

Asian Fisheries Society, Selangor, Malaysia

Optimum Food Type, Feeding Schedule and Prey Density for the Zoea Larvae of the Crucifix Crab, *Charybis feriatus* (Crustacea:Decapoda:Portunidae)

JULIANA C. BAYLON*

Division of Biological Sciences, College of Arts and Sciences University of the Philippines Visayas , 5023 Miagao, Iloilo, Philippines

Abstract

Feeding experiments were carried out on the zoea larvae of the crucifix crab, Charybdis feriatus. The 1st experiment aimed to find out which among the rotifer Brachionus sp., brine shrimp Artemia sp. nauplii, and combination of both, would be a better diet to support survival, development of the zoeal larval stages (Z1-Z5) and metamorphosis to megalopa. In this experiment, the timing of introduction and cessation of rotifers, and co-feeding with brine shrimp nauplii was also determined. The 2nd experiment determined the density of Artemia nauplii that can support up to postmolt and the possibility of reducing prey density as zoeal stage progresses. Results showed high survival of Z1-Z2 larvae when fed purely with Brachionus but none of the larvae reached Z4 stage. When fed purely with Artemia nauplii, all the larvae died at Z3. Highest survival, fastest development and successful metamorphosis to megalopa was obtained on larvae fed with rotifers alone in Z1 and combination of rotifers and brine shrimp nauplii from Z2 onwards. Survival dropped when rotifers were removed from the diet in the later zoeal stages and when density of Artemia nauplii was gradually lowered from 2.0 nauplii ml⁻¹ at Z1 to 0.5 nauplii ml⁻¹ at Z5. Larvae fed with Artemia at a constant density of 2.0 nauplii ml⁻¹ in all zoeal stages (Z1-Z5) resulted to highest number of megalopa produced. These results advance the development of hatchery techniques for the crucifix crab, C. feriatus.

*Corresponding author. Tel. No.: 63-33-3158335 E-mail address: profjbaylon@yahoo.com

Introduction

Charybdis feriatus L. is a highly priced portunid crab found in Philippine waters. It is exported to other Asian countries and commands a good price. There is a high demand for this crab in the local and international markets because of its delicate taste, excellent meat quality, large size and attractive appearance. This species can be easily identified because of its striking red and white colour pattern and the presence of a distinct cross on the median surface of the carapace (Padayatti 1990). This crab was found to be a continuous breeder (Del Norte-Campos and Panes 2004) and the broodstock is available throughout the year, making it an excellent species for aquaculture, domestication and stock enhancement (Williams and Primavera 2001). Interest in the development of the seed production of *C. feriatus* started recently (Parado-Estepa et al. 2002; Parado-Estepa et al. 2005). Ingestion behavior of the zoea larvae and effects of salinity and temperature on larvae and juveniles had been investigated (Baylon et al. 2005; Baylon and Suzuki 2007), but there was no report yet on the food type, feeding schedule and prey density requirement of the zoeal stages of the larvae.

In *Scylla serrata*, a well-studied portunid crab, it has been reported that the zoea larvae survived when fed with either *Artemia* nauplii alone (Brick 1974; Heasman and Fielder 1983; Marichamy and Rajapackiam 1992; Faazaz and Che 1995) or combination of both *Brachionus* and *Artemia* nauplii given at specific stages of zoeal development (Baylon and Failaman 1997; Mann et al. 1999; Quinitio et al. 2001; Fortes et al. 2002; Ruscoe et al. 2004). For *C. feriatus*, it is also possible that each stage of zoeal development requires a specific food type and feeding schedule. To minimize food wastage and maximize survival, the following questions are needed to be answered: "What food type do early zoeal stages require? In what larval stage should rotifers be removed from the diet and shift to *Artemia* nauplii start? Will continued presence of rotifers in the diet provide additional benefit to the larvae? Will lowering of prey density as the larvae develop affect metamorphosis to megalopa? Will rotifers be provided only during the first few days of culture or will it be continued up to the last zoeal stage? If density of brine shrimp nauplii can be reduced in later stages or if rotifers can be removed from the diet in advanced stages, then substantial savings in terms of food cost and microalgal production can be achieved.

This study had the following specific objectives: to find out the appropriate food organism for the early and late zoeal stages; to identify the zoeal stage where rotifers could be withdrawn from the diet and the time to introduce *Artemia*; to determine the optimum density of *Artemia* nauplii that will support development up to megalopa; and to find out if lowering of *Artemia* nauplii density towards the end of zoeal stages will not affect survival. The results obtained from these investigations will provide information that will be valuable in the development of hatchery techniques for the commercial production of *C. feriatus* juveniles.

