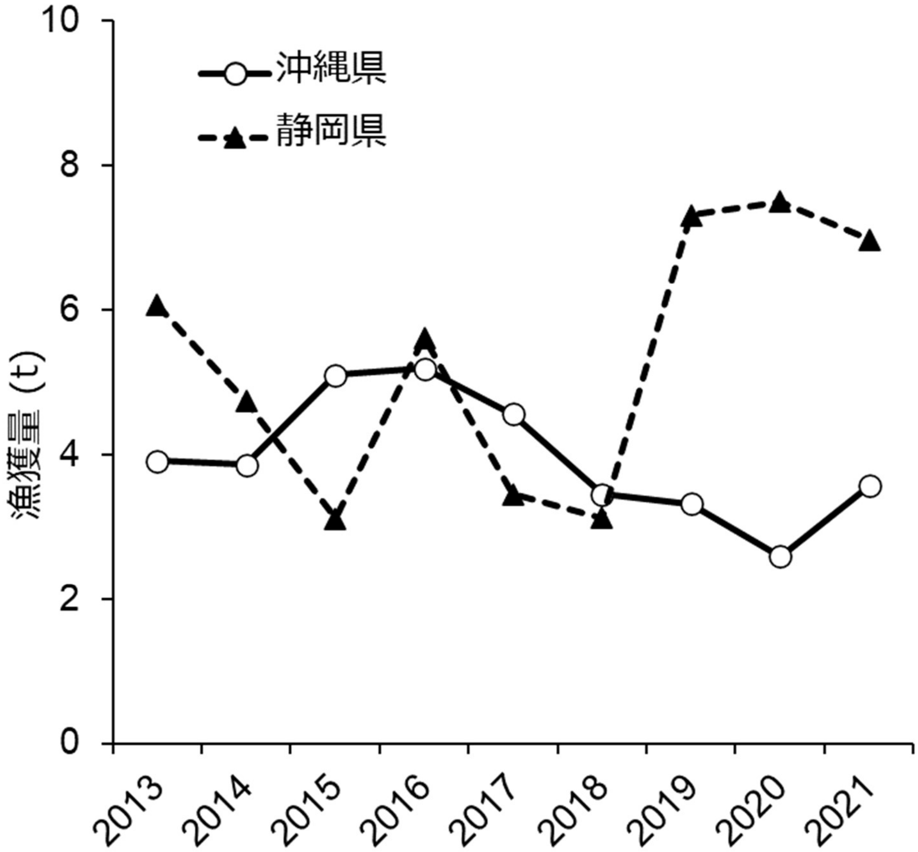

図 1.2. 沖縄県と静岡県におけるノコギリガザミ類の漁獲量の推移

沖縄県のデータは、JF 沖縄魚市場、名護漁協、糸満漁協、与那城町漁協、八重山漁協、那覇地区漁協における「ガザミ類」（ノコギリガザミ類とタイワンガザミ）の水揚げ量の合算。（沖縄県漁業協同組合連合会 HP 市況情報及び、静岡県水産技術研究所浜名湖分場水産・海洋技術研究所広報誌「はまな」より作成）

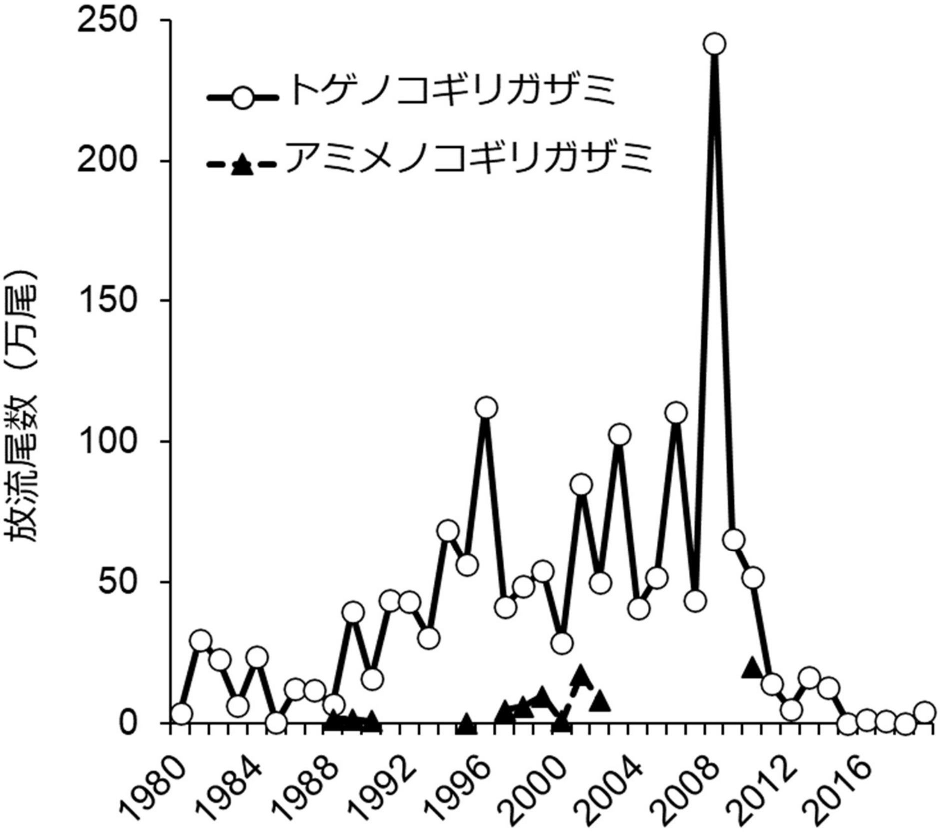

図 1.3. 本邦におけるノコギリガザミ類の放流尾数の推移

（昭和 55 年～平成 11 年度栽培漁業種苗生産・入手・放流実績（全国）. 資料編及び、平成 23 年～令和元年度栽培漁業・海面養殖用種苗の生産・入手・放流実績（全国）. 資料編より作成）

第2章 第1節

アミメノコギリガザミ稚ガニの共食いに及ぼす体サイズ差の影響

Effect of body size on cannibalism in juvenile mud crab *Scylla serrata* (Decapoda: Brachyura: Portunidae) under laboratory conditions

2. 1. 1 Introduction

Cannibalism, defined as killing and consuming either all or part of a conspecific individual, is a common phenomenon in a wide variety of animals (Fox, 1975; Polis, 1981), including brachyuran crabs (e.g. Lovrich & Sainte-Marie, 1997; Moksnes *et al.*, 1997, 1998; Fernández, 1999).

Cannibalistic behavior is commonly found in commercially cultured crabs, such as the mud crab *Scylla serrata* (Forsskål, 1775) (Mirera & Moksnes, 2013, 2015) and the blue swimmer crab *Portunus pelagicus* (Linnaeus, 1758) (Marshall *et al.*, 2005), and it causes serious impacts on the productivity and profitability of the aquaculture industry (Romano & Zeng, 2017). Post-molt crabs with soft shells are the most vulnerable to cannibalism, and large body size differences between individuals facilitate cannibalism even among intermolt crabs with hard shells (Marshall *et al.*, 2005; Mirera & Moksnes, 2013, 2015; Romano & Zeng, 2017).

The mud crab *S. serrata* is an important aquaculture species in subtropical and tropical regions worldwide (Keenan & Blackshaw, 1999). Mud crab aquaculture mainly relies on wild-caught juveniles, and due to the exhaustion of wild crab populations and the limited supply of crab seeds, the expansion of the mud crab aquaculture industry has been considerably hindered since the last few decades (Keenan & Blackshaw, 1999; Shelley, 2008). However, thanks to the development of seed production technology, commercial-scale hatcheries are now producing sufficient amounts of crab seeds for the industry in several countries (Allan & Fielder, 2003). In mud crab hatcheries, nursery cultures grow either megalopae or first crab instar (C1) to third to eighth instar crab (C3–C8) juveniles with carapace width (CW) of 6–40 mm before supplying crabs to farmers (Mann *et al.*, 2007; Quinitio & Parado-Estepa, 2011). Mann *et al.* (2007) conducted nursery culture experiments on mud crabs under different stocking densities for three development phases (phase 1, megalopae to C1/C2; phase 2, C1/C2 to C4/C5; and phase 3, C5 to C7/C8), and reported that cannibalism probably caused higher crab mortality levels, which were associated with increasing crab size, particularly in phase 3.

The supply of sufficient food and shelter and reduction of stocking density are the two recommended measures that can effectively mitigate the agonistic interactions that lead to cannibalism in mud crab nursery cultures (Mann *et al.*, 2007; Quinitio & Parado-Estepa, 2008; Mirera & Moksnes, 2013, 2015). In these environments, substantial body size variations occur within a short period of time (Mann *et al*., 2007); therefore, size grading is also considered as an effective strategy to reduce the occurrence of cannibalism (Mirera & Moksnes, 2013; Romano & Zeng, 2017). Nevertheless, many aspects of how body size differences induce cannibalistic behavior among juveniles remain unknown.

In the present study, we aimed to elucidate the effect of body size on cannibalism in hard-shelled juvenile mud crabs during specific, high mortality culture phases.

2. 1. 2 Materials and Methods

One-to-one match experiment

To examine the effect of body size on cannibalism in hard-shelled juvenile mud crabs, laboratory experiments were conducted in 2018 at the Yaeyama Field Station, Japan Fisheries Research and Education Agency, Okinawa Prefecture, Japan. Sibling hatchery-raised crabs (C5–C9) with a CW of 12.5–33.3 mm were individually cultured in small plastic cups (diameter, 12 cm; height, 10 cm) filled with sand-filtered natural seawater (salinity: 35 ppt; temperature: 27°C–28°C). Crabs were daily fed with formula feeds used for prawns (Higashimaru Co. Ltd., Kagoshima, Japan).

Larger plastic cups (diameter, 14.5 cm; height, 17.7 cm) were used as experimental arenas and were devoid of any structure that could be used as a refuge by the crabs. The experimental arenas containing seawater (salinity; 35 ppt; temperature: 28°C) were set within a water bath system controlled at 28°C with alternating phases of 12 h of light and 12 h of darkness. Crabs without any sign of pre-molt stage (Quinitio & Parado-Estepa, 2011) were selected after examination of the flat dactylus of the fifth pereiopod. Their CW was then measured at the widest portion of the carapace, including the lateral spines, using a digital caliper. The two individuals were then simultaneously placed in the experimental arena and a total of 50 trials were conducted. The body size ratio of small to large crabs (large crab size / small crab size) was within the range between 1.0 and 1.9. The two test crabs were observed 24 h after being placed in the arena, and the occurrence of cannibalism (death) was assessed. We also checked the loss of pereiopods in surviving crabs.

Statistical analysis

Statistical analyses were performed in R (R4.0.2; R Core Team, 2020) using a 5% significance level. We used a generalized linear model (GLM) with a binomial distribution to evaluate the effect of body size (explanatory variable) on binary values defined as death (cannibalism) (1) or survival (0) (response variable). Two models were employed using the absolute body size of large crabs (LCW) and small crabs (SCW) or the relative size difference (RSD) between large and small crabs as the explanatory variables: model 1, response variable ~ LCW + SCW; and model 2, response variable ~ RSD. The RSD was calculated as per earlier studies (Briffa & Elwood, 2001; Zhang *et al.*, 2018), RSD = 1 – SCW / LCW, and it ranged from 0.001 to 0.474. The RSD_{50} with 95% confidence intervals (CI), at which 50% of the small crabs were cannibalized by large crabs, was calculated using a logistic equation based on the binomial GLM results. The RSD_{50} value was then converted into the predator-prey size ratio (predator size / prey size).

In the present study, pereiopod losses were observed in the surviving small crabs, whereas only one large crab was observed to have lost a left third pereiopod. Using binomial GLM analyses, we evaluated the effect of body size (explanatory variable) on the proportion of lost pereiopods (chelipeds, number of lost chelipeds / 2; and walking legs, number of lost legs / 8) in the surviving crabs (response variables). However, we detected model overdispersion (dispersion parameters > 1.6 for chelipeds and > 4 for walking legs) and thereby corrected the standard errors using a quasibinomial GLM (Zuur *et al.*, 2009). The binomial GLM and quasibinomial GLM analyses were performed using the *glm* function (logit link).

2. 1. 3 Results

Cannibalism occurred in 18 (36.0%) out of 50 test trials, and large crabs preyed on small crabs in all cases. The body size influenced the occurrence of cannibalism (Table 2.1.1). The probability of cannibalistic events significantly increased with the increasing LCW ($P = 0.0139$) or with the decreasing SCW ($P = 0.0122$), and thereby it significantly increased with the increasing RSD ($P = 0.0015$), as illustrated by the logistic curve in Fig. 2.1.1. The RSD_{50} was calculated as 0.337 (95% CI: 0.259–0.460) [or predator-prey size ratio = 1.51 (95% CI: 1.35–1.85)].

At the end of the trails, one cheliped and both chelipeds were missing in 12.5% (4/32) and 12.5% (4/32) of the surviving small crabs, respectively, and 21.9% (7/32) and 15.6% (5/32) of the preyed crabs lost 1–4 and 5–8 walking legs, respectively. The body size did not significantly affect the proportion of lost chelipeds ($P = 0.3080$–0.8440) (Table 2.2.1). The proportion of lost walking legs was not significantly influenced from the SCW ($P = 0.4080$), but it tended to increase with the increasing LCW (*P* = 0.0570) and thereby significantly increased with the increasing RSD (*P* = 0.0413) (Table 2.1.1), as illustrated by the logistic curve in Fig. 2.1.2.

2. 1. 4 Discussion

Mirera & Moksnes (2013) conducted laboratory experiments to examine the effects of body size differences and shelter types on cannibalism in juvenile mud crabs, using four CW size classes (21–30 mm, 31–40 mm, 41–50 mm, and 51–70 mm inner carapace width (ICW), measured at the widest portion of the carapace excluding the lateral spines). Each set of four crabs with two different size classes was placed in tanks that served as experimental arenas, and high mortality rates were detected when crabs of the smallest size class (21–30 mm) were exposed to crabs of the largest size class (51–70 mm), suggesting that size difference would be an important factor affecting cannibalism in juvenile mud crabs.

In the present study, we placed two juvenile mud crabs, one small and one large, in the test arena and evaluated the occurrence of cannibalism (i.e., one-to-one match experiment). We demonstrated that body size significantly affected the probability of cannibalistic events between hard-shelled juveniles during nursery culture phases (Table 2.1.1 and Fig. 2.1.1). In our experiments, large crabs always preyed on small crabs. Furthermore, 25% and 38% of the surviving small crabs lost their chelipeds and pereiopods, respectively. The proportion of lost walking legs increased with the increasing relative size difference between large and small crabs, indicating a stronger aggressive behavior displayed by larger crabs (Fig. 2.1.1).

Mann *et al*. (2007) reported higher percentages of harvested juvenile mud crabs in nursery cultures missing either one or both chelipeds in phase 3 (C5 to C7/C8) than in phase 2 (C1/C2 to C4/C5). In addition, the study found higher percentages of juveniles missing chelipeds in younger instars in both nursery culture phases. In the present study using C5–C9 juveniles, the body size did not significantly affect the proportion of lost chelipeds in the surviving small crabs. Our experiments suggest that the small crabs that lost their cheliped weaponry would have certainly died if placed in trials with larger relative size differences.

Molting is a highly variable process in juvenile mud crabs. Mann *et al*. (2007) reported that five instars, C2–C6 and C5–C9, were found during harvest in phase 2 and phase 3 nursery cultures, respectively, resulting in an increased probability of larger instar crabs displaying cannibalistic behavior toward smaller instar crabs. Therefore, size grading has been considered one of the most effective measures to reduce cannibalism in mud crab nursery cultures (Mirera

& Moksnes, 2013; Romano & Zeng, 2017). In fact, Mirera & Moksnes (2015) conducted culture experiments on mud crabs using small net cages for seven days and reported that crab mortality significantly decreased in treatments involving smaller crabs (20–50 mm ICW) compared to those including larger individuals (20–80 mm ICW).

In the present study, the relative size difference at which 50% of the small crabs were cannibalized by large conspecifics was estimated at 0.34 (or predator-prey size ratio = 1.5). Maintaining the relative size difference values (or predator-prey size ratios) between juveniles below 0.34 (1.5) could contribute to reducing cannibalism and ensure high productivity levels in nursery cultures. In fish aquaculture, size grading devices are normally used (Kelly & Heikes, 2013), but the literature does not report that such devices have yet been developed for juvenile crabs. Developing a size grading methodology to reduce cannibalistic events would represent a key improvement for the mud crab aquaculture industry, thereby allowing the establishment of cost-effective nursery cultures that can ensure higher productivity.

We used only hard-shelled crabs in the experiments whereas post-molt crabs with soft shells are known to be the most vulnerable to cannibalism (Marshall *et al.*, 2005; Romano & Zeng, 2017). Therefore, future studies examining cannibalism between juveniles with different molting stages considering their body size difference would also contribute to establishing the cost-effective nursery cultures in mud crabs.

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Table 2.1.1. Evaluation of the effect of body size (explanatory variable) on cannibalism outcome (absence or presence) or the proportions of lost chelipeds and lost walking legs (response variable) in juvenile mud crab *Scylla serrata*. Two models were employed using the absolute body size of large crabs (LCW) and small crabs (SCW) (model 1) or the relative size difference (RSD = 1 – SCW / LCW) between large and small crabs (model 2) as the explanatory variables. Data were analyzed using a generalized linear model (GLM). A binomial GLM (logit link) and a quasibinomial GLM (logit link) were employed to analyze the binary and proportional data, respectively.

Response variable	*N*	Model	Explanatory variable	Coefficient Estimate	SE	*z* or *t* values	*P*
Cannibalism	50	1	(Intercept)	-0.2674	2.7542	-0.097	0.9227
			LCW	0.2331	0.0948	2.459	0.0139
			SCW	-0.3428	0.1368	-2.505	0.0122
		2	(Intercept)	-3.1768	0.9518	-3.338	0.0008
			RSD	9.4202	2.9734	3.168	0.0015
Proportion of lost chelipeds	32	1	(Intercept)	-4.0204	3.0778	-1.306	0.2020
			LCW	-0.0228	0.1146	-0.198	0.8440
			SCW	0.1625	0.1568	1.037	0.3080
		2	(Intercept)	-1.2066	0.7006	-1.722	0.0953
			RSD	-1.3871	3.1398	-0.442	0.6618
Proportion of lost walking legs	32	1	(Intercept)	-3.7360	3.2680	-1.143	0.2620
			LCW	0.1791	0.0903	1.982	0.0570
			SCW	-0.1238	0.1476	-0.839	0.4080
		2	(Intercept)	-2.9834	0.8129	-3.670	0.0009
			RSD	5.8716	2.7539	2.132	0.0413

N, number of observations; SE, standard errors.

