Evaluation of selected commercial aquaculture feeds as substitute for natural feeds in rearing mud crab (*Scylla serrata*) juveniles

Jerome G. Genodepa, Alan N. Failaman

Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo 5023, Philippines. Corresponding author: J. Genodepa, jggenodepa@gmail.com

Abstract. A feeding trial was conducted to assess the potential of commercial feeds for fish and shrimp as substitute for natural feeds in rearing juveniles of the mud crab, *Scylla serrata*. The crablets (2nd crab instar; 0.044 to 0.049 g average body weight (ABW); n = 300) were fed either fish feed, shrimp feed or natural feeds (mussel and *Acetes*) alone, or with the 50-50% combination of the natural feed and each of the commercial feeds. The performance of the feeds and their combinations were evaluated after 11 and 20 days of rearing in 1000 L tanks based on survival, ABW, specific growth rate (SGR) and degree of aggression (indicated by missing appendages). The commercial diets tested were not suitable as complete replacement for natural feeds in rearing mud crab juveniles. Treatments fed with the commercial diets had significantly lower survival (35 to 54%) and ABW (0.186 to 0.399 g) compared to the treatment fed natural feeds alone (74% survival and 0.506 g ABW) after 20 days ($p < 0.01$). The combination of commercial feeds and natural feeds can possibly replace natural feeds during nursery rearing of juvenile crabs. The ABW in the treatment fed the combination of shrimp feed and natural feeds (0.611 g) was significantly higher ($p < 0.01$) than the treatment fed natural feeds only (0.506 g) while it did not differ between the treatment fed natural feeds only and the combination of fish feed and natural feeds (0.535g). In addition, SGR and degree of antagonism did not vary significantly between treatments given the feed combinations and treatment fed natural feeds only.

Key Words: mud crabs, nursery, food types, natural feed, commercial feeds.

Introduction. Crabs belonging to the genus *Scylla* de Haan (Crustacea: Decapoda: Brachyura: Portunidae) are commonly called mud crabs. These crabs are in demand in the local and export markets because they are highly presentable food item with unique pleasing flavor. Global aquaculture production of mud crabs increased from 6,882 tonnes in 1995 to 123,085 tonnes in 2005 (Shelley 2008) and continued to increase to 179,536 tonnes in 2013 (FAO 2015). However, continued, sustainable aquaculture production is a major challenge because the industry depends primarily on seed from the wild (Keenan 1999; Shelley 2008; Quinitio 2015; Wang 2015).

Among mud crabs, *Scylla serrata* is the biggest and the fastest growing of the four species under genus *Scylla* (Keenan 1999). Hatchery production of *S. serrata* has received significant attention in several countries over the past decade (e.g., Australian Centre for International Agricultural Research - Mud Crab Project involving Australia and Philippines; Culture and Management of *Scylla* Project involving European countries, Vietnam and Philippines), yet the technology is still largely confined to research institutions. Despite the notably low survival, the potential for commercial *S. serrata* hatchery production in the Philippines has been reported (Fortes et al 2002; Quinitio & Parado-Estepa 2003). Mud crab seed production in the Philippines involves two stages, the rearing of newly hatched larvae to megalopa stage in the hatchery and then further rearing of megalopa to at least second crab instar (C2) in nurseries. Our experience at the University of the Philippines Visayas hatchery has shown that prolonged rearing of crablets in tanks result in significantly lower survival because of cannibalism. For this reason, nursery operation is usually done in hapa nets installed in brackishwater ponds in order to provide extra space to accommodate lower stocking densities necessary to achieve better survival. Integrating hatchery and nursery operation and producing larger crablets has been suggested to in order to improve the profitability of the mud crab seed production business (Quinitio & Parado-Estepa 2003) but having hatchery and nursery operation in different sites is difficult to manage and requires extra manpower. An ideal system would be to have hatchery and nursery operation in the same site using the existing hatchery facilities, however, technologies for extended rearing of crab juveniles in hatcheries are still lacking.

