BROODSTOCK

Performance of Mud Crab *Scylla serrata* Broodstock held at Bribie Island Aquaculture Research Centre

David Mann[1], Tom Asakawa[1] and Alan Blackshaw[1]

Abstract

Reproductive performance of 104 female mud crabs was assessed. A large degree of variability was found in a range of characteristics related to maturation, spawning and hatching. Seasonal influences were detected for a number of characteristics with highly significant differences in fecundity, time to spawn, egg size, zoea size and proportion of non-viable zoea. Unilateral eyestalk ablated crabs produced larger eggs and had a lower production of non-viable eggs. Highly significant relationships were found within the group of measured characteristics indicating the potential for developing a model of reproductive performance.

IN MOST areas where the larval culture of mud crab, *Scylla* spp., is conducted, the source of eggs relies on gonadal maturation and spawning of broodstock in captivity. Typically, sub-adult or adult female crabs are collected from the wild and maintained in tanks or ponds until ovulation occurs. Male crabs are only required if sub-adult females are used since mating occurs only at the maturity moult and sperm are subsequently stored for long periods by the female (Du Plessis 1971).

Due to the migratory behaviour of female mud crabs in the wild (Hill 1994), knowledge of spawning, brooding and hatching of eggs under natural conditions is lacking. Most information on these processes therefore comes from crabs that are held in captive conditions for the purposes of aquaculture research and production.

Mud crab culture research, particularly larval rearing, has been conducted at the Queensland Department of Primary Industries, Bribie Island Aquaculture Research Centre (BIARC) for a number of years. Mature female crabs obtained from the local environment have been used as the source of eggs for the research. In order to develop the best management practices for captive *S. serrata* broodstock, detailed records have been kept of individual crab reproductive performance since 1994.

[1]Bribie Island Aquaculture Research Centre, Queensland Department of Primary Industries, PO Box 2066, Bribie Island, Qld 4057, Australia

Quality of newly hatched larvae or their inherent viability is regarded as a significant factor influencing the success of hatchery production. Very little is known of the factors that influence larval quality for this species and attempts to consistently reduce the variability and maximise quality of larvae have been largely unsuccessful. If readily measured criteria could be used to predict the subsequent performance of larvae it would improve the consistency of production and reduce the resources expended on larvae of inadequate viability.

The objective of this investigation is firstly, to determine management practices that promote the production of good quality larvae, and secondly, to formulate a model that can be used as a management tool for the selection of broodstock, eggs or larvae for hatchery production purposes. The work detailed here is the first step towards this objective and aims to determine factors influencing larval production and the existence and extent of interactions among biological characteristics of the larval production process.

Materials and Methods

All mud crabs used at BIARC are of the species *Scylla serrata* (Keenan et al. 1998). They were caught using baited traps from the Redland Bay region (27 ° 20′S, 153 ° 15′E) of Moreton Bay near Brisbane, Australia. All crabs collected were

weighed, measured and subjected to ovarian biopsy after capture. Individual oocytes in the ovarian tissue extracted were measured in order to estimate the maturity stage of the crabs (D. Mann, unpublished data). A proportion of the collected crabs was selected for broodstock, based on shell condition and ovarian maturity stage. Crabs with immature ovaries or damaged or necrotic carapace were rejected.

The system used for holding mud crab broodstock consisted of a 12-tonne capacity fibreglass tank equipped with an area of sand covered bottom through which water was circulated by airlifts. Typically, 15–18 crabs were held in this tank at the same time giving a stocking density of 1.25–1.5 crabs/m^2.

The broodstock were fed once per day in the evening. However, when feeding rates were high, feeding occurred in both morning and evening. A varied diet was supplied ad libitum and consisted of crustaceans, molluscs and fish.

The tank was maintained under low light conditions and temperature was controlled at 25 to 28 °C. Salinity ranged between 32 and 36 ppt with infrequent brief periods of lower salinity. Water quality was managed by flow-through of new seawater as well as recirculation through a biofilter.

Unilateral eyestalk ablation was performed on broodstock crabs to promote spawning when the hatchery had a high demand for larvae. The eyestalk ablation method used was the cautery pinch method, which entails clamping the base of the eyestalk with a hot pair of pliers. Following ovulation the crabs were removed from the main tank and maintained individually in 400 L tanks with high inflow of new seawater. Small amounts of the egg mass were excised as necessary for the measurement of eggs and assessment of fertilisation rate.

One or two days prior to hatch, the berried female was transferred to a 1000 L cylindro-conical tank for hatching to occur. The hatch tank had high rates of inflow of new seawater and temperature was controlled at 26–28 °C. After completion of hatching, turbulence in the tank was stopped and observations of larval behaviour and vigour were made. Following this, vigorous aeration was applied to evenly disperse the larvae. Estimates of unhatched eggs, pre-zoea, dead zoea and total zoea numbers were made from volumetric samples taken from the well-mixed tank.

Analysis of variance and correlation analyses were conducted on the maturation, spawning and hatching data. The analyses investigated three main areas:

1. Influence of time (season) of broodstock collection on egg and larval production; Two sets of analyses conducted on data divided into four seasons–spring (Sep., Oct., Nov.), summer (Dec., Jan., Feb.), autumn (Mar., Apr., May), and winter (Jun., Jul., Aug.) and into two seasons–spring/summer (Sep. to Feb.) and autumn/winter (Mar. to Aug.).
2. Influence of eyestalk ablation on egg and larval production;
3. Determination of characters that may be used as a predictive model for larval viability.

Results

From 1994 to the first half of 1996, a total of 200 female mud crabs were collected from the wild and brought to BIARC. Ovarian tissue was sampled from 192 newly caught crabs. The mean oocyte diameter was 218 μm with a range from 98–310 μm.

Of the 200 female mud crabs collected, 104 were selected and held at BIARC for production of larvae. The average size of the broodstock crabs held was 167 mm carapace width (range 148–218 mm) and 785 g (range 498–1594 g). Half of the 104 females held as broodstock were eyestalk ablated to promote ovulation. A total of 92 crabs successfully spawned. The mean values and range for spawning and hatching characteristics measured are listed in Table 1. Fecundity was significantly related to crab size ($P<0.05$) with larger crabs producing a greater number of eggs.

Influence of season on egg and larval production

The results of the seasonal analyses for both 2-season and 4-season groups are listed in Table 2. Several of the measured characteristics were found to vary significantly between batches, and between seasons of both 2 and 4 season groupings. Significant variation was also found between batches (years) within a season in some cases.

The results of the seasonal variation of characteristics of broodstock, eggs and larvae are included in Table 3. Many of the characteristics show highly significant variation ($P<0.01$) by season when the year is divided into the four defined season periods. Crabs collected in autumn were larger in size and weight and had significantly larger, more developed oocytes than those collected in winter or summer. Division of the year into 4 seasons rather than 2 periods better explains the variability exhibited in the characters.

The average time following collection required for a crab to spawn was significantly longer for summer crabs than for those of the other three seasons. Spring crabs spawned significantly smaller eggs than crabs collected in the other seasons. Fertilisation rate of the eggs did not vary significantly by season and rates of greater than 90% were common in all seasons.

Table 1. Mean, standard deviation and minimum and maximum values of eggs and larvae produced per crab.

	Egg size (μm)	No. eggs[1] ($\times 10^6$)	Eggs/ g crab weight	Fert. rate[2] (%)	Pre-zoea[3] (%)	Non-viable[4] (%)	No. zoea ($\times 10^6$)	Zoea size (μm)
Mean	315	4.49	5688	89	2.5	10.3	3.92	860
SD	9	1.94	2445	20	3.0	13.2	1.90	29
Max.	347	8.36	11531	100	13.5	57.7	7.83	928
Min.	271	0.39	543	0	0.0	0.5	0.28	801
n	88	56	53	82	59	58	59	60

[1] Total of unhatched eggs (including unfertilised eggs) and hatched zoea.
[2] Proportion of developing eggs 7 to 9 days after extrusion.
[3] Proportion of pre-zoea stages of total hatched zoea.
[4] Proportion of non-viable eggs/zoea of total number developed eggs; non-viable eggs/larvae = sum of unhatched fully developed eggs and total number zoea hatched.

Table 2. Effect of season on broodstock, egg, and larval characteristics (ns P>0.05; * P<0.05; ** P<0.01). Seasons with like letters are not significantly different.

Characteristic		2-season group			4-season group			
		Sp/Su	Au/Wi		Sum	Aut	Win	Spr
Broodstock								
Crab carapace width (mm)	**	165	170	**	164^a	172^b	167^a	169^{ab}
Crab weight (g)	**	744	820	**	724^a	865^b	770^{ac}	802^c
Initial ova diameter (μm)	ns	222	230	*	220^a	240^b	219^a	224^{ab}
Time to spawn (days)	*	79	61	**	92^a	67^b	54^b	52^b
Fecundity (eggs/g bwt)	ns	5.63	5.74	**	6.89^a	5.34^b	6.23^{ab}	2.56^c
Eggs								
Egg diameter (μm)	**	312	318	**	315^a	318^a	317^a	306^b
Fertilisation rate (%)	ns	86	91	ns	88	92	89	81
Zoea								
Proportion prezoea (%)	ns	2.8	2.4	**	1.7^a	1.8^a	3.1^{ab}	5.6^b
Proportion non-viable (%)	ns	10.3	10.6	ns	7.8	9.2	12.4	16.9
Total no. eggs (10^6)	ns	3.59	4.46	**	4.29^a	4.51^a	4.40^a	1.71^b
Total no. zoea (10^6)	ns	3.47	4.26	**	4.14^a	4.30^a	4.06^a	1.76^b
Zoea width (μm)	**	837	877	**	836^a	877^b	877^b	839^a

Spring consistently scored poorer than other seasons in characteristics related to hatched zoea. It had a higher average proportion of prezoea at hatching, smaller number of eggs and zoea produced. The warmest seasons, spring and summer, had a smaller average zoea size than autumn and winter.

The influence of eyestalk ablation on egg and larval production

Eyestalk ablation was found to have a significant influence ($P<0.05$) on two egg and larval characteristics. Eggs of ablated crabs were on average larger than those of intact crabs, (317 ± 1 μm and 313 ± 2 μm, respectively) and the proportion of non-viable eggs and larvae was lower for ablated crabs (6 ± 1% and 14 ± 3%, respectively).

Determination of characters that may be used as a predictive model for larval viability

As the data set is not yet complete, the full analysis of the data has not yet been performed. Preliminary analysis, however, has determined highly significant correlations between pairs of characteristics related to broodstock, egg and larval data. To provide useful predictive power a factor analysis and multiple regression incorporating several characteristics is required.

Most notable among the correlations were those that indicated relationships between characteristics from either broodstock or egg phase with the following phase. Significance of selected paired characteristic relationships are listed in Table 3. In most cases, the regression explains less than 20% of the variation of the dependant characteristic.

Table 3. Significance of correlations between selected characteristics. (* P<0.05; ** P <0.01).

Correlated characters		P	R^2
Time to spawn	Fert. rate	**	0.11
	Zoea size	**	0.14
Egg diam.	Pre-zoea	**	0.12
	Non-viable	*	0.12
	Zoea size	**	0.32
Fert. rate	No. eggs	**	0.14
	No. zoea	**	0.18
	Prezoea	**	0.21
	Non-viable	**	0.17
	Zoea size	*	0.08
No. eggs	Prezoea	**	0.37
	Non-viable	**	0.30
	Zoea size	*	0.10

Discussion

A very high rate of successful ovulation (spawning) was experienced in this study with 88% of broodstock spawning. As high fertilisation and hatch rates also occurred, it is obvious that production of eggs and larvae is not an issue affecting the hatchery cycle of *S. serrata*. The main issue is related to the production of quality larvae that show at least acceptable performance, in terms of growth and survival, under hatchery conditions. This current work has investigated a range of aspects that may have relevance to the larval quality issue.

The reproductive activity of *S. serrata* in Moreton Bay is highly seasonal, as indicated by the proportion of recently spent female crabs in the wild population (Heasman et al. 1985). Moreton Bay is in a sub-tropical zone and experiences marked differences in temperature between the seasons, ranging from around 16 °C in winter to 28 °C in summer. In winter there is no spawning activity, followed by spring in which a low but increasing level of activity occurs. Peak spawning occurs in summer and then in autumn spawning activity rapidly decreases so that no recently spawned females are present by mid-autumn (Heasman et al. 1985).

The patterns of reproductive activity in the wild, however, do not directly correlate with the performance experienced with wild caught broodstock held at BIARC. Summer is the peak period for spawning activity in the wild so it may be expected that during this period female crabs are closer to ovulation. However, broodstock sourced during the summer period were moderate in developmental stage of the ovary and took far longer on average to spawn following capture. The apparent discrepancy is possibly due to the migratory behaviour of female *S. serrata* as ripe crabs migrate out of estuaries to release larvae (Hill 1994). During the summer months when natural spawning activity is at its peak, the rate of fully mature females moving out of the estuary is at its highest, so there may be a higher proportion of crabs further from spawning in the catch. Heasman (1980) found that the mean gonad-somatic index (GSI) for female crabs in Moreton Bay did not follow a distinct seasonal pattern but the variation in GSI between crabs was highest in the first month of summer.

Spring is associated with a seemingly poorer quality of reproductive output. This is indicated by a reduced number of smaller eggs produced. There is also a tendency for a higher proportion of non-viable larvae; however, this is not significant due to the high variability of this characteristic. It is not clear why this pattern occurs but the knowledge of its existence is important for a hatchery striving to maximise the quality of larvae to be cultured.

A peak of ovarian development recorded in autumn may be related to female crabs having entered the maturity moult and undergone gonadal development during the warmer summer months, but are still available for capture in estuarine areas. Heasman (1980) determined that female *S. serrata* can over-winter in advanced states of ovarian development. These females then apparently contribute to the early rise in spawning activity in spring.

Broodstock crabs are held at the BIARC facility at elevated temperatures and lengthened photoperiod designed to simulate conditions experienced during spring and summer. Using this method, females can be spawned after collection at any time of the year. The majority of female crabs collected during late autumn or winter, outside their normal spawning period, will spawn within 3 months. There is no evidence to suggest that inducing spawning outside of the natural season adversely influences the production of eggs and larvae. Only the size of the eggs and newly hatched larvae were different between the spring/summer and autumn/winter groups. If large size is considered a positive characteristic, then eggs and larvae produced during autumn and winter may be of better quality.

Significant seasonal variation was observed in the characteristics of the eggs and larvae produced, including proportion of pre-zoea, egg and larvae size, and number of eggs produced. The significance of size, number and proportion of non-viable larvae to the success of subsequent culture attempts is poorly understood. However, at BIARC, preference is given to batches that have little or no persisting pre-zoea and eggs and newly hatched zoea of at least average size. Subsequent work at BIARC is intended to investigate these and related aspects.

Aquatic hatcheries generally consider that any abnormalities associated with the eggs or larvae are indicators of a low quality batch of larvae. Elevated levels of abnormal or non-viable eggs or larvae are therefore considered undesirable characteristics. The proportion of non-viable eggs and larvae exhibited high variability that was not related to the seasons and at this stage the influencing factors have not been identified. These factors may be related to the reproductive history of the crab prior to being held in captivity and include time between mating and spawning, quality of the sperm, and nutritional influences during ovigenesis.

Eyestalk ablation was performed on crabs at varying times after initial collection as this procedure was only carried out when the hatchery foresaw an urgent need for larvae. The influence of ablation on the time taken to spawn therefore cannot be derived from the data. A critical evaluation of the effects of eyestalk ablation on spawning time and eggs and larvae is the topic of another report that is in preparation.

This study did not identify any adverse effects of eyestalk ablation on egg and larval production. Published works concerning the influence of eyestalk ablation on Penaeid broodstock have indicated a range of effects on reproductive performance (Browdy and Samocha 1885, Emmerson 1980). In this study, ablation resulted in larger egg size and a lower proportion of non-viable eggs and larvae. While the significance of these two characters is not well understood, it is unlikely that they are undesirable or indicators of poor quality.

The pair-wise correlations reveal that there is a high degree of relatedness between the biological characters of the maturation through to hatching process, and indicates a potential for developing a predictive model of larval quality. The predictive model would seek to process a group of readily measured characters to identify which batches of larvae were worthwhile for investing hatchery resources. This work would also identify the set of conditions most conducive to producing good quality larvae so that recommendations could be made to maximise the chance of producing high quality larvae. Further data are still being accumulated for this work. Once the data set is complete, the potential for development of a model of larval quality will be explored using multi-factor analyses, which account for the relationships between all the measured characters.

A further step will need to be completed before the practical application of the model is possible and entails relating the variability in the measured characters to actual larval performance in culture. This will require quantification of the larval growth and survival in standard culture conditions and will be the subject of subsequent work.

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DIETS

Suitability of Local Raw Materials for Mud Crab Feed Development

Johannes Hutabarat[1]

Abstract

The aims of the study were to identify production and nutritional values of local raw materials available in Central Java, and to develop feeds using selected raw materials for mud crab fattening. The production of local raw materials was determined by using secondary data available from relevant technical institutions followed by direct site surveys in the production centres of agriculture and fisheries by-catch in Central Java. Production levels of local raw materials and their nutritional values were determined. The results indicate that the local raw materials for protein sources are found in abundance in Central Java throughout the year at a relatively cheap price; these include trash fish, mysid, squid, blood meals, worm-meals and shrimp head meals (animal protein) and saga, soy beans (plant protein). The protein levels of selected raw materials are high (41.1–80.3%) and the highest levels are found in blood meal, followed by squid, trash fish and shrimp head meals. The selected raw materials, generally, contained 10 essential amino acids (arginine, lysine, histidine, phenylalanine, leucine, isoleucine, methionine, valine, threonine and tryptophan) and long chain EFA (n-3 HUFA and n-6 HUFA) which are required by mud crabs (crustacean) for their growth. The selected local raw materials are therefore nutritionally suitable for mud crab feed development in Central Java.

THE MUD crab *(Scylla* spp.) is an important fisheries commodity. Recently, the Central Java Government decided that production levels have to be improved, since the demand for both domestic and export markets is increasing yearly (DGF 1993). Mud crabs fisheries in Central Java, Indonesia, have not been intensively developed and still depend on wild crabs caught offshore and in mangrove areas. Development of mud crab farming in brackish water ponds is an alternative approach for increasing production levels.

Theoretically, the potential for increasing mud crab production is vast. There are approximately 20 000 ha of brackishwater ponds (tambak) developed for shrimp culture in Central Java, which have been abandoned and are now available for mud crab culture. In spite of the potential for mud crab culture development, there exist a number problems and constraints. At present, the mud crab farmer practises a fattening culture system found virtually only in Central Java (Demak and Jepara, Central Java Fisheries Bureau 1996). There is a shortage of mud crab feed for fattening, and farmers still depend on trash fish as a main food source. This is inefficient, less precise and liable to cause water quality deterioration (Wartas and Hutabarat 1992). Availability of mud crab feed in good quantity and quality throughout the year is important in order to support mud crab culture development. Raw materials for feed production are available in several agricultural and fisheries production centres in Central Java but these have not been utilised for aquaculture feed production. Therefore, this study was initiated to overcome existing problems and to optimise use of local raw materials

The aims of the study (phase I, 1996/1997) were to identify the suitability of local raw materials with respect to quality (level of nutritional values), quantity and availability and to formulate experimental diets using selected local raw materials, to produce cost-effective diets for mud crab feed development in Central Java. The results derived from this study will be used for grow-out studies (phase II, 1997/1998).

[1]Research Centre for Technology Development, Diponegoro University, J1. Imam Bardjo, SH No. 5, Semarang, Central Java, Indonesia 50241

Materials and Methods

Data on production of local raw materials were collected from several agricultural and fisheries production centres in Central Java (Pekalongan, Kendal, Semarang, Jepara, Pati and Rembang regencies), by using the statistical books available in related technical institutions, confirmed by direct site checking. The potential local raw materials were selected according to quantity (availability throughout the year at low prices), quality (the level of nutritional value) and low competition for human food resources or industrial products.

Determination of nutritional values (proximate analysis, profile and availability of EAA and EFA) of the selected raw materials was performed in the Laboratory of Fish Nutrition, Tokyo University of Fisheries, Tokyo, Minatoku, Japan, using standard procedures (AOAC 1990; Takeuchi 1988).

Formulation of experimental diets was made by varying the level of dietary protein and the ratio of animal and plant proteins contained in the diets. The diets were formulated by a least-cost method using different combinations of protein sources (trash fish, squid, mysid and soybean, saga and flour). Experimental diets contained approximately 30% and 35% of dietary protein. These diets will be used in mud crab grow-out studies (laboratory and pond) in 1997/1998 (Phase II).

Results

Data on the production of the local raw materials available in Central Java, the level of raw materials required (tonne/year), the level of competition, their price (Rp/kg) and seasonal availability are presented in Table 1. Animal protein, either from fisheries by-catch or cold storage by-product, is more abundant than plant protein sources (soybean, saga or groundnut). The protein level of these raw materials varies from 42.0–80.5% (animal origins) and 41.1–45.8% (plant origins) (Table 2). The quality of proteins, determined by their amino acid profiles and availability are presented in Table 3.

The profile and availability of fatty acids in selected raw materials from animal and plant origins are shown in Table 4. The results of nutritional levels contained in selected raw materials were then used for formulating experimental diets. The composition of experimental diets, from animal origin only, and combined animal and plant origins, with protein levels of 30% and 35% are shown in Table 5.

Table 1. Production (tonnes/year) in districts, requirements, competition with human and industrial goods, and price, of local raw materials available in agriculture and fisheries production centres of Central Java.

Raw materials	Jepara	Pati	Rembang	Semarang	Kendal	Pekalongan	Total production	Requirements (tonnes/year)	Competition with human/ industrial	Price per kg (Rp)
Tembang	632.30	7871.50	4216.30	87.40	489.90	8093.50	21 341.23	1067.10	++	345
Leirognathus ssp	656.51	43.03	1934.60	792.40	26.50	179.55	3632.59	181.60	++	299
Trash fish	535.50	817.04	2708.66	294.50	202.16	6544.60	11 102.46	2775.60	+	358
Mysid	1.78	132.30	—	—	—	—	134.09	13.40	+	137
Squid	14.43	11.52	58.45	30.50	18.10	16.17	149.18	7.50	++	1200
Blood meal	—	—	—	360.32	—	139.68	500.00	50.00	+	500
Worm meal	—	—	19.14	40.86	—	—	50.00	10.00	—	300
Shrimp head meal	51.20	4592.04	42.49	163 516.50	1771.90	15.15	169 989.30	16 998.90	+	50
Saga	—	139.76	160.24	—	—	—	300.00	50.00	—	150
Ground nut	11 700.50	4732.60	654.00	5190.00	5416.30	1150.10	28 834.50	1441.70	+++	2500
Soy bean	548.50	3343.60	5741.00	1258.30	1025.00	593.00	12 509.40	3127.40	+++	1200

Table 2. Nutritional levels (proximate analysis) of selected local raw materials.

Selected raw materials	Proximate analysis (%)				
	Protein	Carbohydrate	Lipid	Ash	Moisture
Trash fish	57.46	1.14	7.04	20.80	13.20
Mysid	45.54	2.26	6.20	31.90	14.10
Squid	70.74	2.62	10.90	4.90	11.20
Blood meal	80.55	1.05	2.70	3.70	12.00
Worm meal	41.99	25.41	5.40	16.50	10.70
Shrimp head meal	48.06	8.64	4.80	25.40	13.10
Saga	41.15	30.55	11.80	3.50	13.00
Soy bean	45.82	20.28	19.40	4.20	10.30

Table 3. Profile and availability of essential amino acids (EAA) and non EAA of selected raw materials from Central Java.

Raw materials	EAA (mg/g amino acid)											Non EAA (mg/g amino acid)						
	Arg	Lys	His	Ph	Tyr	Leu	Iso	Met	Va	Thr	Tryp	Tau	Ala	Gly	Glu	Ser	Asp	Pro
Trash fish	75	87	24	42	35	75	45	34	51	42	7	6	67	73	155	41	99	43
Mysid	77	70	21	49	44	78	50	26	56	42	6	9	69	60	151	39	108	44
Squid	75	79	19	44	43	80	48	35	45	42	8	19	61	63	153	38	103	44
Blood meal	54	90	57	66	35	111	41	15	70	47	17	3	86	38	105	38	91	34
Worm meal	52	55	23	2	51	78	47	22	59	47	tr	—	61	74	125	44	93	36
Shrimp head meal	58	58	24	55	47	73	46	27	62	46	10	7	61	62	153	48	112	50
Saga	78	56	16	49	47	75	40	12	50	30	10	—	46	69	177	57	100	45
Soy bean	87	59	25	50	39	76	47	13	49	38	tr	—	43	43	202	48	113	49

Note : Arg = arginine; Lys = lysine; His = histidine; Ph = phenylalanine; Tyr = tyrosine; Leu = leucine; Iso = isoleucine; Met = methionine; Va = valine; Thr =threonine; Tryp = tryptophan; Tau = taurine; Al = alanine; Gly = glycine; Glu = glutamic acid; Se = serine; Asp = aspartic acid; Pro = proline; tr = trace; — = none detected

Table 4. Profile and availability of fatty acids (area %) of selected raw materials available in Central Java.

Profile of fatty acids	Animal origin						Plant origin	
	Shrimp head meal	Mysid	Squid	Trash fish	Worm meal	Blood meal	Soy bean	Saga
12:0	0.2	0.3	0.8	0.1	1.7	0.6	tr	0.3
13:0	2.3	0.8	0.4	0.2	1.6	0.5	0.1	0.1
14:0	3.4	4.0	2.5	3.7	1.3	0.7	0.1	0.1
15:0	1.3	1.2	0.7	0.5	0.7	0.1	—	tr
16:0	30.6	16.6	27.6	19.8	21.3	28.1	11.1	10.5
16:1n-7	2.6	13.7	1.0	6.1	0.5	1.4	0.1	0.2
17:0	1.3	1.8	1.3	0.3	1.0	0.2	0.1	0.1
16:3n-6	0.5	0.9	0.1	0.7	0.4	0.1	tr	tr
16:3n-3	0.4	1.0	1.7	0.4	0.1	0.3	—	—
16:4n-1	—	0.1	0.1	0.4	tr	0.3	—	—
18:0	10.2	8.1	8.3	5.7	5.0	10.1	4.1	6.0
18:1	20.0	10.3	4.7	23.2	15.9	32.3	23.1	33.4
18:2n-6	9.3	3.9	0.3	1.2	8.9	14.3	52.9	41.0
18:3n-6	0.4	0.9	0.2	0.1	0.2	0.2	0.2	0.3
18:3n-3	0.3	3.6	0.1	0.7	0.2	0.2	6.6	3.4
18:4n-3	0.1	0.7	0.1	1.4	0.4	—	0.1	0.1
18:4n-1	0.2	tr	tr	0.1	0.1	—	—	—
20:0	0.8	0.4	0.2	0.1	0.1	0.3	0.4	0.8
20:1	—		—	—	—	—	0.2	0.4
20:2n-6	0.5	0.3	0.3	0.1	0.1	0.2	—	tr
20:3n-6	0.1	0.1	tr	tr	0.1	0.5	—	tr
20:4n-6	0.7	3.7	5.7	1.0	0.1	4.4	—	—
20:3n-3	0.1	0.2	1.1	0.1	0.3	tr	—	—
20:4n-3	0.1	0.2	0.1	0.6	0.1	—	—	—
20:5n-3	1.3	6.8	7.6	9.9	0.1	0.1	—	—
22:0	1.1	0.4	0.1	0.1	tr	0.2	0.5	1.7
22:1	1.7	0.1	0.5	0.7	3.2	—	—	0.1
22:4n-9	0.3	0.2	0.3	0.4	0.9	—	—	0.1
22:4n-6	—	0.1	0.5	0.1	0.2	0.4	—	—
22:5n-6	—	0.4	1.9	0.2	—	0.4	—	—
22:5n-3	0.3	0.4	0.7	1.8	1.1	0.2	—	—
22:6n-3	1.0	1.9	25.9	12.4	2.1	1.5	—	0.1
∑saturate	51.2	43.6	41.9	30.5	32.7	40.8	16.4	19.6
∑monoene	27.4	24.6	8.2	32.0	25.4	34.1	23.4	34.1
∑n-6	11.5	9.8	6.6	3.1	9.8	19.7	53.1	41.3
∑n-3	3.6	14.8	37.3	27.3	4.4	2.3	6.7	3.6
∑n-3 HUFA	2.8	9.5	35.4	24.8	3.7	1.8	0.0	0.1
Lipid (% d.b)[1]	4.8	6.2	10.9	7.4	5.4	2.7	19.4	11.8
Moisture	13.1	14.1	11.2	3.7	10.7	12.0	10.3	13.0

Note : tr = Trace; — = Not detected; [1](% db) = Dry basis (%)

Table 5. Composition of experimental diets with protein levels of 30% and 35% (per 100 gr).

Ingredients (grams)	Protein level of 30%		Protein level of 35%	
	80% animal + 20% plant protein	100% animal protein	80% animal + 20% plant protein	100% animal protein
Trash fish	12.53	15.56	14.62	18.27
Squid	10.18	12.72	11.87	14.48
Mysid	21.08	26.35	24.59	30.74
Soy bean	6.55	—	7.64	—
Saga	7.29	—	8.51	—
Flour	38.87	41.77	29.27	32.65
Lecithin	1	1	1	1
Top Mix	2	2	2	2
CMC	0.5	0.5	0.5	0.5

Discussion

The results indicate that suitable local raw materials, as protein sources (animal and plant origin), are available throughout the year with low competition with human food or industrial products. The requirements of these materials for the feed industries are still below their potential level and the prices are relatively low (Table 1). The production of local materials varies from area to area. Animal protein sources, either from fisheries by-catch or agricultural by-products, are more abundant than plant protein sources (soybean, saga, groundnut). Therefore, some of these raw materials have been selected for use in trial diets. These selected raw materials (indicated in Table 2) contained relatively high animal and plant protein levels, and are suited to the nutritional requirements for aquaculture feed (Hutabarat 1984).

Profiles and availability of amino acids in the materials both of animal and plant origin will also determine the quality of protein sources (Jauncey and Ross 1982). Table 3 shows that the local protein sources (trash fish, mysid, squid, blood meal, worm meal, shrimp head meal, saga and soybean) contain 10 essential amino acids (methionine, arginine, threonine, tryptophan, histidine, isoleucine, leucine, lysine, valine and phenylalanine) which are important for mud crab growth. These cannot be synthesised by the mud crab and must be available in their diet (Halver 1972). Kanazawa (1982) states that beside the availability of EAA, the raw materials should also contain long chain fatty acids (n-3 HUFA) and (n-6 HUFA) which are available in the selected raw materials (Table 4). They cannot be synthesised by the mud crab (Castel 1982), and should be available in the diets in adequate levels for further desaturation and elongation to essential fatty acids (EFA) such as 20:5-n3; 22:5n-3 and 22:6n-3 (Kanazawa 1982).

These analyses show that the selected local raw materials are nutritionally suitable as mud crab feed. Therefore, they were used in designing the experimental diets (Table 5). The protein levels of the experimental diets were formulated to 30% and 35%, according to Djuwito et al. (1992) who showed that protein requirements for 'fattening' and mud crab culture ranged from 30% to 35% and should contain 10 essential amino acids, particularly lysine, arginine, leucine, isoleucine and valine (Akiyama et al. 1991).

Conclusions

Local raw materials for protein sources of animal and plant origin are abundant in Central Java throughout the year, at relatively cheap prices.

The potential raw materials selected for the survey were: trash fish, mysid, squid, blood meal, worm meal and shrimp head meal (animal origin) and saga and soy bean (plant origin).

Nutritional values, profiles and availability of EAA, profiles and EFA composition (n-3 HUFA and n-6 HUFA) of local raw materials are qualitatively suitable for mud crab feed ingredients. Feeding trial experiments conducted during grow-out studies will supply definitive information (Phase II, 1997/1998 fiscal year).

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Reproductive Performance of Pond-sourced *Scylla serrata* Fed Various Broodstock Diets

Oseni M. Millamena[1] and Emilia T. Quinitio[1]

Abstract

Feeding experiments were conducted to determine the effect of diet on reproduction of pond-sourced unablated and ablated *Scylla serrrata* broodstock. Broodstock were fed either natural food (T1) consisting of mussel, squid, fish by-catch, a combination of natural food and formulated diet (T2), or formulated diet (T3). After 120 days of culture, best broodstock response in terms of total spawnings, spawnings with hatchings, number of eggs per g body wt (BW) of female, egg fertilisation rate, and total zoea produced was obtained in T2 and poorest response was in T1. Broodstock in T3 gave intermediate values among the treatments. Larval quality measured as zoea growth index and broodstock survival was also highest in T2. Results showed that combination diet feeding improves the reproductive performance and larval quality of unablated and ablated females compared with those fed on natural food or artificial diet alone. Latency period from stocking to maturation and spawning was shorter in ablated than in unablated females. Rematurations were observed both in unablated and ablated females in all dietary treatments.

THE MUD crabs, *Scylla* sp., are commercially important in the Indo-Pacific countries. In the Philippines, mud crab culture is an important source of income among small-scale fishermen in coastal communities (Laviña and Buling 1977). A major constraint to further develop mud crab culture is insufficient supply of seedstock (Heasman and Fielder 1983, Hill 1994, Robertson and Kruger 1994).

Broodstock nutrition was shown to have a considerable effect on gonadal growth and fecundity, egg hatchability and larval quality (Teshima and Kanazawa 1983, Watanabe 1988). Hence, studies to evaluate the effect of improved broodstock nutrition and management on consistency of performance and larval quality needs to be undertaken. This study aims to evaluate the reproductive performance and larval quality of pond-sourced *Scylla serrata* fed various broodstock diets.

Methodology

Diets

Dietary treatments consisted of natural food (T1), a combination of natural food and formulated diet (T2), and formulated diet (T3). Natural food consisted of squid (*Loligo* sp.), mussel meat (*Perna* sp.) and fish by-catch (*Leiognathus* sp.). The formulated diet is modified Southeast Asian Fisheries Development Centre (SEAFDEC) formulation for prawn broodstock. Table 1 shows the diet composition and Table 2 the proximate composition of natural food and formulated diet. Feeding rate was 6–10% of biomass for natural food, 2–3% for formulated diet, and half of these amounts each for the mixed diet. Feed was given three times daily at 0800, 1300 and 1700, with 40% of the ration given in the morning, 30% at noon and 30% in the afternoon.

Table 1. Composition of broodstock formulated diet (T3) for mud crab *Scylla serrata* (modified from Millamena et al. 1986).

Ingredients	Percentage
Chilean fish meal	20
Shrimp head meal	20
Squid meal	20
Wheat flour	17
Seaweed (*Gracilaria* sp.)	4
Cod liver oil	5
Lecithin	3
Cholesterol	1
Vitamin mix[a]	3
Mineral mix[a]	4
Dicalcium phosphate	3

[a]Vitamin and mineral mix after Kanazawa (1981).

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines

Table 2. Proximate composition of natural food and formulated broodstock diet for mud crab Scylla serrata.

	Natural food			Formulated diet
	Squid	Fish bycatch	Mussel	
Crude protein	78.69	65.04	66.06	46.03
Crude fat	8.07	9.50	3.74	11.64
Crude fibre	0.78	0.78	0.48	4.18
NFE	5.03	5.91	17.58	23.13
Ash	7.43	18.77	12.14	15.02

Culture

Pond-sourced, premated *S. serrata* females, mean body wt of 300–400 g, were used as experimental animals. Crabs were tagged by engraving identification marks on their carapace: nos. 1–8 (Tank 1), 9–16 (Tank 2) and 17–24 (Tank 3). Each female was sampled for egg diameter to determine an index of maturity at stocking. Broodstock were randomly stocked in 3 units of 4 m diameter circular concrete tanks at 8 crabs per tank. Pebbles topped with sand were used as tank substrate. Sand-filtered seawater was supplied in a partial flow-through system from 0900–1300 daily with adequate aeration. Each crab was provided with a 20 × 20 × 10 cm high shelter made of wood and black nylon net to prevent cannibalism. Moulting and mortality were recorded daily.

Two weeks from stocking, even-numbered crabs were unilaterally ablated while odd-numbered crabs were unablated. Broodstock were monitored for spawnings and berried females were transferred to 300 L fibreglass tanks for incubation of eggs. Sampling for egg fertilisation rate was conducted on the sixth and on the tenth day after spawning. Upon hatching of eggs, total numbers of zoea produced were estimated from aliquot water samples taken from the hatching tank. Zoea were cultured in 250 L fibreglass tanks to determine the growth index (Villegas and Kanazawa 1980). Broodstock were returned to experimental tanks for rematuration.

Performance of broodstock was evaluated based on percent spawnings, spawnings with hatchings, number of eggs/g body weight of females, egg fertilisation rate, total number of zoea, zoea growth index and broodstock survival. Four experimental runs were conducted. Culture period lasted for 120 days.

Chemical analyses

Proximate analyses of natural food and artificial diets were made according to AOAC (1984). Water quality parameters (temperature and salinity), were monitored daily while ammonia, nitrite, and dissolved oxygen were measured three times weekly (Monday, Wednesday and Friday). These parameters were within suitable levels for the duration of culture.

Statistics

Data were summarised for the four runs and analysed using two way analysis of variance (Gomez and Gomez 1984) and Duncan's multiple range test ($P = 0.05$) was used to test significant differences among treatment means.