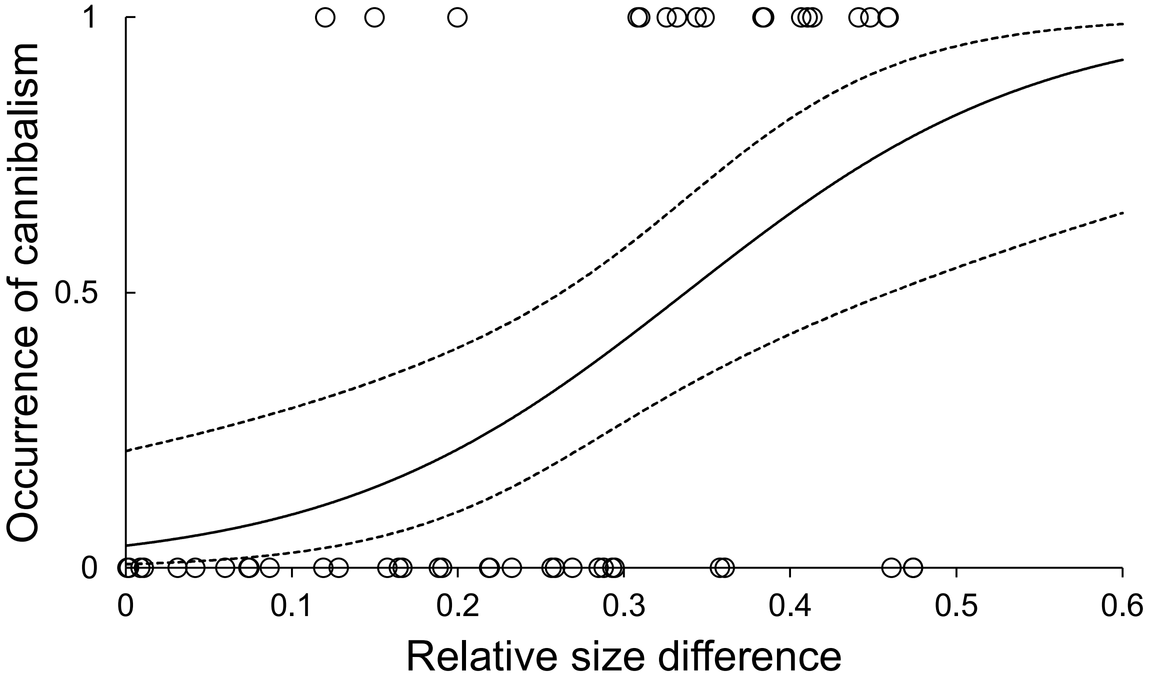

Fig. 2.1.1. Relationship between relative size difference and occurrence of cannibalism in test pairs of mud crab *Scylla serrata* juveniles in one-to-one match experiments. Relative size difference = 1 – (small crab size) / (large crab size). The estimated probabilities of cannibalistic events (solid curve) with 95% confidence intervals (dotted curves) were derived from a logistic equation formulated according to the GLM analysis: $y = 1 / (1 + e^{(3.1768 - 9.4202x)})$ (Table 2.1.1).

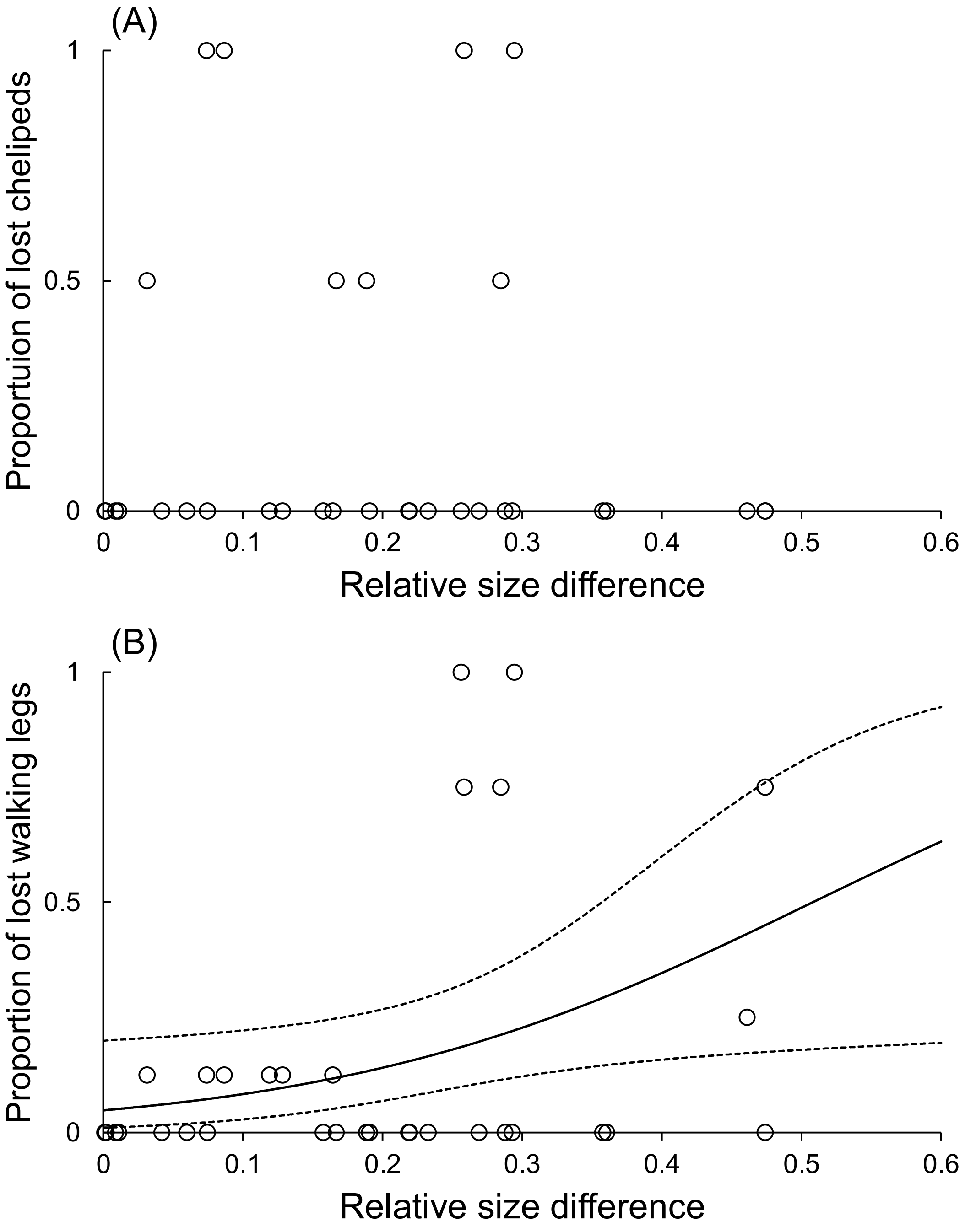

Fig. 2.1.2. Relationship between relative size difference and the proportions of lost chelipeds and walking legs in small juveniles of mud crab *Scylla serrata* at the end of one-to-one match experiments. Relative size difference = 1 – (small crab size) / (large crab size). The estimated proportions of lost walking legs (solid curve) with 95% confidence intervals (dotted curves) were derived from a logistic equation formulated according to the GLM analysis: $y = 1 / (1 + e^{(2.9834 - 5.8716x)})$ (Table 2.1.1).

第2章 第2節

トゲノコギリガザミ稚ガニの共食いに及ぼす体サイズ差の影響

Effect of body size on cannibalism in early juvenile mud crab *Scylla paramamosain* (Decapoda: Brachyura: Portunidae) under laboratory conditions

2. 2. 1 Introduction

Mud crabs in the genus *Scylla* (Decapoda: Brachyura: Portunidae) are important fishery and aquaculture resources in the Indo-Pacific, from warm temperate through subtropical to tropical regions (Keenan & Blackshaw, 1999; Le Vay, 2001; Allan & Fielder, 2004; Ogawa *et al*., 2011, 2012). They consist of four species: *S. paramamosain* Estampador, 1949, *S. serrata* (Forskål, 1775), *S. olivacea* (Herbst, 1796), and *S. tranquebarica* (Fabricius, 1798) (Keenan & Blackshaw, 1999). *Scylla paramamosain* is the main aquaculture target species in China (Ye *et al*., 2010) and Vietnam (Ut *et al*., 2007), and it is also treated as an important target species for stock enhancement programs in Japan (Obata *et al*., 2006; Hamasaki *et al*., 2011).

Mud crab aquaculture production has increased over the past decade (FAO, 2020), but much of the aquaculture relies on wild-caught juvenile crabs, raising concerns about negative impacts on natural stocks (Le Vay, 2001; Shelley, 2008). However, recent advances in seed production technology have allowed aquaculture farmers to use artificially produced crab seeds (Allan & Fielder, 2004). The hatchery-grown juvenile crabs are delivered to aquaculture farms at a size of about 6–40 mm carapace width (CW) (Mann *et al*., 2007; Quinitio & Parado-Estepa, 2011). However, cannibalism is the main cause of juvenile mortality during the nursery culture phase before the crabs are supplied to farmers (Mann *et al*., 2007; Quinitio & Parado-Estepa, 2011; Mirera & Moksnes, 2013), leading to serious impacts on the productivity and profitability of the aquaculture industry (Romano & Zeng, 2017).

In brachyuran crabs, post-molt individuals with soft shells are the most vulnerable to cannibalism, and large body size differences between individuals facilitate cannibalism, even among intermolt crabs with hard shells (Marshall *et al*., 2005; Mirera & Moksnes, 2013, 2015; Romano & Zeng, 2017). Therefore, size grading is considered an effective strategy to reduce the occurrence of cannibalism in aquaculture (Mirera & Moksnes, 2013; Romano & Zeng, 2017).

Sanda *et al*. (2021) examined the effect of body size on cannibalism in hard-shelled juveniles of *S. serrata* during the nursery culture phase. They estimated the threshold size difference at which 50% of the smaller crabs are cannibalized by larger crabs and suggested maintaining a size difference below the threshold value to contain the occurrence of cannibalism in nursery cultures of *S. serrata*. However, little is known about the relationship between body size differences and cannibalism during the nursery culture phase of *S. paramamosain*. In the present study, we aimed to elucidate the effect of body size on cannibalism in hard-shelled juveniles (third to fifth instar crabs, C3–C5) of *S. paramamosain* with 5–11 mm CW.

2. 2. 2 Materials and Methods

Experimental animals

A wild mature female was caught in Urado Bay (33°31'N, 133°33'E), Kochi Prefecture, Japan, in February 2022. The female crab and her larvae were cultured at the Yaeyama Field Station, Japan Fisheries Research and Education Agency, Ishigaki, Okinawa Prefecture, Japan, according to Sanda *et al.*'s (2022) method. Sibling hatchery-raised crabs were individually cultured in small plastic cups with lids (diameter, 6.5 cm; height, 7 cm), which were submerged in tanks with a flow-through water system of 500 L volume at natural temperature (mean ± standard deviation, 30.1 ± 0.5 °C) and salinity (34–35 ppt) conditions. The juveniles were fed *Artemia* nauplii once a day.

One-to-one match experiment

Experiments using juvenile crabs were conducted from July to August 2022. Plastic cups (diameter, 14.5 cm; height, 17.7 cm) were used as experimental arenas and did not have any structure that could be used as a refuge by the crabs. The experimental arenas, which contained seawater (salinity: 35 ppt; temperature: 28.8–29.5 °C) and *Artemia* nauplii at densities of 10 individuals/mL as food, were set within a water bath system with alternating phases of 12 h of light and 12 h of darkness. Crabs with no sign of being in the pre-molt stage (Quinitio & Parado-Estepa 2011) were selected after examination of the flat dactylus of the fifth pereiopod using a profile projector (V-12B, Nikon Corporation, Tokyo, Japan). The experimental C3–C5 juveniles were then measured at the widest portion of the carapace, including the lateral spines, using a digital caliper and profile projector (CW, 5.1–10.9 mm). All selected crabs had not lost any pereiopods, that is, chelipeds and walking legs. Two individuals were then placed simultaneously in the experimental arena, and a total of 60 trials were conducted. The body size ratio of smaller to larger crabs (larger crab size/smaller crab size) was within the range of 1.01

and 2.01. The test arenas were observed for the presence of the two test crabs after 24 h, and the occurrence of cannibalism (death) was assessed. We also checked for the loss of pereiopods in surviving crabs.

Statistical analysis

Statistical analyses were performed with R statistical software (R4.2.1; R Core Team, 2022) using a 5% significance level. We used a generalized linear model (GLM) with a binomial distribution to evaluate the effect of body size (explanatory variable) on binary values defined as death (cannibalism) (1) or survival (0) (response variable). Two models were employed using the absolute body size of the larger crabs (LCW) and smaller crabs (SCW) or the relative size difference (RSD) between the larger and smaller crabs (RSD = 1 − SCW/LCW) as the explanatory variables: model 1, response variable ~ LCW + SCW; and model 2, response variable ~ RSD. In the model 1, positive or negative impacts of body size of the test juveniles on cannibalism could be evaluated. In the model 2, the RSD_{50} with 95% confidence intervals (CI), at which 50% of the smaller crabs were cannibalized by the larger crabs, was calculated using a logistic equation based on the binomial GLM results. The RSD_{50} value was then converted into the predator–prey size ratio (predator size/prey size).

In the present study, pereiopod losses were observed in the surviving smaller crabs. We evaluated the effect of body size (explanatory variable) on the proportion of lost pereiopods (chelipeds, number of lost chelipeds/2; and walking legs, number of lost legs/8) in the surviving crabs (response variables) by using a quasibinomial GLM (Zuur *et al*. 2009). Binomial GLM and quasibinomial GLM analyses were performed using the *glm* function (logit link).

2. 2. 3 Results

Cannibalism occurred in 29 (48.3%) of the 60 test trials, and larger crabs preyed on smaller crabs in all cases. Body size influenced the occurrence of cannibalism (Table 2.2.1). The probability of cannibalistic events significantly increased with increasing LCW (P = 0.0051) or with decreasing SCW (P = 0.0021), and thereby significantly increased with increasing RSD (P = 0.0013), as illustrated by the logistic curve in Fig. 2.2.1. The RSD_{50} was calculated as 0.213 (95% CI: 0.180–0.254) [or predator–prey size ratio = 1.27 (95% CI: 1.22–1.34)].

At the end of the trials, one cheliped and 1–4 walking legs were missing in 6.4% (2/31) and 16.1% (5/31) of the surviving smaller crabs, respectively. Body size did not significantly affect the proportion of lost chelipeds ($P = 0.4576–0.6113$) or lost walking legs ($P = 0.6870–0.8770$) (Table 2.2.1).

2. 2. 4 Discussion

In the present study, we demonstrated that body size differences significantly affect the probability of cannibalistic events between hard-shelled juveniles (C3–C5; 5.1–10.9 mm CW) during the nursery culture phases of *S. paramamosain* (Table 2.2.1 and Fig. 2.2.1). Larger crabs always preyed on smaller crabs, and the relative size difference [RSD = 1− (smaller crab size)/(larger crab size)] at which 50% of the smaller crabs were cannibalized by larger conspecifics (RSD_{50}) was estimated at 0.21 (or predator-prey size ratio = 1.27).

Cannibalism induced by body size differences has also been observed in other portunid crabs, including the mud crab *S. serrata* (Moksnes *et al*., 1997; Marshall *et al*., 2005; Mirera & Moksnes, 2013, 2015; Sanda *et al*., 2021). Sanda *et al*. (2021) conducted one-to-one match experiments, as we did in the present study. They used C5–C9 juveniles (12.5–33.3 mm CW) of *S. serrata* to investigate the effect of body size differences on cannibalism during the nursery culture phase. They reported cannibalistic events similar to those observed in *S. paramamosain*, and the RSD_{50} was estimated at 0.34 (or predator–prey size ratio = 1.51).

Sanda *et al*. (2021) reported that chelipeds and walking legs were lost in 25% and 38% of the surviving smaller crabs of *S. serrata*, respectively, and the proportion of lost walking legs increased significantly as the relative size difference between the larger and smaller juveniles (C5–C9) increased. Furthermore, Mann *et al*. (2007) reported higher percentages of harvested juveniles of *S. serrata* in nursery cultures missing either one or both chelipeds during the C5 to C7/C8 (22–33 mm CW) period compared to the C1/C2 (4.0–4.8 mm CW) to C4/C5 (8.5–15 mm CW) period. In the present study, using C3–C5 juveniles of *S. paramamosain*, 6% and 16% of the surviving smaller crabs lost their chelipeds and walking legs, respectively. Consequently, pereiopod loss in prey crabs is likely to occur in larger instars of mud crabs. This may be because of the stronger aggressive behavior displayed by larger predator crabs and/or the stronger resistance behavior displayed by larger prey crabs: they lost their pereiopod(s) instead of being completely cannibalized. Stronger resistance behavior in larger prey crabs may also explain why the RSD_{50} (or predator-prey ratio) estimate was smaller in *S. paramamosain* (C3–C5, present study) than in *S. serrata* (C5–C9, Sanda *et al*. 2021).

Sanda *et al*. (2022) reared the C1 juveniles of *S. paramamosain* individually and reported that the CW increased approximately 1.2–1.3-fold per molt between C3 and C5 when carbs were reared at 30 °C. This magnitude, when converted to the RSD value, is about 0.20–0.23 between successive molts, which is consistent with the RSD_{50} estimate (0.21) in the present study. Therefore, synchronized molting of juveniles would reduce the chance of cannibalism. However, molting is a highly variable process in juvenile mud crabs; for example, Mann *et al.* (2007) reported that harvested juveniles included five instars in nursery cultures of *S. serrata.* Therefore, size grading to maintain the relative size difference values (or predator–prey size ratios) between juveniles below 0.21 (1.27) could contribute to reducing cannibalism in nursery cultures (C3–C5) of *S. paramamosain*. A practical size grading methodology has not yet been developed for juvenile crabs, which would be key for advancing the mud crab aquaculture industry using juveniles raised from artificial seeds.