Materials and Methods

Newly hatched C. feriatus larvae from one female were distributed in 1,000 L cylindrical fiberglass tank and were fed purely with Brachionus. Green microalgae predominantly Nannochloropsis, were added to the tank as food to the rotifers. The next day, when the larvae were one day old, they were collected from the tank and used in the experiment. The experiments were conducted in 1.5 L plastic containers filled with 1 L seawater (35 ppt). The experimental containers were floated in a water bath where the temperature was maintained at 29-30 °C, by submersion of two 150 w thermostat controlled heaters into the water baths. In the 1st experiment, 30 larvae were placed in each container and three replicates were prepared for every treatment. In the 2^{nd} experiment, 50 larvae were placed in each container with the same number of replicates. Larvae were transferred daily to new containers with fresh seawater (34-35 ppt) and were fed the appropriate food type and food density. During transfer, survival and development were determined by individually collecting the larvae using a glass dropper with a plastic air hose extension. The C. feriatus passed through five zoeal stages (Z1-Z5) before they metamorphosed to megalopa. When larvae were in Z5 stage, they were transferred to bigger, 3 L containers because of earlier observation that larvae that continued to be reared in smaller, 1.5 L containers failed to complete their molting to megalopa. Development to the next zoeal stage takes place every three days and metamorphosis to megalopa starts 15 days after hatch. To prevent cannibalism, newly molted megalopa were promptly collected and transferred to a different container. The experiment was terminated when all the surviving larvae have molted to megalopa. To compute for percentage survival, the number of larvae that survived at the end of each stage was divided by the original number of larvae placed in the container and then multiplied by 100.

Experiment 1. First feed organism and feeding schedule

This investigation aimed to find out the appropriate food organism for the zoea larvae, the feeding schedule that would determine if rotifers would be limited only in the early stages or if it would still be continued up to later stages. Four feeding treatments were tested (Table 1). In treatment 1, larvae were fed purely with rotifers from Z1 onwards. In treatment 2, the larvae were fed purely with *Artemia* nauplii. In treatment 3, the larvae were fed with rotifers alone in Z1 and from Z2 onwards, diet was shifted to pure *Artemia* nauplii. Treatment 4 was similar to treatment 3 except that starting from Z2, instead of pure *Artemia*, a combination diet of both rotifers and *Artemia* nauplii, was given. In all treatments, the density of the *Artemia* nauplii was decreasing: 3.0 *Artemia* ml⁻¹ in Z1, 2.0 *Artemia* ml⁻¹ in Z2, 1.0 *Artemia* ml⁻¹ in Z3 and 0.5 *Artemia* ml⁻¹ in Z4-Z5. *Artemia* nauplii were hatched by incubating cysts (Sanders Brine Shrimp Co., Great Salt Lake, Utah) for 24 h where cysts were disinfected with sodium hypochlorite at a concentration of 5 mg l⁻¹ (5 ppm) active chlorine for 15 minutes prior to incubation. *Brachionus* were given at a density of 20 individuals ml⁻¹.

Table 1. Feeding regimes using either *Brachionus* alone, *Artemia* alone, or a mixture of both, as food for *C. feriatus* larvae.

Treatment 1	Brachionus alone (Z1-Z5)
Treatment 2	Artemia nauplii alone (Z1-Z5)
Treatment 3	Brachionus alone (Z1); Artemia nauplii alone (Z2-Z5)
Treatment 4	Brachionus alone (Z1); Brachionus + Artemia nauplii (Z2-Z5)