Results and Discussion

The relative effects of diet on the reproductive performance of unablated and ablated mud crab females in four runs are summarised in Table 3. Over-all broodstock response showed that the combination diet (T2) gave the best reproductive performance while those fed natural food (T1) gave the poorest response. Although the total number of spawnings was high in T1, spawnings without hatching were significantly lower ($P < 0.05$) than those in T2 or T3. There were no significant differences found ($P > 0.05$) among the treatment means in terms of number of spawnings, fecundity, egg fertilisation rate, and total zoea produced. However, T2 gave the highest numerical values relative to the other two treatments. Lowest values of these parameters were observed in T1. Moreover, mean broodstock survival and larval quality based on zoea growth index was highest in T2.

The effect of dietary treatments on response of unablated and ablated females appeared to be similar, suggesting that ablation did not improve the reproductive performance. Latency period from stocking to first spawning was relatively shorter (10–40 days) in ablated than unablated females (15–63 days). Rematurations were observed in both unablated and ablated females and occurred about a month after the first spawning. There was no decline in reproductive performance and larval quality of rematured females except for a decrease in egg fertilisation rates.

The results further suggest that feeding mud crabs a combination of formulated diet and natural food improves reproductive performance and larval quality. Essential dietary nutrients that are lacking in natural food may have been compensated by giving a formulated diet as supplement (Table 4). Ablation of females did not improve reproductive performance and larval quality but shortened the latency period. The technique may be useful only when there is an immediate need for seed supply in the hatcheries.

Table 3. Reproductive performance and larval quality of unablated and ablated *Scylla serrata* females fed various diets.

Parameter	Treatment		
	1 Natural food	2 1:1 NF to AD	3 Artificial diet
Number of spawnings	35	36	23
with hatching	20 (57%)	29 (81%)	20 (87 %)
without hatching	15 (43%)	7 (19%)	3 (13%)
Mean no. of eggs/g body wt	4780	7534	7369
ablated	4437	7758	9317
unablated	5124	7310	5421
Mean egg fertilisation rate (%)	69	72	73
ablated	58	69	88
unablated	80	76	57
Total no. of zoea	36 171 194	73 089 083	42 546 663
ablated	15 676 528	27 354 416	23 125 048
unablated	20 494 666	45 734 667	19 421 615
Mean zoea growth index	3	4	3.5
ablated	2	4	4
unablated	4	4	3
Megalopa stage achieved	3	6	5
ablated	0	3	3
unablated	3	3	2
Broodstock survival (%)	60	77	57
ablated	42	70	64
unablated	83	84	50

Table 4. Fatty acid composition (% of total lipid) of natural and formulated diets for mud crab broodstock.

Fatty acid	Natural food			Formulated Diet	Mud crab
	Mussel	Fish bycatch	Squid		
14:0	6.24	5.0	2.53	2.89	0.83
16:0	19.69	29.61	26.52	14.14	11.70
16:1n-7	12.56	11.80		6.39	3.01
18:0	3.58	10.61	5.54	2.09	8.02
18:1n	6.34	18.05	7.37	25.16	22.34
18:2n-6	1.81	1.11		21.78	10.18
18:3n-3	0.75			4.26	1.03
18:4n-3	4.32				
20:1n-9	7.32	4.61	2.83	2.88	1.00
20:4n-6	5.49		6.45	0.76	8.05
20:5n-3	15.29	2.97	9.25	7.51	18.05
22:5n-3	1.14	1.28	0.64	0.76	0.69
22:6n-3	9.16	1.07	33.60	9.85	12.46
total n-3	30.66	5.32	43.49	22.38	32.23
total n-6	7.30	1.11	6.45	22.54	18.23
n-3/n-6	4.20	4.79	6.74	0.99	1.77

Biochemical analyses of the diets and *S. serrata* tissues should be conducted to further elucidate the effects of diet on reproductive performance.

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LARVAL REARING

Investigations into the Reproductive and Larval Culture Biology of the Mud Crab, *Scylla paramamosain*: A Research Overview

Shaojing Li[1], Chaoshu Zeng[1], Hong Tang[1], Guizhong Wang[1] and Qiongwu Lin[1]

Abstract

Studies on reproductive biology and larval cultural biology, as well as mass rearing of mud crab seeds, *Scylla paramamosain,* have been carried out by the mud crab research group in the Department of Oceanography, Xiamen University, China, since 1985. The present paper briefly reviews the research conducted in the laboratory to date.

A SERIES of studies aimed to develop reliable cultural techniques for the mud crab, *Scylla paramamosain,* (Keenan et al. 1998) have been carried out by the mud crab research group in the Department of Oceanography, Xiamen University, China since 1985. The research to date has focused on the following 3 aspects:

- Reproductive biology of the crab;
- Larval cultural biology and ecology;
- Mass rearing of the crab seeds.

In addition, some other work relevant to juvenile crab nurseries and growout of adult crab has also been conducted. Following is a brief summary of the research results from the laboratory.

Reproductive Biology of the Mud Crab

Studies on broodstock management, including comparison of effects of single and bilateral eyestalk ablation on ovarian maturation, spawning and hatching of the female crab, diet and feeding rate of the spawner, induction of out-of-season spawning and female fecundity in relationship to body weight and length, were carried out and the results reported (Zeng 1987; Zeng et al. 1991; Lin et al. 1994).

Investigations on the annual reproductive cycle of the crab, embryonic development and the influence of temperature on developmental rates of different embryonic stages were also conducted. The results showed that the local mud crab has two annual spawning peaks and embryo development of the crab can be divided into 10 stages. The temperature range for embryo development was found to be 15–35 °C, while the optimal range was 20–30 °C. The embryonic stage 2 (gastrula) of the crab appeared to be most susceptible to low temperature during which diapause occurred when temperature fell below 15 °C (Zeng 1987; Zeng et al. 1991). Changes in protein, lipid, carbohydrate content and activity of four hydrolytic enzymes during the embryonic development were also measured. The result suggested that protein is the most important energy source for supporting embryo development (Wang et al. 1995). Specific activities of four hydrolytic enzymes increased rapidly at embryo stage 9 (immediately before hatching), reflecting the preparation for upcoming larval feeding. The embryo hydrolytic enzyme activity was suggested as an indicator for the viability of larvae (Li et al. 1995).

Microstructure and ultrastructure of the sinus gland and X-organ of the mud crab were observed with light and electron microscopy. Two types of neurosecretory cells, B and C type, were shown to coexist in the X-organ of the crab and each has different secretory characteristics which may relate to

[1]Department of Oceanography and Subtropical Institute of Oceanography, Xiamen University, Xiamen 361005, Fujian, China

the production of different hormones (Shangguan and Li 1994a; 1995). Based on histological study, oogenesis of the crab was divided into 3 stages and ovarian development divided into 6 stages (Shangguan et al. 1991; Yan et al. 1994). Vitellogenesis in oocytes was also described (Yan et al. 1995). Examining the morphology and ultrastructure of mature sperm of the crab with transmission microscopy showed that the crab sperm consisted of an acrosome, nuclear cup and radial arms (Shangguan and Li 1994b).

Comparative studies on changes of biochemical composition, lipid classes and fatty acid composition in muscle, hepatopancreas and ovary during ovarian development suggested that lipid may be transferred from hepatopancreas to the ovary during crab gonad maturation. Thin-layer chromatography analysis showed that triglycerides and phospholipids were the major lipids in the ovary of the crab. Fatty acid compositions of ovary, hepatopancreas and muscle were analysed by gas-liquid chromatography, the results suggested the importance of the ratio of ω3/ω6 polyunsaturated fatty acids in the diet for ovarian development (Lin et al. 1994; Li et al. 1994).

Larval Cultural Biology and Ecology

Experimental studies on effects of quality and quantity of the diet on larval survival and development of the mud crab showed that the rotifer, *Brachionus plicatilis*, is a suitable diet for early larval development, though its density significantly affected survival and development of the larvae. Larval survival rate was shown to steadily increase with density of rotifers and at 60 ind/mL, the highest survival rate to Z3 could be reached. However, for late larvae, fed with rotifers alone, mass mortality and delay in moult occurred, indicating that rotifers were not a complete diet. In contrast, Z1 larvae fed with *Artemia* nauplii usually resulted in lower survival, but for later zoea, *Artemia* proved to be a good diet. A comparative study on larval diet combinations showed that larvae initially fed with a high density of rotifers, but then shifted to *Artemia* at Z2/Z3 or fed a mixed diet at Z3 had the best overall zoeal survival. Poor nutritional status during the zoeal stages appeared to have a delayed effect on survival of megalopa (Zeng and Li 1992a).

Analysing dry weight (DW), carbon (C), nitrogen (N) and hydrogen (H) content of larvae fed with two different diets, rotifers and *Artemia,* showed that for Z2 larvae, there were no significant differences between them. The results confirmed that the nutritional value of rotifer can meet larval development requirements at early zoeal stages. However, as larvae entered Z3, those fed with *Artemia* have apparently higher dry weight and C, H, N content and the gaps grew wider as larvae developed. This result suggested that diet replacement should take place at this time. During larval development, C, H, N percentages reached their highest levels at late Z5, and newly moulted megalopa had the highest daily growth rate, indicating a critical period of high nutritional demand around the time of first metamorphosis (Zeng 1987).

Histological and histochemical studies of the digestive system showed increasing development of the gastric mill, gland filter and hepatopancreas with larval development. The basic form of the gastric mill appeared at Z3 and was nearly complete at Z5. The cuticle cells of the midgut also showed differentiation at Z3. Histochemical observations suggested that accumulation of glycogen, lipid and protein in larval alimentary tract reached their highest levels at Z5 and the megalopal stage, but generally showed a significant increase at Z3 (Li 1990; Li and Li 1995).

Studies on specific activities of three digestive enzymes (protease, amylase and cellulase) during larval development indicated that the level of larval digestive enzyme activities was associated with both larval developmental stage and nutritional composition of the diets. The specific activity of protease was high in Z1 larvae, indicating an immediate diet requirement after hatching but the low protease activity in Z5 larvae may relate to high mortality at that time (Tang et al. 1995).

The influence of other environmental factors; temperature (Zeng and Li 1992b), salinity (Wang et al. 1997) and starvation (Zeng and Li 1998), on larval survival and development, and larval feeding rate under different dietary densities and conditions were also investigated (Zeng 1987). The results showed that 25–30 °C was optimal temperature range for zoeal development. However, early larvae appeared generally more tolerant to lower temperatures, while megalopa could survive well at temperatures as high as 35 °C (Zeng and Li 1992b). The effect of salinity on survival and development of the larvae showed that for early larvae (Z1–Z3) the most suitable range of salinity was 27–35 ppt. For later stages (Z4–M) the most suitable range of salinity was 23–31 ppt. The most optimal salinity for the duration of larval development appeared to be 27 ppt. Starvation experiments indicated that a short period of starvation after hatching could affect larval survival and development. On the other hand, if larvae were fed for only one day after hatching, there was a possibility of moulting into Z2 without further feeding. The PNR_{50} (Point-of-No-Return) for the Z1 larvae of the crab was estimated to be about 1.3 days, and PRS_{50} (Point-of-Reserve Saturation)

about 2.3 days (Zeng and Li 1998). Daily feeding rates of early larvae were significantly affected by the diet density, and newly metamorphosed megalopa had significantly high daily feeding rate which lasted for 2–3 days after metamorphosis (Zeng 1987).

Finally, other relevant subjects, such as the appearance of an extra zoea-6 larval stage and environmental induction of such larval stage variation, cannibalism between larvae and variation in larval quality among different larval batches were also described and discussed (Zeng 1987).

Mass Rearing of Mud Crab Seeds

Based on experimental studies on larval cultural biology and ecology, small-scale intensive larval rearing trials were carried out in 1 m^3 concrete tanks from 1989–1990: tens of thousands of juvenile crabs were obtained each year. In 1993 and 1994, mass larval rearing trails were conducted in larger concrete ponds ($3 \times 4 \times 1.7$ m) and hundreds of thousands of juvenile crabs were produced, both in Spring and Autumn. In 1995, larvae culture was carried out in 500 m^2 of hatchery tanks and about 50 million Z5 were produced, which were spread to other hatcheries. After moulting to megalopa, some were put into soil ponds for the final moult to crab, while others were kept in hatchery concrete ponds. About 1 million juvenile crabs were finally produced. A preliminary trial of poly-culture of hatchery produced crab seeds with shrimp showed potential.

Other Work

Other work has included diethylstilboestrol effects on juvenile growth and feeding (Wang and Li 1989), sexual differentiation (Lin et al. 1994), isoenzyme phenotype (Wang and Li 1991) in juvenile crabs, and bacterial proliferation in water and sediments of mud crab growout ponds (Li et al. 1997).

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Development of Hatchery Techniques for the Mud Crab *Scylla serrata* (Forskål): Comparison of Feeding Schemes

Emilia T. Quinitio[1], Fe Parado-Estepa[1] and Veronica Alava[1]

Abstract

Scylla serrata larvae were reared in 3 L plastic containers and fed various amounts of artificial diets (AD) with or without natural food (NF: *Brachionus rotundiformis* and newly-hatched *Artemia*). The amounts of AD fed alone to zoea in treatments (T) 1 to 4 were as follows: 1) 2.0 mg/L/day + 0.25 mg/L/day increment/substage; 2) 2.0 mg/L/day + 0.5 mg/L/day increment/ substage; 3) 4.0 mg/L/day + 0.5 mg/L/day increment/substage; 4) 4.0 mg/L/day + 1.0 mg/L/day increment/ substage. NF were given in addition to the respective amounts of artificial diet in T5, T6, T7 and T8. T9 served as the control (NF only). Based on three experimental runs, only larvae in T5, T6, and T9 survived until the megalopa stage. Thus, only these three treatments were compared in succeeding experiments using a commercial shrimp diet in 250 L fibreglass tanks. Of the three runs conducted using a commercial diet, two runs showed significant differences ($P<0.05$) in survival. T5 gave higher survival (3.71% and 1.33%) than T9 (1.84% and 0.45%) and T6 (1.37% and 0.45%). Population development index did not differ among treatments in three runs.

LARVAL rearing of mud crab zoea and megalopa has been achieved but survival rates are very low and inconsistent (Chen and Jeng 1980; Heasman and Fielder 1983). Feeding management may be one area which has to be investigated and modified to improve larval performance. Moreover, larval rearing may be simplified and production costs reduced by partial replacement of natural food with an artificial diet.

This study was conducted to compare larval development and survival from zoea (Z) to megalopa (M) of *Scylla serrata* (based on the identification of Keenan et al. 1998) using natural food and/or artificial diet at different levels..

Methodology

Zoea 1 were stocked at 50 ind/L in 3 L containers. Larvae were fed different amounts of shrimp larval diet available commercially (AD) with or without natural food (NF). The different treatments (T) used were the following:

T1) 2.0 mg/L/day + 0.25 mg increment/substage;
T2) 2.0 mg/L/day + 0.5 mg increment/substage;
T3) 4.0 mg/L/day + 0.5 mg increment/substage
T4) 4.0 mg/L/day + 1.0 mg increment/substage;
T5) 2.0 mg/L/day + 0.25 mg increment/substage +NF;
T6) 2.0 mg/L/day + 0.5 mg increment/substage + NF;
T7) 4.0 mg/L/day + 0.5 mg increment/substage + NF;
T8) 4.0 mg/L/day + 1.0 mg increment/substage +NF;
T9) *Brachionus rotundiformis* and newly hatched *Artemia* as control.

B. rotundiformis were maintained at 10–15 ind/ mL. *Artemia* introduced at the start of zoea 3 were gradually increased from 1 to 5 ind/mL as the crab larval stages progressed. In treatments with the artificial diet, NF were reduced by half.

Survival at each stage was determined by direct counting. The mean population development index (PDI) (Quinitio and Villegas 1980) was determined for each treatment to compare growth.

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021. Philippines

Rearing water (32 ppt) was replaced daily at 50%–80% of the total volume starting on day 2. Dead larvae and uneaten feeds were siphoned out prior to water change.

Three experimental runs with 3–4 replicates for each treatment were conducted using a completely randomised design. Survival rates and PDI were compared using two-way ANOVA and Duncan's Multiple Range Test.

Only those zoea in T5, T6, and T9 (control) reached the megalopa stage; thus, only these three treatments were compared in succeeding experiments using the same shrimp commercial diet (54% protein) in 250 L tanks. The protocol used was the same as in the previous experiment except that salinity was reduced gradually from ambient (32 ppt) to 25 ppt, starting with late zoea 3 and continuing to megalopa. Water samples for physico-chemical parameters and microbial analyses were taken 2–3 times weekly before water change. Survival was estimated at the end of the experiment. Fatty acid composition of feeds and newly hatched zoea were analysed.

Results and Discussion

There was a significant reduction in the survival of zoea 1 three days after stocking in T3 and T4 in three runs (Figure 1A). At zoea 2, survival rate was reduced further to 0–2% in T3 and T4 (Figure 1B). Larvae fed artificial diet alone (T1, T2, T3, and T4) did not survive beyond zoea 2 (Figure 1C). It was also noted that survival decreased as the amount of artificial diet increased. Even in T7 and T8 when natural foods were added in combination with high amounts of artificial diet, larvae did not survive beyond zoea 4 (Figure 1D). High amounts of artificial diet increased particle sedimentation leading to water deterioration and increase in bacterial load. High concentrations of luminous *Vibrio* were detected in T3, T4, T7, and T8 both in rearing water (3.5×10^2 to 2.5×10^3 cfu/mL) and larvae (3.0×10^3 to 5.5×10^4 cfu/mL) while counts were less than 1×10^2/cfu/mL or sometimes not detectable in the larvae in other treatments. According to Colorni (1985), these bacteria proliferate and colonise in the host's digestive tract and become pathogenic, thus causing mass mortality. Only the larvae in T5, T6, and T9 reached the zoea 5 and megalopa stages (Figures 1E, F). No difference in PDI was observed among treatments.

In the succeeding runs, only T5, T6, and T9 were compared using the same amount of artificial diets in 250 L fibreglass tanks. Two-way analysis of variance showed a significant interaction ($P<0.03$) between runs and treatments. Of the three runs conducted using a commercial shrimp larval diet, two runs showed similar trends while the other run did not show significant differences in megalopa survival (Figure 2). In runs 1 and 3, T5 gave significantly higher survival (3.71% and 1.33%) than T9 (1.84% and 0.45%) and T6 (1.37% and 0.45%). PDI did not differ among treatments in three runs.

In general, zoea reached the megalopa stage in 15–17 days. Moulting was not synchronous even within treatments. Ong (1964) reported that the development of zoea 1 to megalopa required a minimum of 18 days. Water temperature ranged from 26.5–29 °C throughout all three runs. The NO_2-N and NH_3-N levels during the experimental runs were 0.0–0.04 and 0.0–0.58 ppm, respectively.

Lipids are important as sources of fatty acids for metabolic energy, and to maintain structural integrity of cellular membranes. Fatty acids, specifically n-3 highly unsaturated fatty acids (HUFA) such as 20:5n-3 (eicosapentaenoic acid; EPA) and 22:6n-3 (docosahexaenoic acid; DHA) are essential components in the diet of crustaceans (Kanazawa et al. 1977; Jones et al. 1979). The EPA and DHA contents of the shrimp commercial diet were close to those of the crab zoeae (Table 1). In contrast, DHA was absent in both *B. rotundiformis* and *Artemia* while EPA was low in *Artemia. Chlorella virginica*, which constituted the feed of *B. rotundiformis*, contained high EPA and this was reflected in the rotifers. Any deficiency in essential fatty acids particularly n-3 HUFA in rotifers and *Artemia* may have been offset by giving supplemental feeds to crab larvae. Artificial diet could also serve as enrichment for rotifers and *Artemia*, which in turn are taken in by the larvae. The supplementation of artificial diets could improve the growth and survival of crab larvae and reduce the requirement for natural food. An additional experiment is being conducted using a crab-formulated diet to further improve the survival of larvae.

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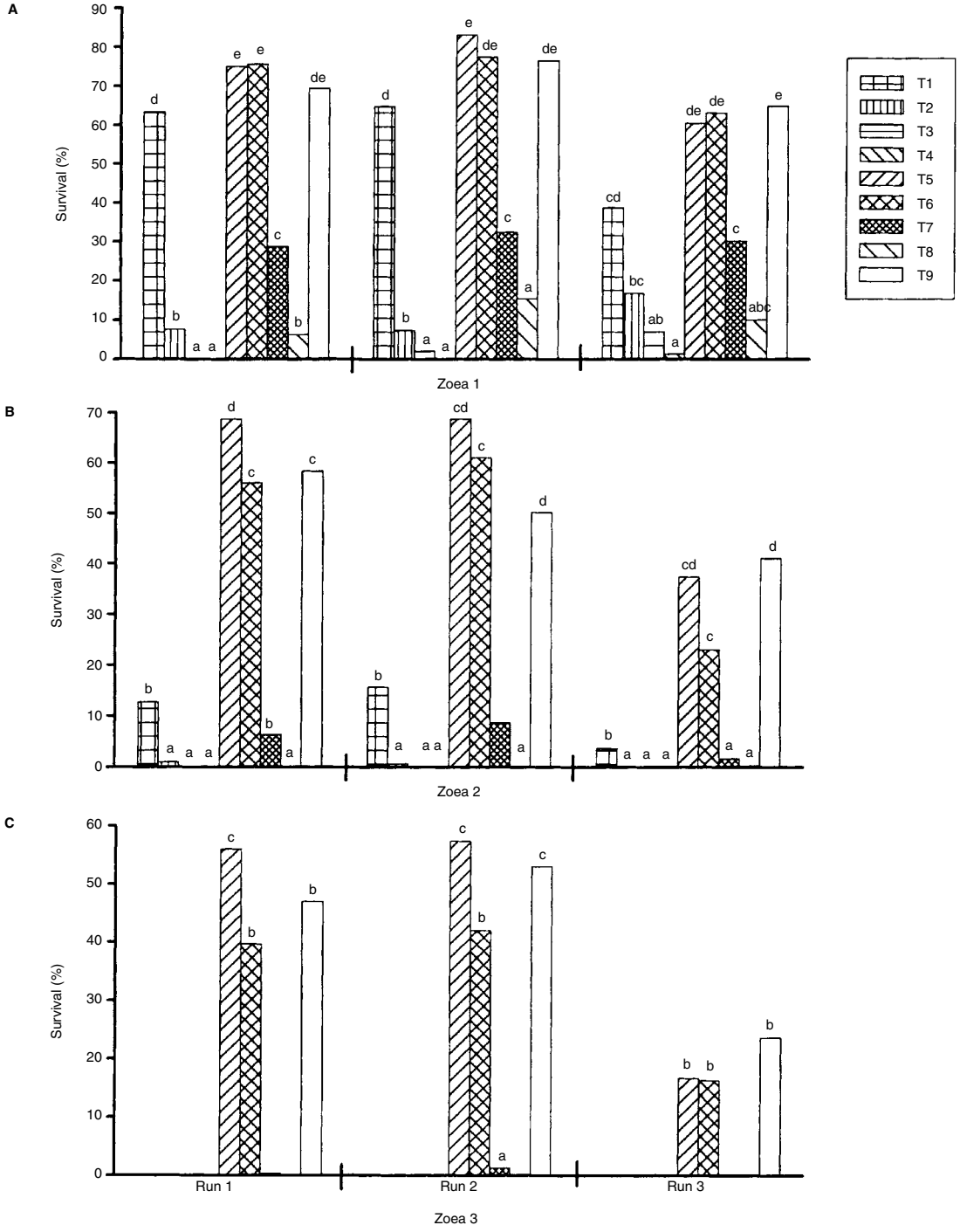

Figure 1. Percentage survival of *Scylla serrata* from zoea to megalopa stage given different amounts of shrimp larval diet reared in 3 L containers. Different letters in the same run are significantly different ($P<0.05$).

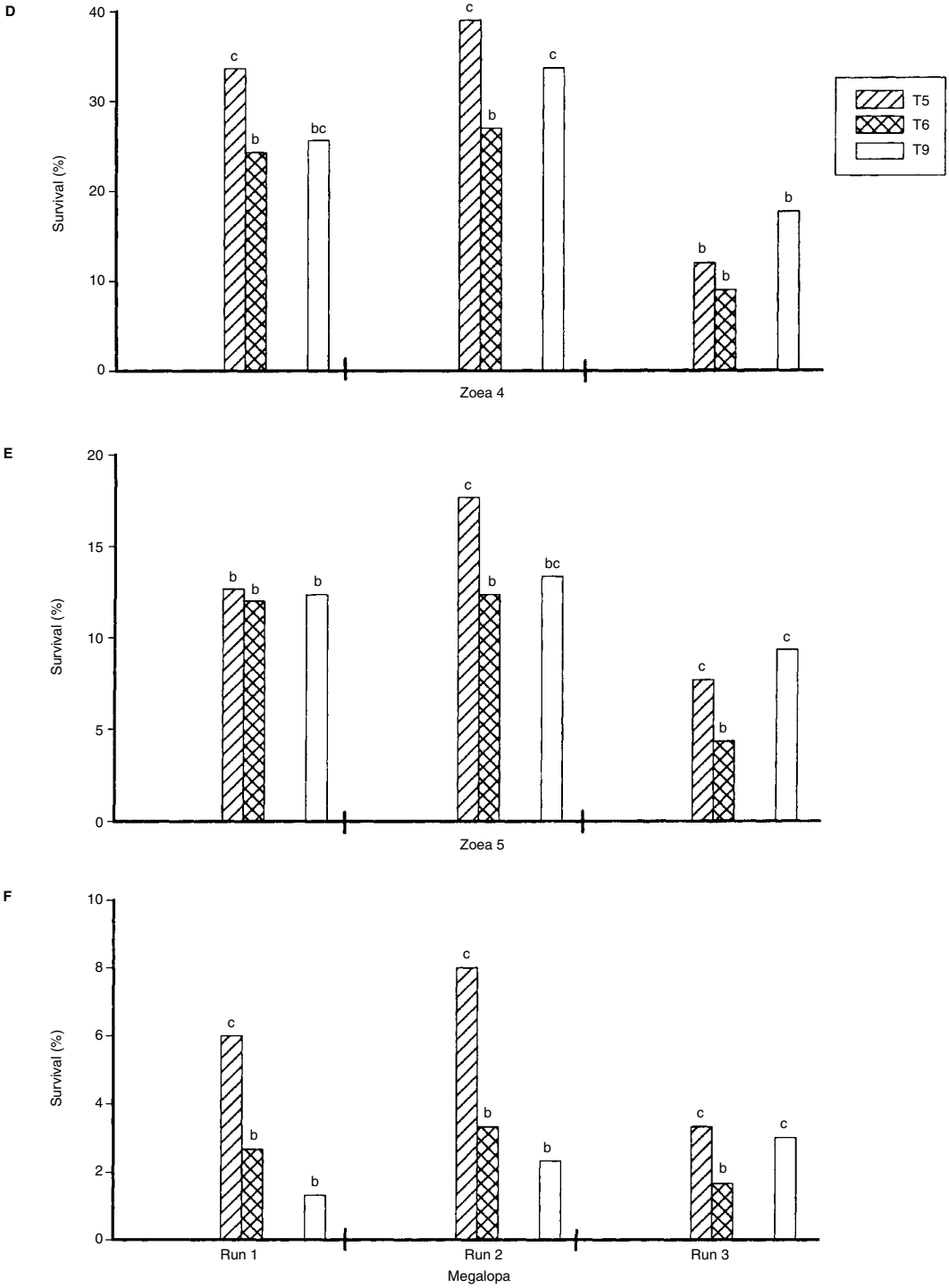

Figure 1 (continued) Percentage survival of *Scylla serrata* from zoea to megalopa stage given different amounts of shrimp larval diet reared in 3 L containers. Different letters in the same run are significantly different ($P<0.05$).

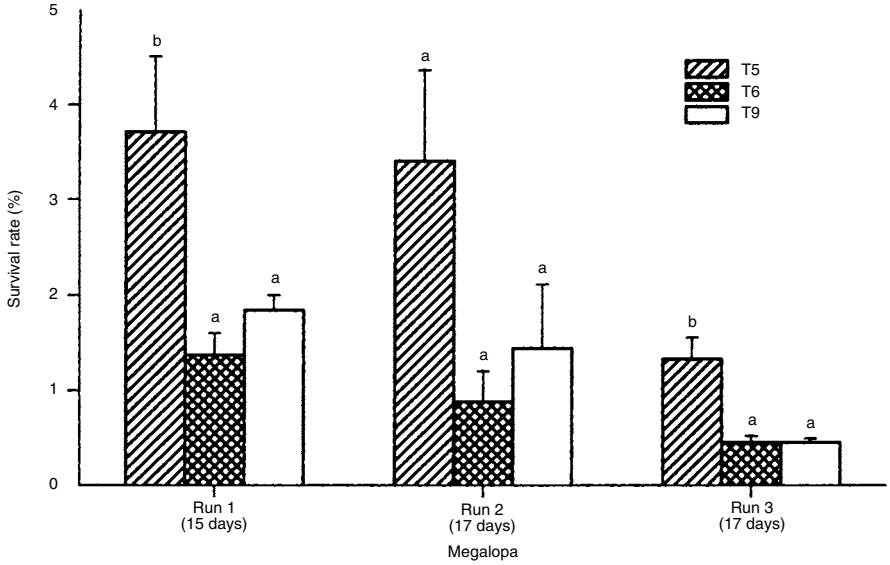

Figure 2. Percentage survival of *Scylla serrata* from zoea 1 to megalopa stage given different levels of commercial larval diet in combination with natural food reared in 250 L tanks. Bars with different letters in the same run are significantly different ($P<0.05$). Error bars indicate standard error of means.

Table 1. Fatty acid composition of natural food, artificial diets and *Scylla serrata* zoea.

Fatty acid	Natural food				Shrimp commercial diet	Crab zoea
	Chlorella	*Tetraselmis*	*Brachionus*	*Artemia*		
14:0	5.09	0.58	3.32	1.37	3.00	0.91
16:0	25.19	19.30	18.20	11.89	15.30	18.44
16:1n-7	20.42	10.10	14.82	7.04	7.95	5.21
18:0	2.56	0.66	3.96	2.05	5.10	8.70
18:1n	13.86	24.87	11.13	31.33	18.15	18.56
18:2n-6	2.32	9.08	5.62	6.98	14.60	1.53
18:3n-3		1.38		0.13	2.30	0.25
18:4n-3		17.43		23.79		1.04
20:1n-9	0.84	5.62	2.19	3.73	1.55	1.39
20:4n-6	2.94	1.11	5.01	1.01	2.70	8.35
20:5n-3	22.25	4.43	21.31	4.52	9.90	15.82
22:5n-3			11.16		1.25	1.50
22:6n-3					11.20	11.10
Total n-3	22.25	23.24	32.47	28.44	23.40	29.71
Total n-6	5.26	10.19	10.63	7.99	17.30	9.88
n-3/n-6	4.23	2.28	3.05	3.56	1.35	3.01
Total n-3 HUFA	22.30	4.40	32.50	4.50	21.10	28.40

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Mud Crab (*Scylla serrata*) Megalopa Larvae Exhibit High Survival Rates on *Artemia*-based Diets

Graham R. Williams[1], John Wood[1], Brian Dalliston[1], Colin C. Shelley[1] and Chris M. Kuo[1]

Abstract

Two trials rearing mud crab (*Scylla serrata*) larvae from the megalopa stage through to the first crab instar (C1) were carried out to investigate the performance of 10 diets. The megalopae were fed diets of enriched *Artemia* nauplii, unenriched *Artemia* nauplii, dried *Acetes* shrimp and dried polychaete as individual feeds or in combination to compare their effects on survival rates from megalopae to C1. The diets that contained *Artemia* produced a significantly higher ($P<0.01$) number of first instar crabs with survival ranging from 38.9%–57.8% whereas the diets without *Artemia* produced survival rates of between 6.1%–12.8%. These results indicate that the diets containing *Artemia* nauplii can produce consistently high survival rates from megalopae to first crab instar. Feeding regimens including live *Artemia* can be used as controls for further investigations into optimal nutrition for megalopa to C1.

THE LARGE variation in survival rates of mud crab (*Scylla serrata*) larvae has been a problem commonly encountered in the authors' previous investigations into mud crab culture. Nutrition has been suggested as a possible cause of the mass mortalities experienced. Other researchers have used various live feeds such as *Artemia*, copepods and rotifers individually or in combination to establish a reliable method of raising mud crab larvae. Heasman and Fielder (1983) reported their highest survival of 26% from zoea 1 (Z1) to the first crab instar (C1) was obtained with larvae fed solely on *Artemia*, whereas Marichamy and Rajapackiam (1991) reported a maximum survival from Z1 to C1 of 15% using a mixed rotifer and *Artemia* diet. Zainoddin (1991) used a combination of rotifers and frozen *Artemia* and obtained a survival from Z1 to C1 of 20% where previously Brick (1974) had found that *Artemia* alone produced higher survival rates than rotifers, diatoms or wild zooplankton alone, or in combination with *Artemia*.

Although it has been demonstrated that rotifers and *Artemia* can sustain all larval stages, the survival rates produced have been inconsistent, perhaps indicating a nutritional deficiency. This may be linked to the findings of Sorgeloos et al. (1991) who found that rotifers and *Artemia* were deficient in certain highly unsaturated fatty acids (HUFA) essential for marine species. The benefits of increased levels of dietary HUFA to *Penaeid* shrimp were demonstrated by Jones et al. (1979) and Kanazawa et al. (1985) who reported that survival and growth of larvae were greatly improved by enriching rotifers and *Artemia* with essential HUFA.

The use of enriched *Artemia* or *Artemia* used with supplements could be expected to produce better survival than *Artemia* used alone, if nutritional deficiencies in the *Artemia* were the cause of the inconsistent survival rates experienced. The two experimental trials described in this paper (Nov. 1996 and Feb. 1997) were carried out to compare the effect of a range of feeds and their combinations on the survival of *S. serrata* larvae from megalopa to C1.

Materials and Methods

In the past, there has been confusion regarding identification of the various species in the genus *Scylla.* This has led to a degree of uncertainty when comparing the work of different authors. The megalopae used in these trials are the offspring of crabs

[1]Darwin Aquaculture Centre, Department of Primary Industry and Fisheries, GPO Box 990, Darwin, Northern Territory, Australia

identified as *S. serrata* according to the description given by Keenan et al. (1998).

The larval rearing methods used in the two experiments were identical. The megalopae used in the trials were raised from Z1 to megalopa stage using rotifers (Z1–Z3) and *Artemia* (Z3–megalopa) as feeds in 7000 L outdoor tanks. Megalopae were stocked into the bowls at 10/L on the first day that the majority of Z5 larvae metamorphosed to megalopae which was day 13 for the Nov. 1996 trial and day 14 for the Feb. 1997 trial. The day that the eggs hatched to become Z1 larvae was termed Day 0.

During the trials, the larvae were held in 5 L, clear plastic, hemispherical bowls containing 3 L of culture water. Initial and replacement culture water was 40 µm sand filtered sea water, which was diluted to the required salinity by town supply water. All water was disinfected with 10 ppm of active chlorine for a minimum of 16 hours and de-chlorinated with sodium thiosulphate before use. Salinity was maintained at approximately 25 ppt for the duration of the trials.

The larval rearing containers were randomly allocated a position in a 7000 L water bath which had a continuous flow-through of ambient temperature sea water. The water bath was in an outdoor shaded area that received no direct sunlight. Gentle aeration was provided through a 1 mL plastic pipette placed in the centre of each bowl.

Larvae were removed from the bowls daily using a large bore pipette and counted. After the bowl was washed in fresh water and the culture water replaced, the larvae were returned to the bowl. The appropriate feeds were then distributed to the bowls. Each treatment was replicated three times.

Feed preparation and treatments

1. Algae

Nannochloropsis oculata, *Chaetoceros muelleri* and *Isochrysis* sp. (Tahitian strain, T-*iso*) were added to all the treatments daily in equal proportions to obtain a combined density of 5×10^4 cells/mL throughout the experiments.

2. *Artemia* nauplii

Artemia cysts (AF Grade, Artemia Systems, Belgium) were disinfected in fresh water containing 200 ppm of active chlorine for 20 minutes then incubated according to the instructions of the producer. All *Artemia* used were thoroughly rinsed with 0.5 µm filtered, UV-treated sea water before use.

Treatment a: Newly hatched *Artemia.* Instar 1 nauplii applied at a daily rate of 0.75/mL.

Treatment b: Boosted *Artemia*. Instar 2 nauplii were enriched for 16 hours with Frippak booster (Frippak, England) at a rate of 1 g per million nauplii. These were applied at a daily rate of 0.5/mL. The instar 2 nauplii were applied at a lower rate than the instar 1 nauplii because of their greater size.

Treatment c: Dried *Acetes* shrimp. Frozen *Acetes* shrimp were thawed out and salted in aerated, saline water (70 ppt) for 1 hour before sun-drying. The dried *Acetes* shrimp were macerated in a kitchen blender for 15–20 seconds then screened through a series of plankton nets to produce a particle size range of 500–800 µm. These particles were mixed in water and fed to the larvae at a total daily rate of 5 mg/L.

Treatment d: Dried polychaete mud worm (*Marphysa* spp.). The preparation and application rate of the dried mud worm was the same as that described in the section on *Acetes* shrimp in Treatment c.

Where feeds were used in combination with other feeds the daily ration for each feed was halved, but where only one feed was used in a treatment the whole daily ration was applied. The ration was divided into three feeds per day which were fed to the larvae at even intervals between 0900 and 1700.

Experimental design

A factorial design was used in both trials with the combinations shown in Table 1.

Table 1. Summary of treatments applied to megalopae.