2. 2. 5 References

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Table 2.2.1. Evaluation of the effect of body size (explanatory variable) on cannibalism outcome (absence or presence) or the proportions of lost chelipeds and lost walking legs (response variable) in juvenile mud crab *Scylla paramamosain*. Two models were employed using the absolute body size of larger crabs (LCW) and smaller crabs (SCW) (model 1) or the relative size difference (RSD = 1 – SCW/LCW) between larger and smaller crabs (model 2) as the explanatory variables. Data were analyzed using a generalized linear model (GLM). A binomial GLM (logit link) and a quasibinomial GLM (logit link) were employed to analyze the binary and proportional data, respectively.

Response variable	*N*	Model	Explanatory variable	Coefficient Estimate	SE	*z* or *t* values	*P*
Cannibalism	60	1	(Intercept)	-1.926	6.633	-0.290	0.7715
			LCW	3.514	1.255	2.800	0.0051
			SCW	-4.190	1.359	-3.083	0.0021
		2	(Intercept)	-7.720	2.387	-3.234	0.0012
			RSD	36.170	11.225	3.222	0.0013
Proportion of lost chelipeds in the surviving smaller crabs	31	1	(Intercept)	-12.183	5.335	-2.284	0.0302
			LCW	0.543	0.899	0.604	0.5510
			SCW	0.522	0.693	0.753	0.4576
		2	(Intercept)	-2.772	0.875	-3.169	0.0036
			RSD	-4.065	7.911	-0.514	0.6113
Proportion of lost walking legs in the surviving smaller crabs	31	1	(Intercept)	-1.016	7.349	-0.138	0.8910
			LCW	0.192	1.227	0.156	0.8770
			SCW	-0.536	1.315	-0.408	0.6870
		2	(Intercept)	-3.852	1.497	-2.572	0.0155
			RSD	3.906	10.462	0.373	0.7116

N, number of observations; SE, standard errors.

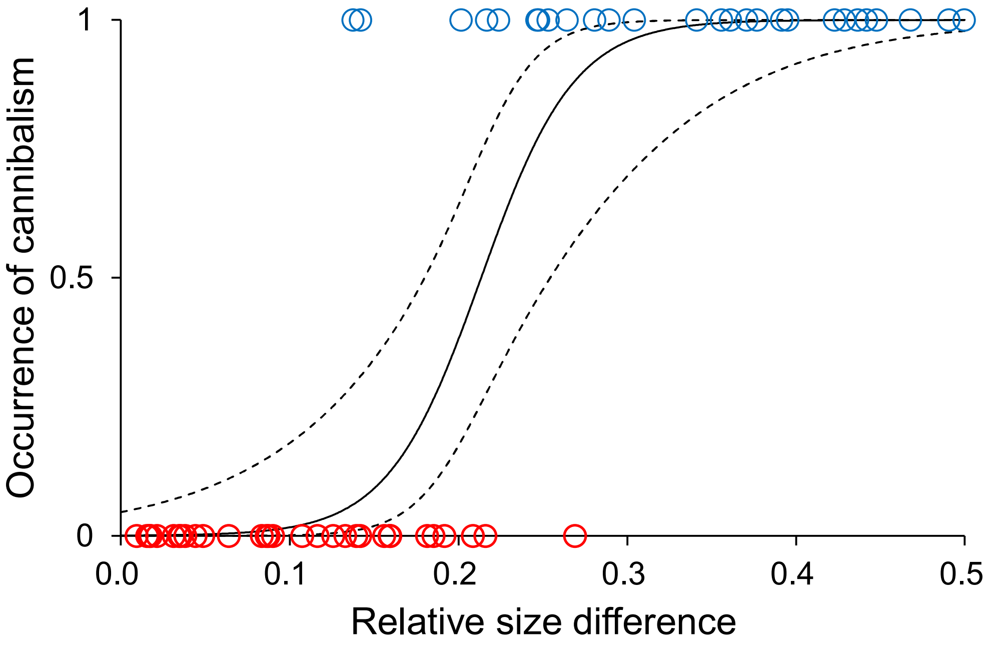

Fig.2.2.1 Relationship between relative size difference and occurrence of cannibalism in test pairs of mud crab *Scylla paramamosain* early juveniles in one-to-one match experiments. Relative size difference = 1− (smaller crab size)/(larger crab size). The estimated probabilities of cannibalistic events (solid curve) with 95% confidence intervals (dotted curves) were derived from a logistic equation formulated according to the GLM analysis: $y = 1 / (1 + e^{(7.720-36.170x)})$ (Table 2.2.1).

第3章

トゲノコギリガザミとアミメノコギリガザミの稚ガニの温度適応

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

3. 1 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 (Portner, 2001; Sunday *et al.*, 2011; Araujo *et al.*, 2013; Hoffmann *et al.*, 2013).

Mud crabs in the genus *Scylla* (Decapoda: Brachyura: Portunidae) are important fishery resources in the Indo-Pacific, from warm temperate through subtropical to tropical regions (Keenan & Blackshaw, 1999; Le Vay, 2001; Ogawa *et al.*, 2011; 2012). They consist of four species: *S. paramamosain* Estampador, 1949, *S. serrata* (Forskål, 1775), *S. olivacea* (Herbst, 1796), and *S. tranquebarica* (Fabricius, 1798) (Keenan & Blackshaw, 1999). In Asian and Oceanian countries, they are also treated as important aquaculture target species (Keenan & Blackshaw, 1999; Azra *et al.*, 2019). The main aquaculture target species are *S. paramamosain* and *S. serrata* (Allan & 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 & 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 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). *S. 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 than *S. paramamosain*'s habitat (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) and Hamasaki (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 & 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.

3. 2 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*. 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* 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 Table 3.1. Thus, low temperatures were not tested at older instar crabs because a limited number of our temperature-controlled incubation chambers could not allow us to use them for further longer periods at low temperature levels. 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^{TM} Conical Centrifuge Tube, 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* nauplius 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 & 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.

3. 3 Results

Survival

The survival rate of juveniles was not significantly different among broods in both species (Table 3.2). In *S. paramamosain*, temperature significantly affected the survival of juveniles and their ability to reach C2, C4, and C5 (Table 3.2). Survival rates decreased at the lowest temperature level (15.3°C) when juveniles molted to C2 although they still showed high values (80%) (Fig. 3.1). 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 survival of juveniles and their ability to reach C2 and C3 (Table 3.2). 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. 3.2). 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* (Table 3.3). The intermolt period decreased exponentially (mode l 1) or linearly (model 2) with increasing temperatures (Fig. 3.3, 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. 3.3, 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 (Table 3.4 and Figs. 3.5, 3.6). 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* (Table 3.4 and Figs. 3.5, 3.6). 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 (Table 3.4). The relationship between temperature and carapace width was expressed by an upward convex curve by model 1 in both species (Figs. 3.5, 3.6), and the carapace width tended to reach peaks at around 26°C in *S. paramamosain* (Fig. 3.5). However, it did not largely decrease even at higher temperatures (> 30°C) in *S. serrata* (Fig. 3.6). Inter-brood variability was evident in carapace width at 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. 3.5, 3.6).

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* (Table 3.5).

3. 4 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. 3.1). 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. 3.2). Survival rates of *S. paramamosain* juveniles declined to 30–40% until C5 at 29.8°C (Fig. 3.1), whereas all *S. serrata* juveniles survived to C5 even at 30.5°C (Fig. 3.2). Additionally, the carapace width of juveniles increased with increasing temperature but became smaller at 27.6–30.2°C in *S. paramamosain* (Fig. 3.5), whereas it appeared to reach a plateau at > 24°C in *S. serrata* (Fig. 3.6). 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 (Figs. 3.3, 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* (Table 3.5). 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 well 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 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 moult 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 & 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 e*t al.*, 2018). *S. 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.

3. 5 References

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Table 3.1. 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
1	15.3 ± 0.4	22 Apr 2020	54	C2	1	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
	24.7 ± 0.2	22 Apr 2020	49	C5		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
2	15.3 ± 0.4	23 Apr 2020	52	C2	2	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
	21.8 ± 0.2	23 Apr 2020	49	C4		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
3	19.0 ± 0.6	29 Jul 2020	35	C3	3	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
	23.3 ± 0.5	28 Jul 2020	25	C4		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

Table 3.2. 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.

		S. paramamosain					*S. serrata*				
Instar	Coefficient	*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

Table 3.3. 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, $y \sim \mathrm{T} + \mathrm{T}^2 + \mathrm{B}$ and model 2, $y \sim \mathrm{T} + \mathrm{B}$. 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 Tables 3.1 and 3.2 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.

		S. paramamosain					*S. serrata*				
Instar	Coefficient	*N*	Estimate	SE	*z* value	*P*	*N*	Estimate	SE	*z* value	*P*
C1	Intercept	164	11.7114	0.4980	23.516	< 0.0001	158	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
	T^2		0.0120	0.0011	10.876	< 0.0001		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
C2	Intercept	144	14.1412	1.2720	11.118	< 0.0001	156	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
	T^2		0.0154	0.0022	6.885	< 0.0001		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
C3	Intercept	101	14.1041	2.5929	5.439	< 0.0001	120	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
	T^2		0.0154	0.0039	3.938	< 0.0001					
	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
C4	Intercept	43	5.0630	0.6360	7.961	< 0.0001	68	2.7240	0.6702	4.064	< 0.0001
	T		-0.0874	0.0246	-3.554	0.0004		-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

Table 3.4. 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, $y \sim \mathrm{T} + \mathrm{T}^2 + \mathrm{B}$ and model 2, $y \sim \mathrm{T} + \mathrm{B}$. 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 Tables 3.3 and 3.4 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.

		S. paramamosain					*S. serrata*				
Instar	Coefficient	*N*	Estimate	SE	*z* value	*P*	*N*	Estimate	SE	*z* value	*P*
C1	Intercept	170	3.4829	0.0627	55.539	< 0.0001	170	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
	T^2										
	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
C2	Intercept	164	-0.1561	0.4767	-0.327	0.7438	158	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
	T^2		-0.0074	0.0009	-8.227	< 0.0001		-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
C3	Intercept	144	-6.9159	1.6019	-4.317	< 0.0001	156	-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
	T^2		-0.0201	0.0027	-7.489	< 0.0001		-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
C4	Intercept	101	-12.1186	5.6778	-2.134	0.0354	120	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
	T^2		-0.0291	0.0085	-3.434	0.0009					
	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
C5	Intercept	43	11.1068	2.3906	4.646	< 0.0001	68	10.3924	0.9338	11.130	< 0.0001
	T		-0.0735	0.0914	-0.804	0.4260		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

Table 3.5. 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	15.4391	0.1564	15.1242	15.7226	98.73	< 0.0001

N, number of crabs.

Appendix Table 3.1. 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, $y \sim \mathrm{T} + \mathrm{T}^2 + \mathrm{B}$ and model 2, $y \sim \mathrm{T} + \mathrm{B}$. 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.

			Model 1				Model 2			
Instar	*N*	Coefficient	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

Appendix Table 3.2. 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).

			Model 1				Model 2			
Instar	*N*	Coefficient	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

Appendix Table 3.3. 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.

			Model 1				Model 2			
Instar	*N*	Coefficient	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

Appendix Table 3.4. 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 *Scylla serrata*. 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).

			Model 1				Model 2			
Instar	*N*	Coefficient	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
		T^2	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
		T^2	-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
		T^2	-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
		T^2	-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
		T^2	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

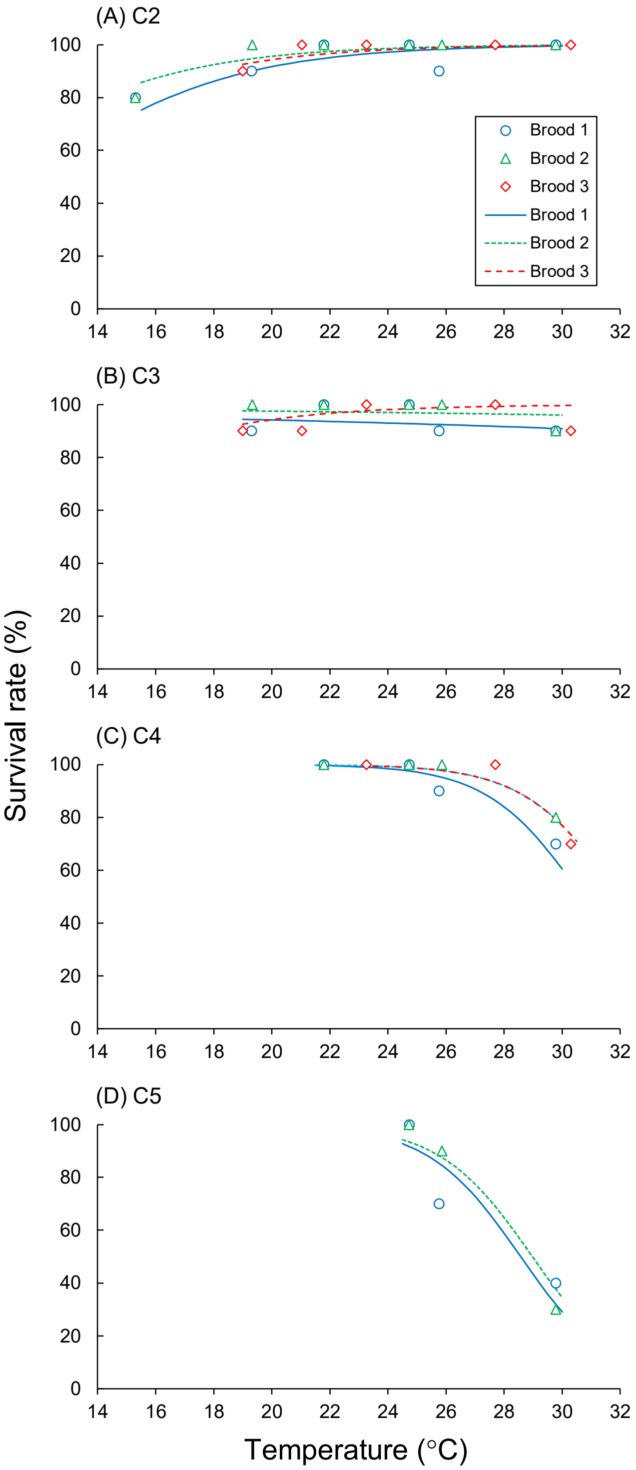

Figure 3.1. Survival rates of *Scylla paramamosain* juveniles under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Table 3.2. Significant differences were not found among broods at all instar crabs.

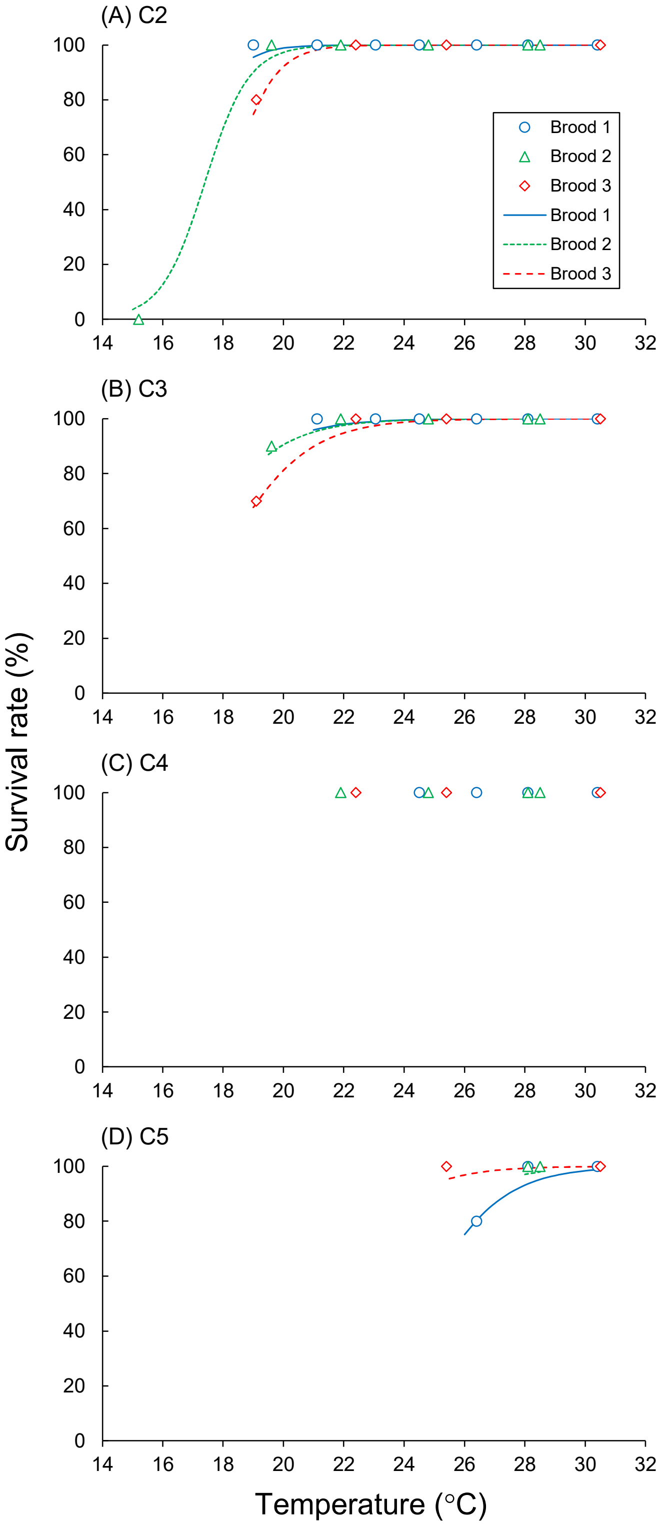

Figure 3.2. Survival rates of *Scylla serrata* juveniles under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Table 3.2. Significant differences were not found among broods at all instar crabs.