Experiment 2. Artemia density

This study aimed to identify the lowest density of *Artemia* nauplii that could support survival, development and metamorphosis to megalopa and to find out if it would be possible to decrease the prey density as larval stage progresses. Z1 larvae were fed with pure *Brachionus* upon hatching up to day one and varying densities of *Artemia* nauplii were added to the larvae starting on day two of Z1. There were five *Artemia* nauplii densities tested (Table 2). In treatment 1, a constant density of 2.0 nauplii ml⁻¹ was given from Z1 onwards. In treatment 2, *Artemia* nauplii density was decreased gradually as the larval stage progresses where 2.0 nauplii ml⁻¹ were given to Z1, 1.0 nauplii ml⁻¹ to Z2, and 0.5 nauplii ml⁻¹ from Z3 onwards. In treatment 3, the larvae were given a constant *Artemia* density of 1.0 nauplii ml⁻¹ from Z2 onwards. In treatment 4, 1.0 nauplii ml⁻¹ were given to Z1, decreased to 0.5 nauplii ml⁻¹ at Z1, 1.0 nauplii ml⁻¹ at Z2, 1.5 nauplii ml⁻¹ at Z3, 2.0 nauplii ml⁻¹ at Z4 and 3.0 nauplii ml⁻¹ at Z5. A constant *Brachionus* density of 20 individuals ml⁻¹ were provided to the larvae in all stages.

Table 2. Artemia nauplii given to C. feriatus larvae, either at a constant density in all zoeal stages or at a decreasing/increasing rate as the larvae developed

Treatment 1	2 Artemia nauplii ml ⁻¹ at Z1-Z5
Treatment 2	2 <i>Artemia</i> nauplii ml ⁻¹ at Z1; 1 <i>Artemia</i> nauplii ml ⁻¹ at Z2; 0.5 <i>Artemia</i> nauplii ml ⁻¹ at Z3-Z5
Treatment 3	1 Artemia nauplii ml ⁻¹ at Z1-Z5
Treatment 4	1 Artemia nauplii ml ⁻¹ at Z1; 0.5 Artemia nauplii ml ⁻¹ at Z2-Z5
Treatment 5	0.5 Artemia nauplii ml ⁻¹ at Z1; 1 Artemia nauplii ml ⁻¹ at Z2; 1.5 Artemia nauplii ml ⁻¹ at Z3; 2 Artemia nauplii ml ⁻¹ at Z4; 3 Artemia nauplii ml ⁻¹ at Z5

Percentage survival taken on the last day of each developmental stage was arcsine transformed and analyzed using one way ANOVA. This was followed by Duncan's Multiple Range Test to find out differences among the treatments at 0.05 probability level of significance. All statistical analyses were computed using SPSS software version 10 (SPSS Inc., 44 N Michigan Avenue, Chicago, Illinois).

Results

Experiment 1. Food type and feeding schedule

The survival rate of Z1 larvae was 100% in feeding treatments which contained *Brachionus* in the diet (Fig. 1). When fed purely with *Brachionus* (treatment 1), survival was very high at Z1 and Z2 but mortalities occurred starting at Z3 and none of the larvae survived to Z4. Larvae fed with *Artemia* nauplii alone (treatment 2) had mortalities in Z1 and Z2 and none of the larvae survived to Z3. In treatment 3, where the larvae were fed with rotifers alone in Z1 and from Z2 onwards, diet was shifted to pure *Artemia*, a good survival was maintained up to Z4, but heavy mortalities took place when larvae were in Z5, causing a significant decline in the number of megalopa produced. On the other hand, larvae that were fed with rotifers alone in Z1 and combination diet of rotifers and brine shrimp nauplii from Z2 onwards (treatment 4), had the highest successful metamorphosis to megalopa. The ANOVA results of the analysis of variance on survival of each stage of larvae reared at different food types and feeding schemes is shown on table 3.

Onset of development to Z2 was synchronous in all treatments that contained rotifers in the diet (Table 4). For larvae fed purely with rotifers, commencement of development was two days delayed and molting duration was extended up to two days, during development to Z3. This delay was extended up to four days during development to Z4. When rotifers were removed from the diet starting at Z2, commencement of metamorphosis to megalopa was delayed by one day and duration of molting was prolonged by one day as opposed to the treatment where rotifers were present in the diet up to the last zoeal stage.

Survival of Z1 larvae was not affected by the varying densities of *Artemia* nauplii when rotifers were present in the diet (Fig. 2). From Z2 to Z4, survival declined in all other treatments where prey density was lowered as opposed to the treatment where *Artemia* density of 2.0 nauplii ml^{-1} was maintained throughout the zoeal development. In Z5, larvae fed with *Artemia* density of 2.0 and 3.0 nauplii ml^{-1} remained to have good survival. Metamorphosis of Z5 to megalopa however was lower in the treatment where *Artemia* density was raised to 3.0 nauplii ml^{-1}

compared to the treatment where *Artemia* density was 2.0 nauplii ml^{-1} . The results of the analysis of variance on survival of each stage of larvae fed with varying densities of *Artemia* nauplii is shown on table 5.