Cannibalism is perhaps the most serious cause of mortalities in nursery culture of mud crabs. Among the factors that can be manipulated to reduce cannibalism are shelters, stocking density and feeds. Studies on formulated feeds for mud crab nursery and grow-out rearing are still limited despite the many advantages of formulated feeds over live feeds (Southgate 2003). In rearing megalopa to first crab instar (C1), Williams et al (1999) found that *Artemia* based diets (containing either newly hatched or boosted *Artemia*) resulted in significantly higher survival compared to diet consisting of *Acetes* shrimp or mud worm (*Marphysa* spp.) or the combination of *Acetes* and mud worm. Rodriguez et al (2005) found no significant differences in growth and survival of megalopa reared up to crab stage in hapa nets fed with either formulated diet or mussel meat or their combination, however, it was also reported that a microbound diet can be a suitable substitute for *Artemia* in rearing megalopa to C1. Genodepa et al (2004b) found that although survival did not differ significantly with microbound diet and *Artemia* as feed, molting of megalopa to C1 was earlier in the former.

For rearing juvenile crabs, natural feeds such fish, crustaceans and mollusks are still commonly used. Marasigan (1999) found that the specific growth rate of crab juveniles reared in individual containers was higher with mussel meat or trash fish as feed compared moist and dry formulated diets, while Ut et al (2005) found that shrimp meat resulted in higher survival and growth rate of crabs compared to those fed fish flesh, and *Artemia* biomass gave results similar to pealed shrimps, but frozen *Artemia* was inferior.

The development of formulated diets for mud crabs has been the focus of research during the last decade as trash fish and other wild resources come under increasing pressure for use as feed for various types of aquaculture and for human consumption. These included studies which evaluated the potential importance of plant based nutrient sources to mud crabs (Tuan et al 2006; Truong et al 2008; Truong et al 2009) and nutrient requirements (Sheen & Wu 1999; Catacutan 2002). Optimal lipid levels for mud crab juveniles were reported by Sheen & Wu (1999) to be between 5.3 to 13.8%. Protein and lipid levels of 32 to 40% and 6 to 12% respectively were also reported to meet the requirements of juvenile mud crabs (Catacutan 2002). Despite these research developments, availability commercial mud crabs diets is still a major concern for mud crab farmers in many countries. Considering that the protein and lipid levels in some commercially available formulated diets for aquaculture (particularly for milkfish and shrimp) are within optimal levels for mud crabs, it would therefore be worth finding out whether these are suitable as complete or partial substitute for natural feeds in *S. serrata* juvenile production.

Material and Method

***Production of crab juveniles**.* Female *S. serrata* (at least 14 cm carapace width) were purchased from local traders and maintained until they spawned at the Multi-purpose Hatchery of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas. Species identity was confirmed using the criteria outlined by Keenan et al (1998). The crabs were held individually in circular concrete tanks with 250 L of seawater (26-29°C temperature and 33-35 ‰ salinity) flowing at the rate of 8-12 L h^{-1}. The crabs were fed once daily in the evening with either fish or mussel meat at a rate of 5-8% body weight. Water quality in the tanks was maintained by cleaning and removal of uneaten food every morning, followed by 20-30% water change.

The crabs were mostly allowed to spawn naturally but some were induced to spawn by bilateral eyestalk ablation in order to get timely supply of larvae for the various experiments. Following ovulation, the crabs were transferred to a new tank and provided with more in-flow of new seawater. One or two days prior to hatching, the berried crabs were disinfected by immersing for 6 h in static seawater with full strength formalin added at a concentration of 50-80 µl L^{-1} (Hamasaki & Hatai 1993; Genodepa 2003) and transferred to a 500 L cylindro-conical hatching tank.