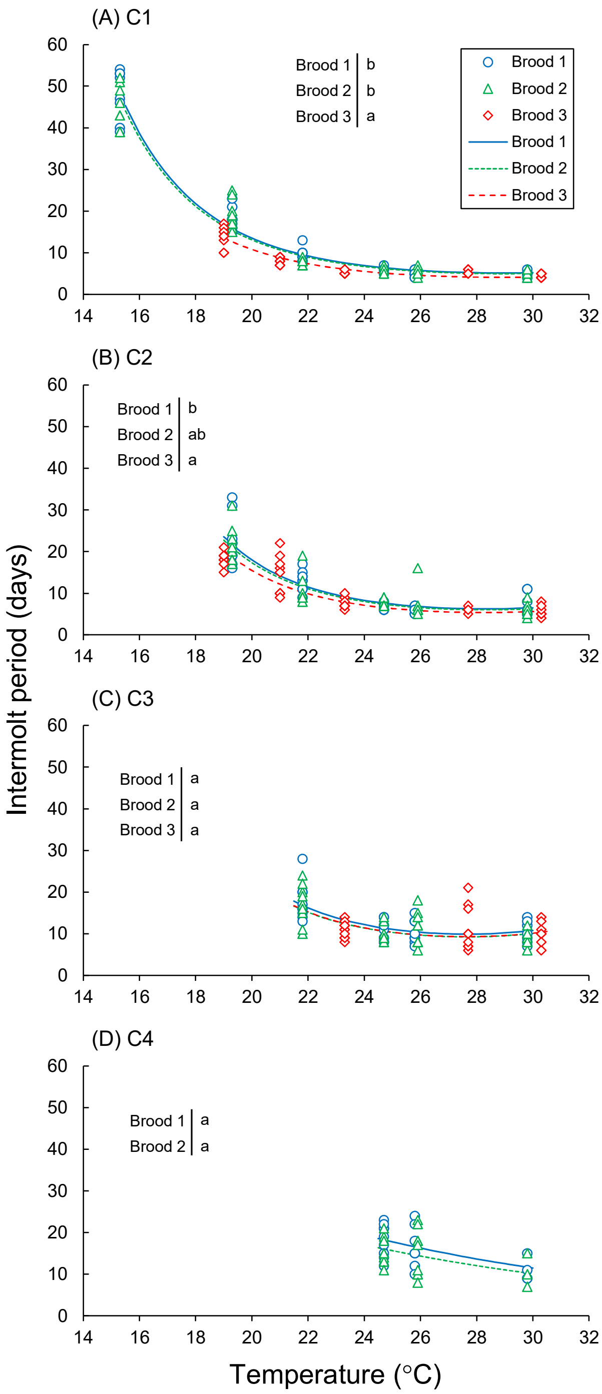

Figure 3.3. Intermolt periods of *Scylla paramamosain* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Table 3.3. 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.

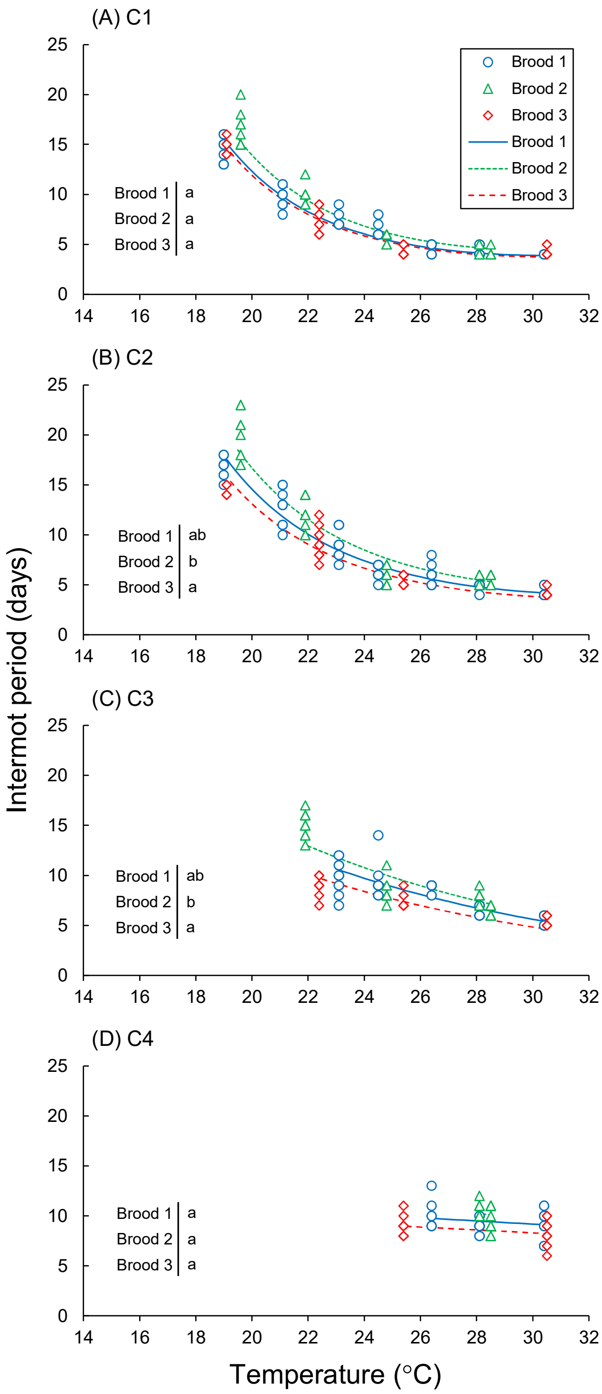

Figure 3.4. Intermolt periods of *Scylla serrata* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Table 3.3. 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.

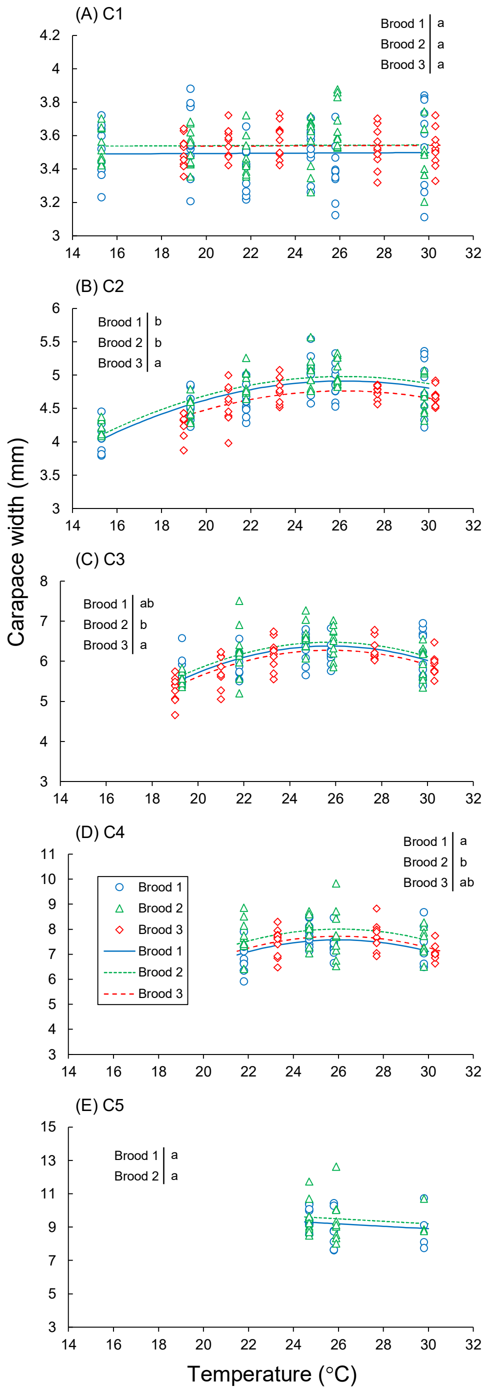

Figure 3.5. Carapace widths of *Scylla paramamosain* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Table 3.4. 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.

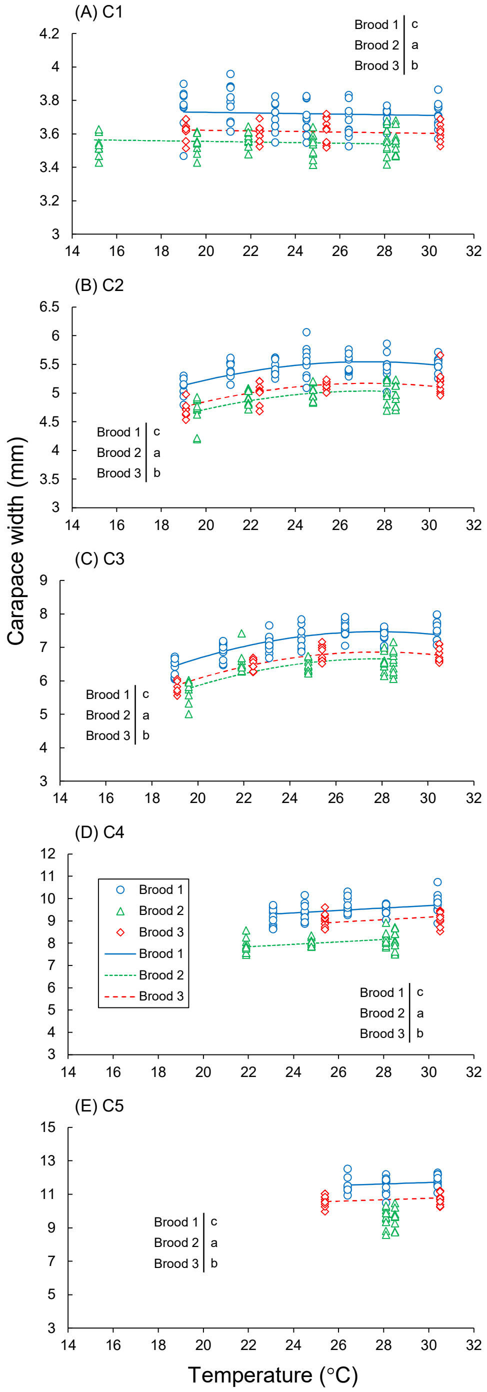

Figure 3.6. Carapace widths of *Scylla serrata* juveniles at respective instars under different temperature conditions. The first instar crabs (C1) were raised from three broods (nos. 1–3) and reared until molting to the second to fifth instar crabs (C2–C5). Curves were drawn using the coefficient estimates shown in Table 3.4. 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.

第4章

トゲノコギリガザミとアミメノコギリガザミ稚ガニの温度耐性

Temperature tolerance of juveniles of two mud crab species, *Scylla paramamosain* and *Scylla serrata* (Decapoda: Brachyura: Portunidae)

4. 1 Introduction

Mud crabs in the genus *Scylla* De Haan, 1833 (Decapoda: Brachyura: Portunidae) are distributed from warm temperate through subtropical to tropical regions of the Indo-West Pacific, and they are important fishery and aquaculture resources (Keenan & Blackshaw, 1999; Le Vay, 2001; Ogawa *et al.*, 2011, 2012). Mud crabs comprise four species: *S. olivacea* (Herbst, 1796), *S. paramamosain* Estampador, 1949, *S. serrata* (Forskål, 1775), and *S. tranquebarica* (Fabricius, 1798) (Keenan & Blackshaw, 1999). They have similar life histories: eggs are incubated under the female's pleon, and larvae grow into the first instar crab (C1) through five zoeal and one megalopal stages; then, juveniles initiate the benthic life after settling on shallow coastal habitats (Keenan & Blackshaw, 1999; Le Vay, 2001).

Three mud crab species, *S. olivacea*, *S. paramamosain* and *S. serrata*, reside in Japanese coastal waters, and the two latter species are abundant and main components of mud crab fishery (Obata *et al.*, 2006; Hamasaki *et al.*, 2011; Ogawa *et al.*, 2011, 2012). These two species exhibit different biogeographical distributions: *S. paramamosain* occurs on the Pacific coast of Honshu, a warm temperate area, and *S. serrata* are mainly distributed in the Ryukyu Archipelago, a subtropical area (Oshiro & Imai, 2003; Ogawa *et al.*, 2011, 2012).

Temperature plays a fundamental role in determining the geographical distribution of ectotherms because the thermal environment strongly affects the biological processes, such as the behaviour, physiology, growth and survival, of ectothermic animals throughout their entire life cycle (Sunday *et al.*, 2011; Araújo *et al.*, 2013; Hoffmann *et al.*, 2013). The sea-surface temperatures have increased at a rate of 1.19°C/100 years in the whole country of Japan under the global warming environment (Japan Meteorological Agency, 2022). Therefore, knowing the temperature tolerance of *S. paramamosain* and *S. serrata* throughout an entire life cycle is key to understanding and predicting future changes in the species compositions and the northern limit of their distribution under a global warming scenario.

We previously investigated the temperature adaptations of *S. paramamosain* and *S. serrata* by culturing C1 juveniles to moult to C2–C5 at different temperatures (~15–30°C) (Sanda *et al.*, 2022). The C1 juveniles of *S. paramamosain* could moult to C2 at 15.4°C and the survival rate was reduced at 29.8°C through C4–C5, whereas the C1 juveniles of *S. serrata* could not moult to C2 at 15.2°C and the survival rate was not affected by temperature thereafter. Thus, it is suggested that juveniles of *S. paramamosain* and *S. serrata* adapt to lower and higher temperature conditions, respectively, reflecting their biogeographical distribution of the former in the temperate area and the latter mainly in the subtropical area, Japan. Nevertheless, the low- and high-temperature tolerance limits of juveniles, particularly after growing to C5, of these two mud crab species are largely unknown. In the present study, to further elucidate the temperature adaptations of *S. paramamosain* and *S. serrata*, we determined the low- and high-temperature tolerance limits for locomotor activity and survival in laboratory-raised juveniles.

4. 2 Materials and Methods

Experimental animals

Temperature tolerance experiments were conducted using laboratory-raised juvenile crabs in 2018–2021. Larvae were cultured to moult to C1 juveniles according to the method of Sanda *et al*. (2022) at the Yaeyama Field Station, Japan Fisheries Research and Education Agency, Ishigaki, Okinawa Prefecture, Japan. Juvenile crabs were kept individually in plastic containers with lids (6.5 cm diameter and 7 cm height or 16 cm length, 10 cm width and 8 cm height), which were submerged in tanks with a flow-through water system of 300 L volume at natural temperature (mean ± standard deviation, 25.2 ± 2.7°C) and salinity (34–35 ppt) conditions. The lids of the culture containers had small holes that allowed seawater to enter the containers from the stock tank. Juveniles were fed with *Artemia* nauplii and formula feeds for prawns (Higashimaru Co. Ltd., Kagoshima, Japan) ad libitum once a day. The crabs that reached or exceeded the C5 juveniles were used for the temperature tolerance experiments.

Temperature tolerance experiments

The temperature tolerance of the mud crabs was evaluated by exposing the juveniles to decreasing or increasing thermal regimes in temperature-controlled incubatory chambers or tanks and determining their locomotor activity and survival. Low- and high-temperature tolerance experiments were performed twice for *S. paramamosain* and four times for *S. serrata*, respectively, using 20 juveniles for the respective trials (Supplementary Table S4.1). The mean CW values of juveniles used for the respective trials were 11.1–16.9 mm for *S. paramamosain* and 11.6–48.4 mm for *S. serrata* (Table S1). We prepared two size classes for *S. serrata* juveniles (11.6–18.1 mm and 35.6–48.4 mm mean CW) to infer if body size affects the temperature tolerance.

In trial no. 1 of the low-temperature tolerance experiment using *S. serrata*, juveniles were stocked individually in 50 mL plastic tubes (Falcon^{TM} Conical Centrifuge Tube, Corning Japan KK, Tokyo, Japan) and kept in a temperature-controlled incubation chamber (MT1-201, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). In other trials, crabs were kept individually in plastic containers with lids (8.7 cm diameter and 4.2 cm height or 13 cm length, 9.5 cm width and 5.5 cm height), which were submerged in 30–100 L test tanks containing 30–70 L of seawater (salinity, 34 ppt) by species, of which the temperature was controlled using aquarium coolers (ZC-200α, Zensui Co. Ltd., Osaka, Japan) or heaters (Protect Heater R-300W, Marukan Co., LTD, Osaka, Japan). The lids of the culture containers had small holes that allowed seawater to enter the containers from the test tank. A piece of formulated feed for prawns was given to juveniles during the experimental period.

Juvenile crabs were directly transferred from the culture vessels to the test containers controlled at a designated initial temperature. In the low-temperature tolerance experiment, initial temperature was set at 25°C in trial no. 1 for *S. paramamosain* and trial nos. 1 and 3 for *S. serrata*, and it was reduced to 20°C in successive trials for both species (Table S1). It was because this temperature did not affect juvenile activity and survival in the former trials and reducing the initial temperature could shorten the duration of the experiment. In the high-temperature tolerance experiment, initial temperature was set at 30°C in all trials for both species. The temperature settings of the incubation chamber and test tanks (i.e. coolers and heaters) were then adjusted to reduce or raise by 1°C for the low- and high-temperature tolerance experiments, respectively. After 24 h, the crabs were observed for locomotor activity (walking or not), survival and moulting. Temperature adjustment (decrease or increase of 1°C) and observation of juveniles were repeated every 24 h until all juveniles had died. After observation, all the culture water was replaced in the culture tubes used in trial no. 1 of the low-temperature tolerance experiment using *S. serrata* or half of the water was exchanged with new water in the test tanks used for other trials. The water temperature was recorded every 30 min with a data logger (Thermochron SL-type; KN Laboratories, Inc., Osaka, Japan) set in the culture tube or container.

Statistical analysis

Statistical analyses were performed using R statistical software (R4.2.1; R Core Team, 2022) at a 5% significance level. The temperature tolerance ability of the juveniles was evaluated by estimating the critical low or high temperatures (CLT or CHT) at which 50% of test juveniles ceased walking behaviour or died. Moulting occurred in some juveniles of both species; however, moulting incidence was not considered for evaluating the temperature tolerance traits of the crabs because the occurrence of moulting depends on the initial moulting stage of the test juveniles, which was not controlled in the present study.

We applied a bias-reduced logistic regression to formulate the relationship between the mean water temperature for 24 h before observation, the proportions of survivors among the test juveniles (survival rate) and the juveniles that exhibited walking behaviour among the survivors (walking rate). Logistic regression analyses were performed with the *brglm* function implemented in the brglm package (Kosmidis & Firth, 2021), and the CLT and CHT with 95% confidence intervals were estimated with the *invest* function in the investr package (Greenwell & Kabban, 2014).