Stage	SV	df	SS	MS	F computed	Significance
Z1	Treatments	3	1,236.604	412.201	221.114 **	.000
	Error	8	14.914	1.864		
	Total	11	1,251.518			
Z2	Treatments	3	129.245	4,312.748	492.235 **	.000
	Error	8	70.092	8.762		
	Total	11	13,008.338			
Z3	Treatments	3	7,984.090	2,661.363	400.200 **	.000
	Error	8	53.201	6.650		
	Total	11	8,037.291			
Z4	Treatments	3	12,880.397	4,293.466	464.506 **	.000
	Error	8	73.945	9.243		
	Total	11	12,2954.341			
Z5	Treatments	3	11,470.255	3823.418	240.083 **	.000
	Error	8	127.403	15.925		
	Total	11	11,597.659			
Megalopa	Treatments	3	8,054.888	2,684.963	61.859 **	.000
	Error	8	347.237	43.405		
	Total	11	8,402.126			

Table 3. Analysis of variance of survival of C. feriatus zoea larvae reared at different diets and feeding schemes.

Survival values were arcsine transformed before analysis. SV=source of variation; df=degrees of freedom; SS=sum of squares; MS=mean squares; *F*=Fisher *F* statistic; **=highly significant

Treatments	To Z2	To Z3	To Z4	To Z5	To megalopa
Brachionus (Z1-Z5)	3-7(5)	8-13(5)	14-20(7)	a	а
Artemia (Z1-Z5)	3-5(3)	a	a	a	a
Brachionus (Z1); Artemia (Z2-Z5)	3-5(3)	6-9(4)	10-12(3)	12-15(4)	16-19(4)
Brachionus (Z1); Brach + Art (Z2-Z5)	3-5(3)	6-8(3)	9-11(3)	12-14(3)	15-17(3)

Table 4. Days of development to next stage and duration of molt (in parentheses) of *C. feriatus* zoea larvae reared at different food types and feeding schedules.

^a all larvae died



Fig. 1. Survival of *C. feriatus* larvae reared at different diets and feeding schemes. Percent survival were arcsine transformed and analyzed using one way ANOVA followed by Duncans Multiple Range Test. Columns with the same letters in each larval stage, are not significantly different (P > 0.05). Survival values are means of three replicates.

Development to Z2 was not affected by the *Artemia* nauplii density (Table 6). In succeeding stages of development however, commencement of molt was delayed and duration of molt was extended by 1-3 days in larvae that were fed with *Artemia* densities lower than 2.0 nauplii ml⁻¹. Metamorphosis to megalopa was most rapid, occurring on the 16th day of rearing, for those fed with *Artemia* density of 2.0 and 3.0 nauplii ml⁻¹. Molt duration however was faster on Z5 larvae fed continuously with a constant density of 2.0 *Artemia* nauplii ml⁻¹ compared to the larvae fed with increasing *Artemia* density of 0.5 to 3.0 nauplii ml⁻¹.



Fig. 2. Survival of *C. feriatus* larvae fed with *Artemia* nauplii at different densities. Percent survival were arcsine transformed and analyzed using one way ANOVA followed by Duncans Multiple Range Test. Columns in each larval stage with the same letters, are not significantly different (P > 0.05). Survival values are means of three replicates.

Table 5. Analysis of variance of survival of C. feriatus zoea larvae fed with Artemia nauplii at different densities. At
Z1, there was no difference in survival between treatments, so ANOVA was not applied.

Stage	SV	df	SS	MS	F computed	Significance
Z2	Treatments	4	700.526	175.132	8.800 **	.003
	Error	10	199.018	19.902		
	Total	14	899.544			
Z3	Treatments	4	927.002	231.750	6.428 **	.008
	Error	10	360.511	36.051		
	Total	14	1287.513			
Z4	Treatments	4	1,572.137	393.034	6.500 **	.008
	Error	10	604.648	60.465		
	Total	14	2,176.785			
Z5	Treatments	4	1,692.875	423.219	9.637 **	.002
	Error	10	439.160	43.916		
	Total	14	2,132.035			
Megalopa	Treatments	4	1,456.637	364.159	7.363 **	.005
	Error	10	494.564	49.456		
	Total	14	1,951.201			

Survival values were arcsine transformed before analysis. SV=source of variation; df=degrees of freedom; SS=sum of squares; MS=mean squares; *F*=Fisher *F* statistic; **=highly significant

