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

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Abstract

Cannibalism is a major cause of mortality in nursery cultures of *Scylla paramamosain,* and size grading is recommended as an effective measure to reduce cannibalism. However, little is known about the relationship between body size differences and the occurrence of cannibalism in early juvenile stages. We conducted one-to-one match experiments (60 trials) using hatchery-raised juveniles with carapace widths between 5.1 and 10.9 mm. Two crabs were placed in an experimental arena for 24 h, and survival was subsequently observed. Cannibalism occurred in 48 % of the trials, and larger crabs always preyed on smaller crabs. The probability of cannibalistic behavior significantly increased with increasing relative size difference [RSD = 1 − (size of smaller crab)/(size of larger crab)]. The RSD threshold at which 50 % of the smaller crabs are cannibalized by larger crabs was estimated at 0.21.

Key words: portunid crab, agonistic behavior, aquaculture, nursery culture

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 and Blackshaw 1999; LeVay 2001; Allan and 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 and 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 (LeVay 2001; Shelley 2008). However, recent advances in seed production technology have allowed aquaculture farmers to use artificially produced crab seeds (Allan and 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 and 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 and Parado-Estepa 2011; Mirera and Moksnes 2013), leading to serious impacts on the productivity and profitability of the aquaculture industry (Romano and 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 and Moksnes 2013, 2015; Romano and Zeng 2017). Therefore, size grading is considered an effective strategy to reduce the occurrence of cannibalism in aquaculture (Mirera and Moksnes 2013; Romano and 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.

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 and 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).

Table 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.

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 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. 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 1).

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 1 and Fig. 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 and 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

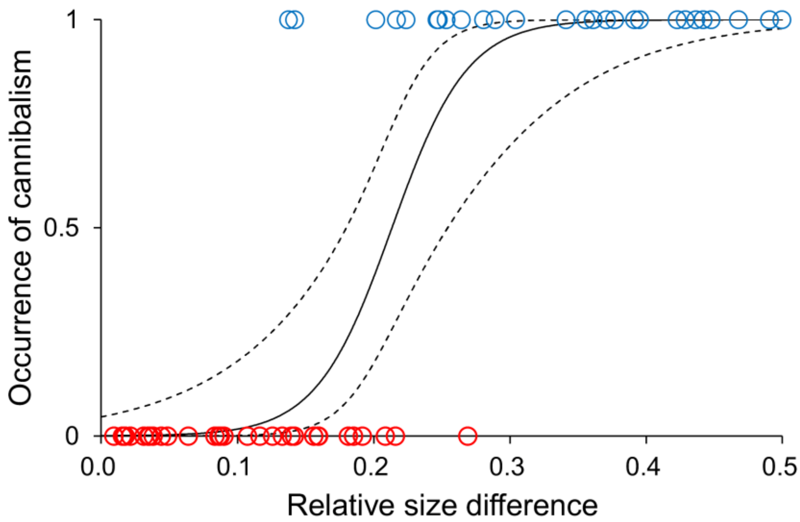

Fig. 1. Relationship between relative size difference and occurrence of cannibalism in test pairs of mud crab *Scylla paramamosain* early juveniles in one-to-one match experiments. Relative size difference = 1 − (smaller crab size)/(larger crab size). The estimated probabilities of cannibalistic events (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 1).

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.

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