4. 3 Results

Low-temperature tolerance

Similar trends were observed for changes in the walking and survival rates of juveniles in relation to decreased temperature regimes in both species. The walking rates of juveniles largely decreased below ~10°C, and almost all juveniles did not walk below 7–9°C; then, their survival rates sharply declined (Supplementary Fig. S4.1). Moulting occurred in 3–5 juveniles at 17.7–22.2°C for *S. paramamosain* and 2–5 juveniles at 17.1–25.5°C for *S. serrata* (Supplementary Table S4.2), and moult-related death was not observed in any juveniles.

CLT values for the walking behaviour and survival of juveniles were successfully estimated in all trials of both species (Fig. 4.1; Tables S4.2 and S4.3). They did not vary in two size classes of *S. serrata* and tended to be lower in *S. paramamosain* than in *S. serrata*, as summarised as follows [mean ± standard deviation (range)]: walking, 8.40 ± 0.69°C (7.91–8.89°C) and 9.63 ± 0.62°C (8.83–10.19°C), and survival, 6.36 ± 0.86°C (5.76–6.97°C) and 7.37 ± 0.44°C (7.00–7.92°C) for *S. paramamosain* and *S. serrata*, respectively.

High-temperature tolerance

The survival rates of juveniles were relatively high below 38°C but largely declined at 39–40°C, and all died above 40°C (Supplementary Fig. S4.2). Almost all surviving juveniles exhibited walking behaviour (Fig. S4.2). Moulting occurred in 4–5 juveniles at 30.3–38.7°C for *S. paramamosain* and 1–5 juveniles at 32.8–38.7°C for *S. serrata* in the respective trials (Table S4.2), and moulting failure did not occur in any juveniles.

CHT values were not estimated for walking behaviour, but they were successfully estimated for survival in all trials of both species (Fig. 4.1; Tables S4.2 and S4.3). CHT estimates for survival were similar in *S. paramamosain* [38.99 ± 0.36°C (38.74–39.25°C)] and two size classes of *S. serrata* [39.12 ± 0.56°C (38.39–39.72°C)].

4. 4 Discussion

The present study evaluated the temperature tolerance ability of two mud crab species, *S. paramamosain* and *S. serrata*, by exposing the juveniles (> C5 with ~11 mm CW) to decreasing or increasing thermal regimes and determining the critical low or high temperatures (CLT or CHT) at which 50% of test juveniles ceased locomotor activity (i.e. walking behaviour) or died. In the low-temperature tolerance experiment, the walking and survival rates of juveniles largely declined below ~10°C in both species, and the estimated CLT values were summarised for locomotor activity as 8.4 ± 0.7°C and 9.6 ± 0.6°C and those for survival as 6.4 ± 0.9°C and 7.4 ± 0.4°C in *S. paramamosain* and *S. serrata*, respectively.

Sanda *et al*. (2022) reported that when C1 juveniles of *S. paramamosain* and *S. serrata* were reared at ~15–30°C, *S. paramamosain* could moult to C2 at 15.4°C, whereas *S. serrata* could not moult to C2 at 15.2°C. Additionally, the lower threshold temperature (LTT) (95% confidence interval) for juvenile development was estimated as 13.7℃ (13.5–13.8°C) for *S. paramamosain* and 15.4℃ (15.1–15.7°C) for *S. serrata*. Thus, interspecific variation in the low-temperature adaptation by evaluating CLT and LTT values was equivalent in two mud crab species: juveniles of *S. paramamosain* adapted to lower temperature conditions than those of *S. serrata*.

Sanda *et al*. (2022) also documented that the survival rate of *S. paramamosain* juveniles declined to 30–40% until C5 at 29.8°C, whereas all *S. serrata* juveniles survived to C5 even at 30.5°C, suggesting that juveniles of *S. serrata* adapt to higher temperature conditions than those of *S. paramamosain*. In our high-temperature tolerance experiments, CHT values for locomotor activity could not be estimated, as almost all surviving juveniles exhibited walking behaviour, whereas the estimated CHT values for survival were summarised as 39.0 ± 0.4°C in *S. paramamosain* and 39.1 ± 0.6°C in *S. serrata*. Thus, upper thermal tolerance ability may be enhanced in larger juveniles of *S. paramamosain*.

The thermal tolerance traits of brachyuran crabs have often been evaluated by estimating the critical thermal minimum (CTmin) and/or maximum (CTmax), which are defined as the temperatures at which a crab cannot right itself after being turned onto its dorsal surface (Azra *et al*., 2018). Syafaat *et al*. (2021) estimated CTmin and CTmax for C1 juveniles of *S. paramamosain*. Juveniles were exposed to decreasing and increasing temperature regimes at a rate of 1°C/min, and the CTmin and Ctmax were determined as 17–18°C and 40°C, respectively. Thus, Japanese *S. paramamosain* appeared to exhibit a lower temperature adaptation compared with Malaysian *S. paramamosain*, probably because of the lower habitat temperature in temperate Japan compared to tropical Malaysia. However, it should be noted that the different decreasing thermal regimes, 1°C/min in Syafaat *et al*. (2021) and 1°C/24h in the present study, may affect the low-temperature tolerance limits of the test crabs.

In Japan, *S. paramamosain* is distributed on the Pacific coast of Honshu (31–35°N), a warm temperate area, whereas *S. serrata* mainly occurs in the Ryukyu Archipelago (< 28°N), a subtropical area (Oshiro & Imai, 2003; Ogawa *et al.*, 2011, 2012). Major fishing grounds of *S. paramamosain* occur in brackish bays and inlets, such as Urado Bay (33°31'N, 133°33'E), Kochi Prefecture, Japan; *S. serrata* also occurs in these fishing grounds and its fishery catch declined largely during the winter season (Ogawa *et al.*, 2012). The main reproductive season extends from spring to summer in *S. paramamosain* (Hamasaki, 2002; Ogawa *et al.*, 2012) and *S. serrata* (T. Sanda, personal observation), and the planktonic larval duration prior to moulting to C1 is about 3–5 weeks at 26–32°C (Hamasaki, 2003). Larvae of *S. serrata* hatched in the Ryukyu Archipelago may disperse to the temperate area via the western boundary current, i.e. the Kuroshio Current, as inferred for marine larvae (e.g. Sanda *et al.*, 2019). After settling in the brackish bays and inlets, *S. serrata* juveniles could moult and grow under the warm water conditions above the threshold temperature for development; then, their mortality may increase during the overwintering periods, depending on their weak low-temperature tolerance ability compared with *S. paramamosain* juveniles.

The present study further highlighted the interspecific variation in thermal adaptation of two mud crab species, *S. paramamosain* and *S. serrata*. In Urado Bay, the composition of *S. serrata* in the total catch of mud crab fishery increased from a small percentage during the late 1980s to 23% in 2008–2009 (Sugimoto & Hasegawa, 1989, 1990; Ogawa *et al*., 2012). The statistics of the lowest sea surface temperatures were available from 2010 to 2019 in central Urado Bay, and they were recorded as 9–13°C (Kochi Prefectural Fisheries Experiment Station, 2022). The survival rate of *S. serrata* juveniles may be further improved during the overwintering period, and mud crab species compositions in temperate brackish bays and inlets may shift to *S. serrata* under global warming conditions.

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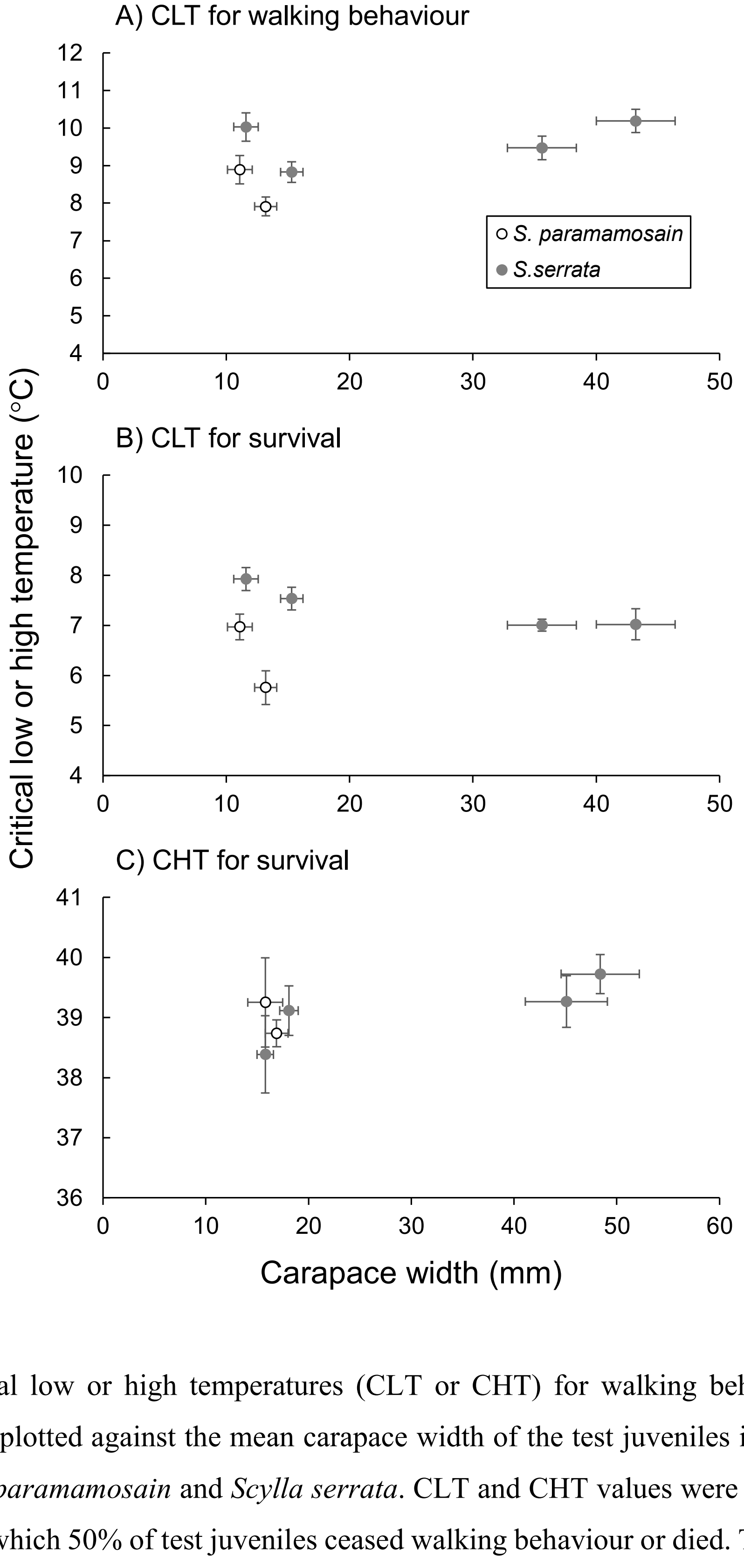

Fig. 4.1. Critical low or high temperatures (CLT or CHT) for walking behaviour (A) and survival (B, C) plotted against the mean carapace width of the test juveniles in two mud crab species, *Scylla paramamosain* and *Scylla serrata*. CLT and CHT values were estimated as the temperature at which 50% of test juveniles ceased walking behaviour or died. The vertical bars represent the 95% confidence intervals of the CLT or CHT estimates, and the horizontal bars show the standard deviations of the carapace widths of the test juveniles.

Table S4.1. Carapace width (mean ± standard deviation) of test juveniles and temperature at the beginning of the experiment to evaluate the low-temperature or high-temperature tolerance of two mud crab species, *Scylla paramamosain* and *Scylla serrata*. A total of 20 juveniles were used for each trial.

Experiment	Species	Trial no.	Date of experiment	Carapace width of test juveniles (mm)	Temperature at the beginning of the experiment (°C)
Low-temperature tolerance	*S. paramamosain*	1	2020/8/13	11.1 ± 1.0	25
		2	2020/10/22	13.2 ± 0.9	20
	S. serrata	1	2018/9/25	11.6 ± 1.0	25
		2	2020/5/13	15.3 ± 0.9	20
		3	2018/10/30	35.6 ± 2.8	25
		4	2020/2/4	43.2 ± 3.2	20
High-temperature tolerance	*S. paramamosain*	1	2020/7/7	15.8 ± 1.7	30
		2	2020/7/7	16.9 ± 1.1	30
	S. serrata	1	2019/10/28	15.8 ± 0.8	30
		2	2020/7/7	18.1 ± 0.9	30
		3	2020/2/5	45.1 ± 4.0	30
		4	2021/2/2	48.4 ± 3.8	30

Table S4.2. Low- or high-temperature tolerance limit evaluated by critical temperatures (CT) at which 50% of test juveniles ceased walking behaviour or died in two mud crab species, *Scylla paramamosain* and *Scylla serrata*. The CT values with 95% confidence intervals (CI) were estimated based on the logistic regression equations to formulate the relationship between the mean water temperature (T), the proportions of survivors among the test juveniles and the juveniles that exhibited walking behaviour among the survivors (see supplementary Table S4.3). CT values were not estimated for juveniles in the high-temperature tolerance experiment because almost all surviving juveniles exhibited walking behaviour (see supplementary Fig. S4.2). A total of 20 juveniles were used for each trial. Number of moulted juveniles during the experiment are also shown for each trial.

Experiment	Species	Trial no.	Number of moulted juveniles	Critical temperature (°C)					
				Walking behaviour			Survival		
				Estimate	95% CI		Estimate	95% CI	
Low-temperature tolerance	*S. paramamosain*	1	5	8.89	8.52	9.26	6.97	6.71	7.23
		2	3	7.91	7.66	8.16	5.76	5.42	6.09
	S. serrata	1	4	10.03	9.65	10.41	7.92	7.70	8.15
		2	2	8.83	8.55	9.10	7.53	7.31	7.76
		3	5	9.47	9.16	9.78	7.00	6.88	7.12
		4	0	10.19	9.88	10.50	7.02	6.71	7.33
High-temperature tolerance	*S. paramamosain*	1	4				39.25	38.51	40.00
		2	5				38.74	38.51	38.96
	S. serrata	1	4				38.39	37.74	39.03
		2	5				39.12	38.71	39.53
		3	3				39.27	38.84	39.69
		4	1				39.72	39.40	40.05

Table S4.3. Coefficient estimates with standard errors (SE) of the logistic regression equations to formulate the relationship between the mean water temperature (T), the proportions of survivors among the test juveniles (survival rate) and the juveniles that exhibited walking behaviour among the survivors (walking rate) in temperature tolerance experiments using two mud crab species, *Scylla paramamosain* and *Scylla serrata*. Coefficients were not estimated for juveniles in the high-temperature tolerance experiment because almost all surviving juveniles exhibited walking behaviour (see supplementary Fig. S4.2).

Experiment	Species	Trial no.	Coefficient	Walking rate					Survival rate				
				N	Estimate	SE	z value	*P*	N	Estimate	SE	z value	*P*
Low-temperature tolerance	*S. paramamosain*	1	Intercept	19	-13.558	2.452	-5.529	< 0.0001	20	-20.461	4.756	-4.302	< 0.0001
			T		1.525	0.271	5.623	< 0.0001		2.936	0.674	4.357	< 0.0001
		2	Intercept	15	-24.862	5.908	-4.208	< 0.0001	17	-10.511	2.317	-4.536	< 0.0001
			T		3.143	0.747	4.207	< 0.0001		1.826	0.388	4.705	< 0.0001
	S. serrata	1	Intercept	18	-13.420	1.873	-7.165	< 0.0001	21	-35.054	11.972	-2.928	0.00341
			T		1.338	0.187	7.175	< 0.0001		4.423	1.464	3.022	0.00251
		2	Intercept	14	-21.928	4.352	-5.039	< 0.0001	16	-21.667	4.373	-4.955	< 0.0001
			T		2.484	0.489	5.077	< 0.0001		2.876	0.581	4.947	< 0.0001
		3	Intercept	19	-17.606	3.074	-5.727	< 0.0001	21	-52.131	18.523	-2.814	0.00489
			T		1.859	0.323	5.758	< 0.0001		7.444	2.635	2.825	0.00472
		4	Intercept	13	-19.528	3.365	-5.803	< 0.0001	16	-14.150	2.949	-4.799	< 0.0001
			T		1.916	0.329	5.832	< 0.0001		2.016	0.413	4.883	< 0.0001
High-temperature tolerance	*S. paramamosain*	1	Intercept						12	23.086	3.471	6.652	< 0.0001
			T							-0.588	0.091	-6.459	< 0.0001
		2	Intercept						12	151.540	37.543	4.036	< 0.0001
			T							-3.912	0.971	-4.031	< 0.0001
	S. serrata	1	Intercept						12	29.562	4.036	7.324	< 0.0001
			T							-0.770	0.108	-7.154	< 0.0001
		2	Intercept						12	46.994	7.889	5.957	< 0.0001
			T							-1.201	0.203	-5.928	< 0.0001
		3	Intercept						11	66.292	12.931	5.127	< 0.0001
			T							-1.688	0.332	-5.083	< 0.0001
		4	Intercept						11	81.427	18.382	4.430	< 0.0001
			T							-2.050	0.463	-4.430	< 0.0001