Larval rearing protocol was basically adopted from Genodepa et al (2004a). Briefly, the newly hatched larvae were collected soon after hatching and stocked in static rearing tanks (1000 L) at a density of 100-150 larvae L^{-1}. The larvae were fed primarily rotifers (*Brachionus* sp.) and *Artemia* nauplii. Rotifers were introduced into larval rearing tanks only once (40-60 individuals mL^{-1}) on the first day of larval culture. Rotifer populations were then maintained in the larval rearing tanks by daily addition of microalgae (*Nannochloropsis* sp.). As the larvae grew older, the rotifer density was gradually reduced to a negligible level by the time the larvae had reached the Zoea V stage. *Artemia* nauplii were first introduced into larval rearing tanks (0.5 individuals mL^{-1}) on the second day after the larvae moulted to the Zoea II stage. *Artemia* density was gradually increased to 3-5 individuals mL^{-1} by the time the larvae reach the Zoea V stage. A microbound diet for shrimp larvae was fed daily at 2-3 g ton^{-1} from Zoea V onward. Minced mussel meat and *Acetes* were given as feed from late megalopa or first crab stage (C1) onward. Daily water exchange in the larval culture tanks ranged from 20-25% between Zoea I and Zoea II stages to 50-70 % from Zoea III onward.

Feeding experiments. Commercial feeds for fish and for shrimp were tested as complete or partial substitutes for natural feeds in rearing juvenile crabs in concrete nursery tanks in April 2006. Each 1000 L tank was stocked with 300 C2 juveniles and were fed with the following: Trt 1 - 100% formulated diet for milkfish, *Chanos chanos*; Trt 2 - 100% formulated diet for prawn, *Penaeus monodon*; Trt 3 - 100% natural feeds (50% mussel, *Perna viridis* meat + 50% *Acetes* sp.); Trt 4 - 50% milkfish feed + 50% natural feeds; Trt 5 - 50% prawn feed + 50% natural feeds. The natural feeds were given at 30% of the wet biomass while the fish and prawn pellets were given based on the equivalent dry weight of the natural feeds. In treatments given natural feeds alone, the ration was divided into two and given at 08:00 H and at 17:00 H, while in treatments fed the combination of pellets and natural feed, the pellets were given at 08:00 H while the natural feed was given at 17:00 H. Water quality in the tanks was maintained by changing 50% of water volume daily after siphoning out the feces and uneaten food.

Complete inventory of the remaining crab juveniles were done at Day 11 and at Day 20 to monitor survival and average body weight (ABW) under the various treatments. The number of crabs with missing appendages was also monitored at Day 20. Proximate analysis of the natural feeds was also conducted for comparison with the proximate analysis of the commercial feeds indicated in their product label. Protein and lipid were analyzed using Kjeldahl (AOAC 1975) and Bligh & Dyer (1959) methods respectively, while moisture and ash were analyzed using oven and furnace method (AOAC 1975) respectively. Specific growth rate (SGR) was computed using the formula:

$$\text{Specific Growth Rate (g day}^{-1}) = \frac{\text{Ln Final Weight (g)} - \text{Ln Initial Weight (g)}}{\text{Duration of Culture (days)}}$$

Statistical analysis. Survival, ABW, SGR and percentage of crabs with missing appendages were compared using ANOVA and specific differences among treatments were determined using Duncan's Multiple Range Test. Arcsine transformation was applied on values expressed as percentage prior to analysis. Statistical analyses were performed using SPSS for Windows Version 12.0. Data are presented as mean, plus or minus (±) standard error (SE), and results were considered significantly different at $p \leq 0.05$.

Results and Discussion. The survival of *S. serrata* fed different types of food is presented in Figure 1. Survival at both sampling periods was always highest in the treatments fed natural feeds only, followed by the combination of prawn feed and natural feed, thirdly by the combination of fish feed and natural feed, then by prawn feed alone, and lastly by fish feed alone. Survival at Day 11 was significantly lower ($p < 0.01$) in the treatment fed fish feed compared to the rest of the treatments which did not differ significantly. Survival at Day 20 differed significantly among treatments ($p < 0.01$) except between the combination feeds. Based on survival at Day 20, combining formulated feeds with natural feeds significantly improved the performance of both formulated feeds, however the levels were still significantly lower than the level attained using 100% natural feeds ($p < 0.01$).