Treatments	Artemia	Boosted *Artemia*	Dried *Acetes* shrimp	Dried mud worms
Artemia = a	a,a	a,b	a,c	a,d
Boosted *Artemia* = b	–	b,b	b,c	b,d
Dried *Acetes* shrimp = c	–	–	c,c	c,d
Dried mud worms = d	–	–	–	d,d

Data collection and analysis

Any C1 present were removed from the rearing containers at the time of the daily count (approx. 0800). Trials were terminated when all the megalopae had metamorphosed to crabs or died. The survival rates to C1 were expressed as mean percent of the initial number of megalopae stocked into each bowl. Water temperature, pH (HI 8424, Hanna Instruments, Italy) and salinity (Atago, Japan) were recorded daily.

Survival rate data were analysed by ANOVA followed by Fisher's protected LSD test if significant differences were indicated. (StatView® 1992, Macintosh, CA, USA). Data expressed as percentages were transformed to proportions prior to analysis (Underwood 1981).

Results

For both the trials, a one factor ANOVA showed there was a significant difference ($P<0.01$) in the survival rate of megalopae to crab 1 when testing the effect of the diets on survival. A two factor ANOVA (batch = random factor, diet = fixed factor) showed no significant difference ($P>0.05$) between the batches and no interaction between batches and diet. The pooled results from the two trials are shown in Table 2 and Figure 1. Although there were significant differences ($P<0.05$) in survival between the treatments containing *Artemia*, the results could be divided into two groups: those with *Artemia* and those without *Artemia.* The treatments containing *Artemia* as a live feed gave a significantly higher ($P<0.01$) survival than those that contained only inert foods, i.e., *Acetes* and/or mud worm (see Table 2 and Figure 1).

Table 2. Summary of pooled results (Nov. 1996 and Feb. 1997) – Mean % survival of megalopa to crab 1. *Means with the same letter in parentheses are not significantly different (P>0.05).

Diet	Treatment	Pooled mean* ± S.E.
Newly hatched *Artemia*	a,a	38.9 ± 3.5 (A)
Boosted *Artemia*	b,b	46.7 ± 6.4 (A)
Acetes shrimp	c,c	12.8 ± 2.0 (B)
Mud worm	d,d	6.7 ± 1.2 (B)
Newly hatched *Artemia* + Boosted *Artemia*	a,b	49.4 ± 6.4 (A)
Newly hatched *Artemia* + *Acetes* shrimp	a,c	40.0 ± 5.6 (A)
Newly hatched *Artemia* + Mud worm	a,d	57.8 ± 3.8 (A)
Boosted *Artemia* + *Acetes* shrimp	b,c	45.6 ± 5.5 (A)
Boosted *Artemia* + Mud worm	b,d	41.7 ± 4.7 (A)
Acetes shrimp + Mud worm	c,d	6.1 ± 1.6 (B)

Instar 1 crabs first appeared on day 19 in the Nov. 1996 trial whereas in the Feb. 1997 trial a few appeared on day 20 with most of the treatments not producing a substantial number of crabs until day 21 (see Figures 2 and 3). In both trials, all of the treatments containing *Artemia* (excepting treatment b + d, Feb. 1997) produced the majority of crabs within one day of them first appearing, whereas in the treatments not containing *Artemia,* this took two days (see Figures 2 and 3).

Water temperatures ranged from 29.0–30.7 °C in the Nov. 1996 trial and from 25.0–28.6 °C in the Feb. 1997 trial. The differences in the temperature range could explain the extra two days it took the megalopae in the Feb. 1997 trial to metamorphose to crab 1 or die when compared to the Nov. 1996 trial (see Figures 4 and 5).

Discussion

The results of the two trials showed that the treatments containing live *Artemia* nauplii gave significantly higher survivals ($P<0.01$) from megalopae to C1 than those that contained only inert feeds. The inert feeds clouded the water slightly and because only very gentle aeration was used, the particles tended to drop out of suspension making them less available to the megalopae. Poor nutritional quality, deleterious effects on water quality or simple feed unavailability are all possibilities that would explain the lower survival produced by the inert feeds.

There was no significant difference ($P>0.05$) in survival rates produced by the boosted *Artemia* and the unboosted *Artemia*, showing that there was no benefit in boosting this grade of *Artemia.*

Although there was no significant difference ($P>0.05$) between the survival rates produced by any of the treatments containing *Artemia,* the combination of mud worm and *Artemia* gave the highest survival in both trials (55.6% in Nov. 1996 and 60.0% in and Feb. 1997). This suggests that while *Artemia* fed at the rates used in these trials is an adequate feed on its own, the use of a supplement may give improved results. The highest survival from megalopa to C1 (60%) obtained in these trials is higher than the maximum 50% obtained by Brick (1974) using 15 *Artemia*/mL, but lower than the 87% achieved by Heasman and Fielder (1983) using 30 *Artemia*/mL. Marichamy and Rajapackiam (1991) used minced clam and shrimp with copepods and frozen *Artemia* to achieve a result similar to the highest survival (60%) obtained in these trials. The densities of *Artemia* used in the two trials described in this paper were 0.5/mL (instar 2) and 0.75/mL (instar 1) which are considerably lower than that used by the other authors.

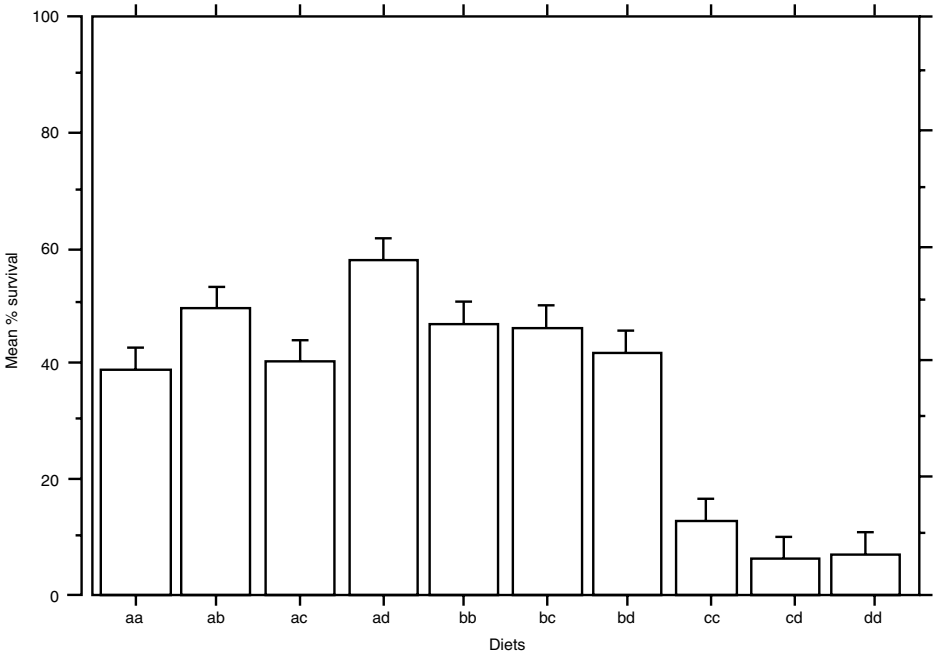

Figure 1. Mean % survival from megalopa to crab 1. Combined Nov. 1996 and Feb. 1997 data. Error bars: ± 1 standard errors.

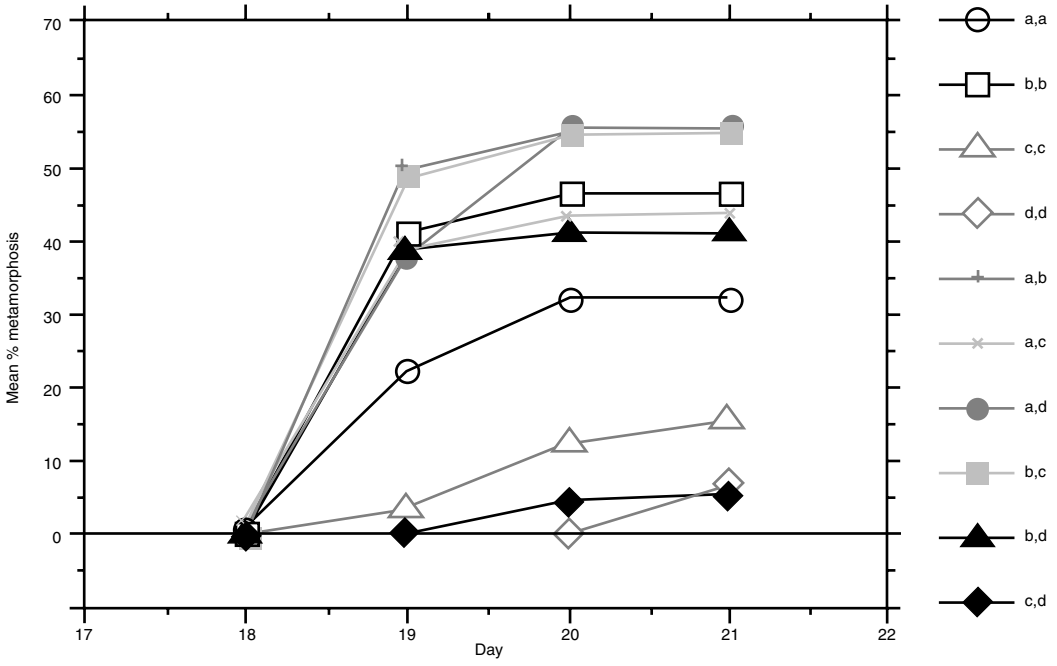

Figure 2. Mean % metamorphosis from megalopa to crab 1 (Nov. 1996).

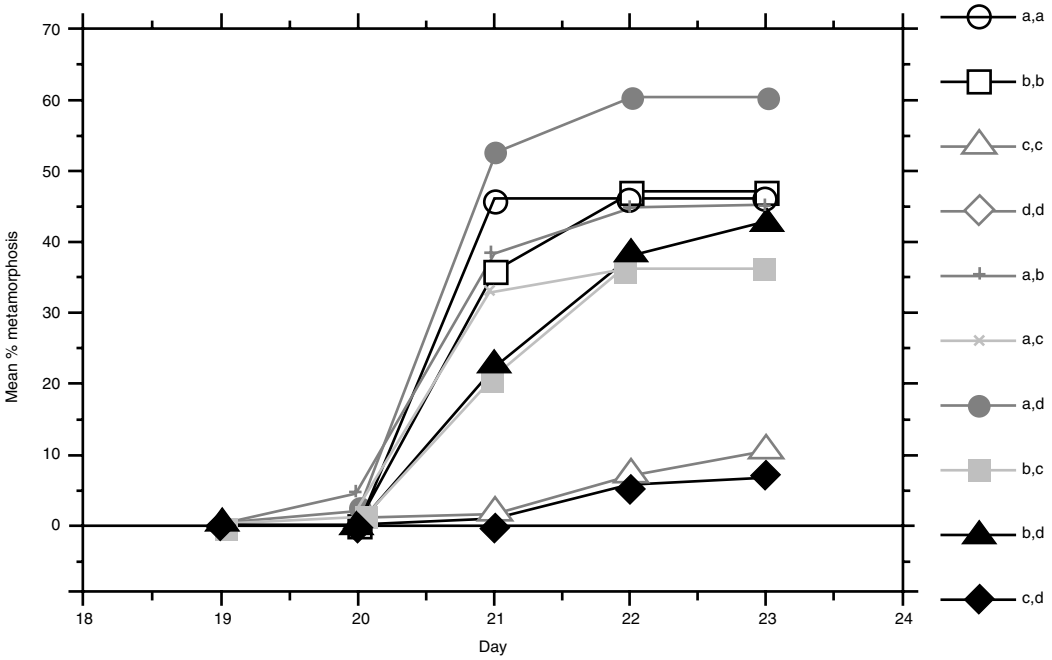

Figure 3. Mean % metamorphosis from megalopa to crab 1 (Feb. 1997).

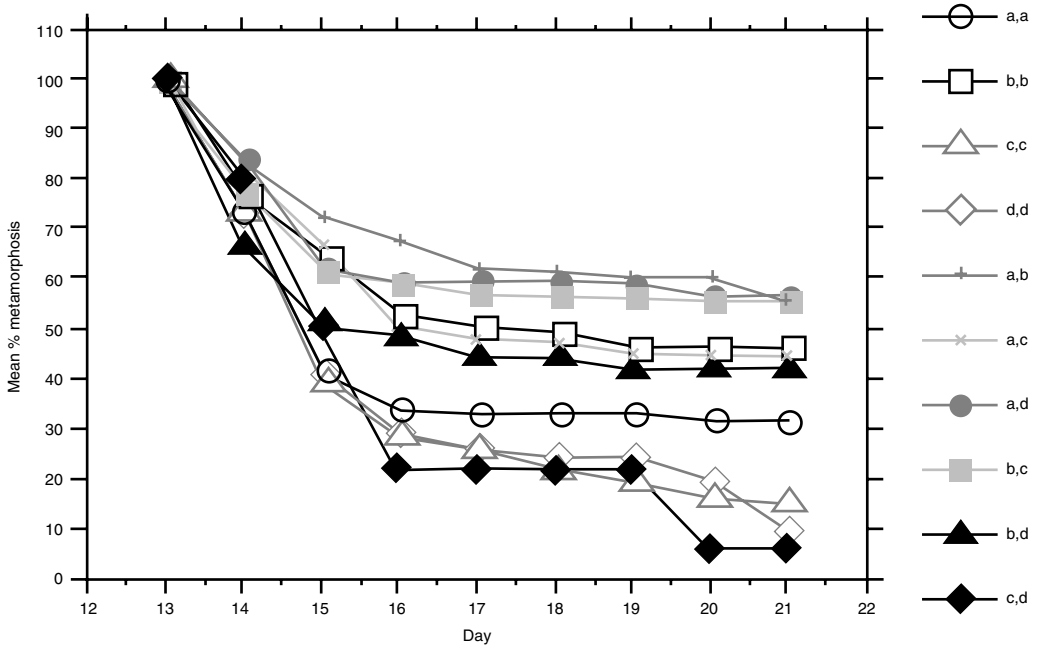

Figure 4. Mean % survival from megalopa to crab 1 (Nov. 1996).

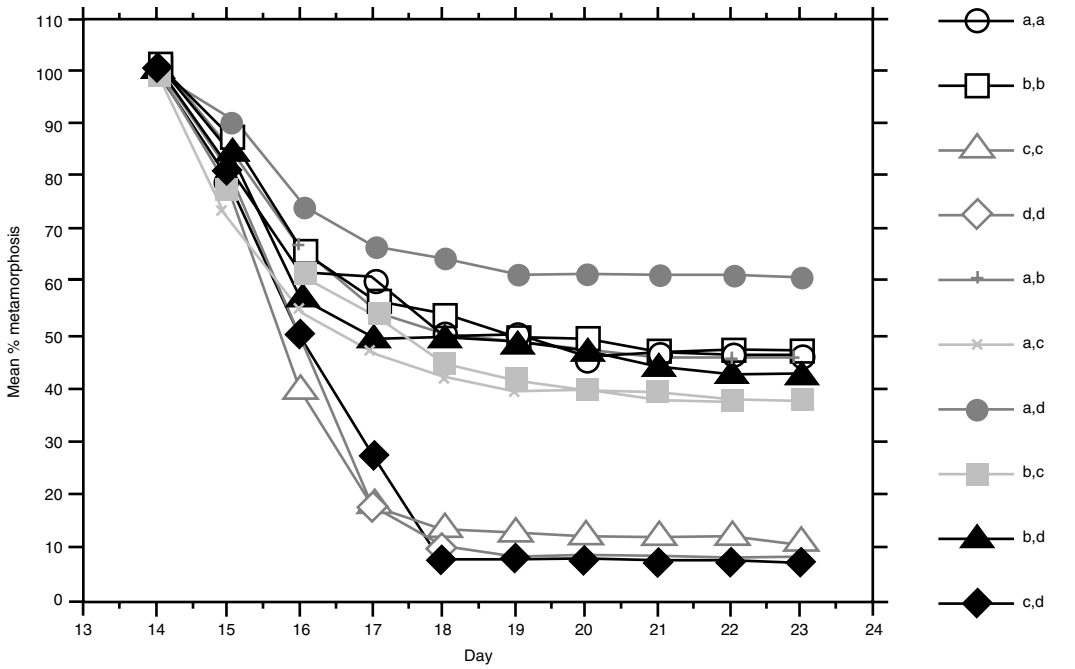

Figure 5. Mean % survival from megalopa to crab 1 (Feb. 1997).

Although an increase in *Artemia* density or supplementation may be required to maximise larval survival, feeding *Artemia* at these lower densities would be more economical, particularly at commercial scale production. The results of the two trials described show that diets containing *Artemia* are suitable for rearing megalopa to C1. In future, such diets can act as controls in experiments seeking to optimise megalopa to C1 survival rates.

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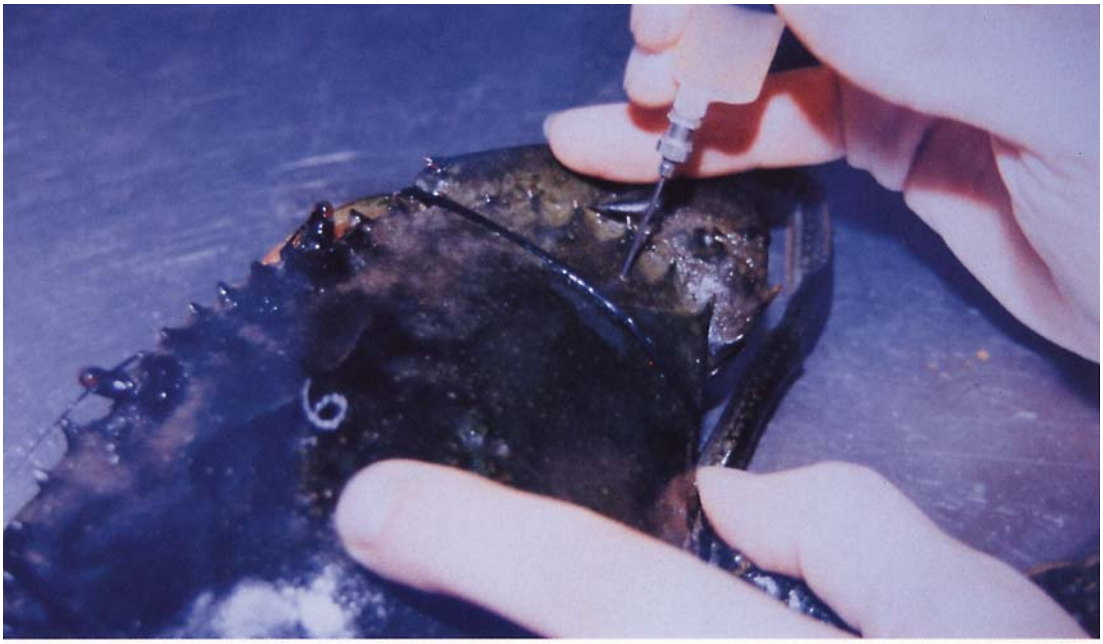


Figure 5. Broodstock mud crabs need to be matured in captivity. Ovarian biopsy through a small hole drilled into the carapace allows accurate determination of oocyte diameter and stage of ovarian maturation. Photo: David Mann.

Figure 6. Spawning of female mud crabs in captivity requires a loose substrate for successful attachment of the eggs to the pleopods. Photo: Glen Smith.

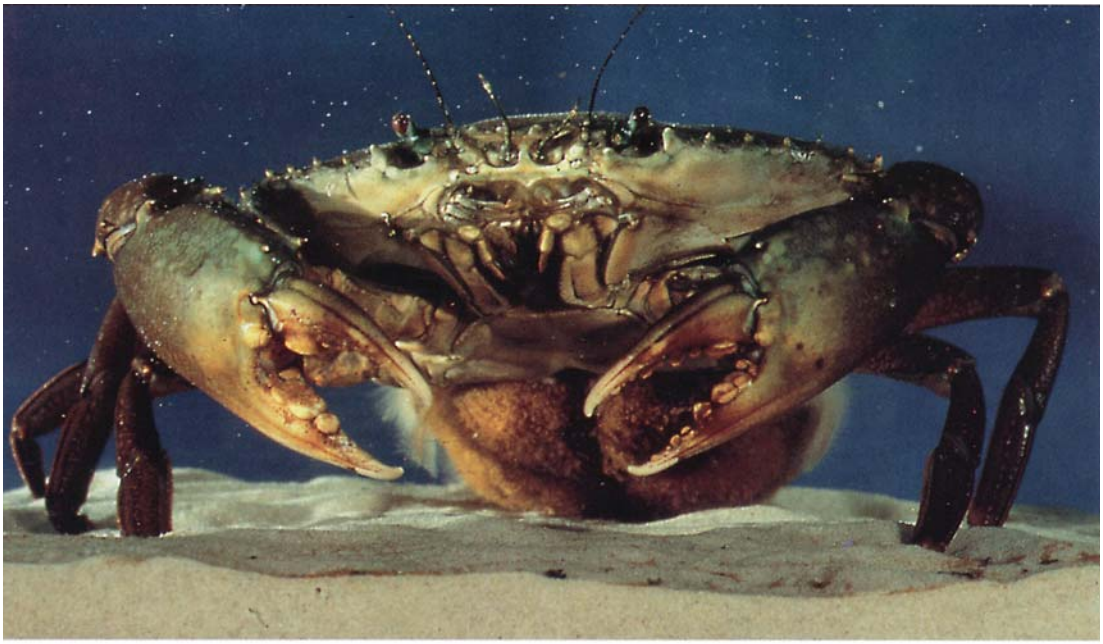


Figure 7. Berried female mud crabs (*Scylla serrata*) commonly carry between 2 – 5 million eggs. During the incubation period of 10 – 14 days, the eggs change colour from orange to black. Photo: Glen Smith.

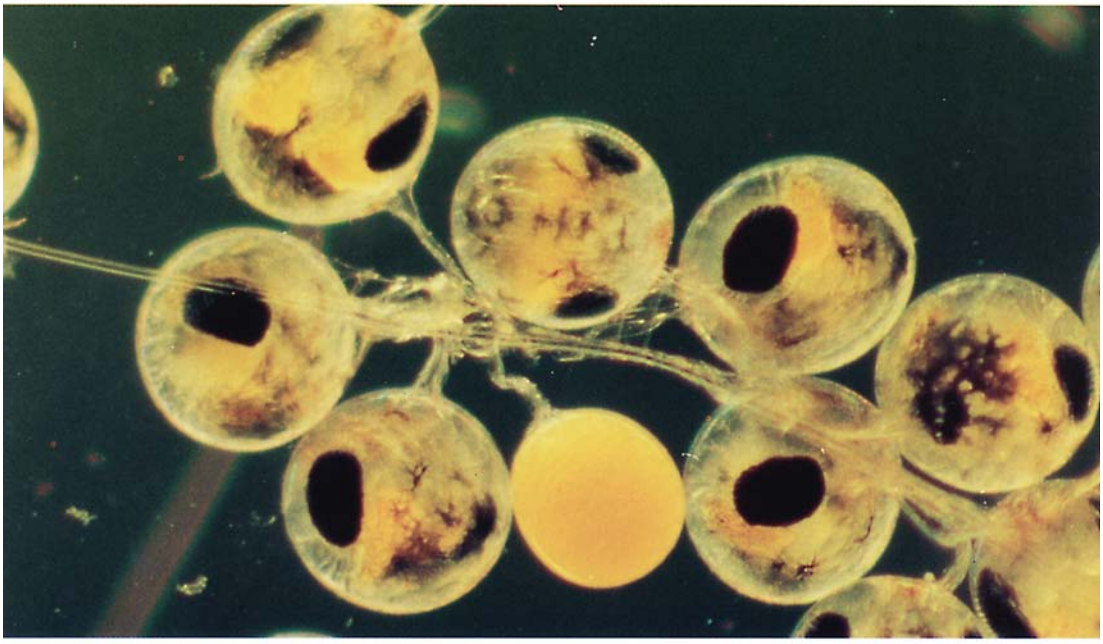


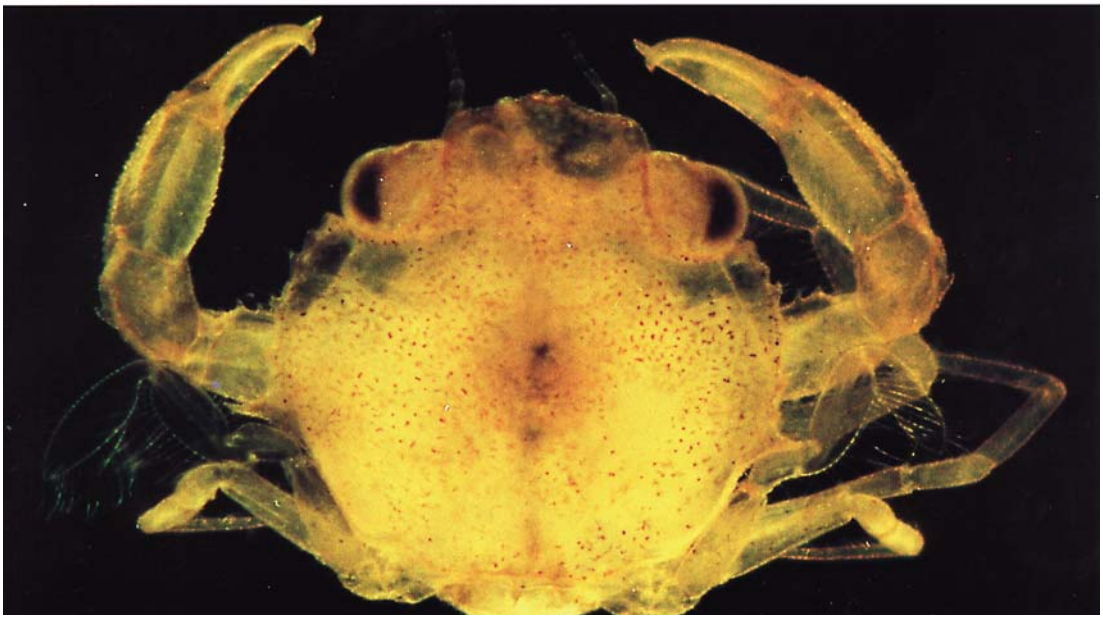
Figure 8. Close up of 12-day-old eggs (≈ 330 µm diameter) attached to setae, showing pigmentation of eyes and body. Note undeveloped egg in the centre of the photograph. Fertilisation rates are typically greater than 90%. Photo: David Mann.

Figure 9. (above) Newly hatched first zoeal stage (Z1) of *S. serrata* next to unhatched egg. Zoea hatch as non-motile pre-zoea which moult within 15 minutes in the free swimming and actively feeding Z1 stage. Photo: David Mann.

Figure 10. (right) The fifth zoeal stage (Z5) of *S. serrata*. The Z5 stage appears between days 11 and 14 of the culture cycle at 28 °C. Note the pleopods on the abdomen, which have developed since the Z1 stage. Photo: David Mann.

Figure 11. (left) Megalopae metamorphose from the Z5 stage, developing large claws. During the megalopal stage they change from a planktonic to a benthic existence. Problems with cannibalism are first experienced at this stage. Photo: David Mann.

Figure 12. (below) The first crab stage (C1) of *S. serrata*, after metamorphosis from the megalopal stage. The carapace width of a C1 is slightly less than 4 mm and wet weight is approximately 13 mg. Photo: David Mann.



Larval Rearing of the Mud Crab *Scylla serrata* in the Philippines

Juliana C. Baylon[1] and Alan N. Failaman[1]

Abstract

Sexually mature crabs collected from mangrove areas were individually maintained in concrete tanks filled with seawater and provided with 10 cm of sand substrate and strong aeration. Eyestalks were bilaterally ablated to induce spawning. After they had spawned, berried crabs were then transferred to a tank without substrate and 100% water change was carried out on a daily basis. It took 10–12 days of incubation before hatching, which usually occurred in the early morning. Larval rearing experiments were then conducted. The zoea, megalopa and crablets were fed and the effects of stocking density, green water, substrate and shelter on survival and metamorphosis to the next larval stages were observed.

STOCKING of mud crabs in ponds for growout and for fattening is dependent on supply from the wild. However, with the increasing destruction of mangroves which are the natural habitat of juvenile crabs, there is a great need to develop a hatchery technology for the mass production of seed to meet the demands of the farming of mud crabs.

Although several studies had already been reported on larval rearing of mud crab in Malaysia (Ong 1964; Zainoddin 1992), Hawaii (Brick 1974), Philippines (Simon 1974; Laviña 1980), India (Marichamy and Rajapackiam 1992), Africa (Hill 1974) and Australia (Heasman and Fielder 1983), consistently low survival of 1% to 30% from zoea stage up to megalopa were reported.

High mortalities were attributed to inappropriate food and feeding density, salinity and light requirement, stocking density and type of substrate; high sensitivity of zoea to water turbulence and sudden change in temperature.

Materials and Methods

Study 1. *Artemia* and *Brachionus* as food for zoea

The experiment was conducted in 4 L capacity flat-bottomed circular plastic containers at a stocking density of 10 larvae/L. The three treatments were: Treatment I, zoea fed newly hatched *Artemia* nauplii only at 10/mL; Treatment II, fed *Brachionus* alone at 25/mL; Treatment III, fed a combination diet of *Artemia* (5/mL) and *Brachionus* (12/mL). There were trial runs with three replicates per treatment. The water used was sand-filtered seawater with a salinity of 30–35 ppt settled for at least a day. Larvae were individually transferred to a new culture container by a large bore pipette. Survival and metamorphosis were then monitored.

Study 2. Shrimp, squid and worm as food for megalopa

The experiment was conducted in 8 L capacity circular plastic containers at a stocking rate of 1 megalopa/L. The three treatments were: Treatment I — megalopae were fed with *Artemia* nauplii only at 20/mL; Treatment II — *Artemia* nauplii supplemented with minced squid; Treatment III — *Artemia* nauplii supplemented with minced worm and Treatment IV — *Artemia* nauplii supplemented with minced shrimp. Each treatment was replicated three times. The sand-filtered seawater was maintained at 28 ppt salinity, provided with strong aeration and 100% daily water change. There were three replicates per treatment and three trial runs were conducted.

Study 3. Shrimp, squid and worm as food for crablets

Crablets produced from megalopa feeding experiment were fed different diets: Treatment I — crablets

[1]Division of Biological Sciences, College of Arts and Sciences, University of the Philippines in the Visayas, 5023 Miagao, Iloilo, Philippines

were fed with *Artemia* nauplii alone at 20/mL; Treatment II — crablets were fed with minced mussel supplemented with *Artemia* nauplii; Treatment III — crablets were fed with minced shrimp supplemented with *Artemia* nauplii; Treatment IV — crablets were fed with formulated diet and *Artemia* nauplii and Treatment V — crablets were fed with formulated feed alone.

The experiment was conducted in 8 L capacity circular plastic containers at one crablet per container to prevent cannibalism and to enable collection and measurement of exuviae in every moult. Each treatment was replicated six times and one trial run was conducted. Salinity of the water was maintained at 26 ppt, there was daily water change and strong aeration was provided. Feeding was ad libitum.

Study 4. Effect of stocking density on survival and metamorphosis of zoea 1 to zoea 2

Three different stocking densities of 10, 25 and 50 larvae/L were tried to find out if stocking density has an effect on survival of zoea 1 larvae and on their metamorphosis to zoea 2. The set-up was similar to that of Study 1. Each treatment was replicated three times and three trial runs were conducted.

Study 5. Mass rearing of larvae using green water

The experiment was conducted in 100 L capacity circular flat-bottomed plastic containers using sand-filtered seawater with a salinity of 35–36 ppt. Water was changed at a 50% rate daily and very mild aeration was provided.

The green algae provided was *Nannochloropsis* at a density of 5×10^4 cells/mL. Zoea were fed a combination of *Brachionus* and *Artemia* starting on the first day. There were three replicates per treatment. Unlike previous larval rearing experiments where the experimental set-up was inside an enclosed building, this trial was carried out in an open space provided only with plastic roofing.

Study 6. The effect of substrate and shelter on the survival of the crablets

Six treatments were prepared: Treatment I — without mud substrate and without shelter; Treatment II — without mud substrate and with coconut leaves as shelter; Treatment III — without mud substrate and with mangrove twigs as shelter; Treatment IV — with mud substrate and without shelter; Treatment V — with mud substrate and coconut leaves as shelter; Treatment VI — with mud substrate and mangrove twigs as shelter.

Each treatment was replicated three times.The experiment was conducted in 54 L capacity aquaria filled with 30 L of water. Six crablets were maintained in each aquarium, strong aeration was provided and water salinity was maintained at 25 ppt with 90% daily water change.

Results and Discussion

Study 1. *Brachionus* and *Artemia* as food for zoea

Figure 1 shows that zoea fed with *Brachionus* alone had high survival up to 96% in the early zoeal stages but this type of food was not enough to sustain survival in the later zoeal stages and to promote metamorphosis up to megalopa stage. Survival of zoea fed with *Artemia* alone was comparatively high in the early zoeal stages (zoea 1 to zoea 4). However, survival became significantly low at zoea 5. Also, megalopa production was very low (0%–24%) in larvae fed with *Artemia* alone. On the other hand, feeding the zoea with a combination of *Brachionus* and newly-hatched *Artemia* nauplii resulted in megalopa production as high as 82% if based on premetamorphic survival or, 56% megalopa production if based on initial number (Table 1). Apparently, the combined nutritional content of these two types of food complement each other, hence promoting survival and metamorphosis to the next zoeal stage up to megalopa.

The results of this study reveal that a combination diet of *Artemia* and *Brachionus* is ideal for the rearing of mud crab larvae, giving a high survival (69%) up to zoea 5 stage, high metamorphosis to megalopa (56%) and the shortest time to produce megalopa (17 days from hatching). More studies, however, need to be done to establish feeding density and feeding scheme of *Artemia* and *Brachionus.*

Table 1. Mean percent metamorphosis of zoea to megalopa in 21 days of culture.

Treatment	Mean % survival	% Metamorphosis to megalopa based on initial number	% Metamorphosis to megalopa based on premetamorphic number	No. of days for megalopa production to occur
I *Artemia*	47.78	0	0	No production
II *Brachionus*	36.67	3.33	25	21
III *Artemia* + *Brachionus*	68.89	55.56	81.52	15

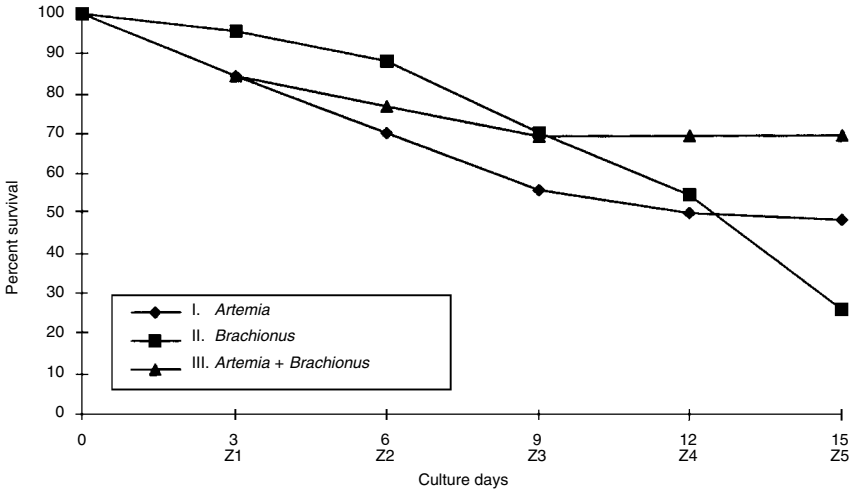


Figure 1. Mean percent survival of mud crab larvae fed with *Brachionus* alone, *Artemia* alone and combination of *Brachionus* and *Artemia.*

Study 2. Shrimp, worm and squid as food for megalopa

Figure 2 shows that megalopa fed with *Artemia* alone gave the highest rate of survival and metamorphosis to crab instar 1 compared with combination diets. The advantage of giving purely live feed is that unconsumed feed did not pollute the water. The combination diet of minced squid, minced worm and minced shrimp supplemented to *Artemia* nauplii did not vary from each other significantly on their effect on survival and metamorphosis to crablets.

The presence of *Artemia* in all treatments may have masked the possible effects of the supplemental diet. All feed combinations were able to support metamorphosis of megalopa to crab instar 1. This means that larvae that were able to metamorphose to megalopa stage are most likely to reach crablet stage. Also, the duration to reach crablet stage did not vary between diets (Table 2).

It is recommended, however, that since megalopa are already benthic in behevior and stays in the bottom of the container most of the time, minced or frozen *Artemia* should be given instead of live *Artemia* which actively swim about and hence are difficult for the megalopa to catch.

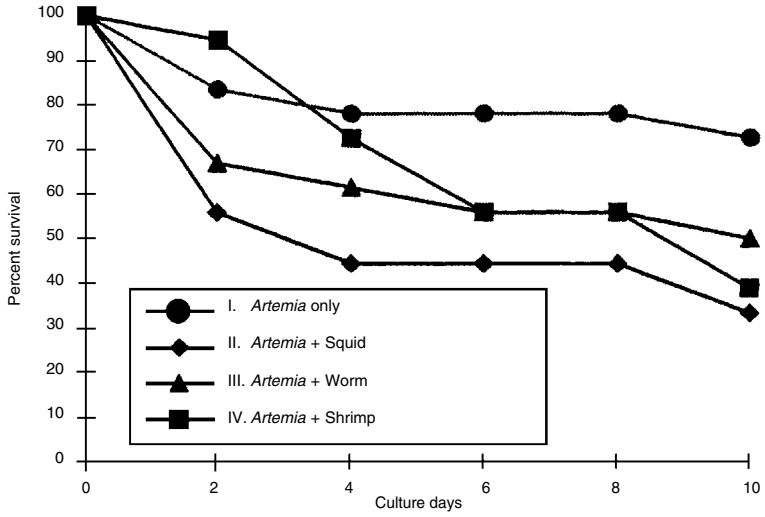


Figure 2. Survival of mud crab megalopa fed with different diets.