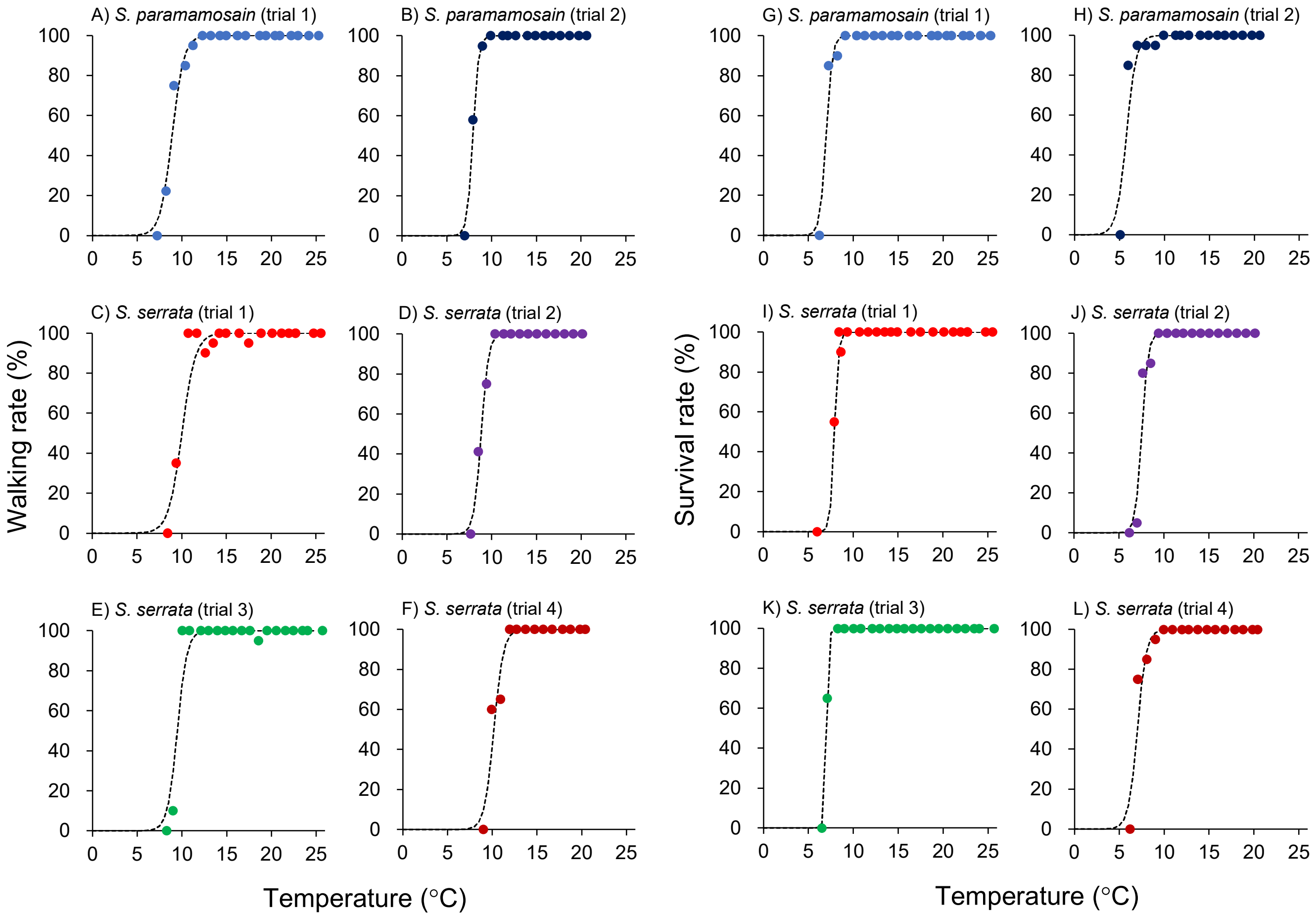

Fig. S4.1. Changes in walking rate (A–F) and survival rate (G–L) in relation to temperature when the low-temperature tolerance limits were evaluated for early juveniles of two mud crab species, *Scylla paramamosain* (A, B, G and H) and *Scylla serrata* (C–F and I–L). Experiments were conducted using 20 juveniles from two broods for *S. paramamosain* and four broods for *S. serrata*. The survival rate was calculated as (number of survivors)/(initial number of juveniles). The walking rate was calculated as (number of crabs that exhibited walking behaviour)/(number of surviving crabs) × 100. Curves were drawn based on the coefficient estimates of the logistic regression equations shown in Table S4.3.

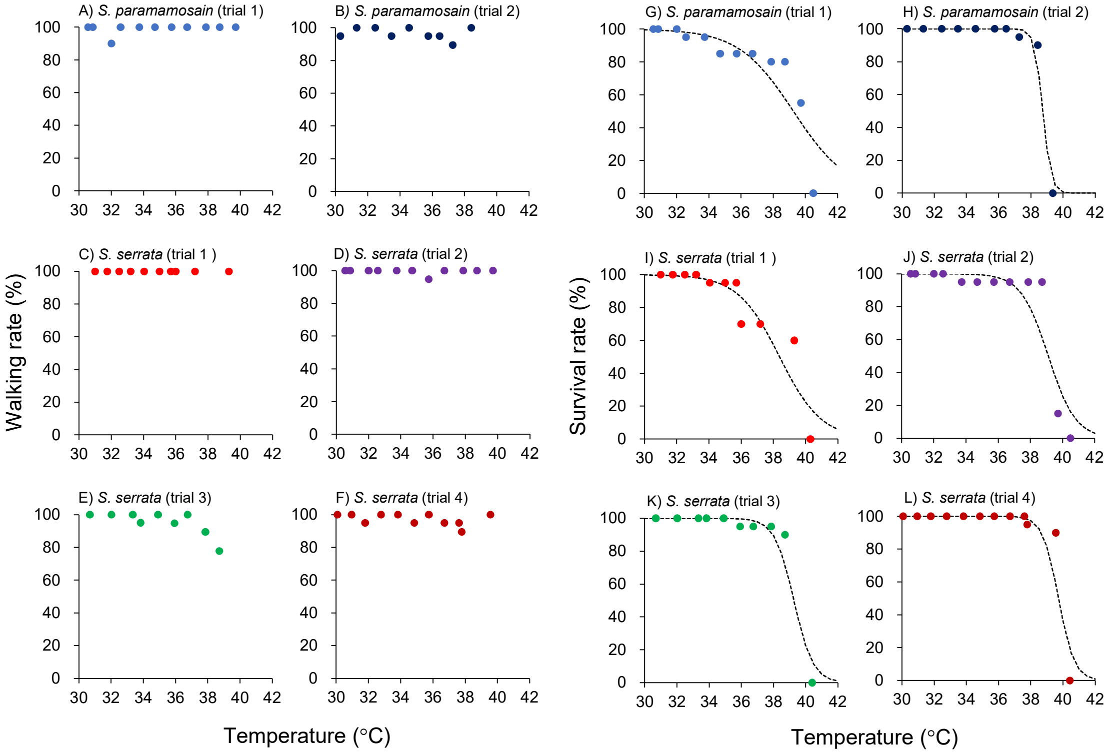

Fig. S4.2. Changes in walking rate (A–F) and survival rate (G–L) in relation to temperature when the high-temperature tolerance limits were evaluated for early juveniles of two mud crab species, *Scylla paramamosain* (A, B, G and H) and *Scylla serrata* (C–F and I–L). Experiments were conducted using 20 juveniles from two broods for *S. paramamosain* and four broods for *S. serrata*. The survival rate was calculated as (number of survivors)/(initial number of juveniles). The walking rate was calculated as (number of crabs that exhibited walking behaviour)/(number of surviving crabs) × 100. Curves were drawn based on the coefficient estimates of the logistic regression equations shown in Table S4.3.

第５章 総合考察

5. 1　ノコギリガザミ類稚ガニの共食い防除技術の高度化に向けて

第 2 章では、体サイズ差が稚ガニの共食いや胸脚欠損に与える影響について明らかにした。第 1 節では、アミメノコギリガザミ（5 齢から 9 齢：甲幅 12.5–33.3 mm）の稚ガニ 2 匹を体サイズ比率別に組み合わせ、2 L のポリエチレン製白色ビーカー（直径 14.5 cm、高さ 17.7 cm）で 24 時間飼育し、共食いの有無と胸脚の欠損について 50 組観察した。その結果、共食いは 18 回（36%, 18/50）発生し、常に大型のカニが小型のカニを捕食した。一般化線形モデルによる解析の結果、共食いは、大型個体（捕食者）の甲幅が大きいほど、小型個体（被捕食者）の甲幅が小さいほど増加し（モデル 1)、体サイズ相対サイズ差：RSD = 1 −（小型カニのサイズ）/（大型カニのサイズ） が大きくなるほど増加した。共食いが起こらなかった 32 組では、鋏脚と歩脚の欠損はそれぞれ 25% （8/32）と 38% （12/32） 発生し、歩脚の欠損率のみが RSD の増加とともに有意に増加した。歩脚欠損で 1 例の例外がみられたが、共食いと同様に大型個体が小型個体を常に攻撃していた。共食いが 50%の確率で発生する RSD の推定値（以下 RSD_{50}）は 0.337（0.259–0.460）となり、小型個体に対する大型個体の甲幅に換算すると 1.51（1.35–1.85）倍となった。このサイズ差は概ね脱皮 2 回分に相当する。以上から、アミメノコギリガザミの 5 齢から 9 齢にかけての共食いの防除には、飼育水槽内の RSD を 0.34 以下に維持することが有効であると考えられた。

第 2 節では、トゲノコギリガザミ（3 齢から 5 齢：甲幅 5.1–10.9 mm）の稚ガニを用い、第 1 節と同様の実験を 60 回行った。その結果、共食いは 29 回（48.3%, 29/60）発生し、常に大型のカニが小型のカニを捕食した。一般化線形モデルによる解析の結果、共食いは、大型個体（捕食者）の甲幅が大きいほど、小型個体（被捕食者）の甲幅が小さいほど増加し（モデル 1)、また、体サイズ相対サイズ差：RSD = 1 −（小型カニのサイズ）/（大型カニのサイズ） が大きくなるほど増加した。共食いが起こらなかった 31 組では、鋏脚と歩脚の欠損はそれぞれ 6.5% （2/31）と 16.1% （5/31） 発生し、すべての事例で大型個体が小型個体を

攻撃していた。胸脚欠損の発生は第 1 節の実験よりも少なく、いずれも体サイズ差による影響は見られなかった。RSD$_{50}$ は 0.214（0.180–0.254）と推定され、小型個体に対する大型個体の甲幅に換算すると 1.27（1.22–1.34）倍となり、概ね脱皮 1 回分の成長差に相当した。

第 1 節のアミメノコギリガザミ（5 齢から 9 齢：甲幅 12.5–33.3mm）と第 2 節のトゲノコギリガザミ（3 齢から 5 齢：甲幅 5.1–10.9 mm）で RSD$_{50}$ の幅や胸脚欠損率に差がみられた、すなわち前者でいずれも大きい値が得られた原因として、実験に用いた稚ガニの体サイズの違いに着目すると、より大型稚ガニの攻撃性が高いこと、また大型になると捕食者に対する防御性が高くなり、脚を失うことがあっても完全には共食いされないことに起因するものと考えられる。

共食いはノコギリガザミ類を始めとした甲殻類養殖における最大の減耗要因の一つであり、その対策法について多くの実験が行われてきた。その結果、体サイズ選別（Mirera & Moksnes, 2013; Romano & Zeng, 2017）、シェルターの設置（Mirera & Moksnes, 2015）、低密度での飼育（Shelley & Lovatelli, 2011; Ut *et al*., 2007）などが有効であることが明らかになっている。本研究では、サイズ選別の指標として RSD$_{50}$ 以下の体サイズ差に保つことを提案したが、小型の種苗は脱皮周期が短く、短期間でサイズ差がつきやすいため、体サイズ選別のみでの対策は困難であることが予想される。Takeshima *et al*., (2017) は、ガザミ *Portunus trituberculatus* (Miers, 1876) のメガロパから 5 齢稚ガニまでの行動を水槽実験により観察し、人工海藻へ付着する行動が 2–3 齢稚ガニ期にピークに達すること、3 齢稚ガニ以降から潜砂し始め、成長とともに潜砂する割合が増加したことを報告している。また、同時期の胸脚の形態変化について相対成長解析を行い、1 齢から 5 齢の間に、第 2 から第 4 胸脚の指節・前節の長さが劣成長を示したこと、第 5 胸脚は指節・前節の幅・長さともに優成長を示したことから、初期に稚ガニが海藻類への付着に適した形態を有していたものが、成長するにつれて潜砂に適した形態への変化、すなわち底生生活への移行が生じたと推測している（Takeshima *et al*., 2018）。このことから、ガザミの場合は、初期の稚ガニには付着基質として人工海藻類を、潜砂行動を示し始めた時期に砂を導入することが

共食いの防除に有効であると考えられる。その後は成長に応じ、定期的な体サイズ選別やシェルターを設置することで、共食い防除の効果を最大限得られるものと考えられる。現状では、ノコギリガザミ類では同様の研究は行われていないが、稚ガニ期の生態や形態を調べ、個体発生に応じた方法を適応することで、従来より高度な共食い対策が可能になると考えられる。これと並行し、体サイズ選別には時間と労力を要することから、産業規模での実装には短時間で大量に処理が可能な選別方法の開発が望まれる。魚類では選別器が実装されているが（Kelly & Heikes, 2013）、カニ類では専用の選別器具は実装されておらず、実験規模でタラバガニ *Paralithodes camtschaticus* (Tilesuis, 1815) の甲幅 2–4.5 mm の種苗を 3.3 mm のネットによって選別した事例がある程度である（Daly *et al*., 2012）。ネットによる選別はノコギリガザミ類においても取り上げ直後の小型種苗には適応可能な方法であると考えられるが、より大型の種苗に適応する場合には、形態や雌雄の成長差などを踏まえた詳細な条件を把握することが必要であろう。

5. 2　ノコギリガザミ類の温度適応と温暖化による資源構成の変化予測

第 3 章では、トゲノコギリガザミとアミメノコギリガザミの稚ガニの成長・生残に与える水温の影響を明らかにした。トゲノコギリガザミの 1 齢稚ガニは 15.4℃で 2 齢に脱皮することができたが、29.8℃では 4 齢稚ガニの生残率が低下した。アミメノコギリガザミの 1 齢稚ガニは 15.2℃で脱皮できず、2 齢稚ガニも低水温で生残率が低下したが、その後は温度による影響は受けなかった。脱皮間隔は両種ともに高水温で短縮され、甲幅も水温の上昇に伴って大きくなったが、トゲノコギリガザミでは 27.6–30.2℃で小さくなり、アミメノコギリガザミでは 24℃で成長は一定値に達した。1 齢稚ガニの臨界発育水温は、トゲノコギリガザミでは 13.65℃（13.51–13.77℃）、アミメノコギリガザミでは 15.44℃（15.12–15.72℃）と推定され、両種の卵の臨界発育水温である 13.99℃（Hamasaki, 2002）と 15.70℃（Hamasaki, 2003）に近似した値となった。以上より、トゲノコギリガザミとアミメノコギリガザミの稚ガニはそれぞれ低温と高温に適応しており、

本邦における地理的分布を反映した温度適応を有していることが明らかになった。

第 4 章では、トゲノコギリガザミとアミメノコギリガザミの稚ガニの低温及び高温に対する耐性について明らかにした。24 時間ごとに飼育水温を 1℃ずつ下降または上昇させ、実験個体の 50%が歩行を停止する水温、または死亡する水温を、低温と高温それぞれで臨界低水温または臨界高水温（CLT：Critical low temperature または CHT：Critical high temperature）として推定した。歩行と生存の CLT の推定値（平均値±標準偏差）は、トゲノコギリガザミでは 8.4±0.7℃と 6.4±0.9℃、アミメノコギリガザミでは 9.6±0.6℃と 7.4±0.4℃と推定された。生存に関する CHT の推定値はトゲノコギリガザミで 39.0±0.4℃、アミメノコギリガザミで 39.1±0.6℃と推定されたが、歩行に関する CHT は、稚ガニが死亡前日まで歩行行動を示したため推定できなかった。

トゲノコギリガザミは東シナ海からジャワ海を中心に分布し（Keenan *et al*., 1998）、本邦では浜名湖や浦戸湾など本州の温帯域が主要な生息地であるが（Ogawa *et al*., 2012; 大城，1988）、宮城県松島湾（矢倉，2021）や京都府舞鶴沖など（本尾・長澤，2007）でも観察例がある。ノコギリガザミ類は過去に分類の混乱があったため判然としないが、酒井（1976）や三宅（1983）における利根川河口域や伊豆下田、南紀南部、紀伊長島、土佐湾、有明海、八代海などの分布記録の多くはトゲノコギリガザミであると推察される。アミメノコギリガザミはノコギリガザミ類の中で最も広い分布域を持ち、東南アジアの熱帯域を中心にインド太平洋のアフリカ大陸東岸からオセアニアまで分布し（Keenan *et al*., 1998）、本邦では南西諸島を中心に生息している（Ogawa *et al*., 2011; 大城, 1988）。国内の主要な産地における漁獲割合を参照すると、浜名湖ではトゲノコギリガザミが 81.9–99.9%（伊藤，2000）、浦戸湾ではトゲノコギリガザミが 74%、アミメノコギリガザミが 23%、アカテノコギリガザミが 3%（Ogawa *et al*., 2012）、西表島ではアミメノコギリガザミが 95%以上、残りはアカテノコギリガザミであり（Ogawa *et al*., 2011）、本州の温帯域においても一定数のアミメノコギリガザミの生息が確認されている。

水温は生物の成長・生残などに大きな影響を及ぼし、分布を規定する要因の一つである。浜名湖（静岡県水産技術研究所浜名湖分場，2022）と浦戸湾（高知県水産試験場，2022）、および各沿岸域（仙台管区気象台，2022；東京管区気象台，2022；大阪管区気象台，2022）の 2019 年と 2020 年の平均水温を参照すると、両種が混在する浜名湖と浦戸湾では、最寒月の平均水温はそれぞれ 13.7℃と 15.4℃であり、第 3 章で示した稚ガニの臨界発育水温と概ね一致する。産地別にみると、浜名湖で 12 月から翌年 4 月までトゲノコギリガザミの臨界発育水温を下回ることはないが、アミメノコギリガザミの臨界発育水温をわずかに下回る水温が続く。一方、浦戸湾ではアミメノコギリガザミの臨界発育水温を下回ったのは 1 月のみであった。このことから、両産地の最寒月の水温が種の構成を制限する要因であるものと考えられる（図 5. 1)。

また、太平洋側の北限となる利根川河口近辺の水温を参照すると、トゲノコギリガザミは茨城県北部以南で臨界発育水温を上回るが、矢倉（2021） による報告がある宮城県では 12 月から翌年 4 月までトゲノコギリガザミの臨界発育水温を下回り、さらに最寒月の 2 月には 8.3℃に達し、半数致死水温に近づく。アミメノコギリガザミでは千葉県北東部以北で臨界発育水温を下回る（図 5.2)。日本海側でのトゲノコギリガザミの報告（本尾・長澤，2007）がある京都府沿岸と能登半島南部・加賀沿岸では、いずれも 2 月から 3 月にかけてトゲノコギリガザミの臨界発育水温を下回る（図 5.3)。以上から、臨界発育水温を下回る地域においても一部の個体は生存することができるが、安定した繁殖のためには最寒月の平均水温がその種の臨界発育水温以上である必要があると考えられる。