Treatments	To Z2	To Z3	To Z4	To Z5	To megalopa
2 <i>Artemia</i> ml ⁻¹ continuous	3-4(2)	7-8(2)	10-12(3)	13-15(3)	16-19(4)
2 Artemia ml ⁻¹ decreasing	3-4(2)	7-9(3)	10-12(3)	13-18(6)	19-25(7)
1 <i>Artemia</i> ml ⁻¹ continuous	3-4(2)	7-9(3)	10-12(3)	13-16(4)	17-21(5) ^a
1 Artemia ml ⁻¹ decreasing	3-4(2)	7-10(4)	11-13(3)	14-17(4)	19-23(5) ^a
Increasing Artemia density	3-4(2)	7-9(3)	10-12(3)	13-15(3)	16-21(6)

Table 6. Days of development to next stage, of *C. feriatus* larvae fed with *Artemia* nauplii at different densities. Days in parentheses represent duration of molt.

^a molt duration was shorter because there were only few surviving larvae

Discussion

Brachionus is recommended first food organism for the Z1 larvae

Larvae fed purely with rotifers Brachionus, demonstrated high survival in the early zoeal stages (Z1-Z2). This is probably because rotifers are slow moving small organisms which could be easily captured and ingested by the crab larvae. Rotifers are reported to be more digestible than Artemia (Watanabe et al. 1978) and early crustacean larvae exhibit low levels of enzyme activity and digestive capacity (Ribeiro and Jones 2000; Le Vay et al. 2001). Heavy mortalities however started to occur when in the intermediate zoeal stages and none of the larvae survived up to Z4 when raised in pure rotifer diet. This present result is similar to that was reported in other portunid crabs. For example, the larvae of the blue crab Callinectes sapidus raised on pure Brachionus diet were not able to develop to megalopa (Sulkin 1978). Similarly, the larvae of the xanthid crab Rhithropanopeus harrisii raised on rotifer diet suffered high mortality and prolonged intermolt duration with the greatest effect occurring during the latter half of zoeal development (Levine and Sulkin 1979). For the mud crab S. serrata larvae, there was also high survival at early zoeal stages when fed with rotifers but the larvae failed to develop to megalopa (Baylon et al. 1999; Zeng and Li 1999). These studies indicate that the nutrients provided by a diet of pure rotifers were not sufficient in meeting the requirements of the larvae to sustain development up to megalopa. On the other hand, none of the larvae survived to Z3 when fed

purely with *Artemia* nauplii. Perhaps the small size of the early stage crab larvae made it difficult for them to capture the nauplii which are vigorous swimmers and almost twice their size.

Combination diet of Brachionus and Artemia nauplii from Z2 onwards

There was high survival of C. feriatus larvae when fed purely with rotifers in Z1 and combination of rotifers and Artemia from Z2 onwards, where 75% of the larvae successfully metamorphosed to megalopa. Sulkin (1975) obtained 30% metamorphosis to megalopa of the blue crab Callinectes sapidus larvae raised on a diet of rotifer at Z1-Z2 followed by Artemia nauplii. For the Japanese blue crab, Portunus trituberculatus (Rho 1976) and Uca pugilator (Christiansen and Yang 1976), the rotifer Brachionus plicatilis was efficient as food in the early zoeal stages but for the 3rd, 4th and 5th zoeal stages, Artemia salina was more efficient. On the other hand, Z1 and Z2 larvae of Thalamita crenata gave the highest survival when fed with Brachionus plicatilis alone but later stages (Z3-Z5) survived and developed on Artemia-rotifer combination (Godfred et al. 1997). This suggests that availability of rotifers sustained survival of early zoeal stages while ingestion of the brine shrimp in the later stages provided the larvae with sufficient nutrients both for metabolic expenditure and for tissue build up in preparation for metamorphosis to megalopa. Sorgeloos (1986) reported that Brachionus has a calorific value of 800-1920 ucal and contains proteins and lipids while Artemia nauplii contain 2-3 times more lipid per dry weight than rotifers. The success of newly hatched Artemia nauplii as food could be attributed to its high amino and fatty acid content (Suprayudi et al. 2002).

