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

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**Abstract.**—Cannibalism is considered a major cause of mortality among juveniles in nursery cultures of the mud crab *Scylla serrata*. Size grading is recommended as an effective measure to mitigate cannibalistic behavior; however, little is known about how body size differences influence cannibalistic episodes between juvenile individuals. We conducted one-to-one match experiments using hatchery-raised juveniles with carapace width between 12.5 and 33.3 mm. Two crabs were placed in an experimental arena for 24 h, and survival and pereiopod loss were subsequently observed. Large crabs always preyed on small crabs, and the probability of cannibalistic behavior significantly increased with the increasing relative size difference [RSD=1−(size of small crab)/(size of large crab)]. The loss of chelipeds and walking legs in the surviving small crabs amounted to 25% and 38%, respectively, and the proportion of lost walking legs increased with the increasing RSD, indicating the stronger aggressive behavior of larger crabs. This study suggests that maintaining size differences below the RSD threshold (0.34) at which 50% of the small crabs are cannibalized by large crabs would be an effective strategy to contain the occurrence of cannibalism in mud crab nursery cultures.

**Key words:** portunid crab, aquaculture, agonistic interaction, limb loss, nursery culture

**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, 2004). 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.

### 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 RSD50 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 RSD50 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).

### 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 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

| 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.
The RSD 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 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 1), as illustrated by the logistic curve in Fig. 2.

**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 1 and Fig. 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).

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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