Table 2. Mean percent metamorphosis of megalopa to crablet stage.

Treatment	Mean % survival	Mean % crablet production based on premetamorphic survival	Mean % metamorphosis to crablet based on initial number	Number of days to produce crablets
I *Artemia* only	72.22	100	72.22	7
II *Artemia* + Squid	33.33	100	33.33	7
III *Artemia* + Worm	50	100	50	7
IV *Artemia* + Shrimp	38.89	85.7	33.33	7

Study 3. Minced shrimp, mussel and formulated diet on the growth of the crablets

Growth measurement of the crablet was based on the increase in the width of the carapace instead of weight. Growth of mudcrab in terms of size can only take place during ecdysis, the process of shedding off of old exoskeleton. According to a study made by Laviña (1980), body weight may either increase or decrease after moult or ecdysis hence, it is a less reliable factor for the growth measurement. Carapace length and width on the other hand, increase in every moult regardless of whether there is a decrease in weight prior to moult.

Table 3 shows that a high rate of growth from crab instar 1 to crab instar 2 was obtained on crablets fed with formulated diet alone and mussel and shrimp in combination with *Artemia.* From crab instar 2 to 4, moult increment became significantly higher on those crablets fed with shrimp and mussel supplemented to *Artemia* compared with those fed with *Artemia* alone and formulated diet alone. These results clearly suggest that from instar 1 to instar 4 stage, crablets prefer a diet composed mainly of bivalves and crustaceans, which must also be the diet of adult crabs. Hill (1979) has identified molluscan remains and crustacean remains as major stomach contents of adult *Scylla serrata* in Queensland. The study of Jayamanne and Jinadasa (1991) revealed the presence of small crustaceans, bivalves, gastropods, fish, plant matter, crab remains and sand in the food of juveniles and sub-adults S*cylla serrata* in the Negombo Lagoon in the west coast of Sri Lanka. Table 4 shows almost 100% survival in crablets reared from instar 1 to 4.

Table 3. Mean carapace width, intermoult duration and moult increment from Crab 1 to Crab 4.

Treatment	Crab 1		Crab 2		
	Carapace width (mm)	Intermoult duration (days)	Carapace width (mm)	Growth increment (CW, mm)	Intermoult duration (Days)
I *Artemia* only	3.18	4.67	4.72	1.54	4.67
II Mussel + *Artemia*	3.22	4.2	4.88	1.66	4.4
III Shrimp + *Artemia*	3.18	4.67	4.68	1.5	4.83
IV Formulated feed + *Artemia*	3.35	4	4.7	1.35	4.83
V Formulated feed only	3.12	4.67	4.83	1.71	6.2

Treatment	Crab 3			Crab 4	
	Carapace width (mm)	Growth increment (CW, mm)	Intermoult duration (days)	Carapace width (mm)	Growth increment (CW, mm)
I *Artemia* only	6.25	1.53	5	7.78	1.53
II Mussel + *Artemia*	6.5	1.62	5	8.98	2.48
III Shrimp + *Artemia*	6.22	1.53	4.5	8.41	2.19
IV Formulated feed + *Artemia*	6.37	1.77	5.67	8.87	2.5
V Formulated feed only	5.59	0.98	5.33	7.83	2.24

Table 4. Mean percent survival of crablets from Crab 1 to Crab 4, reared in individual containers and fed different diet.

Treatment	Crab I	Crab II	Crab III	Crab IV
I *Artemia* only	100	100	100	100
II Mussel + *Artemia*	100	100	100	100
III Shrimp + *Artemia*	100	100	100	100
IV Formulated feed + *Artemia*	100	100	100	100
V Formulated feed only	100	83.33	83.33	83.33

Study 4. Effect of stocking density on survival and metamorphosis of zoea 1 to zoea 2.

In larval rearing experiments conducted in 4 L containers conducted at UPV hatchery, a survival of up to 96% was obtained on zoea 1 stage prior to metamorphosis to zoea 2 stage, where larvae were fed with a combination diet of *Brachionus* and *Artemia* and reared at a stocking density 10 larvae/L.

This present experiment was conducted to find out if increasing the density of up to 50 larvae/L would affect survival of larvae in zoea 1 stage and their metamorphosis to zoea 2 stage. Three stocking densities of 10, 25 and 50 larvae/L were tested. Results in Table 5 show that there was no significant difference in the survival of zoea 1 stage and on their metamorphosis to zoea 2 in all stocking densities tested.

It is recommended therefore that a higher density of 50 larvae/L could be used in larval rearing of the mud crab and further studies should be done to determine effect of higher stocking densities of up to 200 larvae/L, on survival of larvae reared in bigger containers.

Table 5. Mean percent survival of zoea 1 and mean percent metamorphosis to zoea 2 in three stocking densities.

Stocking density	Mean percent survival of zoea 1 (Day 3)	Mean percent metamorphosis to zoea 2 (Day 5)
10 larvae/L	85.00	68.33
25 larvae/L	48.00	45.33
50 larvae/L	81.67	77.00

Study 5. Mass rearing of the larvae using green water

Mass rearing of the mud crab larvae was carried out in 100 L plastic containers to find out if adding *Nannochloropsis* algae on the culture water would improve survival of the mud crab larvae from zoea 1 stage up to megalopa, using *Brachionus* and *Artemia* as food. It has been reported that phytoplankton added to the culture water seemed to have a 'beneficial' effect in larval fish cultures in terms of survival by releasing oxygen into and removing certain metabolites like ammonia, from the culture medium. It was even suggested that phytoplankton also releases antibiotic substance into the culture medium. Brick (1974) tested the effect of *Chlorella* on the larviculture of the mud crab *Scylla serrata* and his results showed that addition of phytoplankton did not affect survival of the zoea although it stimulated production of megalopae.

Larvae in this present experiment were observed to be very active and they appear to like the high salinity of the culture water (35–36 ppt). The location of the experiment was an outdoor shed with plastic roofing, which provided enough light to culture containers. However, fluctuations in water temperature resulted in a sudden drop in the larval survival during the first 3 days of culture. This prompted a transfer of the set-up to a large rectangular fibreglass tank provided with water, to serve as a water bath. The condition was further aggravated by the very low dissolved oxygen concentration (3.0 mg/L) in the culture water due to very mild aeration provided on the first day. Results in Table 6 show no significant difference in larval survival and on metamorphosis to megalopa, in treatments without *Nannochloropsis* (3.5%) compared with treatment with *Nannochloropsis* (3.0%). This could be attributed to the collapse of *Nannochloropsis* culture, caused by the very high increase in salinity. The collapse of the microalgae also contributed to the fouling of the culture water. It is therefore recommended that *Tetraselmis* be used in subsequent experiments because these are easier to culture than *Nannochloropsis.*

Table 6. Percent survival of mud crab larvae cultured in green algae and without green algae.

Treatment	Culture days						% Metamorphosis to megalopa
	0	3	6	9	12	14	
I w/ green algae	100	12.1	7.2	6.0	4.0	3.0	1.0
II w/o green algae	100	28.0	13.6	10.0	6.5	3.5	0.3

Study 6. Effect of substrate and shelter on survival of crablets

Table 7 shows that crablets maintained in treatments with mud substrate and without shelter have higher survival (100%) compared with those crablets maintained in aquaria without substrate and with substrate and shelter.

The presence of shelter contributed to the fouling of the mud substrate. It is therefore recommended that mud substrate be provided to prevent cannibalism while the addition of shelters is no longer necessary.

Table 7. Effect of substrate and different kinds of shelter on survival of crablets.

Treatment	Substrate	Shelter	Day 0	Day 10	Day 20
I	None	None	100	83	33
II	None	Coconut leaves	100	100	67
III	None	Mangrove twigs	100	100	67
IV	Mud	None	100	100	100
V	Mud	Coconut leaves	100	83	83
VI	Mud	Mangrove twigs	100	83	33

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Preliminary Studies on Rearing the Larvae of the Mud Crab (*Scylla paramamosian*) in South Vietnam

Hoang Duc Dat[1]

Abstract

Larval rearing of the mud crab (*Scylla paramamosain*) was carried out in the COFIDEC hatchery (Cangio District, Ho Chi Minh City) and Hiep Thanh hatchery (Bac Lieu town, Minh Hai Province). The developmental period from Z1–crab 1 took 30 (29–31) days. The incubation period of the berried female crab is 10 days (9–11). The most suitable range of temperature is 28–30 °C, salinity 30 ppt (29–31) for the period of embryonic development and larval zoea stages; megalopae adapted to a salinity of 22–25 ppt. In these experiments, the food for zoea from stages 1 to 3 was diatoms, chlorella, rotifer (*Brachionus plicatilis*) and, in the final stages, it was rotifers and *Artemia* nauplii. The maximum survival rate of larvae attaining the first crab stage was 24%.

AMONG portunid crabs, the mud crab, genus *Scylla,* is subject to intensive fishing in areas where they are concentrated, such as estuaries and contiguous brackish water mangrove shores. Over-fishing has stimulated mud crab culture in some Southeast Asian countries.

Experiments on rearing larval stages to seed crabs under controlled conditions have been conducted in Malaysia, Australia, Philippines, China and India with varying degrees of success.

Review of the literature shows that there have been a few efforts in recent years to culture the larvae of mud crabs in other regions using a variety of techniques (Heasman and Fielder 1983; Marichamy and Rajapackiam 1992; Jamani 1992).

This report gives results obtained from crab larval culture conducted at the Department of Ecology and Development, Institute of Tropical Biology, in the COFIDEC hatchery, Cangio district, Ho Chi Minh city and Hiep Thanh hatchery in Bac Lieu town, Minh Hai province.

[1]Institute of Tropical Biology, 85 Tran Quoc Toan St. District 3, Ho Chi Minh City, Vietnam

Materials and Methods

Sea water supply and quality

Seawater was pumped from a 150 m deep well. The water was passed through a sand filter and settled overnight in receiver and sedimentation tanks. The water was then filtered through sand and active charcoal filters. In the hatchery tanks, the water was disinfected also by ultraviolet light. The parameters of water quality at the COFIDEC hatchery and Hiep Thanh hatchery were: salinity 29–32 ppt; pH 7.5–8.0; D.O. ≥ 5 ppm; temperature 28–31 ^{o}C.

In the controlled laboratory experiment, 103 female mud crabs were used for spawning. Female mud crabs were purchased from gill-net fisherman at the Can Thanh market (Can Gio district) and Bac Lieu town. The mud crabs were kept in tanks in filtered seawater. The weight of the crabs varied from 170–790 g per crab. The tank volume was from 4–8 m^3 and the depth of water 0.6–0.8 m. The water in the tank was changed 20–50% daily and replaced 100% weekly. Crabs were fed twice daily, once in the morning and once in the evening (at 0700 and 1900) with a diet of fresh clam meat (*Meretrix meretrix*), at a rate of 3–5% crab weight.

Eyestalk ablation was applied for stimulation of ovarian development and spawning. Berried crabs were kept isolated in separate tanks with volumes of 0.5–1 m^3 each, in seawater which was disinfected by

ultra-violet light and aerated. During the first few days, crabs were fed daily with fresh clam meat (*Meretrix meretrix* at 3–5% body weight).

However, later, crabs ate less so the feeding times were reduced to once every two days.

Larval rearing

1. Zoea

After hatching, the number of larvae was estimated and they were transferred to rearing tanks with a tank volume of 180–1800 L.

The environmental conditions were:

salinity 30 ppt ± 1;
temperature 29 °C ± 1;
pH 7.5–8.0;
DO ≥ 6 ppm.

Seawater was settled, filtered, and disinfected by ultra-violet light.

The stocking densities of the larvae were 60, 80, 100 and 150/L (optimum 80/L).

Zoea 1 (Z_1) to Zoea 3 (Z_3) were fed *Chlorella, Skeletonema costatum, Chaetoceros* and rotifers *(Brachionus plicatilis)* at a density of 15–25 pcs/mL. Z_4–Z_5 were fed *Artemia* nauplii (1 day-old) at a density of 5/mL.

2. Megalopa

The megalopae were reared in tanks with volumes of 2000–8000 L, and a salinity of 22–25 ppt.

Initial stocking density was 5–10/L. Megalopa and first crab (C_1) were fed *Artemia* nauplii (2–3 days-old) at a density of 3/mL and processed food with particle sizes of 300–500 μm.

Results and Discussion

Laboratory spawning

Of 103 female crabs selected with yellow eggs, 54 spawned (52.4%) (Table 1).

The spawning crabs had different egg qualities. In the experiment carried out in April and May 1994 (spawning season) at Hiep Thanh hatchery, 10 of 12 selected female crabs spawned after being kept in the tank 7–13 days.

These berried crabs had good eggs which 'stuck regularly and deeply in lower abdomen'.

In this experiment, it was also recognised that factors such as temperature and salinity had an important role in spawning and egg quality.

In this experiment, a female crab could spawn from 1 to 3 times per season.

Rearing berried crabs for collection of zoea 1 larvae

In embryonic development, the colour of fertilised eggs changed from light yellow to dark, grey, and finally black.

At the same time, larvae developed large black eyes and had a strong heart beat.

Hatching occurred after 10 days; the hatching period often lasted from 4–8 hours, but was longer than 20 hours in some cases.

The quality of zoea was not good in these cases and they often fell to the bottom of the tank.

The berried mud crab females (170–790 g) produced from 350 000 to 1 800 000 zoea.

Larval rearing

Shortly after hatching, the zoea are photosensitive and swim vigorously.

Zoea moulted four times to zoea 5, and then zoea 5 moulted into megalopa.

The metamorphic process of zoea 1 to megalopa lasted 17 days (16–19).

Megalopa swam but stuck easily to the sides of the tank wall or bed.

They actively fed, either on *Artemia* nauplii or processed food.

The salinity used for megalopa was 22–25 ppt. After 10 days (8–11), megalopa moulted and metamorphosed into the first crab stage.

These crabs swam quickly but their speed was less than for megalopa.

The crabs lived in the mud on the tank bottom and their carapace length was 2.5–3.0 mm.

Larval rearing experiments showed that zoea were highly sensitive to environmental factors.

In some of the first experiments, seawater was not sterilised by ultraviolet light and as a result, a lot of larvae were infected by *Zoothamnium* which parasitised the shell and gills and reduced the ability of Z1, 2 and 3 larvae to catch food.

After the zoea 5 metamorphose into megalopa, they may feed on younger larvae.

This may explain why the survival rate of zoea is reduced in the last zoeal period.

Therefore, survival may be improved if zoeal density is reduced, by increasing water volume in the tank and supplying more natural food.

There were 22 trials of larval rearing, 12 of them were unsuccessful, others had a 2–24% survival rate (Figures 1–7).

Table 1. Results of laboratory spawning.

No.	Size (cm)	Wt (g)	Stage of gonad	Date of culture	Date of spawn	Days of culture	Hatch date	Days to hatch	Number of zoea (1000s)
1	12.8	360	2	21/6/93	23/9/93	62	03/9/93	10	820
2	16.0	640	1	21/6/93	28/7/93	37	07/8/93	10	1600
3	13.3	350	1	21/6/93	20/8/93	60	31/8/93	11	650
4	13.3	350	1	21/6/93	30/7/93	39	10/8/93	10	710
5	13.0	490	2	21/6/93	15/8/93	53	26/8/93	11	580
6	13.2	375	2	21/6/93	23/7/93	32	3/8/93	10	620
7	16.9	790	2	5/7/93	24/10/93	79	4/11/93	10	960
8	14.9	450	1	5/7/93	10/8/93	35	21/8/93	11	740
9	14.2	430	2	5/7/93	15/8/93	40	25/8/93	10	1800
10	14.0	450	2	5/7/93	29/8/93	54	9/9/93	10	1400
11	14.0	450	2	5/7/93	18/9/93	68	29/9/93	11	860
12	13.0	390	2	27/8/93	16/9/93	20	26/9/93	10	570
13	14.0	480	2	27/8/93	20/10/93	54	30/10/93	10	1100
14	12.5	320	1	15/9/93	23/9/93	14	9/10/93	10	650
15	13.5	400	2	16/9/93	12/10/93	26	23/10/93	11	750
16	13.8	450	2	14/10/93	15/11/93	32	26/11/93	11	1300
17	14.2	490	2	17/10/93	30/10/93	13	10/12/93	10	800
18	13.6	400	1	17/11/93	10/12/93	18	21/12/93	11	720
19	12.5	250	1	22/12/93	5/1/94	14	16/1/94	11	640
20	14.6	590	1	22/12/93	20/1/94	29	31/1/94	11	840
21	12.9	350	2	15/1/94	15/2/94	31	26/2/94	11	650
22	13.5	450	2	15/1/94	12/3/94	56	23/3/94	11	420
23	13.8	450	2	28/2/94	21/3/94	21	1/4/94	10	500
24	13.8	450	1	5/3/94	28/4/94	53	8/5/94	10	1200
25	10.2	190	2	25/4/94	3/5/94	8	14/5/94	11	850
26	10.5	220	2	25/4/94	9/5/94	14	20/5/94	11	600
27	10.0	185	2	25/4/94	4/5/94	9	15/4/94	11	500
28	12.6	400	2	25/4/94	3/5/94	8	14/5/94	11	500
29	10.6	215	2	25/4/94	5/5/94	10	17/5/94	12	500
30	9.6	160	2	25/4/94	5/5/94	10	17/5/94	12	1500
31	10.0	165	2	25/4/94	1/5/94	7	11/5/94	10	1000
32	10.3	215	1	25/4/94	3/5/94	8	13/5/94	10	400
33	9.7	170	2	25/4/94	28/5/94	34	8/6/94	10	350
34	11.0	230	2	25/4/94	18/5/94	23	26/5/94	9	450
35	13.0	380	1	17/6/94	7/7/94	20	17/7/94	10	460
36	14.0	500	1	17/6/94	26/6/94	9	7/7/94	11	50
37	14.2	550	2	20/6/94	1/7/94	11	11/7/94	10	500
38	12.8	325	2	15/7/94	25/8/94	40	5/9/94	11	560
39	14.2	540	1	15/7/94	30/8/94	45	10/9/94	11	750
40	13.6	450	2	20/8/94	18/10/94	57	29/10/94	11	800
41	14.1	500	2	20/8/94	10/9/94	20	21/9/94	11	1100
42	13.5	440	2	20/8/94	12/10/94	53	24/10/94	12	950
43	14.6	480	1	18/9/94	30/9/94	12	10/10/94	10	650
44	12.3	310	1	18/9/94	25/10/94	37	5/11/94	11	450
45	13.8	490	1	18/9/94	5/10/94	17	15/10/94	10	740
46	13.5	420	2	26/10/94	12/11/94	17	23/11/94	11	860
47	13.7	450	1	26/10/94	18/11/94	22	29/11/94	11	540
48	13.7	490	1	15/10/94	10/12/94	45	21/12/94	11	600
49	11.2	250	1	15/10/94	18/12/94	33	29/12/94	11	640
50	13.2	410	2	15/11/94	24/12/94	39	4/1/95	11	750
51	11.8	260	2	20/11/94	3/1/95	44	13/1/95	10	550
52	12.5	350	2	15/12/94	16/1/95	32	27/1/95	11	840
53	15.2	670	1	15/12/94	30/12/94	15	10/1/95	11	1100
54	14.2	560	2	27/12/94	25/1/95	29	5/2/95	11	680

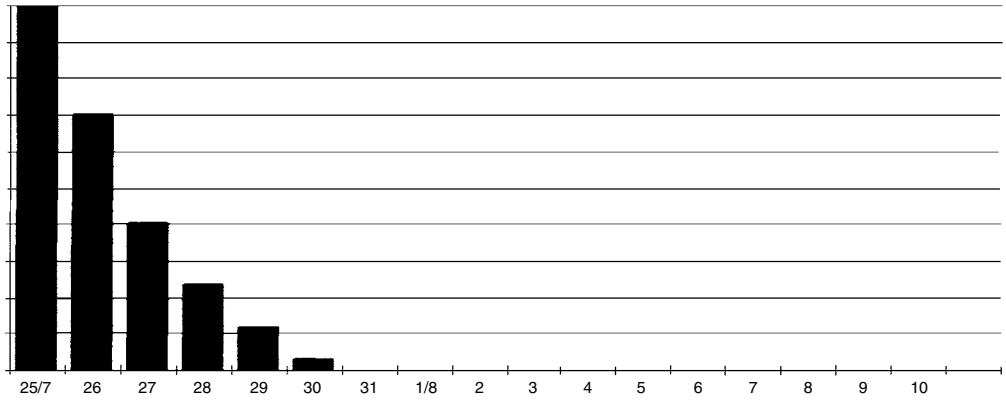

Figure 1. Survival of zoea (%) from experiment started 25/7/93.

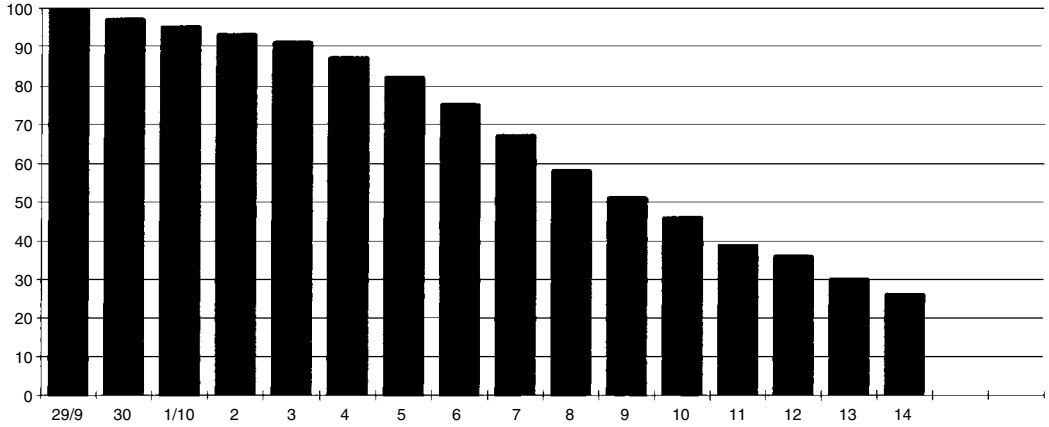

Figure 2. Survival of zoea (%) from experiment 29/9/93–14/10/93 (from zoea 1 to zoea 5).

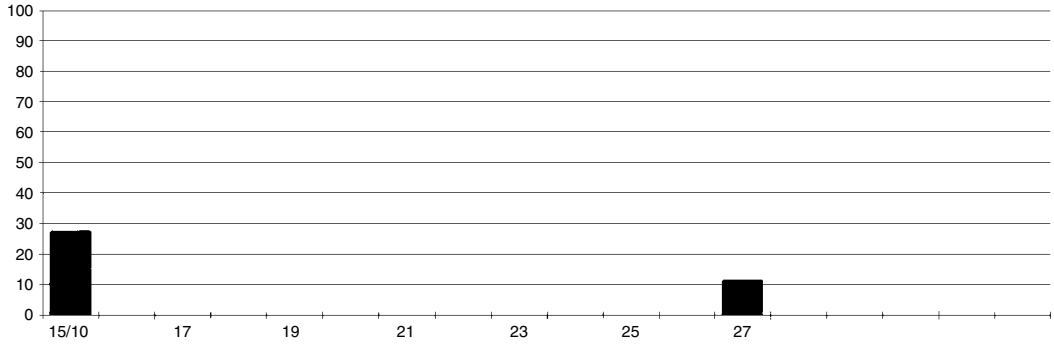

Figure 3. Survival to crab 1 (%) from 15–27/10/93 (from megalopa to crab 1).

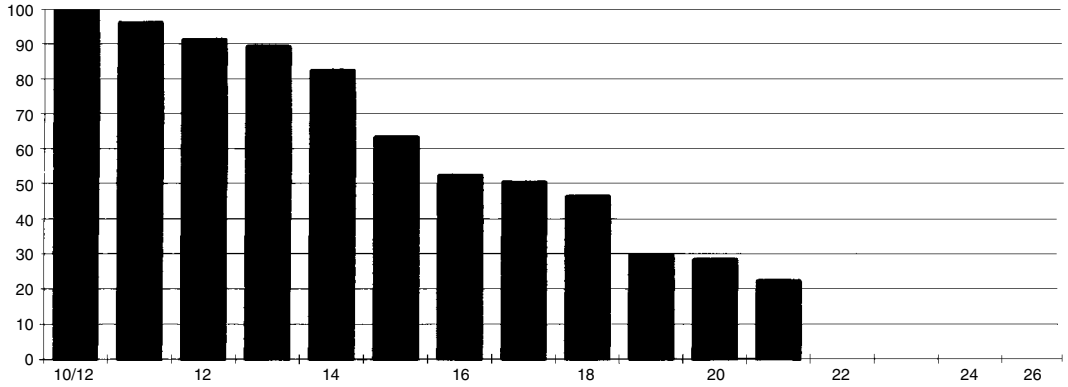

Figure 4. Survival of zoea (%) from experiment started 10/12/93 (from zoea 1 to zoea 5).

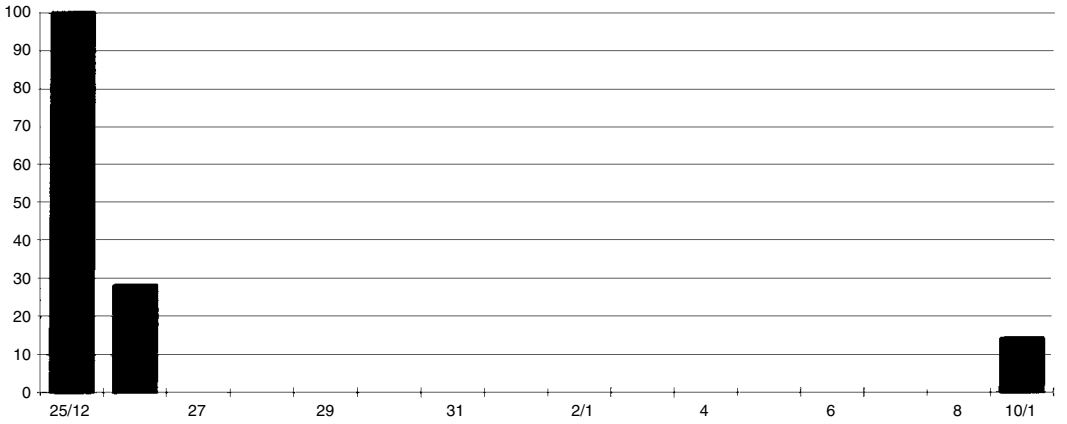

Figure 5. Survival to crab 1 (%) from 25/12/93–10/1/94 (from megalopa to first crab).

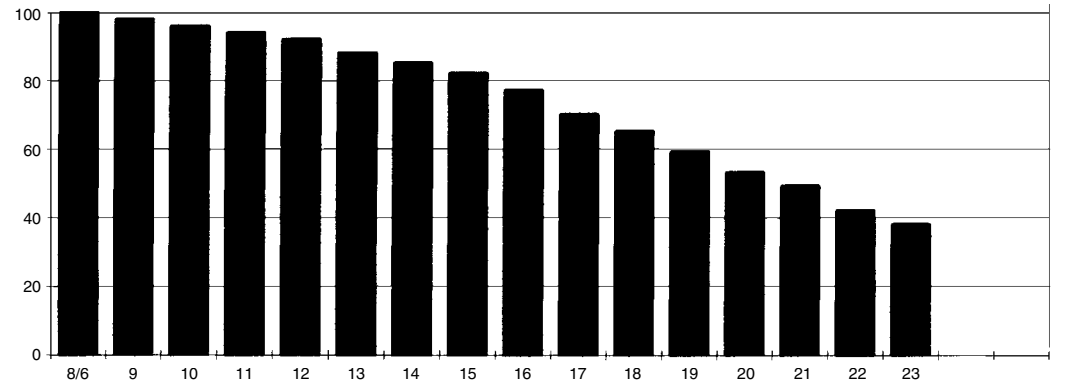

Figure 6. Survival of zoea (%) from experiment started 8/6/94 (from zoea 1 to zoea 5).

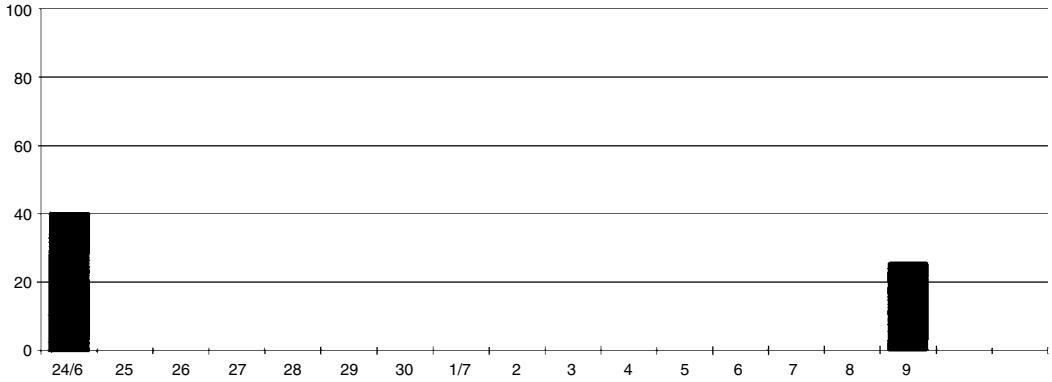

Figure 7. Survival of crab 1 (%) from 24/6–9/7/94 (from megalopa to first crab).

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Development of a Hatchery System for Larvae of the Mud Crab *Scylla serrata* at the Bribie Island Aquaculture Research Centre

David Mann[1], Tom Asakawa[1] and Morris Pizzutto[1]

Abstract

A practical hatchery system for the mass culture of the mud crab *Scylla serrata* has been developed at the Bribie Island Aquaculture Research Centre. The system encompasses the main phases of the hatchery cycle from broodstock management through to harvest of megalopae or juvenile crabs from culture tanks. This report is a brief description of current techniques and equipment used throughout the hatchery cycle.

HATCHERY production of mud crab, *Scylla* sp., crablets has been the subject of intensive research around the world for several decades. It is apparent, however, that still more research is required as production from hatcheries remains low and unreliable (Surtida 1997; Smullen 1997). With increasing interest around the Indo-West Pacific in growing mud crabs and problems with accessing juvenile crabs from the wild for growout in some areas, there is renewed impetus to develop a reliable practical method for hatchery production (Overton and Macintosh 1997).

The hatchery system for mass culture of mud crab (*Scylla serrata*) larvae currently used at the Bribie Island Aquaculture Research Centre (BIARC) evolved as research results and experience at BIARC accumulated and results from other centres became available. This process of evolution is continuing as further detailed research is undertaken.

The BIARC hatchery system outlined in this report represents a practical working model for the production and culture of mud crab larvae, but recognises that it forms a basic structure that will be further modified as current and future research further refine current knowledge. The term 'hatchery system' in the context used here encompasses five main phases of hatchery production.

[1]Bribie Island Aquaculture Research Centre, Queensland Department of Primary Industries, PO Box 2066, Bribie Island, Qld, 4507, Australia

This report outlines the basic methods employed for each of the five phases: broodstock; egg incubation; hatch; larval culture; and harvest. It also outlines the reason for adopting particular techniques. Exchange of results and experience with other researchers working on mud crab larval culture has made obvious the considerable variability of conditions under which larval culture is conducted. The techniques outlined here are developed for those conditions experienced at BIARC. While requirements and constraints differ between sites, for example, temperature maintenance, it is believed the general principles are broadly applicable.

The work conducted at BIARC within the current ACIAR project PN 9217 has clearly demonstrated the role of bacteria in larval mortality events of unknown aetiology and highlights the critical importance of hygiene. An experiment investigating the pre-treatment of raw seawater determined that disinfection or aging of even apparently high quality seawater is necessary to achieve acceptable survival (Figure 1).

Chlorination of 1 µm filtered seawater for at least 16 hours (overnight) at 10 ppm active chlorine was chosen as the standard seawater treatment method for BIARC. This method is the most effective, is relatively simple and cheap, and is already an accepted practice among prawn hatcheries. Following the water pre-treatment experiment, it was determined through experimentation that the dominant cause of larval mortality in cultures was bacterial in

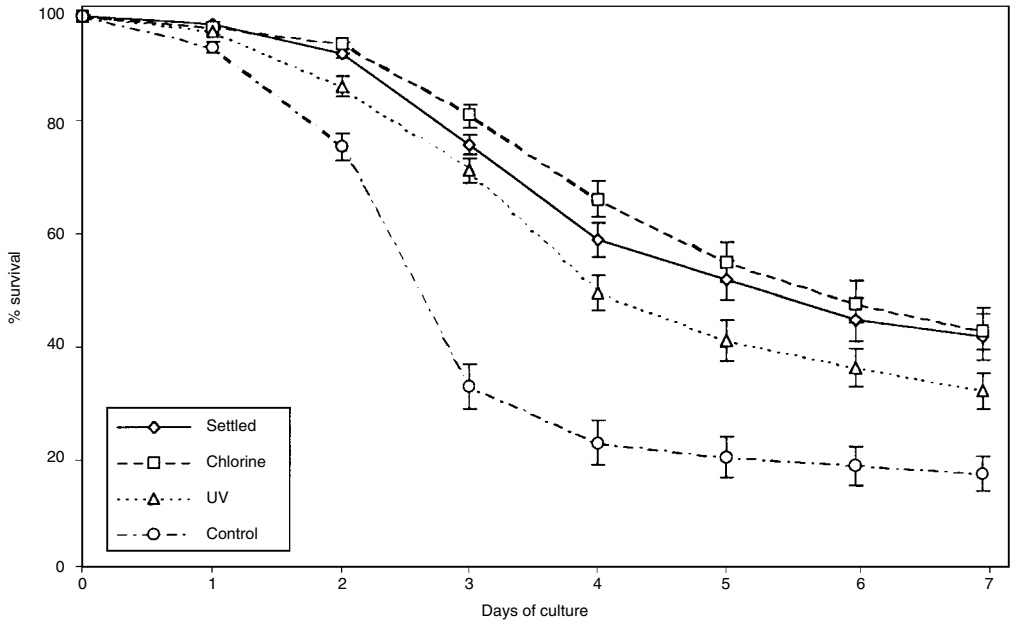

Figure 1. Influence of seawater pre-treatment on the survival of early stage mud crab larvae. Settled = seawater settled for 9–16 days; Chlorine = seawater disinfected with 5–10 ppm active chlorine overnight; UV = seawater passed through an ultraviolet radiation column; Control = seawater taken directly from the supply pipes.

origin. Both experiments combined demonstrate the importance of reducing the potential for contaminating larvae and cultures with pathogenic bacteria.

Highlighting the involvement of bacteria reinforced the commitment to reducing the risk of introducing potentially pathogenic contaminants into the entire hatchery system from broodstock to hatchery. To do this, there is the need to exercise a high degree of control over all inputs into the system. Many of the methods employed in the BIARC hatchery and outlined here are aimed at achieving this goal of greater microbial control.

At BIARC, regular bacterial monitoring of all the phases of the hatchery system is carried out to assess the efficiency of the systems in place. Both TCBS and marine agar plate media are used to estimate *Vibrio* and total heterotrophic bacterial numbers. Following is a brief description of each of the five phases of the mud crab hatchery system used at BIARC.

Broodstock

A standard circular broodstock tank with sand-covered bottom and flow through seawater was originally used to house broodstock through the maturation phase to spawning. This system was then modified to allow greater control over water quality; a diagram of the maturation system is included in Figure 2. Characteristics of the modified system include:

- Chemical pre-treatment of new broodstock. Crabs are disinfected at an average of 100 ppm formalin overnight.
- Reduced volume of sand. This allows for easier cleaning and replacement of the sand substrate, which is important, as crabs extrude eggs while partially buried in the sand.
- Large water volume. The system holds 8 tonnes of water.
- Low stocking density. Broodstock are held at no more than 1.5/m^2.
- Recirculation of water. Dependence on inflowing seawater is reduced by using a recirculation system incorporating a mechanical and biological filter and UV disinfection. Inflow of new seawater is reduced to 0% to 20% per day.
- Controlled feeding. The appropriate feeding level is assessed daily to prevent over-feeding and fouling of the system.
- Varied diet. The broodstock are fed a varied diet containing food groups that reflect their natural diet and includes prawns, bivalves, fish and squid.