Brachionus needs to be present in the diet up to last zoeal stage

There was a decrease in the survival and metamorphosis to megalopa when rotifers were removed from the diet from Z2 onwards. This suggests that at Z2 up to Z5 stages the larvae continue to prey on the rotifers and hence they must remain in the diet. Baylon et al. (2005) compared the ingestion of *C. feriatus* larvae on *Artemia* nauplii with or without *Brachionus* and found higher consumption of *Artemia* nauplii by crab larvae when *Brachionus* was present in the diet. These results were in contrary to what Ruscoe et al. (2004) have reported that the continued presence of rotifer in the later zoeal stages of the mud crab *S.serrata* no longer provides additional benefit to the larvae.

Optimum Artemia density is 2.0 nauplii ml^{-1}

Artemia nauplii given to the larvae in all zoeal stages at a constant density of 2.0 individuals ml^{-1} , supported highest metamorphosis to megalopa. These densities were much lower compared to the densities reported in *S. serrata* larvae which ranged from 5-30 nauplii ml^{-1} (Heasman and Fielder 1983) and 5-10 nauplii ml^{-1} (Baylon and Failaman 1997) but comparable to the results of Mann et al. (1999) where they provided *S. serrata* larvae purely with rotifers at a density of 10-15 individuals ml^{-1} from Z1-Z3 and pure *Artemia* nauplii (0.5-3.0 individuals ml^{-1})

from Z3 to megalopa and newly hatched *Artemia* nauplii (0.5 -0.75 individuals ml⁻¹) to megalopa, where a survival of up to 60% to crablet juveniles was obtained. In earlier experiments with *C. feriatus*, it was observed that larvae fed with an *Artemia* density higher than 3.0 nauplii ml⁻¹ resulted in mass mortality, which indicated high sensitivity of the larvae of this crab species to elevated density of prey.

Survival declined when density of Artemia nauplii was lowered in later stages

When density of Artemia was gradually reduced from 2.0 nauplii ml⁻¹ in Z1 to 0.5 nauplii ml⁻¹ in Z5, there was a high drop in survival as opposed to the larvae that were fed continuously with the same prey density. The lowering of prey density was based on the hypothesis that as the larvae grow, they become stronger and more efficient in capturing the prey, and could be classified as 'active' feeders. However in the present study, the decline in survival when the prey density was reduced suggests that late stage C. feriatus larvae remain to be 'passive' feeders. During the experiment, the C. feriatus crab larvae were never observed pursuing the Artemia nauplii. Instead, they only fed on the prey organisms when there were accidental encounters with the nauplius, which the larvae capture and pin between spines of their telson and rostrum. To promote accidental encounters, higher densities of Artemia nauplii are needed to be provided to the 'passive' feeders. Prey density however must not be too high that can stress the larvae and compromise survival. On the other hand, larvae which can be classified as 'active' feeders are not affected by the lowering of prey density because their efficiency in capturing the prey improves as the larvae grow. This was probably the case in the Portunus pelagicus where all Artemia nauplii provided to the larvae even when density was reduced to 0.5 nauplii ml⁻¹, were wiped out at the end of the day (Baylon 2007).

High Artemia density at Z5 resulted to 'moult death syndrome'

The Z5 larvae fed with *Artemia* density more than 2.0 nauplii ml⁻¹ resulted to high incidence of deaths. When examined, dead Z5 larvae were observed to have developed morphological characteristics of a megalopa such as huge chelipeds, but they were unable to complete their molting to megalopa. This observation may be similar to the 'moult death syndrome" reported by Blackshaw et al. (1999) on the mud crab *S. serrata* larvae, which they suspected to be caused either by inappropriate nutrition or bacteria. Hamasaki et al. (2002) also reported mass mortality during metamorphosis to megalopa in the seed production of *S. serrata*, which they attributed to pollution caused by high concentrations of *Nannochloropsis* in the rearing water.

Conclusion

The optimum food organism to newly hatched zoea larvae of the crucifix crab *C. feriatus* is pure *Brachionus* at Z1 and a combination diet of *Brachionus* and *Artemia* nauplii from Z2-Z5. *Artemia* density maintained at 2.0 nauplii ml⁻¹ from Z1 onwards supported the best survival, rapid development and highest metamorphosis to megalopa.

Acknowledgement

This study was made possible through the support of the University of the Philippines Visayas PhD Dissertation Grant and the Philippine Council of Aquatic and Marine Research Development Dissertation Grant. My sincere gratitude to Alan Failaman, Geoffrey Banihot, Maricel Falguisana, Germin Noquera and Lourdes Cayetano for their dedicated assistance in the rearing of the larvae.