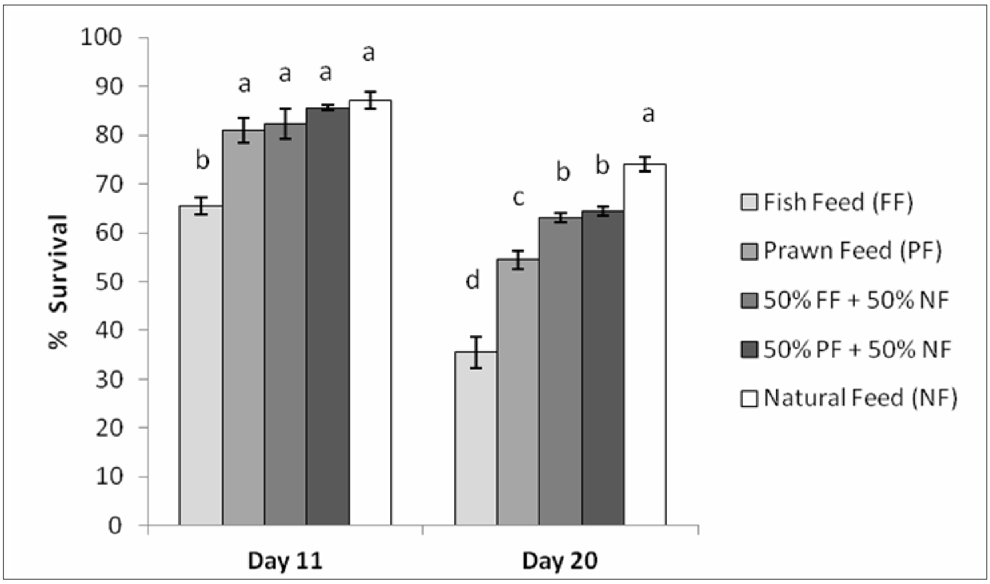

Figure 1. Mean (±SE; N = 3) survival of *S. serrata* fed different types of food. Bars within the same day of culture having the same letter superscript are not significantly different ($p > 0.01$).

In the culture of carnivorous species such as crabs, the use of appropriate feeds is considered as one of the schemes to reduce mortality due to cannibalism (Ut et al 2005; Zmora et al 2005). The results of this study showing highly significant differences in survival with different types of feed indicate that mortality can indeed be lesser when suitable feeds are used. The higher survival in treatments fed natural feeds imply that natural feeds are highly preferred by crabs over the other types of feed. The natural feeds used in this study consist of mussel meat and *Acetes* which are highly preferred by crablets since the natural food of crabs consist mostly of mollusks and crustaceans (Lee 1991; Jayamanne & Jinadasa 1991). Fish feed was poorly accepted by crabs since it resulted in significantly lower survival compared to the other treatments at both sampling periods. In fact, only the treatment fed fish feed differed in survival from the other treatments at Day 11, indicating that crabs were poorly accepting fish feed right from the start.

The percentage of juvenile crabs with limb loss or missing appendages (Figure 2) did not differ between the treatment fed natural feeds only and treatments that had proportions of natural feeds or the treatment fed with prawn feed only. However, the treatment fed with fish feed only had significantly higher percentage of limb loss compared to all treatments that had proportions of natural feed ($p < 0.05$) but did not differ from the treatment given prawn feed only. The poor acceptance of the fish feed was clearly indicated by the highest percentage of limb loss among the treatments.