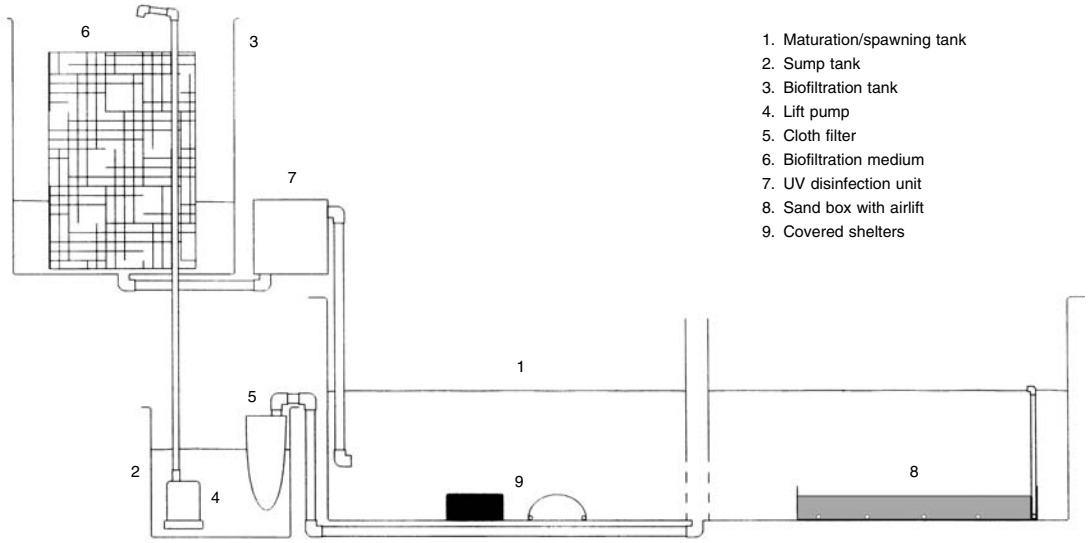

Figure 2. Maturation and spawning system for mud crabs used at BIARC.

Egg incubation

Spawned crabs are removed from the main broodstock tank immediately upon detection. The newly spawned crabs are placed into a system with a high rate of flow-through of UV disinfected water. This procedure has reduced the bacterial growth that occurs on the egg surface. No feed is supplied to the crabs during the 13–14 day incubation period to reduce the amount of particulate and dissolved organic material in the tank.

Hatch

The embryological development of the eggs is monitored so that the time of hatch can be predicted. One or two days prior to hatching, the berried crab is transferred to a hatch tank. The hatch tank holds 1000 L of water and receives a constant inflow of 1 μm filtered, UV-disinfected seawater at a high rate of between 700 and 1000% exchange per day. This provides a clean environment for the larvae to hatch into and reduces the potential for contamination with pathogens. The efficiency of the filtration and disinfection treatment of the water entering the tank is tested by bacterial plating and consistently contains no viable colonies on TCBS or marine agar plates.

Even with a high exchange rate it was found that the bacterial numbers in the tank begin to rise once hatching has occurred (Figure 3). To reduce the exposure of larvae to high bacterial levels, it is standard practice at BIARC to remove the required larvae from the hatch tank within the first hour of hatching.

Larval culture

Stocking culture tanks

After hatching, the aeration in the hatch tank is turned off for several minutes to allow the vigorously swimming, photo-positive larvae to aggregate at the surface, where they are collected. The larvae are then transferred to a plankton mesh screen, where they are slowly flushed for 20 to 30 minutes with culture tank water. The flushing reduces the amount of potentially contaminated hatch tank water that will be eventually transferred into the culture tank and also slowly acclimates the larvae to the new water.

Care is taken to reduce the physical and chemical shock, and therefore stress, that the larvae encounter in the handling and transfer process. Hatch tank and culture tank temperature is manipulated so that temperatures are within half of a degree and other water quality parameters are similar. Gentle handling reduces the physical turbulence that the larvae are subjected to. Although there are no supporting data available, it is expected that reducing the stress to the larvae at all times will reduce the incidence of stress-mediated susceptibility to disease. Temperature shock causing larval stress and mortality has been surmised when unintentional temperature fluctuations due to equipment failure has lead to abnormally high mortality rates. Temperature fluctuations of 5 °C over the range 23–28 °C within a daily cycle have been typically followed by dramatic mortality events.

To ensure larvae are stocked into the culture tank at the required density, counts are made of larval

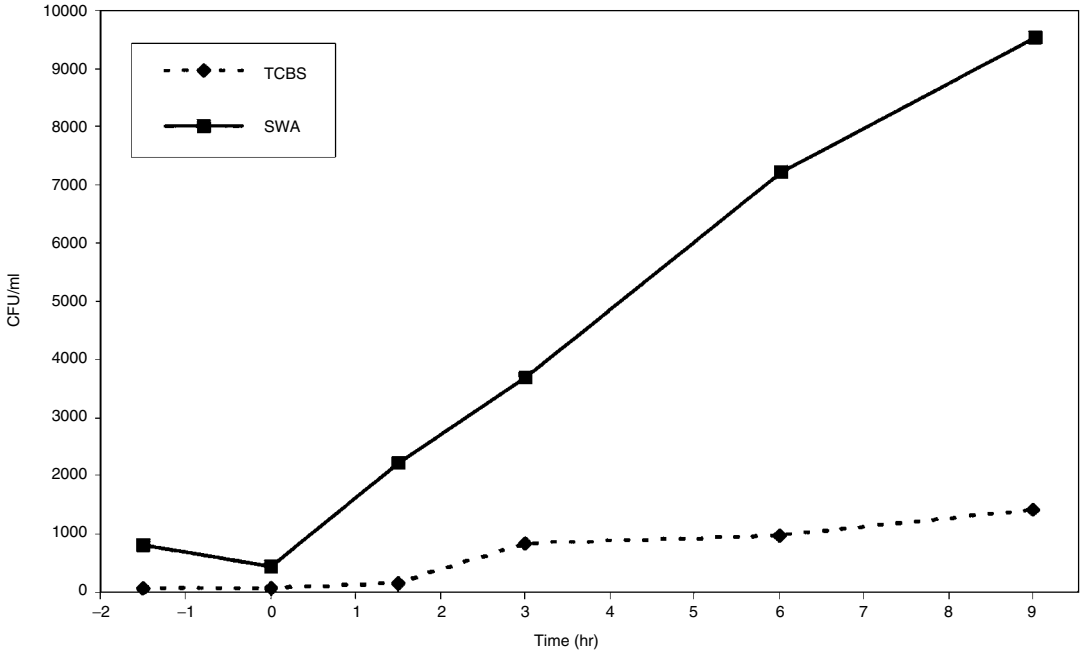

Figure 3. Changes in the bacterial flora of the seawater during hatching of mud crab larvae. Bacterial colony forming units (CFU) counted on seawater agar (SWA) and TCBS agar.

density in the flushing screen. The required volume of larvae concentrate is then transferred directly to the culture tank.

Culture tank design

The larval culture unit used at BIARC incorporates a primary culture tank and a recirculation tank. Seawater is circulated between the primary tank and the recirculation tank via an airlift and gravity flow. Figure 4 illustrates the system. The advantages of using the double tank system are:

- Manipulation of water quality takes place remote from the larvae and changes can be slowly infused into the culture environment.
- Heating of the culture takes place remote from the larvae. Experiments at BIARC demonstrated that even low watt density heaters (<35 k W/m^2) positioned in the culture tank cause larval mortality.
- Addition or removal of live feeds takes place in the recirculation tank, reducing the disturbance to the culture tank. For example, uneaten *Artemia* can be collected in a plankton bag secured over the inlet to the recirculation tank.
- Water exchanges can be introduced gradually into the culture tank to lessen shock to larvae and reduce the possibility of stress mediated infection and mortalities. The recirculation tank is 20% of culture tank volume, which allows up to 20% water exchange to be made at a time. When water exchanges are performed, the tank can be completely drained and cleaned if necessary.
- The recirculation tank acts as a settlement area for particulate material which can then be easily removed.
- A surface skimmer to remove organic pollutants or a biofilter can be added to the recirculation tank to treat water before it returns to the culture tank.
- The airlift pipe between the two tanks provides for extra gas exchange and no electrical pumps are required in the system.
- In smaller culture tanks of 1000 litres or less, aeration rates can be relatively low with most turbulence coming from a predominantly lateral flow generated by water flowing into the tank from the recirculation tank. This allows larvae more stability within the water column and the larvae are able to aggregate in areas where food density is highest. Water velocity can be adjusted for different larval stages.

Algae

A green water culture is maintained throughout the culture cycle. *Nannochloropsis oculata* is maintained at 5×10^5 cells/mL by addition of new algae on a

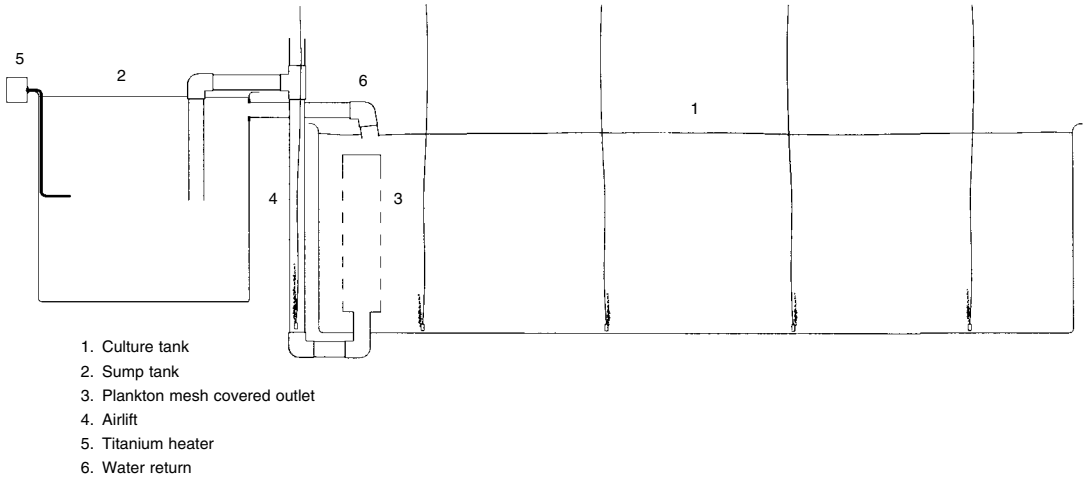

Figure 4. Larval culture system used at BIARC.

daily basis. The algae are added for their water 'conditioning' properties and for continuous enrichment of the live food in the system.

Tahitian *Isochrysis* is added to supplement *Nannochloropsis* in the culture from Z3 onwards to give further enrichment to the *Artemia* nauplii. T. *Isochrysis* was chosen as it is complementary to *Nannochloropsis* in its levels of the two critical fatty acids DHA and EPA, (T. *Isochrysis* is high in DHA and low in EPA while *Nannochloropsis* is the opposite) (Dunstan et al. 1993). All algae in mass production are cultured in filtered seawater disinfected with chlorine overnight. This reduces the potential for contaminants affecting the algae and of introducing bacterial or other potential pathogens into the culture system.

Food

Rotifers are the sole diet fed to the larvae up to the Z3 stage and are maintained in the culture system at 10–15 /mL. While rotifers remain in the system at lower densities beyond this stage, experiments conducted in the Philippines demonstrated that addition of artemia at least by Z3 improves the growth and survival of larvae. From the onset of Z3 newly hatched *Artemia* nauplii are fed to the tank at 0.5 to 3 /mL on a daily basis.

Artemia nauplii are fed to cultures at a level in excess of what the larvae will consume in one day. *Artemia* of 24 hours age or more are larger and more difficult to catch than newly hatched nauplii and if inadequate food is available for them their nutritional value to the larvae is greatly reduced. Prior to addition of new feed each day the *Artemia* remaining after 24 hours in the culture are removed from the system by collection in the recirculation tank.

At the end of the Z5 stage, larger on-grown *Artemia* are fed to the system to serve as food for the megalopae immediately following metamorphosis. The megalopa stage is readily able to catch and consume sub-adult *Artemia* and therefore *Artemia* of up to 7 days old are suitable prey.

Culture management

Bacteriological surveys of larval cultures at BIARC have determined that the bacterial community is very unstable during the first days of culture. Typically, levels of both total heterotrophic and presumptive *Vibrio* (TCBS counts) rapidly increase from culture initiation to day 2. From day 2 to around day 3 or 4, bacterial levels rapidly decline to reach a low base level. A typical pattern of change in bacterial numbers over time in a standard larvae culture is shown in Figure 5. Based on these results, it was decided to start the cultures 3 days before hatching of the crab larvae to be used. This way the larvae were not stocked into the cultures until the bacterial community had stabilised. The influence of this procedure on larval growth and survival has not yet been investigated through experimentation.

Further management strategies used in the culture of larvae are designed to maintain stability of the culture environment and reduce the potential for opportunistic pathogens to invade the system. While cultures are running well, water exchanges are kept at a low level that is sufficient to keep ammonia and nitrite within safe limits and is typically 10% to 20% per day. Exchange rates need to be increased up to

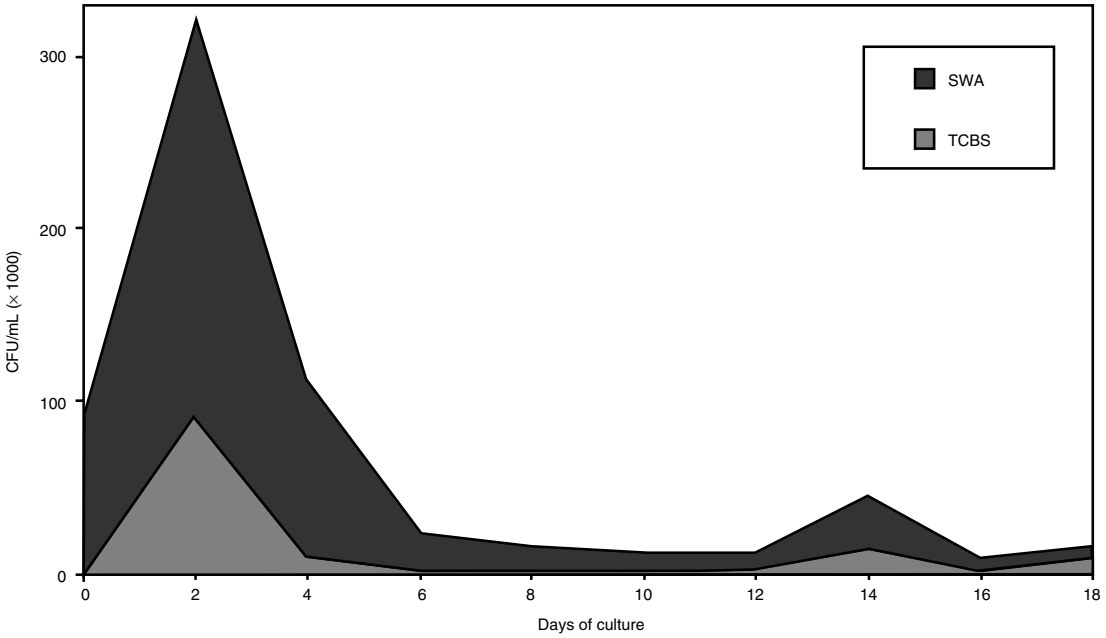

Figure 5. Changes in the bacterial flora throughout a mud crab larval culture cycle. Bacterial colony forming units (CFU) counted on seawater agar (SWA) and TCBS agar.

40% per day near the end of the larval cycle in order to maintain acceptable water quality.

The use of a biofiltration medium in the recirculation tank was investigated and found to act as an accumulator of particulate organic material. This is an advantage as it increases the ability to remove polluting organic material from the system and improve culture quality. The nitrification ability of the substrate was, however, limited due to the need to regularly clean it.

After larvae have metamorphosed to the megalopa stage, additional substrate is suspended within the culture tank to provide a surface for settlement of megalopa and crabs. The substrate is constructed of strips of shade cloth and flyscreen mesh, which is suspended vertically along a length of twine weighted at one end and with a float at the other.

Harvest

The settlement substrate added to the culture tank during the megalopa stage also acts as a convenient way to harvest settled megalopae and first stage crabs from the tank. Late stage megalopa and crab stages tend to remain clinging to the fibres as the substrate is gently removed from the tank and placed in a nursery tank. The remaining megalopae or crabs are drain harvested into a mesh cage.

The most appropriate time for harvesting from the larval culture tank that maximises survival has still not been rigorously investigated. Whether to harvest at megalopa or crab stage will also depend on the conditions to which they will be transferred. More work on this aspect is required.

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Effects of Density and Different Combinations of Diets on Survival, Development, Dry Weight and Chemical Composition of Larvae of the Mud Crab *Scylla paramamosain*

Chaoshu Zeng[1] and Shaojing Li[1]

Abstract

Studies on the effects of diet on survival, development and growth of larvae of the mud crab *Scylla serrata* showed that for early larvae (Z1 and Z2), rotifers (*Branchionus plicatilis)* are a suitable diet although their density significantly affected larval survival and development. All trials showed that larval survival and development steadily increased with density and at 60/mL, the highest survival of 94.7% to Z3 could be reached. However, for the later zoea (Z4 and Z5), feeding with rotifers alone resulted in mass mortality and delayed moult. When fed with *Artemia* only, newly-hatched larvae suffered from low survival, but for late larvae, it proved to be a good diet. A comparative study of replacing rotifers with *Artemia* at every zoeal stage showed that larvae initially fed with rotifers but then substituted with *Artemia* at Z2/Z3 or mixed at Z3 gave best overall zoeal survival. It is noteworthy that in treatments where rotifers were fed at late stages, some Z5 moulted to an extra Z6 stage before metamorphosis. Poor nutritional status during the zoeal stages may have delayed effects on the survival of megalopa. Daily measurement of larval dry weight (DW), carbon (C), nitrogen (N) and hydrogen (H) content showed that at Z2, there were no significant differences between larvae fed with rotifers or *Artemia*. However, as larvae entered Z3, those fed with *Artemia* had significant higher DW and C, H, N values. The gaps grew wider as larvae developed further and when fed with rotifers alone, DW and C, H, N of newly moulted megalopa were only 60–70% of those when *Artemia* was introduced at Z2 or Z3. As C, H, N percentages peaked at late Z5 and the newly moulted megalopa had the highest daily DW and C, H, N increase, a critical period of high nutritional requirements around first metamorphosis was indicated.

THE MUD crab, *Scylla* sp., a commercially important crab, is found throughout the southeast coasts of China and farming of the crab has a long history in the region. In recent years, growing market demands have sustained an increasing interest in expanding crab farming in the area. However, crab farming practice in China has so far depended exclusively on wild seed supply. Since the annual recruitment of natural seeds varies significantly and has limitations, development of a reliable hatchery seed production technique is clearly critically important for sustainable growth of the industry.

Although descriptions of larval morphology (Ong 1964; Huang and Li 1965) and studies of the effects of diet, temperature, salinity, water quality and antibiotics on survival and development of larvae of mud crab have been reported (Duplessis 1971; Brick 1974; Heasman and Fielder 1983; Zheng and Chen 1985), the available literature on the field is by no means extensive. Moreover, probably due to adopting different experimental protocols and/or working on different populations/species, these reports often did not agree, and this was particularly true for larval diets. There was clearly a need for further systematic investigation of larval cultural

[1]Department of Oceanography and Institute of Subtropical Oceanography, Xiamen University, Xiamen 361005, Fujian, P. R. China

biology and ecology with emphasis on nutritional requirements and metabolic mechanisms. This laboratory set such a goal some years ago and the current paper presents parts of the work on the effects of quality and quantity of diet on survival, development and growth of the larvae of the mud crab.

Materials and Methods

Survival and development experiments

Healthy newly-hatched zoea from female crabs, most likely *Scylla paramamosain* (Keenan et al. 1998), spawned in the laboratory were selected for the experiments. Three replicates were set up for all treatments and each replicate consisted of 25 larvae kept in a finger bowl (9 cm) filled with 150 mL sand-filtered seawater. No aeration was provided throughout the experiments. Larvae were transferred to a new container filled with fresh seawater and food daily when the number of dead and moulted larvae were recorded.

For treatments in which diets were changed at specific larval stages, larvae were transferred to another container and fed with the new diet as soon as they moulted to the designated stage; the remaining larvae were reared with the old diet until all had moulted or died. To avoid cannibalism, after larvae metamorphosed to megalopa, they were maintained individually in 60 mL plastic bottles and all were fed with *Artemia* ad lib. During the experiments, salinity varied between 26–31 ppt and temperatures ranged between 26–30 °C in May and June and 27–32 °C in August.

A series of three trials were carried out. In Trial 1, seven rotifer (*Brachionus plicatilis*) densities were used. Larval survival was best at 40/mL, the highest density set, but mass mortality occurred in all treatments at late larval stages. Therefore in Trial 2, density was increased up to 80/mL, along with a treatment of changing from rotifers to *Artemia* at Z4. Based on the first two trials, Trial 3 was more comprehensive and comprised 14 treatments which compared both density and diet combinations. For all trials, rotifers were cultured with *Chlorella* sp. while *Artemia* nauplii were hatched daily from Tianjing, China strain cysts. The cumulative survival rate (%) of a particular larval stage was calculated as the number of larvae moulted to the next stage/total larval number at the beginning of the experiment. Larval development was expressed as the mean intermoult duration of a larval stage and also as the mean cumulative development time for the stage.

Analysis of variance was performed to compare survival and development data (survival data were arcsin-transformed before statistical analysis) among the treatments; if the difference was significant ($P<0.05$), then Duncan's multiple range test was conducted to find out which treatments were different.

Measurement of dry weight (DW) and elemental content

Based on survival and development trials, three different feeding regimens were designed and larvae from each feeding condition were sampled daily for DW and C, H, N analysis. Larvae hatched from the same female were maintained in a series of 2.5 L glass containers (29 cm) and were fed with one of the following 3 diets:

A. Rotifers throughout all zoeal stages.
B. Z1, rotifers only; from Z2 on, *Artemia.*
C. Z1 and Z2, rotifers only; from Z3 on, *Artemia*.

For all three treatments, rotifer density was set at 60/mL and *Artemia* at 10/mL. After larvae moulted to megalopa, they were all fed with *Artemia*. Throughout the trial, water and food were changed daily when larvae were checked for moulting. Larvae that moulted on the same day were collected and transferred to new containers to allow sampling of larvae of the same developmental day. During the experiment, water temperature was maintained at 26–28 °C, and salinity at 25–28 ppt. Larvae sampled for analysis were first rinsed with membrane filtered (0.45 μm) seawater and then in re-distilled water. They were dried at 60 °C in an oven for 24 h prior to analysis. A Perkin-Elmer electronic balance and a model 240 Automatic Element Analyser were used for DW and C, H, N analysis.

Results

Survival and development experiments

In all three trials, larval survival and development showed a consistent relationship with rotifer density (Table 1). In Trial 1, Z1 survival increased from 0% to 68.3% as rotifer density increased from 2 to 40/mL, the trend remained the same for Z2 and cumulative survival to Z3 was highest at 40/mL, the highest density set for the trial. Analysis showed that differences between treatments were significant ($P<0.05$ or $P<0.01$).

In Trial 2, although larval survival was generally high, as density increased from 20 to 60/mL, cumulative survival to Z3 increased from 74.4% to 94.7%; these differences were significant (40 vs. 20, 30/mL, $P<0.05$; 60 vs. 40, 30, 20/mL, $P<0.01$). As density further increased to 80/mL, survival rate dropped slightly, possibly due to water quality deterioration caused by excess rotifers.

Trial 3 had the poorest survival, nevertheless, larval survival increased with density again, and Z1

survival jumped from 24.0% to 73.3% as density increased from 40 to 60/mL (Table 1); the results indicated that rotifer density was particularly critical for larvae of poor hatch quality.

Apart from survival, development of early larvae was also generally enhanced by an increase in rotifer density (Table 2). For Trial 2, at densities ≥60/mL, mean Z1 duration was about 0.5 day shorter than for those reared at densities ≤30/mL while cumulative development to Z3 was about 1 day shorter ($P<0.01$). The difference was also significant in Trial 3; at a density of 60/mL, Z1 mean duration was 1 day shorter than those at densities of ≤40/mL ($P<0.01$). Thus, rotifer density not only significantly affects survival, but also the development of early larvae of the mud crab.

Fed with rotifers alone, a few larvae could metamorphose to the megalopa stage, but mass mortality occurred at late zoeal stages (Table 1). Even when rotifer density was increased up to 200/mL, larval survival was not significantly enhanced (Trial 3, Table 1); thus, nutritional deficiency may be the reason for the mass mortality of late larvae.

Since rotifers were not a good diet for late larvae, in Trial 2 a treatment of replacing rotifers (40/mL) with *Artemia* at Z4 was established. Compared to treatments in which rotifers continued to be the larval diet, the mortality of late larvae was significantly reduced and overall zoeal survival rates reached 58.7% (Table 3), showing that *Artemia* was a suitable diet for late larvae. However, this raised the question as to when a diet change should take place. To answer this question, Trial 3, comprising 14 treatments, was designed and included replacement of rotifers by *Artemia* at each zoeal stage. The result of the trial showed that among all diet treatments, rotifer replacement by *Artemia* at Z2 or Z3, or mixed rotifer and *Artemia* from Z3 had the best overall zoeal survival (Table 3). Statistical analysis showed that survival using these three feeding regimens was significantly higher than others ($P<0.01$) while differences between them were not significant. At the same time, larval development using these three diet combination treatments was also significantly enhanced with the average zoeal development generally several days (3–7) shorter than other treatments (Table 4).

It was noted that in Trials 1 and 3, if rotifers were fed at late larval stages, a proportion of Z5 larvae moulted to Z6, an extra larval stage, before metamorphosis to megalopa. However, this did not occur in treatments in which *Artemia* was added at earlier larval stages and also in Trial 2 (Tables 1 and 3). This phenomenon does not appear to have been reported previously for *Scylla* sp.

Although megalopae from all treatments were fed with *Artemia* and maintained in identical conditions, megalopae from treatments to which *Artemia* was added no later than Z4 generally had higher survival rates (>80%) than those in which rotifers were still fed after Z4 (<50%) (Table 5). The results suggested that poor nutritional conditions during the zoeal stages might have delayed effects on megalopa survival.

Larval dry weight and elemental content experiment

Tables 6–9 show daily changes of dry weight and C, H, N content of larvae fed with three different dietary regimens. Comparing Z2 larvae of the same developmental day (Table 7), there were no significant differences in dry weight and C, H, N content of larvae fed with rotifers (Treatment A) and those in which rotifers were replaced by *Artemia* when they moulted to Z2 (Treatment B). However, as larvae entered Z3, DW and C, H, N content of larvae from Treatment B were significantly higher than those from Treatment A. Even for larvae from Treatment C, in which rotifers were replaced by *Artemia* only after they had moulted to Z3, by one day after the diet change, their dry weight and C, H, N were higher than those of Treatment A in which rotifers alone were fed.

However, compared to Treatment B which diet shifted earlier at Z2, the DW and C, H, N of Treatment C larvae were lower (Table 7). As larvae developed further, the gaps of DW and C, H, N content between Treatments A and B grew wider while those between Treatments C and B gradually closed up (Table 8). For newly moulted Z5 (day-0), the DW and C, H, N content were highest with larvae from Treatment B, lowest with larvae from Treatment A, with larvae from Treatment C in between but rather close to those of Treatment B (Table 8). For newly metamorphosed megalopa, the difference of larval DW and C, H, N content between Treatments C and B was negligible, but for Treatment A megalopa, their DW and C, H, N trailed far behind and were only 60–70% of those of Treatments B and C (Table 9).

During larval development, C, H, N percentages reached their highest level at late Z5 when metamorphosis to megalopa was about to take place (Tables 6–9). Meanwhile, the daily increase of DW and C, H, N was highest (70% increase), for newly moulted megalopa. The results indicated a particularly high nutritional demand around the time of first metamorphosis. It was also noted that the C, H, N percentages of newly extruded eggs were nearly double those of newly hatched Z1 (Table 6), suggesting that a high proportion of yolk reserves were consumed during embryonic development.

Table 1. Cumulative survival rates (%) of zoeal larvae of mud crabs fed at different rotifer densities.

Larval stage**	Rotifer density (individuals/mL)																				
	Trial 1							Trial 2						Trial 3							
	0	2	5	10	20	30	40	0	20	30	40	60	80	0	10	20	30	40	60	$60Z_3$ 100*	$60Z_3$ 200*
Z1	0	0	0	3.3	30.3	60.0	68.3	0	84.0	86.7	90.2	96.0	89.3	0	0	14.7	29.3	24.0	73.3		
Z2				1.7	10.0	45.0	55.0		74.4	78.2	87.3	94.7	89.3			2.7	9.3	10.7	57.3		
Z3				0	6.7	35.0	32.5		72.0	60.0	83.3	88.0	84.0			0	2.7	1.3	22.7	33.2	35.4
Z4					3.3	15.0	10.0		22.7	17.3	45.3	48.0	38.0				0	1.3	14.0	13.4	14.6
Z5(M)					1.7	3.4	3.3		1.3	2.7	9.3	17.8	20.0					0	5.1	8.6	7.3
Z5(Z6)					—	1.7	1.7		—	—	—	—	—						3.1	1.6	1.2
∑Z					1.7	5.0	3.3		1.3	2.7	9.3	17.8	20.0						6.6	8.5	8.5

*$60Z_3$100, $60Z_3$200: Larvae fed with 60/mL rotifer at Z1 and Z2, after larvae moulted to Z3, density increased to 100/mL and 200/mL respectively.
** Z5(M) : Percentage of larvae moulted to megalopa directly from Z5; Z5(Z6) : Percentage of larvae moulted to Z6 from Z5; ∑Z: Cumulative zoeal stage survival, including larvae moulted from megalopa from both Z5 and Z6.

Table 2. Mean intermoult duration and cumulative development time (mean and range) of zoea fed at different rotifer densities.

Mean stage duration and cumulative development time (day)		Rotifer density (individuals/mL)										
		Trial 2					Trial 3					
		20	30	40	60	80	10	20	40	60	$60Z_3$100*	$60Z_3$200*
Z1	Duration	4.7±0.1	4.7±0.2	4.4±2.0	4.2±0.1	4.2±0.1	5.0±0	5.3±0.3	5.4±0.8	4.1±0.2		
	(range)	(4-5)	(3-7)	(3-7)	(3-5)	(3-7)	(4-7)	(3-8)	(4-9)	(3-6)		
Z2	Duration	3.5±0.2	3.4±0.1	3.1±0.1	3.0±0.0	3.0±0.1	5.5	5.0	4.1±0.3	3.4±0.4		
	Cum. develop.	8.2±0.2	8.1±0.1	7.4±0.1	7.2±0.1	7.2±0.2	9.5	9.1±0.3	8.3±0.8	7.7±0.4		
	(range)	(7-12)	(6-10)	(6-9)	(6-8)	(6-9)	(9-10)	(7-10)	(7-11)	(5-11)		
Z3	Duration	3.4±0.2	3.3±0.1	4.2±0.1	4.1±0.2	3.9±0.2		5.0	5.0	5.7±1.0	4.3±0.2	4.3±0.3
	Cum. develop.	12.5±0.4	12.0±0.3	11.7±0.2	11.2±0.2	11.2±0.2		13.5	13	13.4±0.9	12.0±0.2	11.9±0.4
	(range)	(10-16)	(10-14)	(9-14)	(9-15)	(9-13)		(13-14)	—	(9-17)	(8-15)	(8-13)
Z4	Duration	5.4±0.9	4.8±0.6	4.3±0.4	4.9±0.7	5.5±0.3			6.0	4.1±0.6	3.5±0.5	3.3±0.5
	Cum. develop.	17.8±1.1	16.8±0.6	16.0±0.4	16.2±0.7	16.7±0.1			19	17.5±0.8	15.6±1.1	15.2±0.3
	(range)	(15-20)	(14-18)	(14-20)	(14-19)	(14-21)			—	(13-21)	(13-18)	(14-16)
Z5	Duration	—	—	5.6±0.3	4.8±0.20	4.1±0.1				7.7±1.1	5.7±1.0	5.3±0.2
	Cum. develop.	22	21.0	21.0	21.6±0.7	20.7±0.0				24.7±2.1	21.6±1.1	20.4±0.1
	(range)	—	—	(20-22)	(19-24)	(19-22)				(23-24)	(18-23)	(14-23)
Z6	Cum. develop.	—	—	—	—	—				26	19	20

*See legend to Table 1.

Table 3. Cumulative survival rates (%) of zoeal larvae of the mud crab under different dietary regimens.

Larval Stage**	Trial 2	Trial 3				
	Z1–Z3: Rotifer 40/mL Z4: *Artemia* (10/mL)	Z1: *Artemia* (10/m)	Rotifer 60/mL*			
			Z2A	Z3A	Z3A+B	Z4A	Z5A
Z1	90.2	4.0	73.3				
Z2	87.3	1.3	52.3	57.3			
Z3	83.3	1.3	41.9	52.6	50.2	22.7	
Z4	69.3	1.3	39.0	41.5	38.5	16.3	14.0
Z5(M)	58.7	1.3	33.0	27.7	32.3	8.7	4.7
Z5(Z6)	—	—	—	—	—	3.9	7.0
∑Z	58.7	1.3	33.0	27.7	32.3	10.9	10.4

*Z2A, Z3A, Z4A, Z5A: Larvae initially fed with 60/mL rotifer, changed to *Artemia* (10/mL) at Z2, Z3, Z4, Z5 respectively. Z3A+B: Started from Z3, larvae fed with 5/mL *Artemia* + 40/mL rotifer.
**See legend of Table 1.

Table 4. Mean intermoult duration and cumulative development time (mean and range) of zoea under different dietary regimens.

Mean stage duration and cumulative development time (day)		Trial 2	Trial 3					
		Rotifer 40/mL Z4: *Artemia* (10/mL)	Z1: *Artemia* 10/mL	Rotifer 60/mL (see legend to Table 3)				
				Z2A	Z3A	Z3A+B	Z4A	Z5A
Z1	Duration	4.4±0.2	4	4.1±0.2				
	(range)	(3–7)	(3–5)	(3–6)				
Z2	Duration	3.1±0.1	3	2.7±0.1	3.4±0.4			
	Cumulative develop.	7.4±0.1	6	7.0±0.2	7.7±0.4			
	(range)	(6–9)	—	(5–11)	(5–11)			
Z3	Duration	4.2±0.1	4	3.1±0.1	3.1±0.2	2.9±0.2	5.7±1.0	
	Cumulative develop.	11.7±0.2	10	10.1±0.0	10.8±0.1	10.6±0.2	13.4±0.9	
	(range)	(9–14)	—	(8–11)	(8–13)	(8–12)	(8–15)	
Z4	Duration	3.7±0.1	3	3.2±0.1	3.0±0.1	2.9±0.4	3.4±0.5	4.1±0.6
	Cumulative develop.	15.5±1.0	13	13.3±0.1	13.8±0.2	13.5±0.2	16.7±1.0	17.5±0.8
	(range)	(13–18)	—	(11–14)	(11–17)	(12–18)	(15–21)	(13–21)
Z5	Duration	4.3±0.0	4	4.6±0.1	4.1±0.6	3.6±0.5	4.7±1.6	4.7±1.4
	Cumulative develop.	19.9±1.1	17	17.9±0.2	17.9±0.5	17.1±0.3	22.2±2.0	22.8±0.3
	(range)	(17–22)	—	(14–23)	(15–23)	(15–19)	(21–23)	(18–24)
Z6	Cumulative develop.	—	—	—	—	—	22–25	21–22
	(range)						27–29	25–27

Table 5. Survival and development of mud crab megalopa after different dietary treatments during the zoeal stages.

Dietary regimen at zoeal stage*	Trial 2						Trial 3								
	Rotifer density (ind/mL)					Z1–3: Rotifer Z4A	Z1A 10 ind/mL	Rotifer 60 ind/mL							Rotifer 60/mL
	20	30	40	60	80			Z2A	Z3A	Z3A+B	Z4A	Z5A	$Z_3$100	$Z_3$200	
No. of megalopa	1	2	7	12	15	4	1	26	21	25	8	8	7	6	4
% survival	0	0	14.3	50.0	33.3	95.4	100	88.5	71.4	84.0	87.4	50.0	28.6	60.7	50.0
Develop. time			7	7.6±0.8	8.3±0.1	7.6±0.8	11	11.2±1.2	11.1±1.6	10.9±1	11.6±0.9	11.5±1	11.5	11.5±0.6	—
Range (days)			—	7–9	8–10	6–9	—	9–15	9–15	9–13	11–13	11–13	11–12	11–12	9–15

*Z2A, Z3A, Z4A, Z5A: Larvae initially fed with 60/mL rotifer, changed to *Artemia* (10/mL) at Z2, Z3, Z4, Z5 respectively.

Z3A+B: Started from Z3, larvae fed with 5/mL *Artemia* + 40/mL rotifer.

60$Z_3$100, 60$Z_3$200: Larvae fed with 60/mL rotifer at Z1 & Z2, after larvae moulted to Z3, density increased to100/mL and 200/mL, respectively.

Table 6. Dry weight and C, H, N content of newly extruded eggs and Z1 larvae of the mud crab.

	Egg	Zoea 1						
Develop. day	0	0	1	2	3	4	5	6
No.of larvae	—	100	80	80	80	80	80	60
DW (µg/ind)	—	10.7	15.2	16.5	17.3	16.5	15.6	16.9
C (µg/ind)	—	2.80	4.33	4.88	5.69	6.05	4.81	5.06
C % DW	54.06	26.19	28.49	29.56	32.88	30.61	30.86	29.95
H (µg/ind)	—	0.36	0.48	0.68	0.71	0.72	0.44	0.66
H % DW	7.78	3.36	3.17	4.12	4.12	4.33	2.83	3.89
N (µg/ind)	—	0.65	1.02	1.09	1.34	1.28	1.19	1.24
N % DW	10.4	6.08	6.73	6.63	7.76	7.75	7.61	7.33

Table 7. Dry weight and C, H, N content of Z2 & Z3 mud crab larvae under different dietary regimens.