References

- Baylon, J.C. and A.N. Failaman. 1997. Survival of mud crab *Scylla oceanica* from zoea to megalopa when fed the rotifer *Brachionus* sp. and brine shrimp *Artemia* nauplii. UPV Journal of Natural Sciences 2:9-16.
- Baylon, J.C., A.M. Bachoco, M.P. Huyo and J. Gallo. 1999. Prey density and feeding scheme using rotifer (*Brachionus plicatilis*) and brine shrimp (*Artemia salina*) on survival and development from zoea to megalopa of the mud crab *Scylla serrata* (Crustacea:Portunidae). UPV Journal of Natural Sciences 4:21-31.
- Baylon, J.C., H. Suzuki, J. Abarca and C. Genandoy. 2005. Ingestion of *Brachionus* and *Artemia* nauplii by zoea larvae of *Charybdis feriatus* (Crustacea: Decapoda: Portunidae). UPV Journal of Natural Sciences 10:221-232.
- Baylon, J.C. 2007. Appropriate food type, prey density and stocking density in the larval rearing of the blue crab, *Portunus pelagicus* L. (Crustacea:Decapoda:Portunidae). UPV Journal of Natural Sciences 12: 184-194.

- Baylon, J.C. and H. Suzuki. 2007. Effects of changes in salinity and temperature on survival and development of larvae and juveniles of the crucifix crab *Charybdis feriatus* (Crustacea:Decapoda:Portunidae). Aquaculture 269:390-401.
- Blackshaw, A., D. Mann and C. Keenan. 1999. Quality control using hazard analysis principles for mud crab culture. In: Mud Crab Aquaculture and Biology, Proceedings of an International Scientific Forum. Darwin, Northern Territory, Australia, 21-23 April 1997 (ed. C.P. Keenan and A. Blackshaw), pp. 169-173. Australian Centre for International Agricultural Research, Canberra, ACT, Australia.
- Brick, R.W. 1974. Effects of water quality, antibiotics, phytoplankton and food on survival and development of larvae of *Scylla serrata* (Crustacea:Portunidae). Aquaculture 3:231-244.
- Christiansen, M.E. and W.T. Yang. 1976. Feeding experiments on the larvae of the fiddler crab *Uca pugilator* (Brachyura, Ocypodidae), reared in the laboratory. Aquaculture, 8: 91-98.
- Del Norte-Campos, A.G.C., J.B. Panes. 2004. Reproductive cycle and fecundity of the crucifix crab *Charybdis feriatus* (Linnaeus) from Capiz and Pilar Bays, Northern Panay. UPV Journal of Natural Science 9(1), 1360146.
- Faazaz, A.L. and U.C.M. Che. 1995. The biology of mud crab and its hatchery production at NAPFRE, Pulau Sayak, Kedah. Fisheries Bulletin No. 102. Department of Fisheries, Ministry of Agriculture, Kuala Lumpur, Malaysia, 20 pp.
- Fortes, R.D., J.C. Baylon, E.T. Marasigan, A.N. Failaman, J.G. Genodepa, S. Santos-Garibay and G.A.A. Mamon. 2002. A guide to hatchery and nursery production of mud crab (*Scylla serrata*) juveniles. College of Fisheries and Ocean Sciences, University of the Philippines in the Visayas, Miagao, Iloilo, Philippines and Philippine Council for Aquatic and Marine Research and Development, Department of Science and Technology, Los Banos, Laguna, Philippines, 48 pp.
- Godfred, J., A. Ravi and T. Kannupandi. 1997. Larval feed preference of the estuarine edible portunid crab *Thalamita crenata* (Laterille). Indian Journal of Fisheries 44(1):69-74.
- Hamasaki, K., M. Suprayudi and T. Takeuchi. 2002. Mass mortality during metamorphosis to megalops in the seed production of mud crab *Scylla serrata* (Crustacea:Decapoda:Portunidae). Fisheries Science 68, 1226-1232.
- Heasman, M.P. and D.R. Fielder. 1983. Laboratory spawning and mass rearing of the mangrove crab, *Scylla serrata* (Forskal), from first zoea to first crab stage. Aquaculture 34:303-316.