気象庁によると日本近海の海水温は 2021 年までの 100 年間に平均 1.19℃上昇したとされている（気象庁 2022：海面水温の長期変化傾向（日本近海))。各海域と四季ごとの平均値上昇値を参照すると、冬季（1–3 月）には関東の東（+1.08℃）や日本海中部（＋1.63℃）、日本海南西部（＋2.03℃）、四国東海沖（+1.44℃）で上昇値が大きく、冬季の水温上昇は本来熱帯性であるノコギリガザミ類の越冬の成否に大きく影響することが予想される。既知の分布記録のある各地域において今後も同様の水温上昇が起こると仮定すると、浜名湖では最低水温が 15.4℃

となり、アミメノコギリガザミが安定的に繁殖する可能性がある（図 5.4)。また、トゲノコギリガザミでは茨城県北部までが臨界発育水温を上回る水温となり、利根川以北でも繁殖可能になることが示唆され、アミメノコギリガザミでは、千葉県北東部から茨城県南部にかけて臨界発育水温を上回る（図 5.5)。日本海側では、京都沿岸から富山湾付近でトゲノコギリガザミの臨界発育水温を上回り、繁殖する可能性がある（図 5.6)。

以上のように、本研究で得られた成果から、温暖化環境下におけるノコギリガザミ類の分布拡大及び資源構成の変化について推察した。実際の天然環境においては、水温以外にも塩分や底質など様々な要因が分布を規定していると考えられ、これらの影響について引き続き検討していくことが望まれる。

5. 3　引用文献

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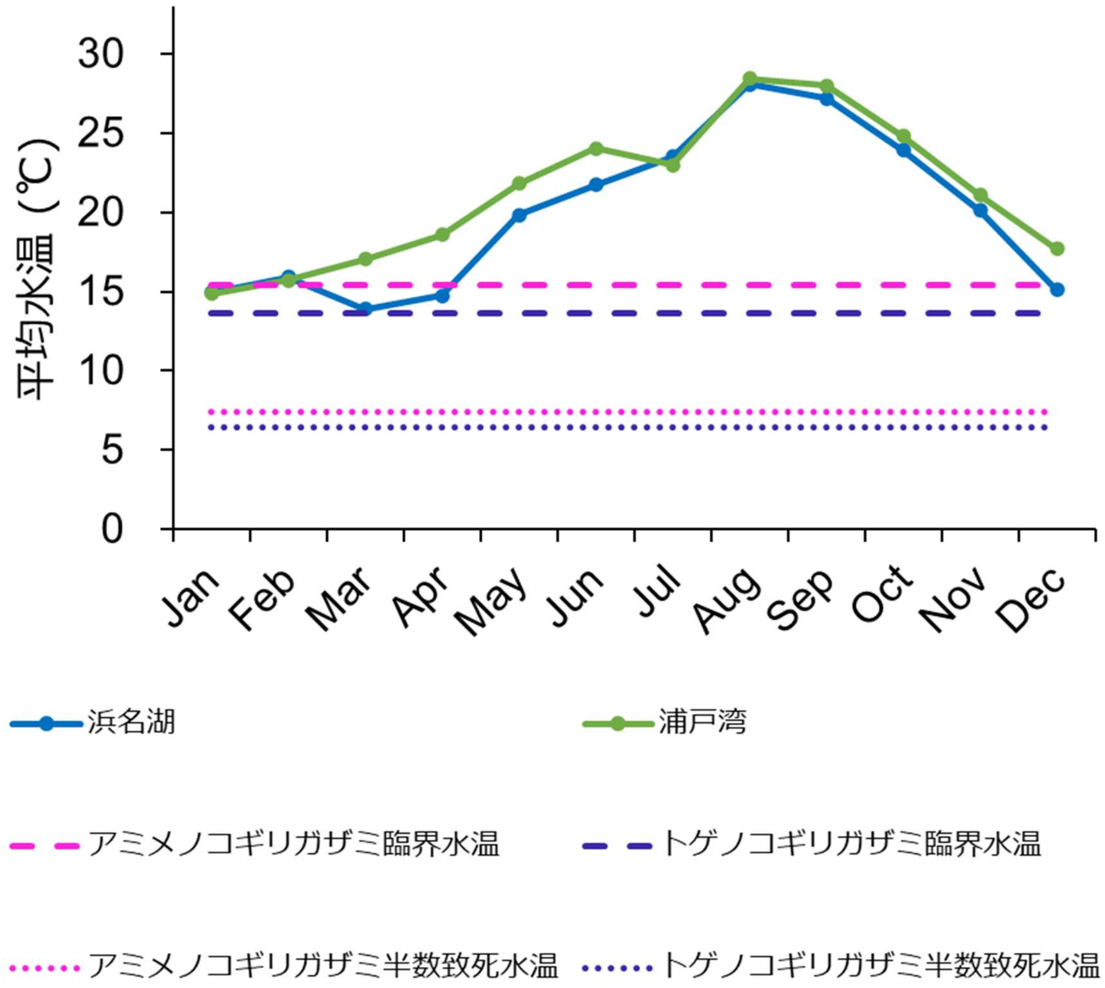

図 5.1 浜名湖と浦戸湾における2019年と2020年の月ごとの平均水温とノコギリガザミ類の臨界水温及び半数致死水温。静岡県水産技術研究所浜名湖分場「浜名湖の水温」(https://fish-exp.pref.shizuoka.jp/hamanako/1_kaikyo/1wt_hamanako.html) 及び、高知県水産試験場環境調査結果(赤潮情報)（https://www.pref.kochi.lg.jp/soshiki/040409/）より作成。

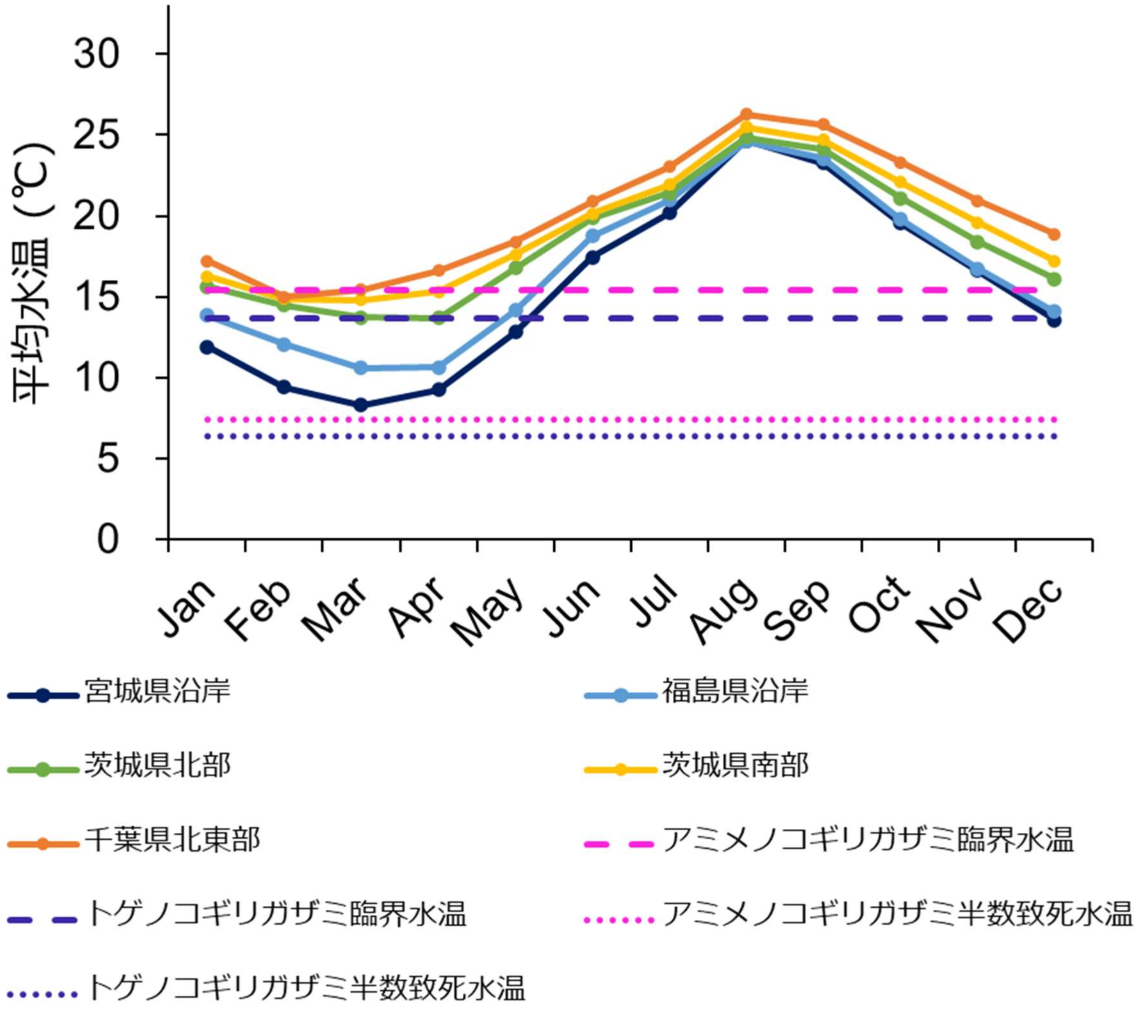

図 5.2　千葉県から宮城県にかけての2019年から2020年の月ごとの平均水温とノコギリガザミ類の臨界水温及び半数致死水温。東京管区気象台沿岸域の海面水温情報（関東・東海・北陸周辺）（https://www.data.jma.go.jp/tokyo/shosai/umi/kaiyou/sst/index.html）及び仙台管区気象台沿岸域の海面水温情報（東北周辺）（https://www.data.jma.go.jp/sendai/data/marine/coast_sst/coast_sst.html）より作成

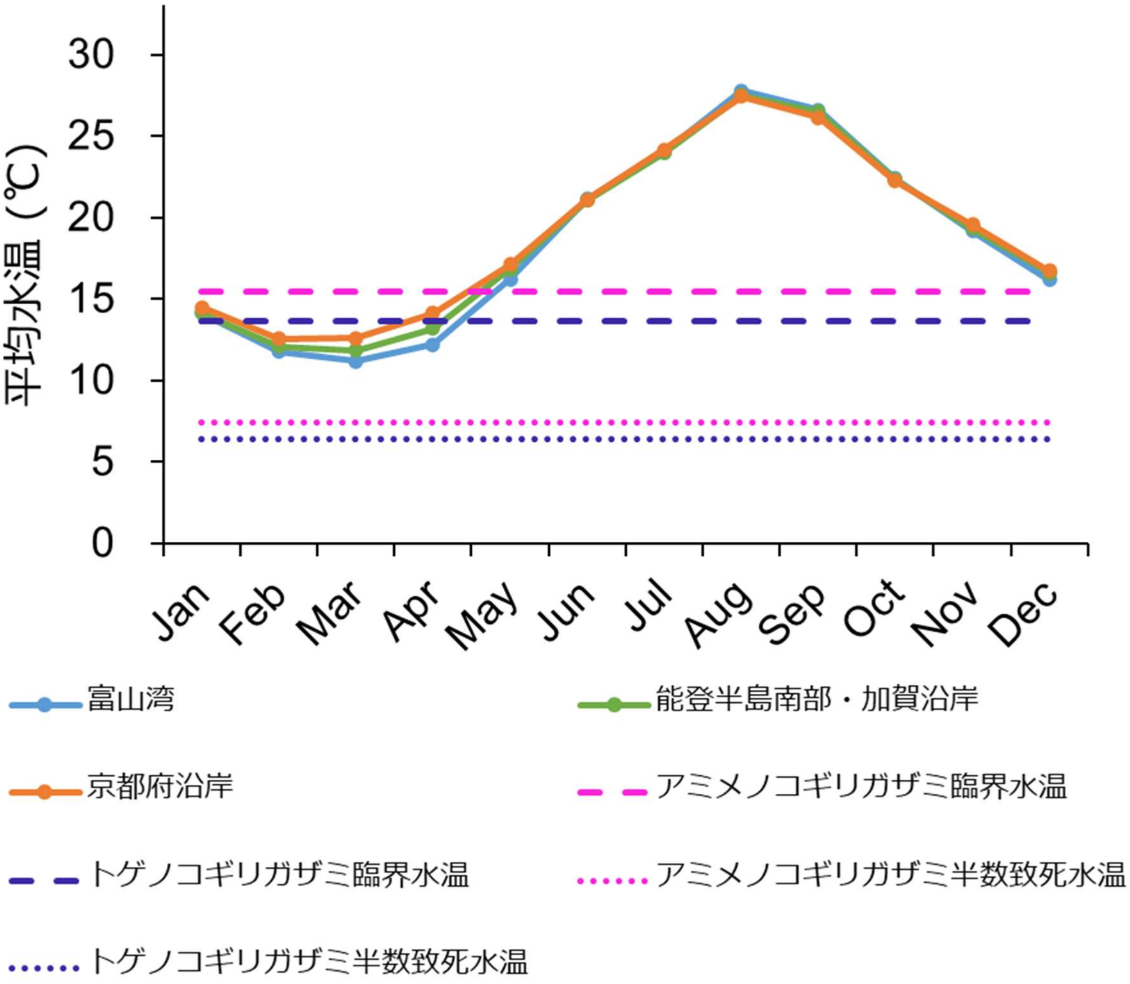

図 **5.3** 京都府沿岸、能登半島南部・加賀沿岸、富山湾における 2019 年と 2020 年の月ごとの平均水温とノコギリガザミ類の臨界水温及び半数致死水温。大阪管区気象台沿岸域の海面水温情報（近畿・中国・四国）（https://www.data.jma.go.jp/osaka/kaiyo/sst/sstin.html）及び、東京管区気象台沿岸域の海面水温情報（関東・東海・北陸周辺）（https://www.data.jma.go.jp/tokyo/shosai/umi/kaiyou/sst/index.html）より作成

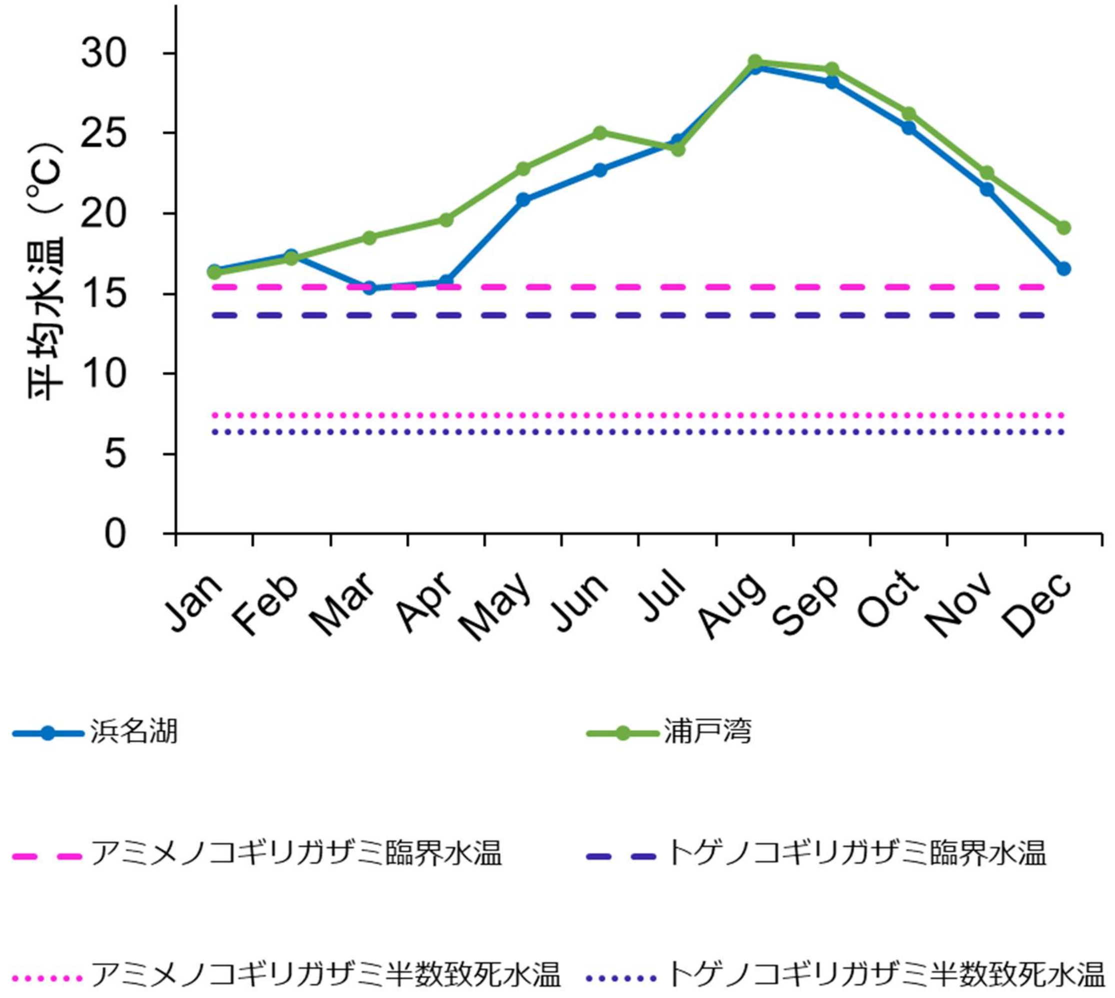

図 5.4 浜名湖と浦戸湾における2019年と2020年の月ごとの平均水温に気象庁海面水温の長期変化傾向（日本近海）（https://www.data.jma.go.jp/gmd/kaiyou/data/shindan/a_1/japan_warm/japan_warm.html）による水温上昇を加えた水温とノコギリガザミ類の臨界水温及び半数致死水温。静岡県水産技術研究所浜名湖分場「浜名湖の水温」(https://fish-exp.pref.shizuoka.jp/hamanako/1_kaikyo/1wt_hamanako.html) 及び、高知県水産試験場環境調査結果(赤潮情報)（https://www.pref.kochi.lg.jp/soshiki/040409/）より作成。