Larval stage	Zoea 2							Zoea 3									
Diet regimen*	A				B			A				B			C		
Develop. day	0	1	2	3	1	2	3	0	1	2	3	0	1	2	1	2	3
No. of larvae	60	50	50	45	45	45	45	30	30	30	30	30	25	25	25	25	25
DW (µg/ind)	22.0	24.9	26.2	27.5	22.4	24.5	29.7	34.7	39.8	41.2	47.1	53.9	59.6	68.4	45.7	57.6	50.6
C (µg/ind)	6.14	7.42	8.26	8.48	6.72	7.93	8.53	9.92	12.70	13.01	15.17	15.84	20.07	24.32	14.22	20.45	17.62
C % DW	27.89	29.81	31.59	30.83	30.03	32.40	28.72	28.59	31.92	31.57	32.21	29.38	33.56	35.55	31.11	35.51	34.82
H (µg/ind)	0.67	1.03	1.07	1.17	0.92	0.89	1.17	1.36	1.37	1.41	2.14	2.18	2.90	3.56	2.00	2.97	2.47
H %DW	3.04	4.12	4.11	4.27	4.11	3.63	3.95	3.91	3.45	3.42	4.55	4.05	4.85	5.20	4.38	5.15	4.89
N (µg/ind)	1.33	1.73	1.88	2.02	1.57	1.86	2.03	2.48	2.95	3.14	3.64	3.43	4.37	5.62	3.19	4.66	4.08
N % DW	6.06	6.95	7.20	7.33	7.00	7.59	6.82	7.15	7.41	7.61	7.73	6.37	7.30	8.22	6.98	8.09	8.07

*A: Larvae fed with rotifers (60/mL) throughout zoeal stage; B: Z1 60/mL rotifer, Z2 onward 10/mL *Artemia*; C: Z1 and Z2 60/mL rotifer, Z3 onward 10/mL *Artemia*.

Table 8. Dry weight and C, H, N content of Z4 and Z5 mud crab larvae under different dietary regimens.

Larval stage	Zoea 4									Zoea 5							
Diet regimen*	A				B	C				A			B	C			
Develop. day	0	1	2	3	0	0	1	2	3	0	1	3	0	0	1	2	3
No. of larvae	15	10	10	10	15	15	10	10	10	8	5	5	6	6	5	5	5
DW (µg/ind)	69.4	87.8	102.0	90.6	109.9	79.9	112.4	120.6	170.3	151.0	185.0	154.0	241.0	220.5	291.0	304.7	333.7
C (µg/ind)	20.48	27.28	32.37	31.00	34.15	24.11	40.76	43.72	61.15	44.30	59.83	55.06	79.63	71.12	103.5	116.6	123.7
C % DW	29.51	31.07	31.74	34.22	31.07	30.48	36.26	36.25	35.91	29.34	32.34	35.75	33.01	32.26	35.55	38.26	37.07
H (µg/ind)	2.87	3.94	3.21	2.96	4.93	3.43	5.90	6.33	8.92	5.86	6.12	7.02	11.16	10.14	12.34	13.62	18.49
H % DW	4.14	4.49	3.15	3.27	4.49	4.34	5.25	5.25	5.24	3.88	3.31	4.56	4.63	4.60	4.24	4.47	5.54
N (µg/ind)	4.61	6.23	7.90	7.21	7.80	5.56	8.96	9.83	13.83	9.80	13	16.72	17.59	15.46	23.25	27.94	28.80
N % DW	6.64	7.10	7.38	7.96	7.10	7.03	7.97	8.15	8.12	6.49	7.41	8.32	7.30	7.01	7.99	9.17	8.64

*See legend to Table 7.

Table 9. Dry weight and C, H, N contents of mud crab megalopae after different diets during the zoeal stages.

Larval stage	Megalopa										
Dietary regimen*	A	B	C								
Develop. day	0	0	0	1	2	3	4	5	6	7	8
No. of larvae	4	4	4	3	3	3	2	2	2	2	2
DW (µg/ind)	258.0	386.0	373.5	639.5	784.0	807.1	877.0	1002	952.2	1112	1234
C (µg/ind)	81.22	120.59	116.12	202.53	255.04	263.44	299.58	345.89	332.41	394.43	450.53
C % DW	31.48	31.24	31.09	31.67	32.53	32.64	34.16	34.52	34.91	35.47	36.51
H (µg/ind)	11.04	17.02	16.17	25.58	34.42	31.15	35.17	41.48	47.04	52.04	59.85
H % DW	4.28	4.41	4.33	4.00	4.39	3.86	4.01	4.14	4.94	4.68	4.85
N (µg/ind)	19.92	30.19	28.20	44.64	55.19	54.56	66.13	77.35	—	92.30	106.12
N % DW	7.72	7.82	7.55	6.98	7.04	6.76	7.54	7.72	—	8.30	8.60

*See legend to Table 7.

Discussion

Survival and development experiments

The largely passive feeding behaviour of early larvae of the mud crab may explain the significant effect of rotifer density on their survival and development. It was observed that capture of food by early larvae of the crab was basically by chance during their frequent tail flipping behaviour; the food item was then held by the forked tail and passed to the mouth parts for consumption. Apparently, higher food density would increase the chance for larvae to encounter and capture food organisms, thus, enhancing survival and development. Moreover, increasing physical contact between food items and larvae as density increased may also stimulate larvae to increase their tail flipping frequency (Heasman and Fielder 1983).

Evidence from the feeding rate experiment showed that at a low *Artemia* density (2/mL), daily feeding rates of early larvae were substantially lower than those at higher densities (>10/mL). When larvae were kept individually in small bottles filled with only 20 mL water, it was shown that even though at low density when larvae were starved, there was always some *Artemia* left over in the water column the next day. The situation was different for megalopae if they were fed with low density *Artemia*, normally there was no *Artemia* left the next day (Zeng 1987). The result suggested that as larvae developed, their feeding behaviour changed from a passive pattern to a more active pursuit and capture. Thus, for later larvae, the total amount of food available rather than density appeared to be more important.

Although the trend of an increase in larval survival with rotifer density was consistent in all batches of larvae tested, the significance of rotifer density in improving larval survival seemed to vary from one batch to another. It appeared that with high quality batches of larvae, even at low rotifer density, larval survival could be reasonable, thus, any improvement is limited. However, for those larvae hatched with poor quality, maintaining a higher rotifer density appears crucial for larval survival. For example, at a rotifer density of 20/mL, Z1 survival reached 84.0% in Trial 2 but it was only 14.7% in Trial 3. Increasing density to 60/mL led to an increase of survival from 84.0% to 96.0% in Trial 2 and a substantial increase of survival from 14.7% to 73.3% in Trial 3. Also, it appeared that for larvae of Trial 2, if Z3 were fed with a high density of rotifers (>40/mL), there could still be healthy survival (>80%) to Z4 which but did not occur in Trial 3 (Table 1). There were also noticeable variations in development time among different batches of larvae (Tables 2 and 4). Therefore, the vast variation in quality of larvae hatched from different females may partially explain the diverse results reported from previous studies on larval diets of the crab (Ong 1964; Duplessis 1971; Brick 1974; Heasman and Fielder 1983; Zheng and Chen 1985) and such phenomenon should also be taken into consideration in hatchery practice. Broodstock quality, egg incubating conditions and seasonal factors are all possible contributors to this variation.

It has been generally agreed that *Artemia* nauplii are a good diet for later larvae of the mud crab (Duplessis 1971; Brick 1974; Heasman and Fielder 1983; Zheng and Chen 1985). However, they may not be a suitable diet for early larvae. With their relatively larger size and faster swimming ability, *Artemia* as the sole diet for early larvae generally yield unfavorable survival rates compared to rotifers. Early larvae seem unable to capture and digest *Artemia* as effectively as rotifers. It was often found in these trials that Z1 larvae held *Artemia* for a long time but finally abandoned them. The abandoned *Artemia* usually only had the head or appendages removed. Using *Artemia* strains having newly-hatched nauplii of a smaller size may result in better results. Rigid control of water quality seems also to improve survival (Brick 1974; Heasman and Fielder 1983).

Present results also indicated that poor nutritional status during the zoeal stages might have delayed effects on the survival of megalopa. As newly metamorphosed megalopa have the highest growth rates (Table 9), larvae with nutritional deficiencies may not be able to pass this critical point. This may partially explain the often-found mass mortality at this time. The results suggested that more attention should be paid to the nutritional links between consecutive larval stages.

Larval dry weight (DW) and elemental content experiment

Larval dry weight (DW) and elemental content analyses showed that for Z2 larvae, whether fed rotifers or *Artemia*, there were no significant differences in their dry weight and C, H, N content. This suggested that for Z2 larvae, nutritional requirements could still be met by rotifers. However, as larvae entered Z3, DW and C, H, N content of larvae fed with *Artemia* were higher than those fed with rotifers and as larvae developed further, the gaps grew wider. The results indicated that starting from Z3, rotifers gradually lose their ability to fully satisfy larval nutritive demands and should be replaced.

The DW and C, H, N of larvae which were first fed Artemia at Z3 were initially lower than those in which diet shifting took place at Z2. However they caught up during later stages and at the time of metamorphosis there was no significant difference between the two. The results suggested that larval nutritional deficiency can be compensated if a high nutrition diet was provided not too late in development. Evidence from the feeding rate experiment also showed that after their diet change to *Artemia* at Z3, under comparable conditions, larvae fed with rotifers initially, had higher daily feeding rates than those fed *Artemia* since hatching (Zeng 1987).

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LARVAL ECOLOGY AND NURSERY

Quality Control Using Hazard Analysis Principles for Mud Crab Culture

Alan Blackshaw[1], David Mann[1] and Clive P. Keenan[1]

Abstract

A quality control system using hazard analysis principles has been developed for mud crab culture. It is based on the examination of flow charts from which are constructed charts containing critical steps, hazards, risks, critical control points, monitoring systems and corrective actions for each hazard.

A CONCEPT of quality control of processes and products was developed by W.E. Deming (Deming 1986). For the food industry, microbial quality control is of the utmost importance (Sumner 1995).

In mud crab culture, reports of high survival rates of larvae are sporadic and generally unpredictable, reflecting the need for intensive research and quality control. This commentary considers the whole process of mud crab culture and applies quality control concepts to industry development.

During culture, there are many hazards and associated risks, often poorly documented. This lack of knowledge concerning potential and actual hazards is an impediment to quality control, but the ability to quantitatively monitor each phase enables definition of the steps that may be critical to culture success.

Performance is a criterion of quality and sequential culture parameters are the number of fertilised eggs, their hatch rate, larval survival, the production rate of megalopae, young and sale crabs.

A significant development in quality control in the food industry has been the Hazard Analysis Critical Control Point (HACCP) method which examines in sequence and in detail the characteristics of process flow charts and codifies what is happening; when, where, why and how, and specifies corrections as needed.

[1]Bribie Island Aquaculture Research Centre, Queensland Department of Primary Industries, PO Box 2066, Bribie Island, Qld 4057, Australia

Mud crab/prawn culture is too imprecise to set up a true HACCP system. Not all the hazards are known and many critical control points are not clearly defined. HACCP principles are used to maintain 'quality' in the animal production process by locating principle hazards and trying to control them.

Quality is defined as high spawning, fertilisation and survival rates, successful metamorphosis and good growth. Quality assurance is based on good husbandry practices.

HACCP operates under 7 principles:

1. Identify and assess all hazards;
2. Identify the critical control points;
3. Identify the critical limits;
4. Establish monitoring procedures;
5. Establish corrective actions;
6. Establish a record-keeping system;
7. Establish verification systems.

HACCP works through a process flow diagram, identifies critical steps in the system, monitors the process and develops preventive and/or corrective strategies. It also develops specifications or requirements for the stages of the process which in crab culture may include:

- acceptable limits for tank temperatures;
- water quality limits;
- absence of contaminants;
- absence of specific organisms;
- equipment performance.

Specifications or process requirements should possess attributes which are clearly identified:

Attribute	Quality	
	Good	Bad
Record	Written	Verbal
Named	Identifiable	Anonymous
Status	Signed agreement	Nil
Realism	Within resources	Unknown
Continuity	All aspects covered	Erratic flow
Detail	Clear understanding	Unclear
Review	Regular review	None/irregular

In considering mud crab culture, the aim is to identify and assess all potential hazards and to determine the likelihood (risk) that a particular hazard will occur. Hazards can be classified:

1. Biological — presence of viruses, fungi, protozoa, bacteria;
2. Chemical — changes in pH, toxic substances;
3. Physical — changes in temperature, oxygen content, salinity, light intensity;
4. Operational — process failure — heaters, pumps, operator, nutrition.

The complete process of mud crab culture begins with the acquisition of broodstock and ends with the production of saleable crabs. Three hazard groups are identified, in each of which critical control points will determine relative success or failure.

These hazard groups and some associated biological profiles are:

1. Broodstock:
   - physical condition and nutritional state;
   - reproductive state;
   - spawning success and fertilisation of ova;
   - brooding, development of embryos, hatching of prezoea.
2. Larvae:
   - normal structure and viability;
   - normal moulting capacity.
3. Immature crabs:
   - physical condition and nutritional state.

Interacting with the basic biology of the adult crab, eggs, larvae and immature crabs are many factors, only some of which have been investigated. These include:

1. Nutrition:
   - feed form – appearance, texture, live or dead;
   - nutritional content.
2. Physico-chemical aspects of seawater:
   - temperature, salinity, pH, oxygen content;
   - toxins – ammonia, nitrite, pesticides, heavy metals.
3. Light:
   - photoperiod, intensity, wavelength.
4. Facilities:
   - tank design and construction;
   - aerosol and splash protection;
   - filtration of air and water;
   - water treatment.
5. Husbandry:
   - water exchange, air supply;
   - feed type, frequency, density;
   - tank cleaning.
6. Microbiological environment:
   - viruses;
   - fungi, bacteria and protozoa;
   - multicellular parasites.

Flow Chart for Mud Crab Culture

To facilitate the assessment of hazards and their risk, a process flow diagram has been constructed, listing factors interacting with the culture and production process.

Broodstock

1. Capture broodstock

Microbiological samples*	Assess quality	Size/weight**
Samples for viral PCR**		Injuries
Antibiotic/antifungal baths***		

2. Maintain broodstock

Isolate from other broodstock**	Density*
Substrate*	Shelter**
Biofilter(s) ***	UV water**
Salinity *	Temperature**
Photoperiod	Light intensity**
Ovarian biopsy***	Eyestalk ablation*
Microbiological sampling**	Water quality***

3. Spawning

Isolation from other crabs**	Individual water supply**
	Microbiological sampling***
Biopsy of egg mass**	Crab/egg mass disinfection***

4. Hatching

Sterilise hatch tank***	UV water flow-through***
Microbiological sampling**	Disinfection**

5. Larvae

Microbiological sampling**	Evaluation**
Larval washing***	

6. Larval culture

Microbiological sampling***	Tank sterilisation***
UV/Cl water***	
Antisplash covers***	Biological filters***
Individual sterile equipment***	Air filters*
Clean algal/rotifer cultures***	Clean *Artemia**
Water quality tests***	Cultures***
Nutrition***	

7. Immature crabs

Transfer of juveniles**	Substrate, shelters***
Temperature, water quality**	Stocking density**
Initial size**, sex separation**	Nutrition***

8. Harvest of sale crabs

Collection method**	Crab selection***
Packing	Transport**

* estimate of relative importance of procedure

Hazard Analysis Critical Control Point Chart

From the flow chart, a HACCP chart was prepared. As critical points and associated hazards are not well documented in mud crab culture, the HACCP chart must be tentative, particularly when attempting to detail any preventive or corrective action.

1. Broodstock

Critical step	*Potential risk*	*Critical control point*	*Preventive control and monitoring*	*Corrective actions*
		New broodstock		
Landing the catch	Damage to catch	Removal from trap	Exercise care Direct monitoring	Inform /train catchers
Fungal/bacterial contamination	Infection of egg mass and larvae	Before spawning	Direct monitoring	Antibiotic /antifungal bath
		Maintenance		
Light intensity	Ovarian maturation and spawning delayed	Any time	Low light	Shelter, substrate, low light
Crab density	Cannibalism	Any time	Allow at least $0.5m^2$/crab	Reduce number, remove damaged and dead
Low water temperature	Affects ovarian development	25–30 °C	Thermostatic control, regular monitoring	Adjust water temperature
Water quality	Stress followed by infection	Limits exceeded for each parameter	Daily monitoring	Clean tank, water exchange, biofilter
Substrate quality	Infection of egg mass	Spawning	Flushing	Clean regularly
Proximity of other crustacean species	Introduction of pathogens	Broodstock tank	Avoid cross-contamination	Maintain effective isolation
Ovarian development	Maintenance of slow/ non developing crabs	Persistence of immature eggs	Ovarian biopsy	Eyestalk ablation/ discard crab
Nutrition	Delayed maturation, poor egg quality	Broodstock tank	Adequate diet	Improve food variety

		Spawning		
Spawning	Fertilisation failure	Spawning	Nutrition Biopsy of egg mass	Maintain/discard crab
Post spawning	Bacterial/fungal contamination	Egg incubation	Bacterial count and identification Improve water quality	Disinfection of crab and egg mass. Increase water flow
		Hatching		
Hatch tank	Bacterial infection of larvae	Immediately prehatch	Check tank water for bacteria. Maintain hygiene	Sterile sea water, high exchange. Sterilise tank

2. Larvae

Critical step	*Potential risk*	*Critical control point*	*Preventive control and monitoring*	*Corrective action*
Hatch tank	Low viability of larvae	Immediately post hatch	Monitor for activity and structural defects	Accept only normal highly active larvae
Pretransfer to culture tank	Carryover of pathogens to culture	Immediately before transfer	Reduce bacterial load. Culture for bacteria	Wash larvae with sterile seawater. Treat with anti-microbial bath
		Larval culture		
Transfer to culture tank	Stress and transfer of pathogens	Transfer to culture tank	Minimise stress and check for pathogens	Compatible temperature and salinity. UV/Cl water/sterile tanks. Gradual introduction to new water
Culture tank	Introduction of pathogens from external sources	Throughout culture	Reduce bacterial load	Clean anti-splash cover
Culture tank	Multiplication of pathogens	Throughout culture	Environment less favourable to pathogens	Biological filter, water exchange, remove debris
Culture tank	Stress from poor water quality	Throughout culture	Reduce stress. Monitor water quality	Increase water exchange. Use biofilter
Culture tank	Unsuitable feeds	Throughout culture	Determine best feeds for each zoeal stage	Use only clean algal/ rotifer and artemia cultures, supplement with artificial diets. Boost rotifer/artemia with algae or specific formulations
Moult from zoea 5 to megalopa	Moult death syndrome (MDS)	Final zoea 5 moult	Bacterial control Food type selection	Maintenance of 'good' tank micro-environment. Selection of appropriate food(s)

3. Immature crabs

Critical step	*Potential risk*	*Critical control point*	*Preventive control and monitoring*	*Corrective actions*
Nursery and growout	Cannibalism	Throughout culture	Shelters, stocking density. Appropriate food, improve quality. Check size range of crabs	Separate sexes, improve shelter and substrate. Feed more regularly. Reduce stocking density, grade crabs
Nursery and growout	Stress from sub-optimal water quality	Throughout culture	Check stocking density, monitor water quality	Reduce stocking rate, improve water flow

The hazard analysis chart contains five principles of the system. Important final principles are:

4. Record keeping

This must be comprehensive and should include records of those risks, preventive control, monitoring and corrective actions which are critical to the success or failure of the enterprise.

5. Verification

This is an extension of record keeping and requires on-the-spot inspections. Supervisors must know what was or was not done and the immediate consequences of changes in the culture process.

This compilation is a preliminary presentation of a systematic overview of the culture of mud crabs and some of the many hazards to which they are exposed. At present, there are many uncertainties in the culture process, and much research is needed to clarify responses to potential and actual hazards. Quality control systems using HACCP principles should lead to a relatively common program of culture, with suitable detection, control and correction methods readily available.

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Larval Survival and Megalopa Production of *Scylla* sp. at Different Salinities

Fe D. Parado-Estepa[1] and Emilia T. Quinitio[1]

Abstract

Salinity tolerance was determined for each zoeal stage of *Scylla* sp. Larvae from ablated pond-grown females were abruptly transferred to salinities of 12, 16, 20, 24, 28 and 32 ppt. Spawning salinity or previous rearing salinity was 32 ppt, except for Z5 which were previously reared at 26 ppt. The mean median lethal time or LT50 values were compared between salinities. For Z1 and Z2, highest values were obtained at 20–32 ppt. Z3 had highest LT50 values at 20–24 ppt and Z4 at 24–32 ppt. For Z5, highest LT50 values were obtained at 20–32 ppt. Another batch of Z3 and Z4 were subjected to the same abrupt salinity transfers and reared to the megalopa stage. Significantly higher percentages of larvae metamorphosed to the megalopa stage at salinities of 20–28 ppt when transfer to test salinities was at Z3. When transfer was at Z4 or Z5, the highest percentage of larvae moulted to the megalopa stage at 24–28 ppt or at 28 ppt, respectively.

THE MUD crab *Scylla* sp. is becoming a commercially important species, especially as a possible alternative culture species to prawns. Present culture techniques involve growing of wild caught juveniles that are becoming scarce. Thus, there is a need to develop hatchery-rearing techniques to provide steady and reliable supplies of seeds. To achieve this, optimal rearing conditions must be determined. This paper aims to define optimal salinity levels for each larval stage.

Materials and Methods

Larvae from ablated pond-grown females were abruptly transferred to salinities of 12, 16, 20, 24, 26 (only for Z5), 28 and 32 ppt. Spawning salinity or previous rearing salinity was 32 ppt, except for Z5 which were previously reared at 26 ppt. Separate tests were conducted for each stage and were terminated when most of the animals had moulted to the succeeding stage. Mortalities at 1, 3, 12 and 24 hours after stocking and every 24 hours thereafter were noted.

The time at which 50% of larvae died or the median lethal time (LT50) was determined for each replicate. LT50 values at each stage were compared through one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was conducted whenever significance was detected.

Separate batches of larvae were used to determine the percentage that will reach the megalopa stage after abrupt transfer to different salinities. Z3 or Z4 were subjected to the same abrupt salinity transfers and reared to the megalopa stage. The number of megalopa produced during the salinity tolerance test for Z5 was also determined. The percentages of megalopa produced were transformed to arcsin values and compared through ANOVA and DMRT.

Results and Discussion

For Z1 and Z2, highest LT50 values were obtained at 20, 24, 28 and 32 ppt (Figure 1A). Z3 had highest LT50 values at 20 and 24 ppt and Z4 at 24, 28 and 32 ppt (Figure 1A, B). At Z5, highest LT50 values were obtained at 20, 24, 26, 28 and 32 ppt.

Significantly higher percentages of larvae metamorphosed to the megalopa stage at salinities of 20, 24 and 28 ppt when transferred to test salinities at Z3, and 24 and 28 ppt when transferred at Z4 (Figure 2). The numbers of megalopa produced from the Z5 salinity tolerance test were also compared. The highest percentage of larvae moulted to the megalopa stage at 28 ppt, followed by 20, 24, 26 and 32 ppt.

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines

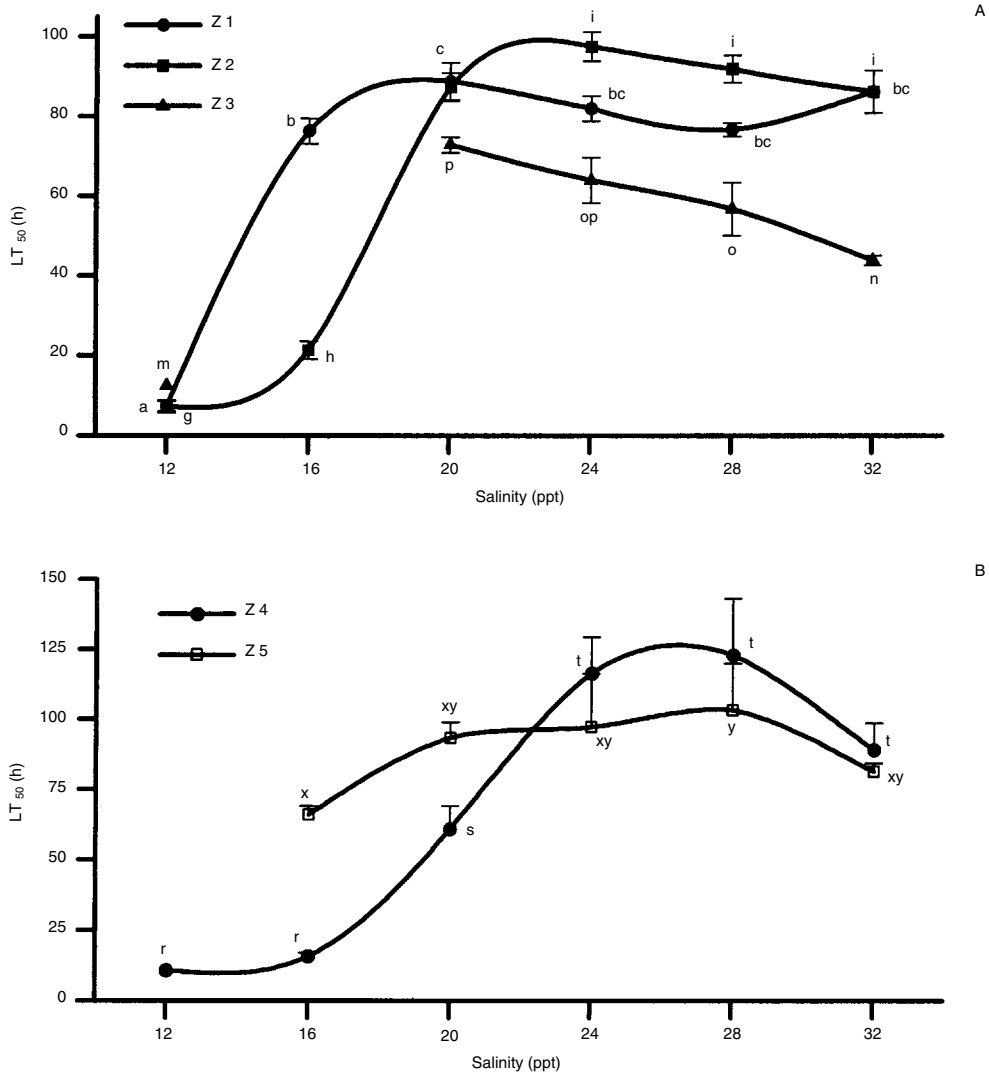

Figure 1. LT50 values for *Scylla* sp. larvae (A: Z1, Z2 and Z3; B: Z4 and Z5) abruptly transferred to different salinity levels. Each bar indicates the standard error of the mean. Z1 to Z4 larvae were previously reared in 32 ppt and Z5 larvae in 26 ppt seawater. Symbols that lie in the same line and have different letter labels have significantly different means.

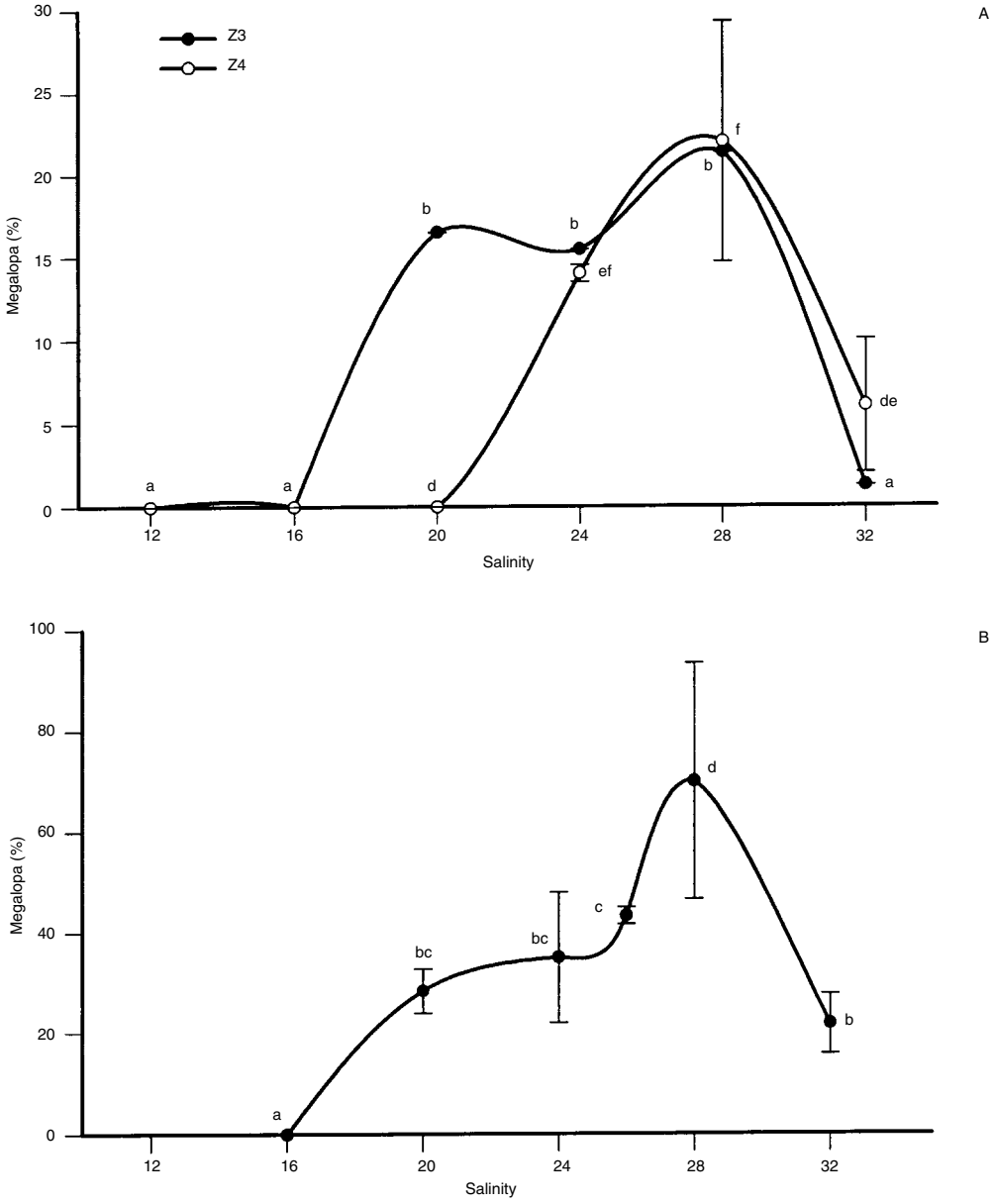

Figure 2. Megalopa produced after abrupt transfer of *Scylla* sp. larvae (A: Z3 and Z4; B: Z5) to different salinity levels. Each bar indicates the standard error of the mean. Symbols which lie in the same line and have different letter labels have significantly different means.

Higher LT50 values indicate better survival of larvae. Results generally suggest that low survival is obtained if mud crab larvae are reared at salinities of 12–16 ppt. Z1 and Z2 can tolerate an abrupt transfer to a wide range of salinities as indicated by the similar LT50 values obtained at 20–32 ppt. Z3 can also survive in 32 ppt but are best reared at 20–28 ppt to have better survival and megalopa production. LT50 and percentage megalopa production consistently indicate that at a temperature of 27 °C, 24 and 28 ppt are optimal for Z4 which had been previously reared at 32 ppt. Results at Z5 indicate that similar survival can be obtained with a salinity increase or decrease of up to 6 ppt but that a higher production of megalopa may be obtained at 28 ppt (2 ppt increase from initial rearing salinity).

Hill (1974) determined the salinity tolerance of Z1 at different temperatures but the test was only for the initial 24 hours. However, his results agree with the present study. At a temperature of 27–29 °C, about 50–90% Z1 survived at salinities higher than 17 ppt (interpolated from the surface response curve).

Most of the work on larval rearing of *Scylla* sp. has employed salinity levels ranging from 30–34 ppt (Ong 1964; Brick 1974; Heasman and Fielder 1983). Results from the present study indicate that salinity levels can be varied to obtain better survival and megalopa production. However, these should be verified in actual larval rearing runs.

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Transport Mechanisms of Crab Megalopae in Mangrove Ecosystems, with Special Reference to a Mangrove Estuary in Ranong, Thailand

D.J. Macintosh[1], F. Gonçalves[2], A.M.V.M. Soares[3], S.M. Moser[1] and N. Paphavisit[4]

CRUSTACEANS, particularly brachyuran crabs, are prominent in the macrofauna associated with mangrove ecosystems (Macnae 1968; Jones 1984; Macintosh 1988). In common with other brachyurans, dispersal from and recruitment into adult habitats are important characteristics of the larval phase of mangrove crabs.

Larval transportation is facilitated through numerous behavioural adaptations: these (with examples) include (a) the timing of larval release by ovigerous crabs to particular phases of the tidal cycle (Christy 1982; Wehrtmann and Dittel 1990); (b) migration by females to more suitable locations for larval dispersal and survival (Queiroga et al. 1997); (c) selective vertical movement by larvae in the water column to exploit different tidal currents (Queiroga et al. 1994; Zeng and Naylor 1996); and (d) use of floating materials as a transport mechanism (Kingsford and Choat 1985; Wehrtmann and Dittel 1990).

Larval release and dispersion

Larval release by most intertidal mangrove crab species occurs during the lunar phases associated with spring high tides. This allows the larvae the greatest chance of being transported out of the mangroves, an adaption presumed to be related to their salinity requirements. Mangrove estuaries commonly feature periods of very low salinity that would be below the larval salinity tolerance of many brachyuran species (Macintosh 1988).

Ocypodid crabs, e.g., the *Uca* spp., and many mangrove grapsid crabs release their larvae at high tides either fortnightly or monthly (Table 1) and thereby optimise the chance for larval dispersion to more saline water conditions. The timing of egg hatching is controlled by endogenous rhythms, which are synchronised with lunar, tidal and light-dark cycles (Morgan 1995).

Figure 1 illustrates this adaptive response for one of the most common mangrove fiddler crabs in Southeast Asia, *Uca rosea*, a species which was found to release its larvae at both full moon and new moon high tides in a mangrove estuary in Malaysia, the great majority of hatchings occurring during the night-time. The release of larvae at nocturnal high tides has also been hypothesised to minimise the risk of predation by predators of egg-bearing females, embryos, and larvae (Morgan 1995).

In contrast to mangrove ocypodid and sesarmid crab species, mangrove portunid crabs of the genus *Scylla,* migrate offshore to release their larvae. In Australia, berried female crabs have been reported to migrate up to 50 km off shore to release their larvae and thereafter return to the mangrove (Hyland et al. 1984).

Larval ingression

Selective vertical migration at different tidal currents is an important crab larvae transport mechanism (Tankersley et al. 1995). The migration of megalopae into the adult estuarine habitat against the net seaward flow of water is accomplished by taking advantage of the tidal currents; i.e., by ascending

[1]Centre for Tropical Ecosystems Research, Dept. of Ecology and Genetics, Building 540, University of Aarhus, DK-8000 Aarhus C., Denmark
[2]Departamento de Biologia da Universidade de Aveiro, Campus Universitario de Santiago, 3810 Aveiro, Portugal
[3]Instituto do Ambiente e Vida–Department of Zoology of University of Coimbra, Portugal
[4]Department of Marine Science, Chulanlongkorn University, Bangkok 10500, Thailand

Table 1. Examples of egg-hatching rhythms of mangrove crabs.

Shore level and species	Family	Lunar/Tidal	Tidal phase	Diurnal phase	References
Supratidal-High Intertidal					
Cardisoma guanhumi	Gecarcinidae	Monthly/Biweekly	—	—	Henning (1975)
Aratus pisoni	Grapsidae	Biweekly	—	—	Warner (1967)
High Intertidal					
Sesarma rhizophorae	Grapsidae	Monthly	High tide	Night (Late)	Morgan and Christy (1995)
Chiromanthes onychophorum	Grapsidae	Monthly	High tide	Night	Macintosh (1984)
Uca rosea	Ocypodidae	Monthly	High tide	Night	Macintosh (1984)
Intertidal					
Uca dussumieri	Ocypodidae	Biweekly	High tide	Night	Macintosh (1984)
Metaplax elegans	Grapsidae	Biweekly	High tide	Night	Macintosh (1984)

Modified from Morgan (1995)

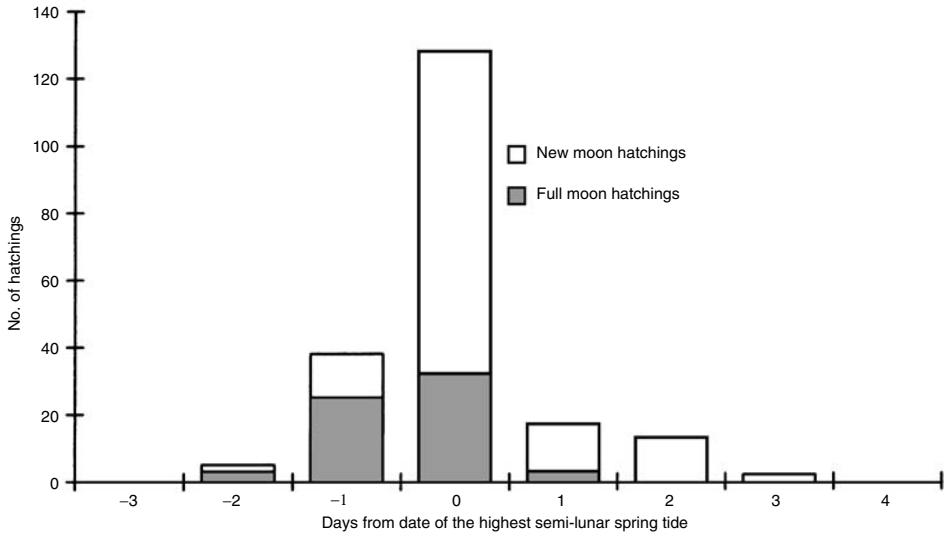

Figure 1. Timing of hatching of egg batches of the mangrove fiddler crab *Uca rosea* from Kuala Selangor, peninsular Malaysia in relation to lunar and tidal cycles (redrawn from Macintosh 1979).

into the water column during flood tides and descending during ebb tide (Dittel et al. 1991; Olmi III 1994; Lochmann and McEachran 1995). Dittel et al. (1991) found all *Uca* spp. and grapsid crab larval stages, except stage 1, to be more abundant in the top layers during flood tide. They calculated the net transport of larvae into the estuary using the estimated tidal volume flux for the mangrove creek. Likewise, the abundance of the *Callinectes sapidus* (Portunidae) and *Uca* spp. (Ocypodidae) megalopae in a riverine estuary in North Carolina was found to peak during nocturnal rising tides (DeVries et al. 1994); this can be interpreted as an adaptation to escape visually-dependent predators.