- Le Vay, L., D.A. Jones, A.C. Puello-Cruz, R.S. Sangha and C. Ngamphongsai. 2001. Digestion in relation to feeding strategies exhibited by crustacean larvae. Comparative Biochemistry and Physiology. A128:623-630.
- Levine, D.M. and S.D. Sulkin. 1979. Partitioning and utilization of energy during the larval development of the xanthid crab, *Rhithropanopeus harrisii* (Gould). Journal of Experimental Marine Biology and Ecology 40:247-257.
- Mann, D., T. Akasawa and M. Pizzutto. 1999. Development of a hatchery system for larvae of the mud crab *Scylla serrata* at the Bribie Island Aquaculture Research Centre. In: Mud Crab Aquaculture and Biology, Proceedings of an International Scientific Forum. Darwin, Northern Territory, Australia, 21-23 April 1997 (ed. C.P. Keenan and A. Blackshaw), pp. 153-158. Australian Centre for International Agricultural Research, Canberra, ACT, Australia.
- Marichamy, R. and S. Rajapackiam. 1992. Experiments on larval rearing and seed production of mud crab *Scylla serrata* (Forskal). In: The mud crabs: a report on the seminar convened in Surat Thani, Thailand, 5-8 November 1991(ed.C.A. Angell). pp. 135-141. Bay of Bengal Project Report 51, India.
- Padayatti, P.S. 1990. Notes on population characteristics and reproductive biology of the portunid crab *Charybdis feriatus* (Linnaeus) at Cochin. Indian Journal of Fisheries. 37(2):155-158.
- Parado-Estepa, F.D., E.T. Quinitio and E.M. Rodriguez. 2002. Seed production of the crucifix crab *Charybdis feriatus*. Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines. July-September, VIII(3), a one page report.
- Parado-Estepa, F.D., E.T. Quinitio and E.M. Rodriguez. 2005. Seed production of *Charybdis feriatus* (Linnaeus). A paper presented during the International Workshop on the Culture, Fisheries and Stock Enhancement of Portunids. Jan 20-22, 2005. Iloilo City, Philippines.
- Quinitio, E.T., F.D. Parado-Estepa, O.M. Millamena, E. Rodriguez and E. Borlongan. 2001. Seed production of mud crab *Scylla serrata* juveniles. Asian Fisheries Science 14:161-174.
- Rebeiro, F.A.L.T. and D.A. Jones. 2000. Growth and ontogenetic change in activities of digestive enzymes in *Fennero Penaeus indicus* post larvae. Aquculture Nutrition 6(1):53-64.
- Rho, S. 1976. Studies on the propagation of blue crab, *Portunus trituberculatus* (Miers) 2. The food and food intake of the zoeal larvae. Bulletin of Fisheries Research Development Agency, Busan, Korea 15:57-72.

- Ruscoe, I.M., G.R. Williams, and C.C. Shelley. 2004. Limiting the use of rotifers to the first zoeal stage in mud crab (*Scylla serrata* Forskal) larval rearing. Aquaculture 231:517-527.
- Sorgeloos, P. 1986. Manual for the culture and use of brine shrimp, *Artemia* in aquaculture. *Artemia* Reference Center.195 pp.
- Sulkin, S.D. 1975. The significance of diet in the growth and development of larvae of the blue crab, *Callinectes sapidus* Rathbun, under laboratory conditions. Journal of Experimental Marine Biology and Ecology 20:119-135.
- Sulkin, S.D. 1978. Nutritional requirements during larval development of the portunid crab, *Callinectes sapidus* Rathbun. Journal of Experimental Biology and Ecology 34:29-41.
- Suprayudi, M., T. Takeuchi, K. Hamasaki and J. Hirokawa. 2002. Effect of *Artemia* feeding schedule and density on the survival and development of larval mud crab *Scylla serrata*. Fisheries Science 68:1295-1303.
- Watanabe, T., T. Arakawa, C. Kitajima and S. Fujita. 1978. Nutritional evaluation of proteins of living feeds used in seed production of fish. Bulletin of Japan Society of Fisheries Science 44(9):985-988.
- Williams, M. and J. Primavera. 2001. Choosing tropical portunid species for culture, domestication and stock enhancement in the Indo-Pacific. Asian Fisheries Science 14(2):121-142.
- Zeng, C. and S. Li. 1999. Effects of density and different combinations of diets on survival, development, dry weight and chemical composition of larvae of mud crab *Scylla paramamosain*. In: Mud Crab Aquaculture and Biology, Proceedings of an International Scientific Forum. Darwin, Northern Territory, Australia, 21-23 April 1997 (ed. C.P. Keenan and A. Blackshaw), pp. 156-166. Australian Centre for International Agricultural Research, Canberra, ACT, Australia.