Acceptance of prawn feed by crabs at the start of the experiment was comparable to natural feeds based on survival at Day 11 but it later turned out inferior to either natural feeds alone or the combination diets. These results may be related to the findings of Marasigan (1999) that formulated diets were inferior feeds for crabs compared to natural feeds. While survival of crablets was significantly lower when fed formulated diets alone, significant improvement in survival was achieved when natural feeds were combined with the formulated diets. These results suggest that the presence of natural feeds can significantly reduce the cannibalism in crabs. This is likely because the presence of natural feeds in the combination diets provided the crabs with highly acceptable alternative feed which could deter them from resorting to cannibalism.

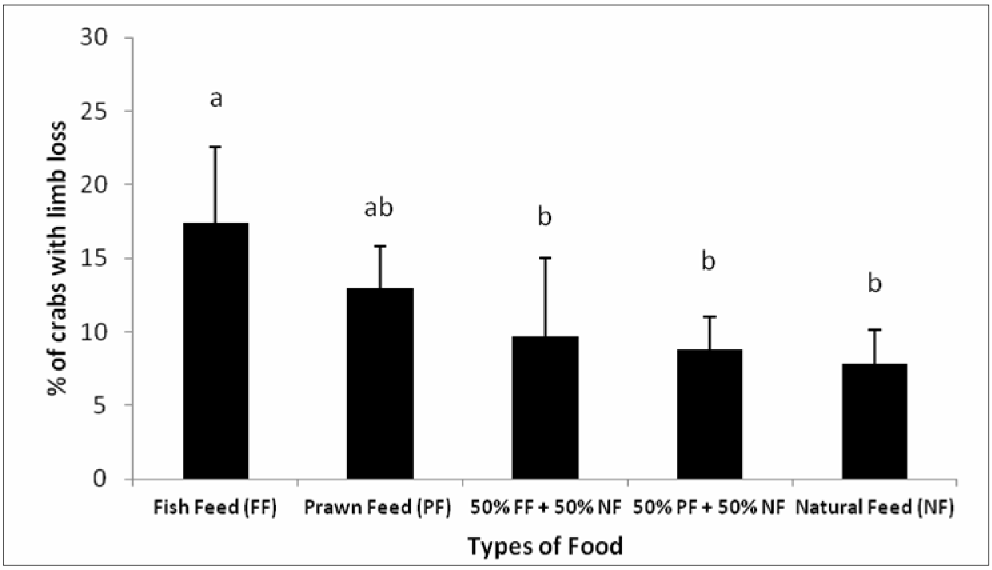

Figure 2. Mean (±SE; N = 3) percentage of crabs with missing limbs harvested after 20 days of culture under treatments fed different types of food. Bars with the same letter superscript are not significantly different ($p > 0.05$).

The proximate analysis of the feeds (Table 1) reveals that survival of crablets was influenced by protein levels in the feeds because survival increased with increasing protein levels. This implies that acceptability of the feed is better if the level of protein is higher and explains why the addition of natural feeds to the formulated diets in the combination treatments resulted in improved survival.

Table 1

Proximate analysis of the feeds used in the experiment

Feed	*% Protein*	*% Lipid*	*% Fiber*	*% Ash*	*% Moisture*
Fish feed*	34.00	6.00	6.00	12.00	12.00
Prawn feed*	45.00	8.00	4.00	16.00	12.00
*Acetes***	64.37	8.46	-	17.10	78.02
Mussel**	54.81	8.36	-	7.52	80.99

* Proximate analysis indicated in the product label; ** Proximate analysis conducted by the Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo 5023, Philippines.

Table 2 shows the ABW and SGR of *S. serrata* juveniles fed different types of food. All treatments that received proportions on natural feeds had significantly higher ABW than treatments fed formulated diets alone ($p < 0.01$). The treatment fed fish feed only had significantly lower ABW than those fed prawn feed only ($p < 0.01$) but the treatments fed the combination feeds still did not significantly differ. ABW at Day11 did not differ significantly between treatments fed natural feeds alone and treatments fed the combination diets, however at Day 20, ABW in the treatment fed combination of prawn feed and natural feeds was significantly higher ($p < 0.01$) compared to treatments fed with commercial formulated feeds only. The SGR after 20 days of rearing was significantly lower in treatment fed fish feed ($p < 0.01$) but did not differ significantly between the rest of the treatments.