Larval transportation using leaves and other floating substrata

An association between decapod larvae and floating leaves and clumps of algae has been observed in several studies (Kingsford and Choat 1985; Wehrtsmann and Dittel 1990). Kingsford and Choat (1985) consistently found crab megalopae associated with sampled drift algae from near-shore and open-shore localities using a plankton-mesh purse seine net. In a study of the associated fauna on mangrove leaves in the Gulf of Nicoya, Costa Rica, mangrove crab larvae and juveniles were found to cling onto leaves (Table 2), and in numbers which were much higher during flood tides than during ebb tides, irrespective

of day or night. This behaviour could (a) reduce predation, (b) save energy when close to the water surface and (c) function as a transport mechanism (Wehrtmann and Dittel 1990).

Table 2. Species composition and number and stage of crab larvae found to be associated with mangrove leaves in the Gulf of Nicoya, Costa Rica.

Crabs	No. of larvae	Stage	%
Ocypodidae			
Uca sp.	1530	Megalopae	93.5
Grapsidae			
Unidentified Grapsidae	42	Megalopae	2.5
Grapsus sp.	8	Juvenile	0.5
Portunidae			
Callinectes sp.	49	Juvenile	3.0
Xanthidae			
Unidentified Xanthidae	1	Juvenile	0.1
Pinnotheridae			
Pinnixia sp.	2	Zoea IV	0.1
Pinnotheres sp.	1	Juvenile	0.1
Unidentified	1	Megalopae	0.1
TOTAL	1635		100.0

Modified from Wehrtmann and Dittel (1990)

During the transportation of megalopae from offshore to the estuarine environment, it is advantageous if they can delay metamorphosis to prevent settlement in an unsuitable environment (Pechenik 1990). Brumbaugh and McConaugha (1995) reported that megalopae in the offshore population of *Callinectes sapidus* were almost entirely in the postmoult or intermoult stage, in contrast to the megalopae found in the estuary, which had already begun their premoult development. Cues in the estuarine environment, which might cause the megalopae to initiate the premoult stage could include contact with a suitable substratum; in mangrove ecosystems, mangrove leaves could play this role.

Objectives of the study

Although there have been many studies of mangrove crabs in the Southeast Asian region, very little is known about their larval recruitment. As part of an on-going research project on the relationships between mangroves and fisheries/aquaculture production in Southeast Asia, the possible role of mangrove leaves as a transport mechanism for *Scylla* and other mangrove crab species is being investigated in a mangrove delta in Ranong, southern Thailand.

Study Site

The largest continuous area of mangroves left in Thailand fringes the delta of the Kra Buri River which borders Thailand and Myanmar on the Andaman Sea coast. Situated in the Province of Ranong, this mangrove system features many interconnecting waterways, one of the larger ones being the Ngao Estuary ('Khlong Ngao'), an extensive shallow creek system supporting 1150 hectares of mangrove wetland surrounded by a further 1880 ha of low hills (Chunkao et al. 1985).

Rainfall in Ranong is the highest in Thailand, averaging more than 4 metres annually (Meteorological Department of Thailand records: 1966–1995), but exceptionally reaching almost 5 to 6 m (Figure 2). This means that there is very high freshwater drainage into Khlong Ngao seasonally during the wet southwest monsoon period from May to October/November.

Khlong Ngao and the surrounding area continues to support traditional fishing activities, including crab catching using traps and nets. In recent years, local catches are reported to have declined (Macintosh et al. 1993), whereas coastal aquaculture has become increasingly important. There is now commercial scale production of shrimps, crabs and fish, the main species being *Penaeus monodon* (tiger shrimp), mud crab (*Scylla olivaceous*), sea perch (*Lates calcarifer*) and groupers and snappers (*Epinephelus* and *Lutjanus* spp.). Mud crabs are farmed both for meat crab and soft-shell crab, but all the crabs used in aquaculture come from the natural mangrove population. Thus, there is considerable importance attached to research on the recruitment and habitat requirements of larval and early crab stages of *Scylla*.

Methodology

Study site and sampling method

In March 1995, floating mangrove leaves were collected in Khlong Ngao hourly over a 24-hour period on two occasions during full moon (11 March) and first-quarter lunar phases (21 March). Two sites were sampled each time, one in the mouth of Ngao Estuary, near the village of Hat Sai Kao (site 2) and the other located 8 km upstream near the Mangrove Forest Research Centre (MFRC, a research facility of the Royal Thai Forest Department) where the estuary becomes a narrow mangrove-fringed channel (site 1). Leaves were sampled directly using a hand-net with 500 µm mesh dipped into the water. Netted leaves were transferred immediately into a bucket with filtrate water and washed thoroughly to dislodge any attached organisms. The washing water was filtered

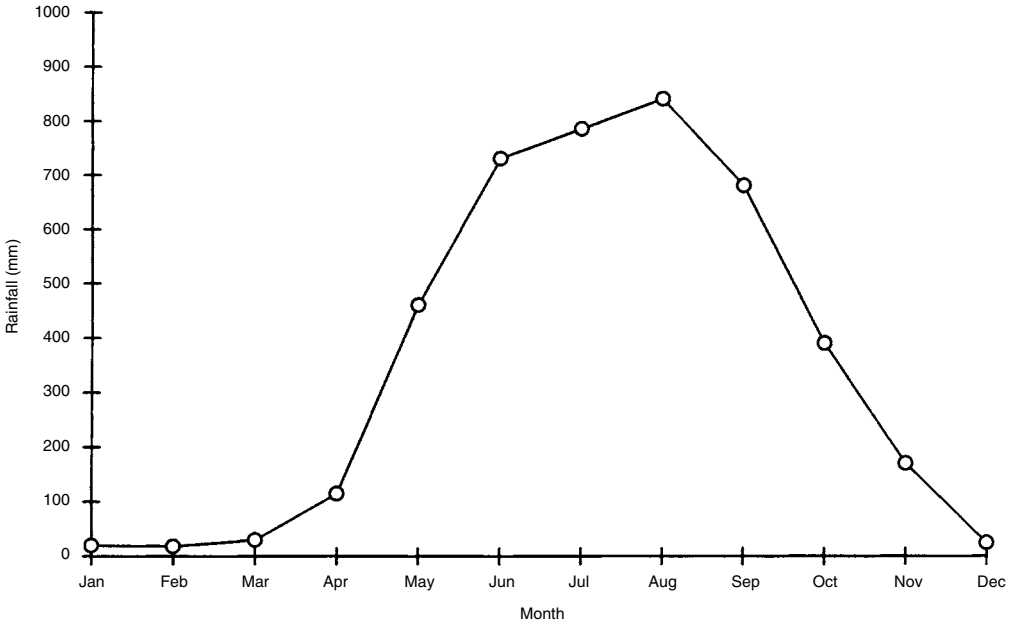

Figure 2. Average monthly rainfall for Ranong (based on meteorological records for 1966 to 1994).

through a second 500 µm net, then 4% buffered formaldehyde was added as preservative. The sample was transferred after two days into 90% ethanol.

The megalopae present in each sample were identified to the lowest *taxa* possible and counted. Mangrove leaf area per sample was calculated by counting the collected leaves and drawing them. The number of megalopae per unit area of leaf was then estimated.

Statistical analysis

Analysis of variance (ANOVA) was used to test the significance of the effects of diurnal period and lunar phase upon the density of megalopae within the taxa considered. The effects of tidal condition were minimised by comparing the data only during the flood tides.

Results

Brachyura were the largest component of the Decapod crustacean larvae obtained from the mangrove leaves (62.6% numerically of the collected organisms). The Brachyura were represented by four major families: Ocypodidae (*Uca* spp.) formed 44.0%, followed by Leucosiidae (12.9%), Portunidae (3.6%) and Grapsidae (2.2%).

The second most abundant group after the Brachyura was the Caridea (21.7%), with Alpheidae and Palaemonidae being the most important families. The Penaeidea (7.4%) were represented chiefly by shrimp of the family Penaeidae. Thalassinidea (5.2%) and Anomura (3.0%) made up the remainder. These groups were represented predominantly by *Thalassina anomala* Herbst and species of Porcellanidae, respectively.

During the full moon lunar phase, significantly higher densities of megalopae (number of individuals/dm^2) at Hat Sai Kao occurred during the flood tide, particularly in the daytime (Figure 4; $F = 6.072$, d.f. = 11, $P<0.05$). Brachyuran larvae dominated in these conditions, whereas species of the Thalassinidea and Caridea showed a preference for the night-time flood tide period (Figure 4).

During the first lunar quarter, there were distinct differences in larval recruitment between the day and night flood tide periods. Densities of brachyuran megalopae were significantly higher for the flood-day tide ($F = 5.885$, d.f. = 10, $p<0.05$), while the Caridea showed significant selection of the flood-night tide ($F = 31.353$, d.f. = 10, $P<0.001$). As during the full moon phase, Brachyura formed the main group of leaf-attached crustacean larvae, followed by species of Caridea, but at Hat Sai Kao the differences between first-quarter and full moon were not significant ($F = 0.002$, d.f. = 23, $P>>0.05$) (Figure 5).

In contrast to the high abundance of decapod crustacean larvae on mangrove leaves collected at

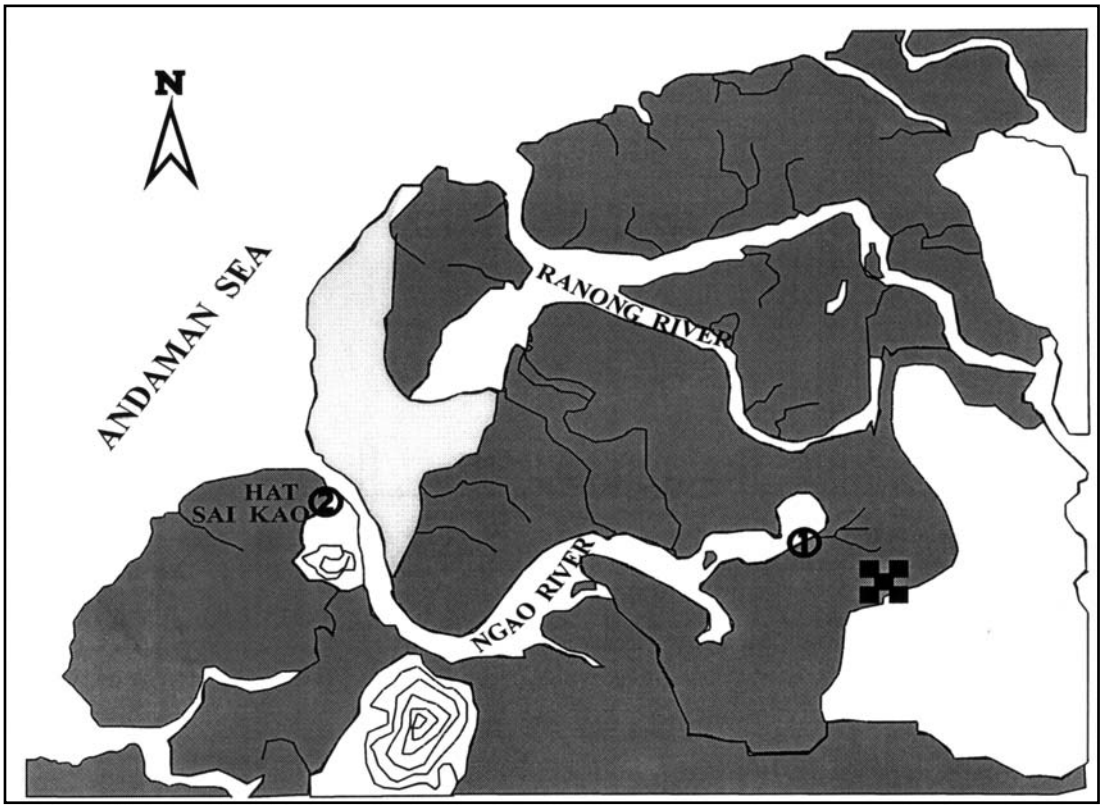

Figure 3. Ngao River Estuary and the location of sampling stations (1) the channel near MFRC and (2) at the estuary mouth opposite Hat Sai Kao.

site 2 near the estuary mouth, sampling at site 1 near the upstream limit of the estuary revealed very low densities of larvae; these data are not shown, but Penaeidea were most numerous, followed by Caridea and Brachyura.

Discussion

This very limited initial study of mangrove crab larval recruitment into the Ngao Estuary confirms that brachyuran megalopae of at least four families, including the Portunidae, utilise floating mangrove leaves as an apparent transportation mechanism. It is also reasonable to conclude that mangrove leaves help to protect megalopae from predators and may also provide a cue for their development into the benthic crab stage. Since mangrove leaves are repeatedly settled and refloated by the tides (Macintosh et al. 1991), megalopae could have a good opportunity for ingression followed by settlement within mangrove forests using such a mechanism. This may be particularly important in the Ranong mangrove ecosystem where the tidal range exceeds four metres during spring tides.

Because the study was confined to only two lunar phases within a single month of the year (March), conclusions cannot be made about the possible significance of mangrove leaf transportation for particular brachyuran species. In the leaf samples studied, *Portunus* but not *Scylla* larvae were recorded. However, as an hypothesis to be tested by further research, leaf transport is proposed as a possible recruitment mechanism for *Scylla* megalopae and early crab stages in Khlong Ngao. Moreover, from information already known about the reproductive cycle of *Scylla* in the Ranong mangroves and the physical environment of the estuary, the authors can suggest the probable season for *Scylla* larval recruitment and it is proposed to design an intensive larval sampling program to target this period.

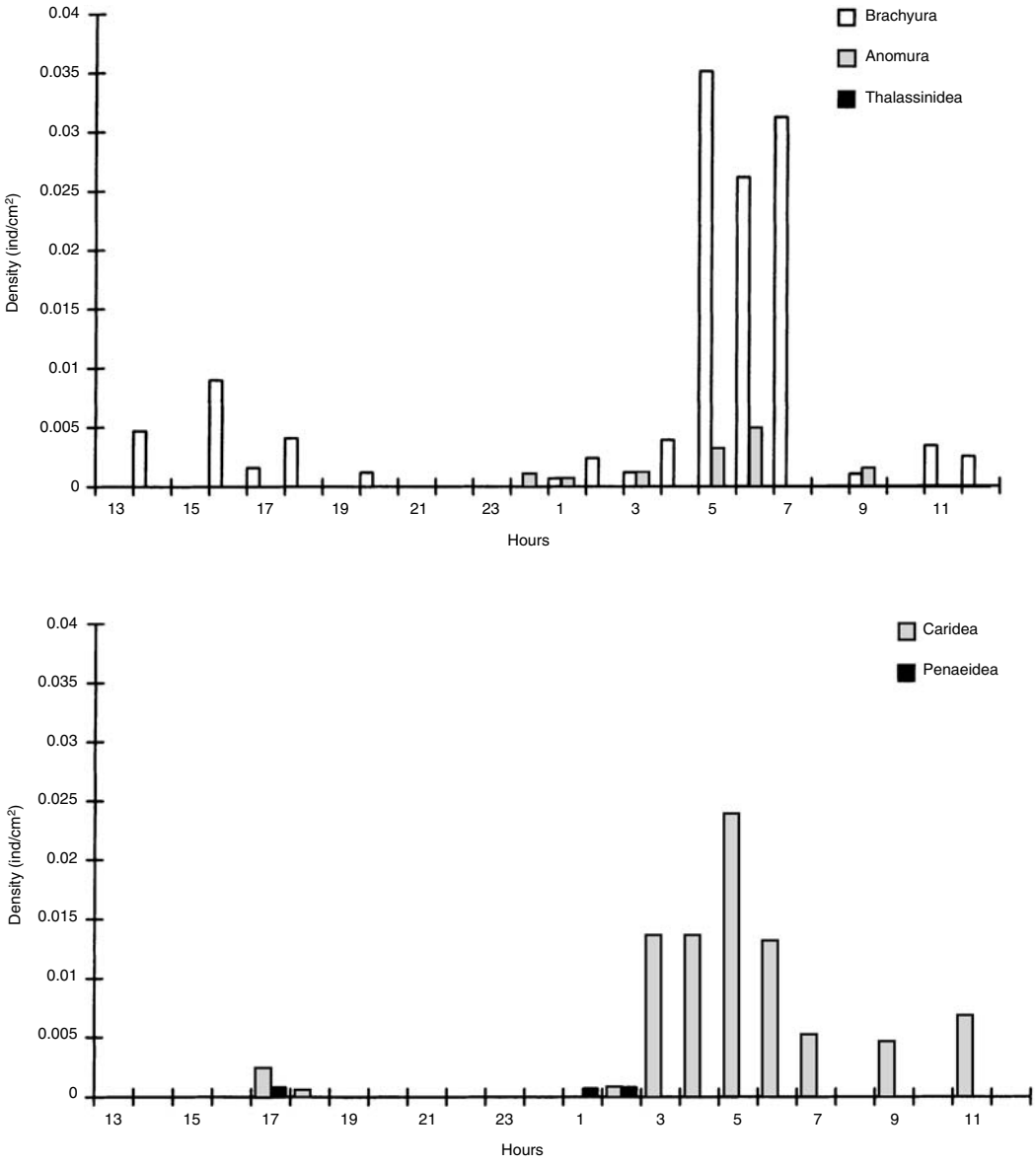

Figure 4. Density of brachyuran and other crustacean larvae on floating mangrove leaves collected hourly at sampling station 2, Ngao River Estuary mouth during a full moon lunar phase (11 March 1995). Dawn and dusk were approximately 06.00 and 18.00 h, respectively.

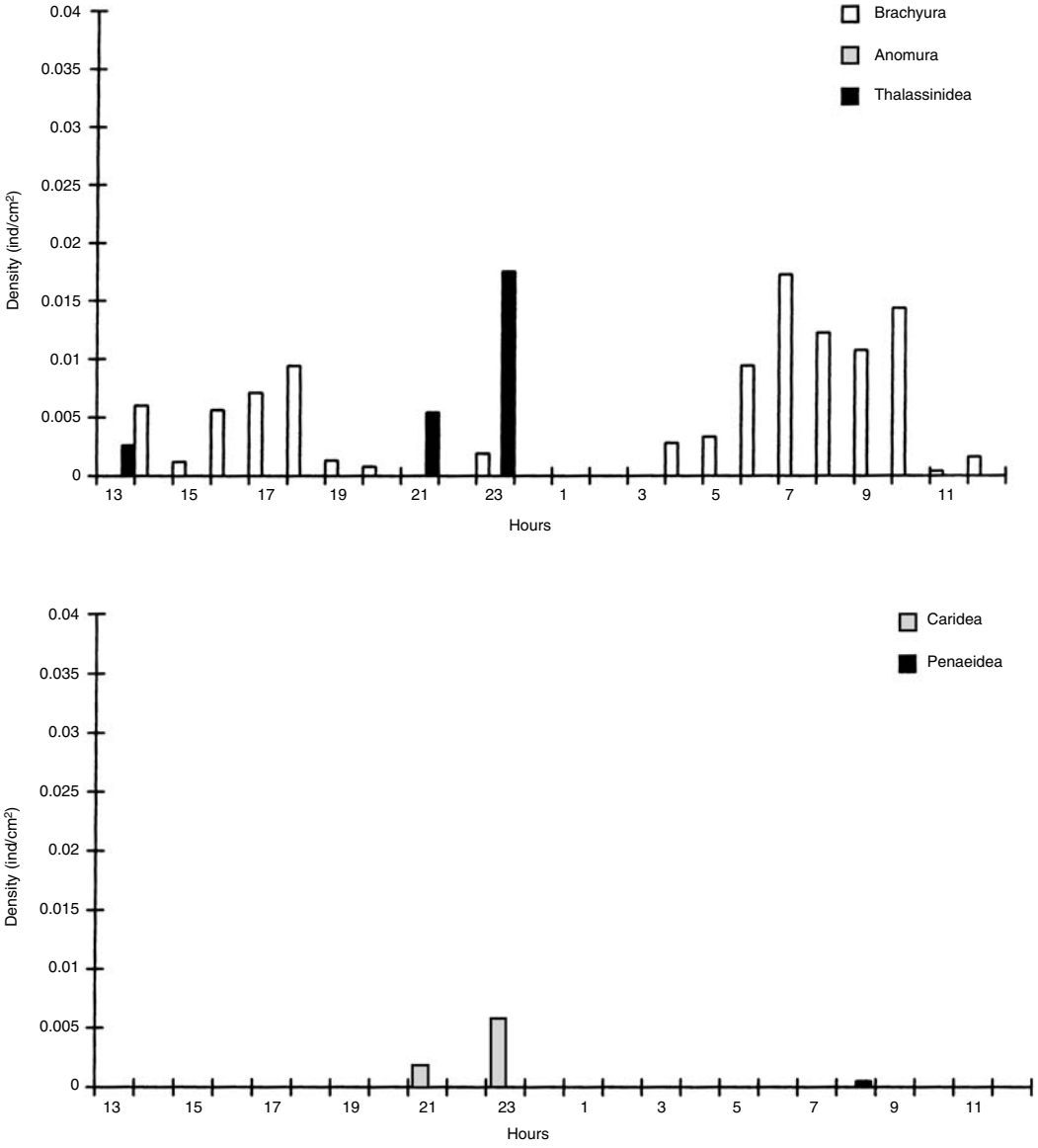

Figure 5. Density of brachyuran and other crustacean larvae on floating mangrove leaves collected hourly at sampling station 2, Ngao River Estuary mouth during a first quarter moon lunar phase (21 March 1995). Dawn and dusk were approximately 0600 and 1800 h, respectively.

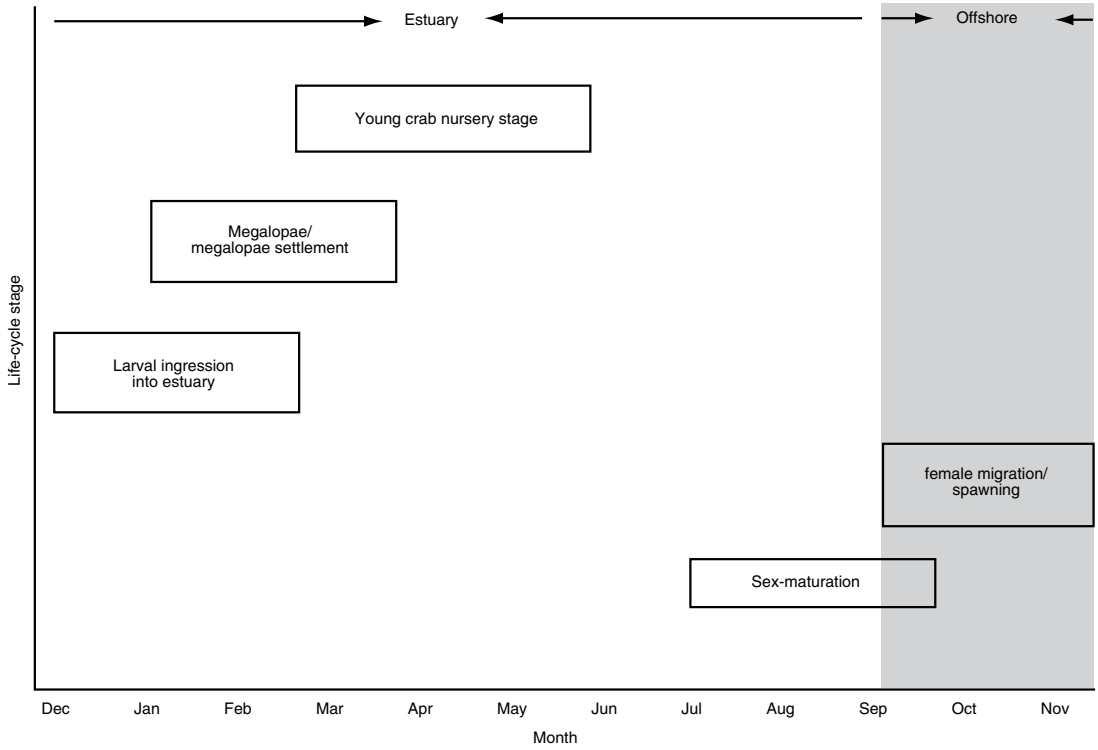

Figure 6. Major stages in the life cycle of *Scylla* in the Ranong mangrove ecosystem. Boxes indicate presumed peak of activity for each life-cycle stage (to be tested by further research).

Mud crab populations in tropical latitudes are known to have protracted breeding seasons, but show distinct peaks of reproductive activity (Heasman et al. 1985). In Khlong Ngao, Cheewasedtham (1990) found from experimental crab trapping that the GSI of adult *Scylla* increases markedly from July to September.

Data he collected from the commercial crab fishery in the area revealed a sudden scarcity of female crabs from September to December, suggesting that sexually maturing female *Scylla* migrate out of the estuary at this season. This contrasted with the months from January to August, when there were no significant differences in sex ratio among the crabs caught by the same group of fishermen (Macintosh et al. 1993). When these observations are combined with the available data on climatic and hydrological conditions in Khlong Ngao, it becomes clear that the most likely period for *Scylla* larval recruitment is from November to February, i.e., from the end of the wet season into the start of the dry season (Figure 6).

It is unlikely that mud crab larvae would not be able to tolerate the low salinities in Khlong Ngao associated with the extremely rainy season in Ranong. Salinity conditions in Khlong Ngao change considerably in response to the effects of the extremely high rainfall in Ranong. Freshwater run-off into the estuary was estimated to increase from almost zero in April at the end of the dry season, to 20 m^3/s at the end of the wet season in September (Macintosh et al. 1991). Within the estuary this results in a salinity drop from 30–32 ppt (dry season) to 15–27 ppt (wet season), the decrease being more pronounced with distance upsteam (Macintosh et al. 1991). Thus, it is speculated that ingression into Khlong Ngao by *Scylla* larvae occurs as a peak in the period December to February (Figure 6) when salinities are most favourable.

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Development of Practical Diet for Grow-out of Mud Crab Species *Scylla serrata* and *S. tranquebarica*

Evelyn T. Marasigan[1]

Abstract

Two experimental runs were conducted to compare the effects of five diets on the growth of mixed species of wild caught mud crab, *Scylla serrata* and *Scylla tranquebarica* (Experiment 1) and hatchery produced *S. serrata* (Experiment 2). The dietary treatments tested for the two experiments were; moist prawn pellet, dry prawn pellet, squid, mussel meat (*Perna viridis*), and trash fish (*Alepes* sp). Each feeding experiment covered 90 days of culture under hatchery conditions. There were significant differences ($P<0.05$) in specific growth rate (SGR) of mixed species of crabs fed with mussel meat compared to crabs fed with moist prawn pellet, and highly significant differences ($P<0.01$) in the SGR of single species of crabs fed with mussel meat compared to crabs fed with moist and dry prawn pellets and squid. The SGR values obtained for mixed mud crab species fed with mussel meat, squid, trash fish and dry prawn pellets were not significantly different ($P>0.05$). The SGR for *Scylla serrata* fed with mussel meat were not significantly different ($P>0.05$) from *S. serrata* fed with trash fish. The differences in the SGR values most likely were influenced by the moulting frequency that varied with the five diets for the two experiments. The diet containing mussel meat resulted in the highest SGR and highest average moulting frequencies.

MUD CRABS are widely distributed in the Philippine coastal areas and considered a delicacy with high market value. Collection of mud crabs and to some extent backyard fattening provides income and livelihood in coastal communities. The development of mud crab culture in the Philippines, however, is hampered by lack of basic knowledge on growth and survival, feeding requirements, stocking rate and other information related to culture systems. It is seen that studies on nutritional requirements of mud crabs in captivity are critical to the development of the mud crab industry.

To date, there are few references available on mud crab nutrition (Cajilig 1995; Heasman and Fielder 1983). Further studies are deemed necessary to optimise the quality of both natural food and artificial feeds needed for maintenance and growth of different species of mud crabs at different life stages, stocking densities and environmental conditions. Besides defining the effects of individual constituents in a diet, it is equally important to formulate combinations of commonly available ingredients for optimum performance with respect to growth and survival. Studies on feed form, texture, size, odour, method of feed distribution and on feeding behaviour of mud crabs at different life stages need to be undertaken (Heasman and Fielder 1983).

Lijauco et al. (1980) observed that mud crabs could not be reared on a diet composed solely of fish since this diet resulted in slow growth rate and poor condition. In a similar study, Jayamane and Jinadasa (1991) noted that mud crabs required both molluscan and crustacean material in their diets.

In this light, the present study was conducted with the general objective of determining the physical and chemical characteristics of practical diets for grow-out of the mud crab while its specific objectives were to compare the effects of diets composed of fresh individual ingredients and commercially available prawn feeds on the growth of the mud crab.

Materials and Methods

Two experimental runs were conducted at the UPV Institute of Aquaculture hatchery facility at Miagao,

[1]University of the Philippines in the Visayas, College of Fisheries, Institute of Aquaculture, 5023 Miagao, Ibilo, Philippines.

Iloilo from June 14, 1996 to October 20, 1996. In Experiment 1, mixed species of crablets, *Scylla serrata* and *S. tranquebarica,* caught from the wild were used, since hatchery produced *S. serrata* were not yet available. However, Experiment 2 was conducted when hatchery *S. serrata* became available.

Experiment 1

Wild caught crab juveniles were purchased from the Bicol region. They were mixed species of *Scylla serrata* and *S. tranquebarica* (as identified by C.P. Keenan). Twenty crablets of about 2 grams weight each were stocked into 200 L capacity circular concrete cement tanks with a sand filled bottom. The tanks were provided with continuous aeration and a flow-through sea water supply at approximately 300% water change daily. After 45 days of culture however, high mortality was observed among the treatments reaching almost 50% in most of the tanks. Mortalities were mostly observed in newly moulted crabs that were attacked and cannibalised by other crabs. Inevitably, the feeding trial was aborted and terminated.

Another feeding run was conducted and designated as Experiment 1. Modifications were made in the culture tanks. For example, to minimise cannibalism among the mud crabs stocked in the culture tanks, plastic netting was used to subdivide the tank into compartments. These compartments were then stocked with individual crabs with mean weight range of 7.48–12.87 g. The feeding trial was begun on 14 June 1996 and was terminated on September 11, 1996 after 90 days of culture. Continuous aeration was provided and seawater was supplied in a flow-through system to the individual tanks.

Experiment 2

A parallel run to the wild caught juveniles was conducted with *Scylla serrata* crab instar 3 and 5. These crablets were produced from the larval production and rearing activity of the ACIAR project in the study carried out by Prof. Juliana C. Baylon. Each crablet was placed in a cage (9 cm dia. × 13 cm high) made of circular nylon screen to avoid interaction among them and to easily monitor the growth increment. Fifty plastic cages were placed in a 2.4 m × 1.2 m × 0.3 m water bath (with water level of about 13 cm) provided with continuous aeration and a flow-through system. The flow rate was adjusted to 25 L/hour or approximately 500% water exchange daily.

Experimental diets

Five diets were formulated and used as feed in the culture of mud crabs: moist prawn pellet for Treatment I, dry prawn pellet for Treatment II, fresh squid for Treatment III, fresh mussel meat (*Perna viridis)* for Treatment IV, and trash fish (*Alepes* sp.) for Treatment V. Moist and fresh diets were stored in a freezer until use.

Experimental design

The two experiments followed a completely randomised design. Experiment 1 had three replicates per treatment while Experiment 2 had 10 replicates per treatment

Feeding, sampling and gathering of data

Crabs were fed at 15% body weight during the initial 30 days which was reduced to 10% body weight for the next 30 days and finally to 6% body weight until the termination of the study. Initial weight and carapace lengths were recorded at the start of the experiment and every 15 days thereafter.

Analysis of data

Weight data for each sampling period were converted to specific growth rate (SGR) and together with the carapace length and moult data were analysed by single factor ANOVA using Microsoft EXCEL. Significantly different treatments were grouped using Duncan's multiple range test (SYSTAT).

Results

Experiment 1

Crablets grew from their initial mean weight range of 7.48–12.87 g to mean weights ranging from 21.28–55.63 g after 90 days of culture (Table 1). Periodic sampling of SGR data showed significant differences ($P>0.05$) among crabs fed the five diets (Table 1). Crabs fed with mussel meat and squid had consistently better SGR than crabs fed with moist pellet ($P<0.05$). However, SGR of mussel meat and squid fed crabs were not significantly different ($P>0.05$) from crabs fed trash fish and dry pellets. No significant differences were observed ($P>0.05$) in the SGR of crabs fed moist and dry pelleted diets.

Significant differences in the SGR of crabs fed with the five diets were observed starting on the 45th day of culture. Crabs fed with mussel meat also had significantly higher frequency of moulting compared to crabs fed with the other four diets and resulted in significantly longer carapace length though the carapace length of crabs fed with mussel meat was not significantly different than crabs fed with squid (Table 1). A graph of the specific growth rate of mud crabs shows a higher SGR at the beginning of the culture period than towards the end (Figure 1).

Water physico-chemical parameters monitored during the culture period showed that water quality in the rearing tanks were within tolerance limits of the crabs (Figure 1a).

Table 1. Mean weights (g) of mud crab fed with five types of diets during the 90-day culture period, Experiment 1.

Treatments	0 day	15 days	30 days	45 days	60 days	75 days	90 days
I. Moist pellet	11.17	16.10	17.17	18.69	19.97	21.38	22.44
SD	2.00	2.21	1.10	2.49	0.55	0.46	0.62
II. Dry pellet	8.99	14.03	14.09	16.28	17.20	19.19	21.91
SD	3.63	4.70	4.48	6.65	5.03	5.95	9.14
III. Squid	12.87	19.11	24.65	32.56	35.38	49.63	55.63
SD	2.22	5.95	8.79	12.05	10.78	25.30	25.91
IV. Mussel	9.74	15.33	19.89	23.58	28.87	39.78	43.98
SD	2.87	2.25	6.36	2.16	18.45	10.67	6.92
V. Trash fish	7.48	12.15	13.08	15.83	16.73	20.28	21.28
SD	1.60	1.19	0.64	0.86	0.18	6.45	3.91

Table 1a. Specific growth rate (g/day) of mud crab fed with five types of diets in the 90-day culture period, Experiment 1.

Treatments	15 days	30 days	45 days	60 days	75 days	90 days
I. Moist pellet	0.0235^{a}	0.0143^{a}	0.0114^{c}	0.0097^{c}	0.0087^{b}	0.0073^{b}
SD	0.006	0.0018	0.0006	0.0012	0.0015	0.00134
II. Dry pellet	0.0319^{a}	0.0162^{a}	0.0137^{bc}	0.0119^{bc}	0.0105^{b}	0.0101^{ab}
SD	0.0116	0.0062	0.0031	0.0027	0.0032	0.0036
III. Squid	0.0306^{a}	0.0235^{a}	0.0219^{a}	0.0179^{a}	0.0185^{a}	0.01675^{a}
SD	0.0044	0.0033	0.0024	0.0013	0.0032	0.0021
IV. Mussel	0.0318^{a}	0.0241^{a}	0.0199^{ab}	0.0174^{ab}	0.0189^{a}	0.0169^{a}
SD	0.0117	0.0038	0.003	0.0053	0.0014	0.005
V. Trash fish	0.0332^{a}	0.0191^{a}	0.0169^{ab}	0.0136^{ab}	0.0131^{ab}	0.0117^{ab}
SD	0.0098	0.0084	0.0059	0.0044	0.0064	0.0036

*Values with different superscripts are statistically different at $p<.05$

Table 1b. Periodic mean carapace length (cm) and mean total number of moults of crabs fed with five types of diets in Experiment 1.