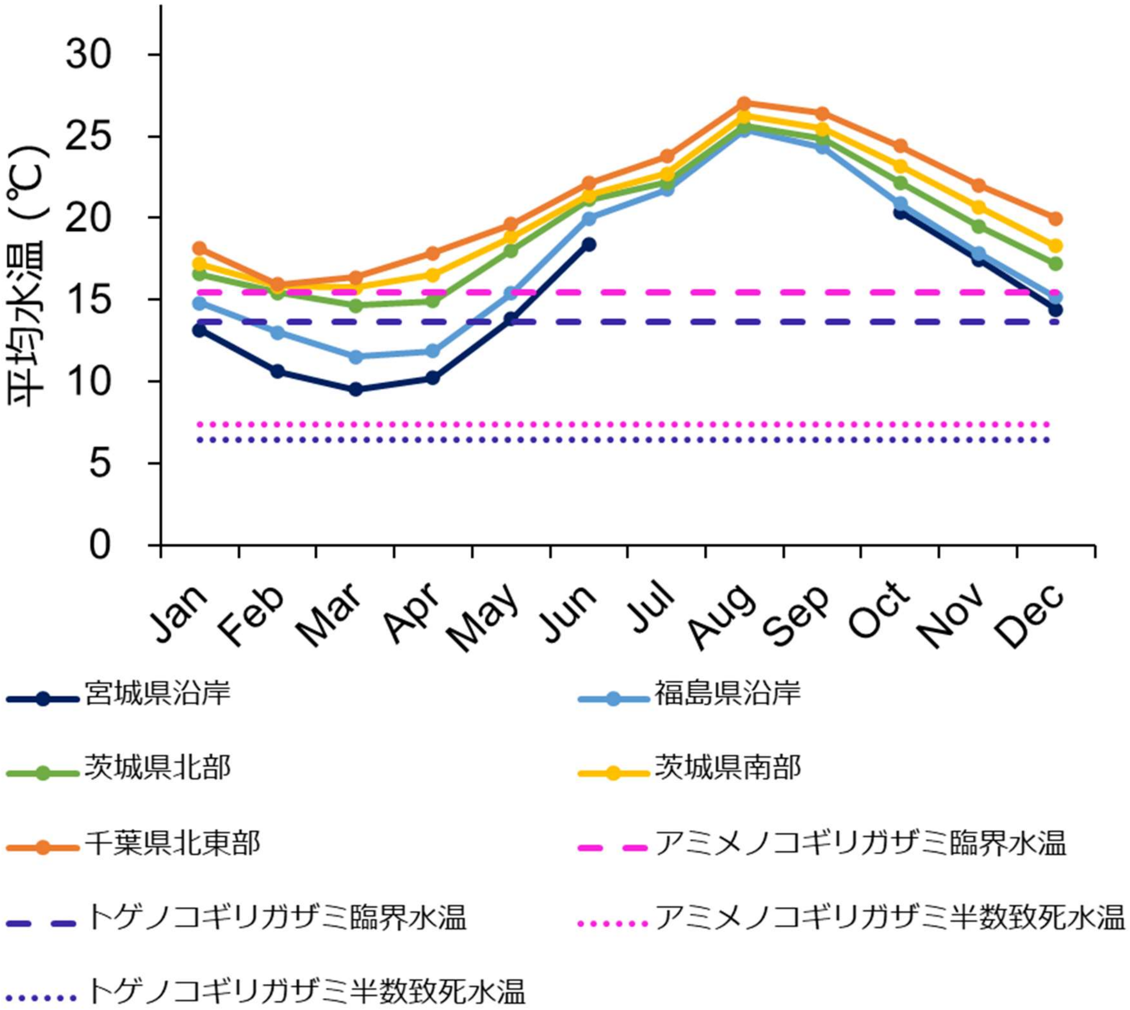

図 5.5 千葉県から宮城県にかけての 2019 年と 2020 年の月ごとの平均水温に気象庁海面水温の長期変化傾向（日本近海）（https://www.data.jma.go.jp/gmd/kaiyou/data/shindan/a_1/japan_warm/japan_warm.html）による水温上昇を加えた水温とノコギリガザミ類の臨界水温及び半数致死水温。東京管区気象台沿岸域の海面水温情報（関東・東海・北陸周辺）（https://www.data.jma.go.jp/tokyo/shosai/umi/kaiyou/sst/index.html）及び仙台管区気象台沿岸域の海面水温情報（東北周辺）（https://www.data.jma.go.jp/sendai/data/marine/coast_sst/coast_sst.html）より作成。三陸沖は 7 月から 9 月かけて長期変化傾向が見られないため、宮城県沿岸の数値は欠損とした。

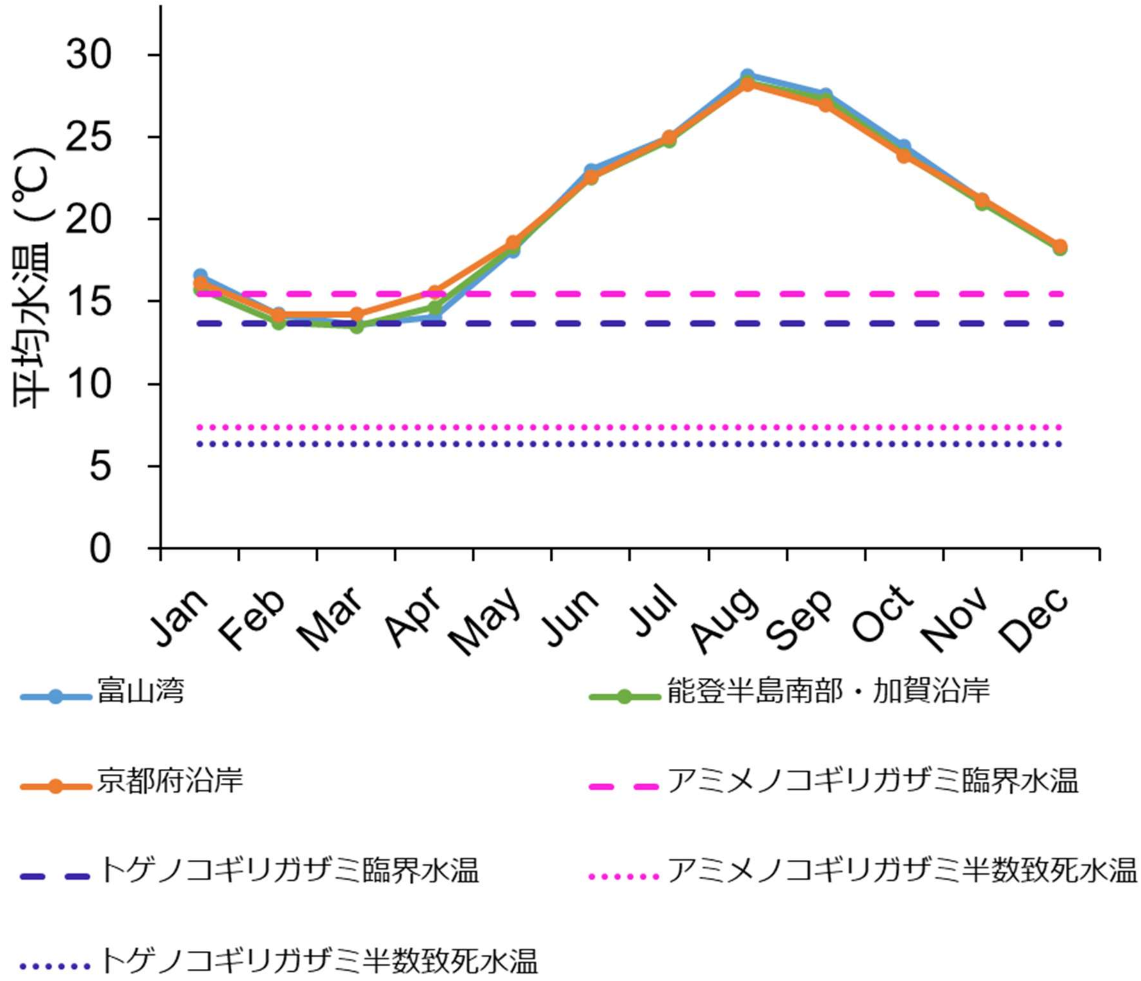

図 5.6 京都府沿岸、能登半島南部・加賀沿岸、富山湾における 2019 年と 2020 年の月ごとの平均水温に気象庁海面水温の長期変化傾向（日本近海）（https://www.data.jma.go.jp/gmd/kaiyou/data/shindan/a_1/japan_warm/japan_warm.html）による水温上昇を加えた水温とノコギリガザミ類の臨界水温及び半数致死水温。大阪管区気象台沿岸域の海面水温情報（近畿・中国・四国）（https://www.data.jma.go.jp/osaka/kaiyo/sst/sstin.html）及び、東京管区気象台沿岸域の海面水温情報（関東・東海・北陸周辺）（https://www.data.jma.go.jp/tokyo/shosai/umi/kaiyou/sst/index.html）より作成

要約

第 1 章　序論

ノコギリガザミ類の分類や生態についての基礎的知見、世界の漁獲量や養殖生産量、国内における栽培漁業の歴史について概括し、ノコギリガザミ類の水産上の重要性、持続可能な養殖産業構築への課題、及び、温暖化による日本国内での分布拡大の可能性について言及した。

第 2 章　ノコギリガザミ類の共食い防除に向けた基礎的知見の収集

共食いはノコギリガザミ類の養殖・中間育成時における最大の減耗要因である。共食いの軽減策として、体サイズ選別が有効であることが知られているが、体サイズ差が共食いに及ぼす影響については知見が乏しい。そこで、アミメノコギリガザミとトゲノコギリガザミの稚ガニの体サイズ差が共食いの発生に及ぼす影響を飼育実験によって明らかにした。

第 1 節 アミメノコギリガザミの稚ガニの共食いに及ぼす体サイズ差の影響

アミメノコギリガザミの甲幅 12.5–33.3 mm（5 齢から 9 齢）の大小 2 個体の人工種苗を体サイズ別（小型個体に対する大型個体の甲幅の比率を 1~2 培）に組み合わせ、直径 14.5 cm のプラスチック製ビーカーで 24 時間飼育し、共食いの状況と胸脚（鋏脚と歩脚）の欠損について 50 回観察した。その結果、共食いは 18 回（36%, 18/50）発生し、常に大型のカニが小型のカニを捕食した。一般化線形モデルによる解析の結果、共食いは相対的な体サイズ差：RSD = 1 −（小型カニのサイズ）/（大型カニのサイズ） が大きくなるほど増加した。共食いが起こらなかった 32 組では、鋏脚と歩脚の欠損はそれぞれ 25%（8/32）と 38%（12/32）発生した。胸脚の欠損は 1 例を除き小型個体で見られ、その頻度は RSD が大きくなるほど増加し、大型個体がより活発な攻撃行動をとることが明らかになった。共食いが 50%の確率で発生する RSD（以下 RSD_{50}）は 0.337（0.259–0.460）と推定され、小型個体に対する大型個体の甲幅に換算すると 1.51（1.35–1.85）倍、概ね脱皮 2 回分の成長差であった。以上のことから、アミメノコギリガザミの 5

齢から 9 齢にかけての共食いの防除には、飼育水槽内の RSD を 0.34 以下に維持することが有効であると考えられた。

第 2 節　トゲノコギリガザミの稚ガニの共食いに及ぼす体サイズ差の影響

トゲノコギリガザミの甲幅 5.1–10.9 mm（3 齢から 5 齢）の人工種苗で第 1 節と同様の実験を 60 回行った。その結果、共食いは 29 回（48.3%, 29/60）発生し、常に大型のカニが小型のカニを捕食した。第 1 節と同様に、共食いは RSD が大きくなるほど増加した。共食いが起こらなかった 31 組では、鋏脚と歩脚の欠損はそれぞれ 6.5%（2/31）と 16.1%（5/31）発生し、すべての事例で大型個体が小型個体を攻撃した。胸脚欠損の発生は第 1 節の実験よりも少なく、いずれも体サイズ差による影響は無かった。RSD_{50} は 0.214（0.180–0.254）と推定され、小型個体に対する大型個体の甲幅に換算すると 1.27（1.22–1.34）倍となり、概ね脱皮 1 回分の成長差に相当した。

第 3 章　トゲノコギリガザミとアミメノコギリガザミの稚ガニの温度適応

本邦の主に温帯域に分布するトゲノコギリガザミと亜熱帯域に分布するアミメノコギリガザミの稚ガニの温度適応解明の一環として、飼育水温が生残と成長に与える影響について調べた。高知県産のトゲノコギリガザミと沖縄県産のアミメノコギリガザミを種苗生産し、稚ガニに変態した当日の個体を 50 ml (容量 40 ml) のプラスチック製遠沈管に個別収容し、毎日換水し、アルテミアを給餌して飼育した。飼育水温は温度勾配器にて管理し、15–30℃で水温に応じて 2 齢から 5 齢まで飼育した。実験は異なる親由来の稚ガニで 3 回ずつ行った。その結果、トゲノコギリガザミは 15.4℃で 80%の個体が 2 齢に脱皮し、29.8℃で 4 齢から 5 齢にかけて生残率が低下したのに対し、アミメノコギリガザミでは 15.2 ℃では 2 齢に脱皮することなく全個体が死亡したが、高水温では生残率の影響を受けなかった。脱皮間隔は両種とも水温が上昇するほど短くなり、甲幅の成長も水温が高くなるほど大きくなる傾向がみられたが、トゲノコギリガザミでは 25℃付近にピークがあり、30℃付近でやや成長が劣った。一方、アミメノコギリガザミでは高水温の影響は見られず、24℃以上で成長は一定値に達した。

有効積算温度則の式による臨界発育水温の推定値は、トゲノコギリガザミで13.65℃（95%信頼区間 : 13.51–13.77）、アミメノコギリガザミで 15.44℃（95%信頼区間 : 15.12–15.72）となり、トゲノコギリガザミが低温への適応を、アミメノコギリガザミが高温への適応をそれぞれ示し、両種の国内分布を反映した結果となった。

第 4 章　トゲノコギリガザミとアミメノコギリガザミの稚ガニの温度耐性

トゲノコギリガザミとアミメノコギリガザミの稚ガニの温度適応解明の一環として低温耐性と高温耐性を評価した。耐性実験は、トゲノコギリガザミでは甲幅 11.1–16.9 mm、アミメノコギリガザミでは甲幅 11.6–18.1 mm と 35.6–48.4 mm の人工種苗でそれぞれ 2 回ずつ実験を行い、飼育水温を 24 時間ごとに 1℃下降または上昇させ、生死、摂餌、脱皮の有無、歩行の可否について観察した。温度耐性の指標として、実験個体の 50%が歩行を停止する水温、または死亡する水温を、臨界低水温または臨界高水温（CLT : Critical low temperature または CHT : Critical high temperature）として評価した。その結果、歩行と生存の CLT の推定値（平均値±標準偏差）は、トゲノコギリガザミでは 8.4±0.7℃と 6.4±0.9℃、アミメノコギリガザミでは 9.6±0.6℃と 7.4±0.4℃、高温側の生存の CHT はトゲノコギリガザミで 39.0±0.4℃、アミメノコギリガザミで 39.1±0.6℃と推定され、トゲノコギリガザミがより強い低温耐性を有していることが明らかになった。

第 5 章　総合考察

総合考察として各章で実施した研究について要約するとともに，今後の課題について言及した。

第 1 節 ノコギリガザミ稚ガニの共食い防除技術の高度化に向けて

共食い防除技術の高度化について、RSD_{50} を指標とした体サイズ選別を提案した。さらに、新たな課題点として、脱皮間隔が短く、RSD_{50} 以下の体サイズ差に維持することが困難であると予想される 5 齢前後のステージでは、潜砂行動の発現や形態の相対成長による生態の変化などを明らかにし、個体発生に応じた

飼育環境やシェルターを提供することが望ましいこと、また、実際の養殖現場に実装することを想定し、大量の個体を効率よく選別可能な器具の開発が求められることが挙げられた。

第 2 節 ノコギリガザミ類の温度適応と温暖化による資源構成の変化予測

温度適応と温暖化による資源構成の変化予測について、トゲノコギリガザミとアミメノコギリガザミの臨界発育水温及び臨界低水温（CLT）と本州温帯域の生息地の水温を比較したところ、ノコギリガザミ類の多産する浜名湖や浦戸湾の冬季の水温がトゲノコギリガザミの臨界発育水温より高く、アミメノコギリガザミよりは低いことから、両種の温度適応の種間差が各地の種組成や分布を規定していることを示した。さらに、気象庁による温暖化による沿岸水温の上昇予想を加味した結果、トゲノコギリガザミの北上・分布拡大と、既知の産地におけるアミメノコギリガザミの資源増加が想定された。今後の課題として、水温以外の環境適応機構について明らかにすることで、より詳細な分布拡大予測が可能になると考えられる。

謝辞

本研究を進めるにあたり、その機会を与えていただき終始懇切丁寧なるご指導を賜った東京海洋大学増殖生態学研究室の濱崎活幸教授に心よりお礼申し上げる。原稿の校閲ならびに貴重なご助言をいただいた東京海洋大学増殖生態学研究室の團重樹准教授、同集団生物学研究室横田賢史准教授、並びにストルスマン・カルロス・アウグスト教授に深く感謝する。大学在学時に大変お世話になった東京海洋大学北田修一名誉教授、国立研究開発法人水産研究・教育機構にて貴重なご指導をいただいた照屋和久氏、小磯雅彦氏、村上恵祐氏、市川卓氏（現東京農業大学准教授）に厚く御礼申し上げる。水産研究・教育機構八重山庁舎の清水智仁氏、岩崎隆志氏には、飼育実験にご助言を頂くとともに絶大なご協力をいただいた。この場を借りて心から感謝を申し上げる。東京海洋大学大学院博士後期課程に同時期に所属した小田健太郎主任研究員とは、ともに励ましあい執筆を進めてきた。ここに記して深謝する。本研究に用いた親ガニの入手には金城吉一氏、新立弘子氏、永野廣氏にお世話になった。また、飼育実験を行うにあたり、井村市氏、鍵山智美氏、神谷秀美氏には飼育管理や実験の補助にご尽力いただいた。ここに記して厚く感謝申し上げる。最後に、在学中常に励まし支えてくれた家族に心から感謝する。