The slower growth of crablets in terms of ABW and SGR in treatments fed formulated diets compared to treatments that received proportions of natural feeds may be related to the higher protein levels of the natural feeds (Table 1), however, the natural feeds possibly had certain nutrients that were lacking in the formulated feeds. For example, mollusks as feed were found by Yalin & Qingsheng (1994) to give better results compared to other feeds. Marasigan (1999) likewise found that mussel (also a mollusk) meat as feed was superior compared to squid, fish or formulated diets. It is then possible that the presence of mussel meat in the natural feeds in this study may have brought about the many advantages to the treatments that had natural feeds. The slower growth of crablets in treatments fed formulated diets compared to treatments that received proportions of natural feeds may also be attributed to cannibalism. In the blue swimmer crab *Portunus pelagicus*, Paterson et al (2005) noted that moulting period was influenced by claw-loss, suggesting the need for regeneration could also delay moulting under some circumstances.

Table 2

Average (±SE; N = 3) body weight (ABW) and specific growth rate (SGR) of *S. serrata* juveniles fed different types of food. Columns within the same day of culture having the same superscript are not significantly different ($p > 0.01$)

Treatment	*ABW at Day 0 (g)*	*ABW at Day 11 (g)*	*ABW at Day 20 (g)*	*SGR at Day 20 (g day^{-1})*
Fish feed (FF)	0.048 ± 0.004^{a}	0.103 ± 0.004^{c}	0.186 ± 0.030^{d}	0.066 ± 0.008^{b}
Prawn feed (PF)	0.046 ± 0.007^{a}	0.164 ± 0.001^{b}	0.399 ± 0.020^{c}	0.108 ± 0.003^{a}
FF + NF	0.044 ± 0.003^{a}	0.196 ± 0.011^{a}	0.535 ± 0.012^{ab}	0.125 ± 0.003^{a}
PF + NF	0.045 ± 0.008^{a}	0.212 ± 0.007^{a}	0.611 ± 0.006^{a}	0.131 ± 0.005^{a}
Natural feed (NF)	0.049 ± 0.011^{a}	0.199 ± 0.003^{a}	0.506 ± 0.025^{b}	0.118 ± 0.006^{a}

The better growth in treatments fed the combination formulated and natural feeds compared to treatments fed natural feeds alone may also imply that certain nutrients are lacking in natural feeds and these are available in the formulated feeds. Combining formulated diet and natural feed was reported by Genodepa et al (2004b) to be more advantageous than natural feed alone. The advantage of combining several types of feed was likewise reflected in the results of Lijauco et al (1980) and Williams et al (1999). Considering that survival of crabs was significantly higher with natural feeds but final ABW was better in the 50-50 combination of prawn feed and natural feeds, a study on the different proportions of natural feed and formulated may yield better results in terms of both survival and growth. Based on the results of this study, total replacement of natural feeds with commercially available formulated diets for fish and shrimps is still not the best option for rearing smaller *S. serrata* juveniles. Considering, however that bigger crab juveniles such as those used in the soft shell crab industry were reported to adapt easily to formulated diets and were even reluctant to eat fresh fish again after a period of feeding with formulated diet (Wilson 2005), it is possible that there are other commercially available aquaculture feeds suitable for mud crabs.

Conclusions. The commercial diets for fish and prawn tested in this study were not suitable as complete replacement for natural feeds (*Acetes* and mussel meat) in rearing mud crab juveniles. The combination of commercial feeds and natural feeds can be a good option for nursery rearing of juvenile crabs but an optimal ratio must be determined. Considering the limited research work on mud crab nursery, information provided by this research can be considered a significant contribution in this field.

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Authors:
Jerome G. Genodepa, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo 5023, Philippines, e-mail: jggenodepa@gmail.com
Alan N. Failaman, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo 5023, Philippines, e-mail: anfailaman@yahoo.com

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