Treatments	Initial	15 days	30 days	45 days	60 days	75 days	90 days	Total number of moults
I. Moist pellet	2.83	3.22	3.35^{bc}	3.47^{b}	3.57^{b}	3.64^{b}	3.71^{b}	2.27^{d}
II. Dry pellet	2.57	3.01	3.05^{c}	3.25^{b}	3.41^{b}	3.50^{b}	3.64^{b}	3.47^{bc}
III. Squid	2.87	3.28	3.65^{a}	3.99^{a}	4.17^{a}	4.58^{a}	4.73^{a}	3.8^{b}
IV. Mussel	2.68	3.09	3.38^{ab}	3.62^{ab}	4.03^{a}	4.28^{a}	4.51^{a}	4.87^{a}
V. Trash fish	2.45	2.89	3.07^{c}	3.29^{b}	3.42^{b}	3.56^{b}	3.69^{b}	3.27^{bc}

*Values with different superscripts are statistically different at $p<.05$

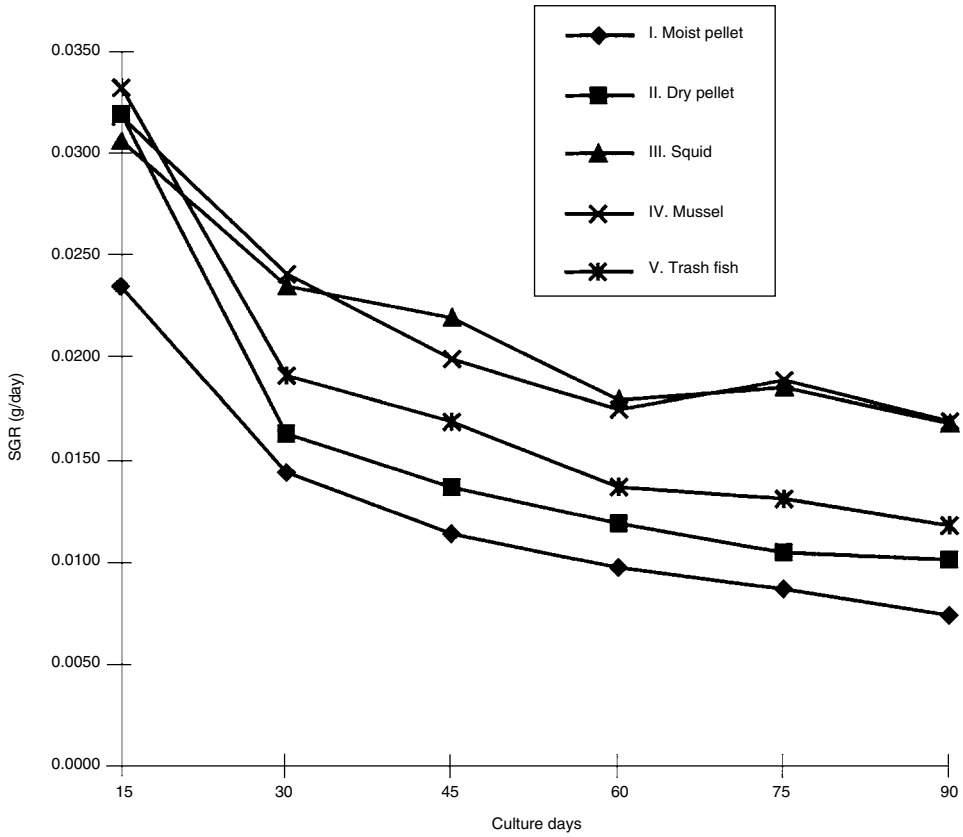

Figure 1. Specific growth rate of mud crab fed with five types of diets in Experiment 1.

Experiment 2

Crablets with initial mean weights ranging from 0.09 g–0.20 g grew to weights ranging from 1.25 g–5.74 g after 90 days of culture (Table 2). Differences in the SGR of crabs fed mussel meat compared to crabs fed moist and dry prawn pellet and trash fish were highly significant ($P<0.01$). However, SGR of mussel fed crabs was comparable with that of trash fish fed crabs (Table 2a). Differences in the specific growth rate of moist and dry pellet fed crabs were also not significantly different ($P<0.05$).

Significant differences in the SGR of crabs fed different diets were observed starting on the 30th day of sampling. Highly significant differences ($P<0.01$) were observed in the total number of moults of crabs fed with the five diets resulting in highly significant differences ($P<0.01$) in carapace length.

Comparison of the moult data show that crabs fed mussel meat and trash fish had higher number of moults compared to crabs fed with moist and dry prawn pellet. Graphs of the SGR of the crabs showed a similar trend to that in Experiment 1 (Figure 2).

Water physico-chemical parameters monitored during the culture period also showed that water quality in the rearing tanks was within the tolerance limits of the crabs (Figure 2a).

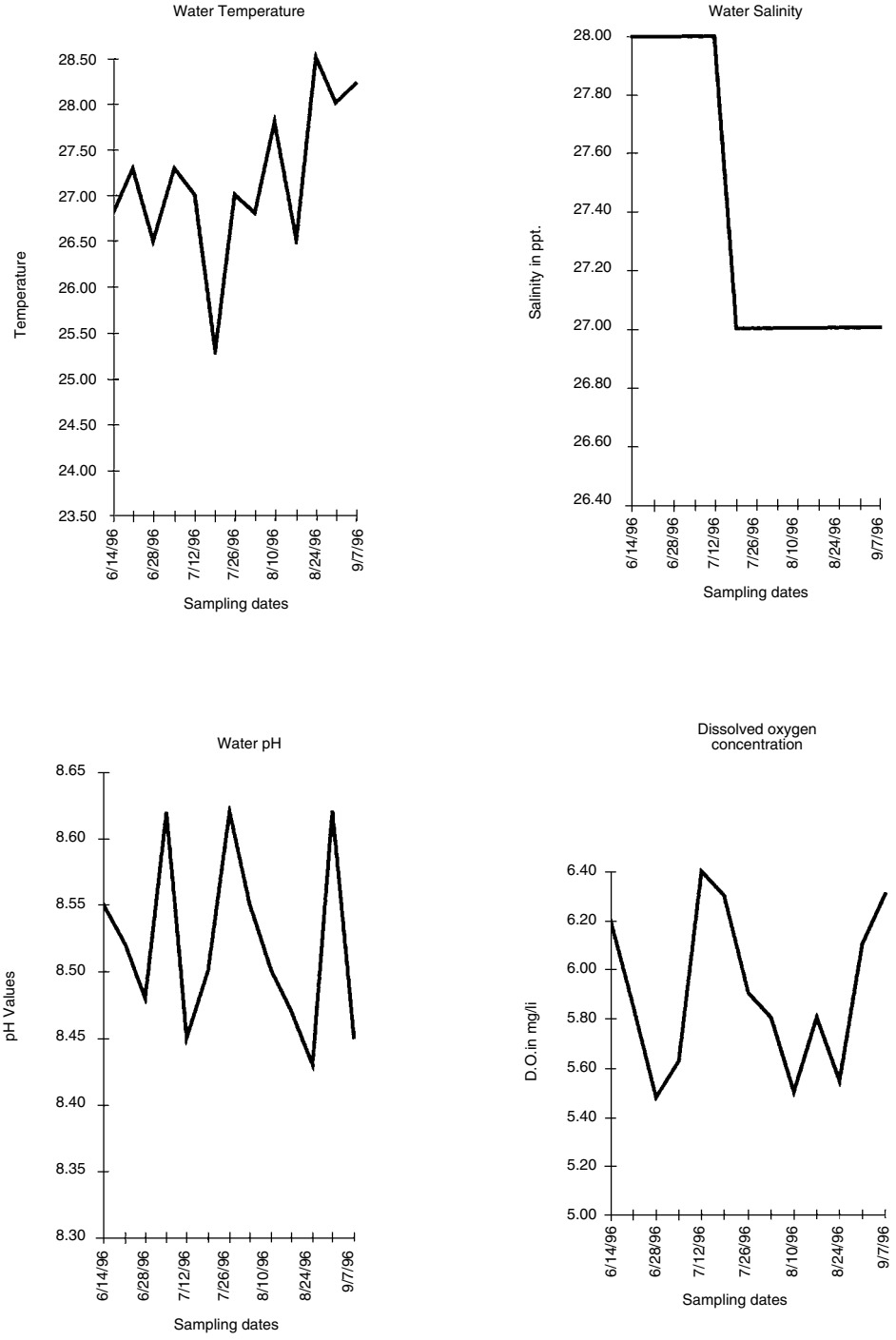

Figure 1a. Water physico-chemical parameters in Experiment 1.

Table 2. Mean weights (g) of mud crab fed with five types of diets during the 90-day culture period, Experiment 2.

Treatment	0 day	15 days	30 days	45 days	60 days	75 days	90 days
I. Moist pellet	0.20	0.40	0.51	0.67	0.78	0.94	1.25
SD	0.14	0.22	0.21	0.20	0.28	0.26	0.29
II. Dry pellet	0.20	0.46	0.62	0.84	1.07	1.71	2.34
SD	0.04	0.13	0.21	0.33	0.35	0.48	0.68
III. Squid	0.20	0.85	1.45	2.07	2.16	3.33	4.02
SD	0.11	0.41	0.62	0.74	0.71	1.04	1.24
IV. Mussel	0.09	0.49	1.09	1.53	2.20	3.46	5.74
SD	0.07	0.45	0.25	0.67	1.21	1.35	2.72
V. Trash fish	0.14	0.50	0.72	1.19	1.97	2.89	4.88
SD	0.10	0.41	0.41	0.63	1.12	1.86	3.55

Table 2a. Mean specific growth rate (g/day) of mud crab fed with five types of diets in the 90-day culture period, Experiment 2.

Treatments	15 days	30 days	45 days	60 days	75 days	90 days
I. Moist pellet	0.0572^{c}	0.0390^{c}	0.0333^{c}	0.0270^{c}	0.0246^{c}	0.0237^{c}
SD	0.0228408	0.0167	0.0145	0.0097	0.0091	0.0084
II. Dry pellet	0.0693^{bc}	0.0466^{bc}	0.0379^{bc}	0.0318^{bc}	0.0331^{bc}	0.0316^{bc}
SD	0.0337	0.0213	0.0150	0.0104	0.0136	0.0122
III. Squid	0.1007^{ab}	0.0685^{a}	0.0543^{ab}	0.0419^{b}	0.0394^{ab}	0.0350^{bc}
SD	0.0224	0.0117	0.0101	0.0091	0.0070	0.0062
IV. Mussel	0.1135^{a}	0.0955^{ab}	0.0699^{a}	0.0569^{a}	0.0528^{a}	0.0494^{a}
SD	0.0171	0.0178	0.0155	0.0084	0.0084	0.0073
V. Trash fish	0.0812^{abc}	0.0591^{abc}	0.0512^{bc}	0.0467^{ab}	0.0413^{ab}	0.0397^{ab}
SD	0.0494	0.0202	0.0169	0.0156	0.0140	0.0112

**Values with different superscripts are statistically different from each other at p<.01

Table 2b. Periodic mean carapace length (cm) and mean total number of moults of crabs fed with five types of diets in Experiment 2.

Treatments	Initial	15 days	30 days	45 days	60 days	75 days	90 days	Total number of moults
I. Moist pellet	0.71	0.91	1.04	1.14^{b}	1.19^{b}	1.28^{b}	1.43^{b}	4.2^{a}
II. Dry pellet	0.67	0.95	1.11	1.19^{b}	1.28^{b}	1.50^{b}	1.68^{b}	4.1^{a}
III. Squid	0.73	1.17	1.43	1.61^{a}	1.65^{a}	1.89^{a}	2.03^{a}	4.4^{a}
IV. Mussel	0.53	0.95	1.35	1.49^{a}	1.62^{a}	1.89^{a}	2.25^{a}	4.6^{a}
V. Trash fish	0.54	0.92	1.10	1.31^{a}	1.54^{a}	1.76^{a}	1.99^{a}	4.8^{a}

**Values with different superscript are statistically different from each other at p<.01

Discussion

Results of both experimental runs showed consistently better performance of mussel meat as feed for crabs in terms of growth compared with the other four diets. The highest growth rate obtained in the present study is similar to the report of Yalin and Qingsheng (1992) who noted that crabs fed with molluscs gave better results compared to crabs given other feeds.

Feeding experiments done by Cheong et al. (1991) also resulted in higher weight gain, survival and feed conversion in animals fed fresh clam meat. This could be attributed to the fact that natural food of crabs consists mostly of molluscs and crustaceans (Lee 1991; Jayamane and Jinadasa 1991). Apparently, mussel meat contains available essential nutrients for the growth of crabs not found or available in prawn feeds.

Further studies should be conducted to verify and elucidate the nutrient profile of mussel meat as a suitable ingredient for mud crab diets. This information is relevant to the establishment of mud crab culture in Panay Island since it is one of the large producers of mussel, making it economical to use and easily available.

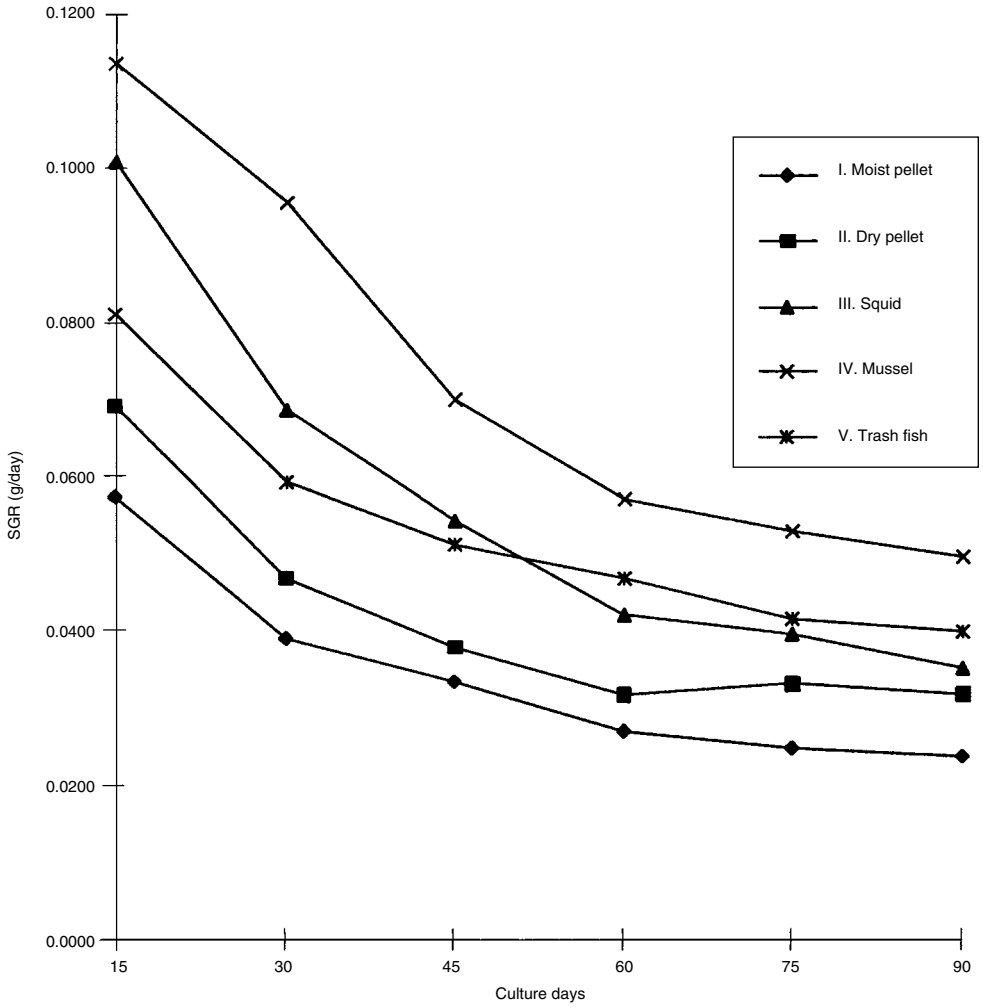

Figure 2. Specific growth rate of mud crab fed with five types of diets in Experiment 2.

The better performance of dry pellet feed could be partly due to the better water stability resulting in higher feed intake compared to moist pellet feed. The moist pellet was observed to disintegrate in the water after only about one hour of immersion while dry pellets were still intact in the water after two hours. This stresses the importance of water stable feed in the culture of mud crab.

The results derived from the two experimental runs compare favourably with the results derived by Ms. Milamena in the feeding of broodstock, on the work carried out by Dr Quinitio on megalopa feeding and the trials by J. Baylon on the feeding of crablets. This shows the necessity of using mussel meat as one of the ingredients in the formulation of crab diets for all stages of crab growth.

A 100% survival was obtained for both experiments through the use of plastic net in the concrete culture tanks to segregate individual crablet. Although the SGR values obtained resulted in a better comparison of the diets, these values were generally low. This could be due to the small compartments where the crabs were confined.

As was observed by Dr Zeng (comments made during these proceedings), the size of the container affects growth increments of crabs, the bigger container resulting in a higher growth increment than smaller containers. The resulting smaller

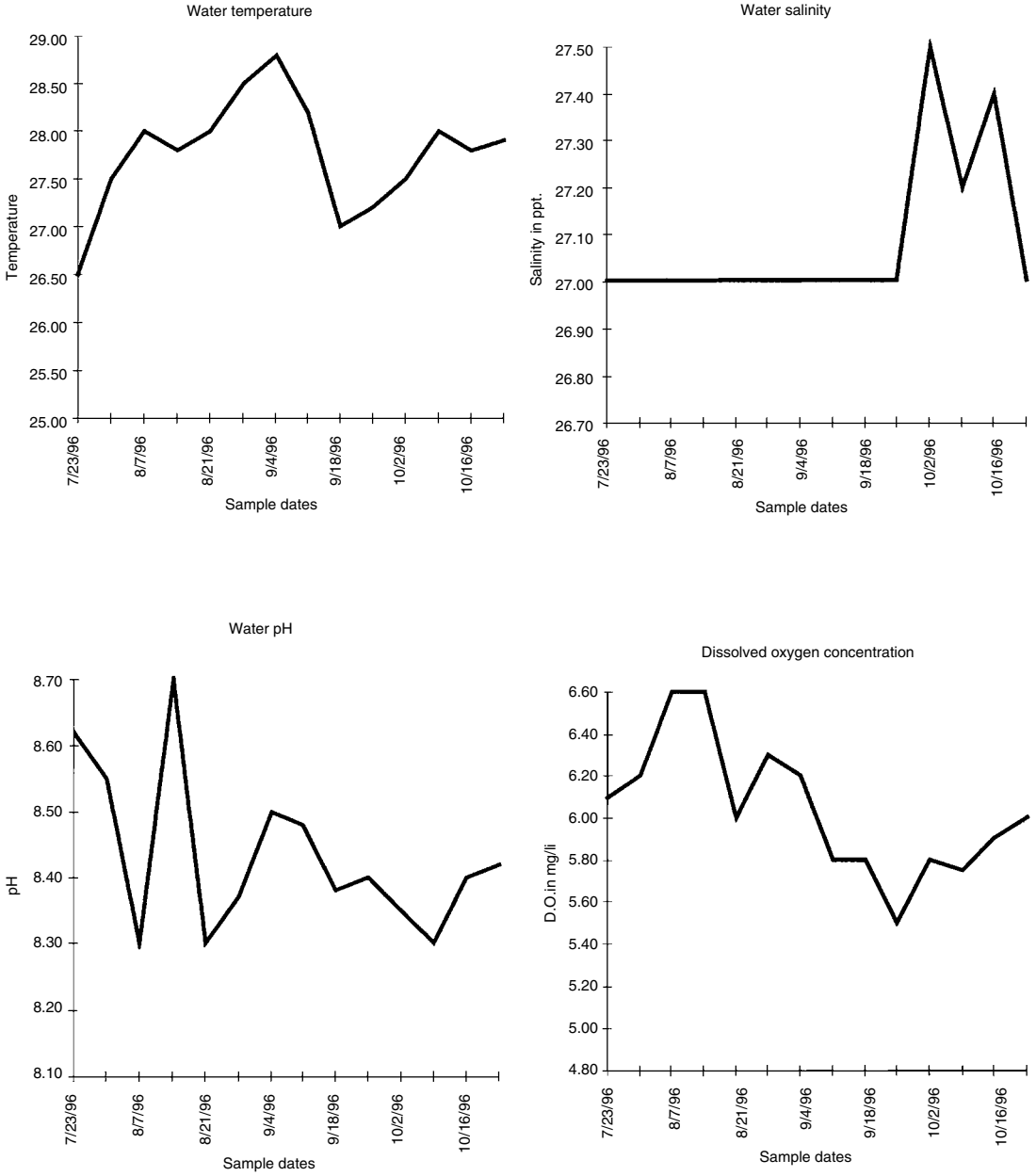

Figure 2a. Water physico-chemical parameters in Experiment 2.

compartments used to segregate the mud crabs used in the present experiments may have resulted to slower growth rate.

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WORKSHOPS

Workshop 1: Farming Systems

Rapporteurs: Donald J. Macintosh[1] and Eddy S.P. Tan[2]

RECOGNISING that crab production is still dependent on wild crab stocks and is based on different culture systems, floating cages for fattening and ponds or pen enclosures for crab grow-out, participants of this workshop have proposed several recommendations and action plans on the following list of factors related to farming systems:

Species selection

There is a need to re-establish the correct species status of mud crabs that have been cultured in different countries. The species in order of priority are:

- *Scylla serrata*;
- *Scylla paramamosain*;
- *Scylla olivacea*;
- *Scylla tranquebarica*.

To facilitate the correct identification of mud crab species, a taxonomic review (Keenan et al. 1998) together with a well-illustrated guide/poster will be prepared by ACIAR. With proper species identification, comparative studies of various experiments will be more meaningful. It was also recognised that the behavioural characteristics of the different species should be defined to facilitate the choice of species for culture.

Production systems

Grow-out systems in ponds in the Philippines are suitable for *S. serrata*, which appears to burrow less, while *S. tranquebarica* is presently being cultured commercially in pens under the canopy of mangrove trees in Sarawak.

There is a need to optimise the production rates in these culture systems through further culture trials, by assessing the effects of different stocking rates, staggered harvesting and restocking schedules. A standardised protocol for estimating production yields has to be established. It has been noted that in some pen enclosures in Sarawak, natural recruitment of crablets into the pens can influence the subsequent yield obtained. Whether *S. serrata* can grow well in pen enclosures, instead of in ponds, needs further investigation.

The economic feasibility of each grow-out system, for mud crabs in different regions, requires a more detailed comparative study, where the farmers' preference for the species being cultured is taken into consideration.

The current methods of production of soft-shelled crab should be strongly discouraged because of the negative impact on the juvenile crab population in the wild. However, such value-added activities may be considered in the future if excess juvenile crabs are being mass-produced from hatcheries.

Nutrition

The nutritional requirements of mud crabs at various phases of their life cycles should be established to enable the development of suitable formulated diets. A more detailed understanding of the physiology of digestion and assimilation of mud crab would further facilitate this.

Recognising that the production cost of formulated diets should be minimised, it is recommended that studies on alternative local materials to replace fish meal should be undertaken, possibly as a component study in a related ACIAR project on fishmeal replacement.

Integrated farming

Farming mud crabs with other commercial aquatic organisms such as fish (milkfish, barramundi), seaweed, or bivalve (*Tapes*) to minimise investment risk and yield enhancement of the culture system need to be addressed, particularly in relation to extensive polyculture farming systems in the Mekong Delta.

[1]Centre for Tropical Ecosystems Research, Dept. of Ecology and Genetics, Building 540, University of Aarhus, DK-8000, Aarhus C. Denmark
[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Environmental management

The development of different culture systems should only be implemented after their environmental impacts have been duly identified together with appropriate utilisation measures. A strategy to exploit the natural crab seed resource on a sustainable basis has to be formulated in parallel with recommendations on how the culture sites should be managed to minimise possible pollution effects. The following issues should be further studied:

- Can mangrove forests act as biofilters?
- What is the impact of aquaculture effluent on the productivity of the mangrove ecosystem?
- How should crab farming be established without damaging the mangrove ecosystem?

ACIAR and DANCED could collaborate to seek solutions to these questions.

Health management

A primary research priority is to establish appropriate culture techniques to ensure that the crabs produced are healthy. A review of the current status of health management of crabs and shrimps in Asia would be useful. AAHRI could be approached to act as the regional coordinator in this study. Expertise in shrimp health management at SEAFDEC should be co-opted.

Marketing

This should be included as a component in the study on the overall aquaculture planning for mud crabs and should include a detailed economic analysis for the region.

Information needs

An exchange of technical information can be initiated by networking and utilising the resources of NACA, DANCED, ACIAR, SEAFDEC and AAHIRI. Communication through newsletters, a networked news group, bulletin board, broadcast e-mail and the publication of occasional papers are possible options.

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Keenan, C.P., Davie, P.J.F. and Mann, D.L. 1998. A revision of the genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). The Raffles Bulletin of Zoology, 46 (1), 217–245.

Figure 13. When moulting from Z5 to megalopa, a commonly observed cause of mortality is an inability of the larvae to completely shed the old carapace before the new carapace hardens, as seen in this photograph. This has been termed "moult-death syndrome" or MDS. Photo: David Mann.

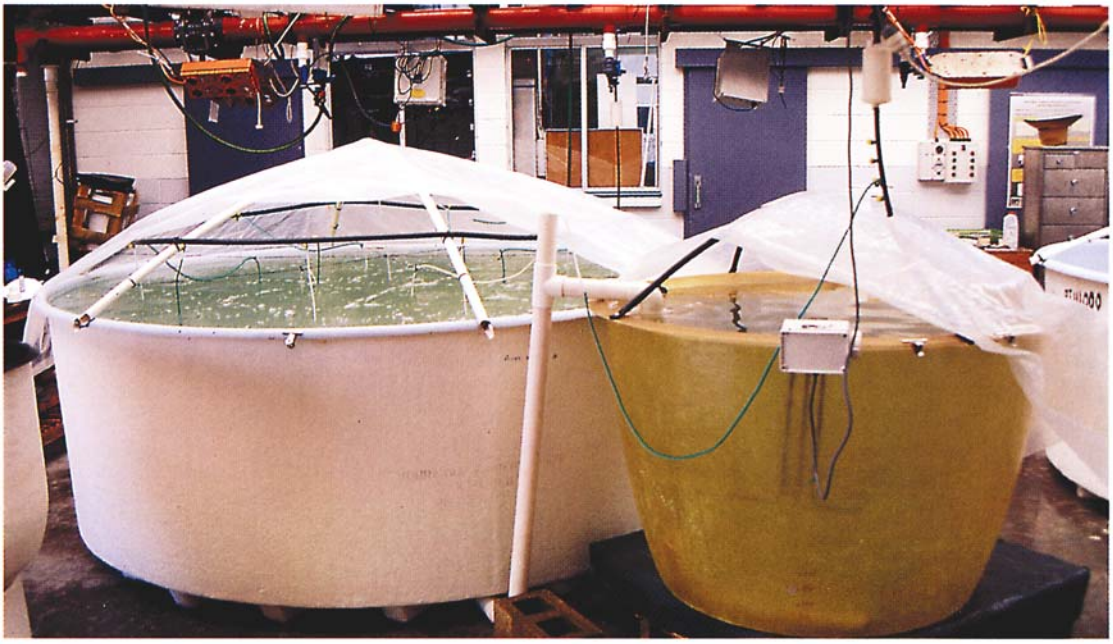

Figure 14. A six tonne mud crab larval rearing tank of the type described by Mann et al. in these Proceedings. Note the plastic covers, heater for maintenance of stable water temperature in the 1 tonne side tank, and the culture water coloured green by the addition of algae. Photo: David Mann.

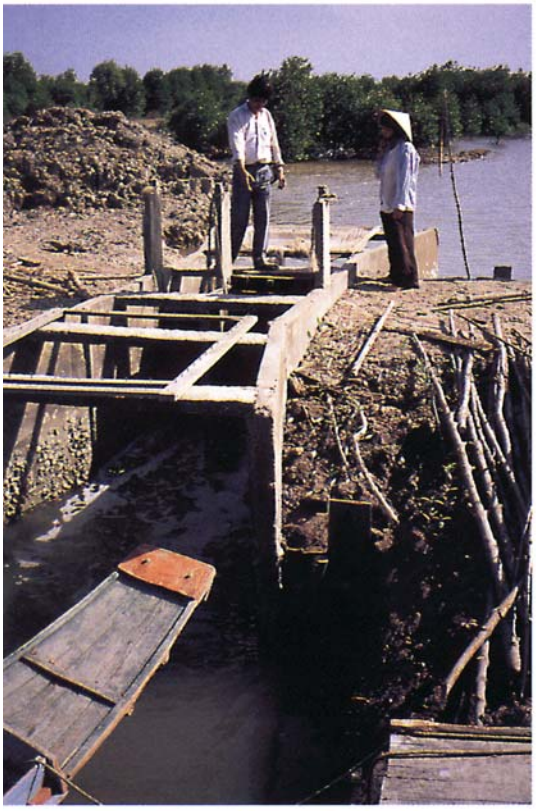

Figure 15. (left) A recently installed, prefabricated, concrete pond gate which are gradually replacing narrow timber gates in the mangrove-shrimp ponds of the Mekong Delta, Vietnam. Photo: Clive Keenan.

Figure 16. (below) A typical shrimp pond of the separate mangrove – shrimp culture style in the Mekong Delta, Vietnam. In the foreground charcoal for cooking fires is being produced from the silvicultured mangrove timber, which can be seen in the background. Photo: Clive Keenan.

Figure 17. (above) A crab growing and fattening pond using the separate mangrove – shrimp culture style in the Mekong Delta, Vietnam. The farmers' house is at the front of the pond on the canal bank. Note the fattening baskets on the bank and in the pond, and the crab traps in the boat. Photo: Clive Keenan.

Figure 18. (right) Smaller canals provide tidal access for water, and shelter for stocked crabs, in the 10 ha of mangrove ponds managed by a single household of a typical Mekong Delta mangrove silviculture farm. These mangroves are approximately 12 years old. Crabs are stocked into the mangrove forest at 500/ha. Photo: Clive Keenan.

Figure 19. (above) Production of mangrove clams within crab enclosures at Sematan, Sarawak, East Malaysia. The farmer was digging up the clams to move them to another site within the enclosure. They provide a natural food source for the stocked mud crabs. Photo: Clive Keenan.

Figure 20. (left) Detail of a channel bank, with the water drained from the channel, within the crab enclosures at Sematan, Sarawak, East Malaysia. Note the many different sized holes and complex environment for shelter. Photo: Clive Keenan.

Figure 21. Planted *Rhizophora* growing in a crab pond at Bone, South Sulawesi, Indonesia. Photo: Clive Keenan.

Figure 22. Crab fattening pen in a crab-growing pond at Bone, South Sulawesi, Indonesia. In the background of the photograph are 10-year-old mangroves that were planted as a conservation effort by the local community. The 500 ha of replanted mangroves are now fished for juvenile mud crabs, which are used to stock the ponds. Photo: Clive Keenan.

Figure 23. Individual crab fattening in a basket in the Mekong Delta, Vietnam. Trash fish is added for crab feed. Photo: Clive Keenan.

Figure 24. Crab fattening cages in a tambak (brackish-water pond) at Timbulsloko, near Semarang, Central Java, Indonesia. The crabs are fed trash fish and also small crabs caught from the tambak. Photo: Clive Keenan.

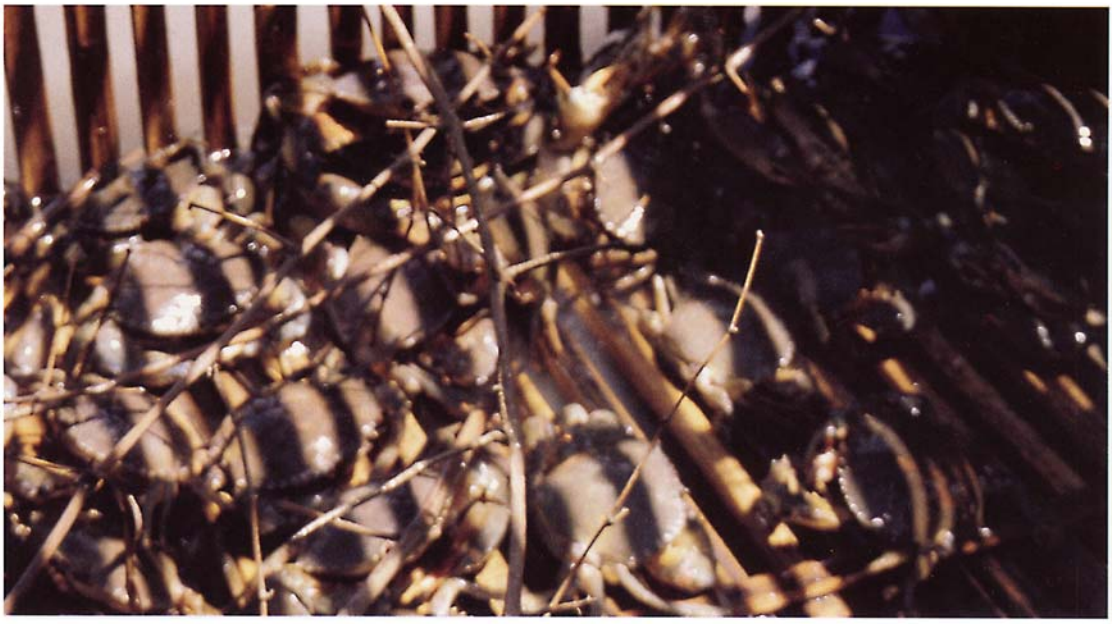

Figure 25. High stocking density of *S. paramamosain* in a crab fattening cage at Timbulsloko, near Semarang, Central Java, Indonesia. Photo: Clive Keenan.

Figure 26. Mud crab catching in Ca Mau, Vietnam using baited lines and a hand net. The lines, supported by a short stick, are placed at intervals around the edge of mangrove shrimp ponds and canals. Photo: Don Macintosh.

Figure 27. Juvenile, 100 g mud crabs (*S. olivacea*), for sale as food at a roadside stall outside of Penang, Malasyia. Photo: Clive Keenan.

Figure 28. An opera-house style of trap used for catching mud crabs from ponds at Kedah, Malaysia. Photo: Clive Keenan.

Figure 29. Carved teak crab table (and chairs in background) from Jepara, Central Java, Indonesia. The identifiable taxonomic features of *S. paramamosain* can be seen in the carving. In this coastal province, crabs provide an important livelihood for many fishers and are a significant component of the diet. Photo: Clive Keenan.

Workshop 2. Larval rearing and nursery production.

Don R. Fielder[1] and Mike P. Heasman[2]

Chair: D.R. Fielder
Rapporteurs: M.P. Heasman, A. Blackshaw, J. Baylon

LARVAL rearing of mud crabs has been attempted by many people for a long time. Most have been successful, but only to a point. One inference from their publications has always been that they were on the verge of solving the numerous rearing problems. Despite rapid progress over the tenure of the ACIAR mud crab project, the above inference has not changed. How close are we really? What problems still require resolution? During the mid-term ACIAR project evaluation meeting in October 1996, much time was spent defining outstanding problems. Topics chosen for discussion in the workshop concerned those problems that still require resolution and/or direction.

Current status of hatchery technology

- Inconsistent survival which has characterised hatchery production for more than 30 years persists.
- Research by Mann et al. at Bribie Island Aquaculture Research Centre (BIARC) has demonstrated that the underlying cause for inconsistent survival of mud crab larvae is vibriosis, with *V. harveyi* and possibly other luminescent species being 'chief suspects'. Experimental use of antibiotics effective against *Vibrio* species virtually eliminated larval mortality.
- The 'quality' of seawater available to the various organisations in hatchery rearing of mud crabs is highly variable necessitating 'customised' pretreatment as a means of combating poor early (Z_1 and Z_2) survival.
- Both chlorination/dechlorination and ageing/settling (9–12 days) in combination with 1 µm filtration have been shown to improve greatly early survival of larvae and have now been widely adopted as a standard protocol by most researchers. An exception is the University of the Philippines in the Visayas (UPV) group which now has access to deep 'high quality' oceanic water of constant high salinity (35 ppt) and low suspended solids and presumably low associated potentially pathogenic bacteria loads.
- The highest and most consistent recent survival rates from Z_1 to C_1 have been achieved by researchers from BIARC, SEAFDEC and UPV. All have used small (3–7 L) experimental rearers, exchanged for new clean rearers on alternate days. It is assumed that consistent survival in the order of 50–60% from Z_1 to megalopa is attributable to minimising the build-up of 'pathogenic' bacteria associated with larger scale rearing systems in which larvae are reared in the same vessel throughout the hatchery cycle.
- Progressive decline in survival of zoeal stages has also been linked with continuous use of wet floor hatchery areas and equipment, highlighting the need for (a) intermediate 'dry out' and disinfection between successive hatchery operations and (b) isolation of successive steps in hatchery operations from broodstock conditioning through spawning, incubation, rearing etc.
- In the absence of bacterial disease problems, several refinements to hatchery rearing protocols have been demonstrated to improve significantly survival and/or growth. The significance of these results is especially important in relation to survival through the critical Z_5 to Meg and Meg to C_1 metamorphic moults. Beneficial refinements can be described under the five topic headings which were used to structure the workshop, i.e.,
  1. Food and feeding (a) Z_1 to Meg, Meg to C_1, Nursery production.
  2. Physical parameters of seawater: (a) salinity, (b) temperature, (c) pH, (d) turbulence, and (e) light.
  3. Provision of substrates for metamorphic moults.
  4. Hygiene and quarantine protocols.
  5. Rearing systems; (a) recirculation in a 'clean' system, (b) flow through system.

[1]Department of Zoology, The University of Queensland, Brisbane, Qld 4072, Australia
[2]N.S.W. Fisheries, Port Stephens Research Centre, Salamander Bay NSW 2316, Australia

1. Feeding regimens

- Replicated small scale experiments conducted by UPV demonstrated very significant advantages of using a combined *Brachionus* (12/mL) *Artemia* nauplii (5/mL) diet rather than *Brachionus* (25/mL) or *Artemia* (10/mL) alone. Although all researchers supported this finding there were considerable differences of opinion as to 'optimal' feeding rates. Indeed, it seemed that variation in rotifer feeding rates (see Table 1) over the range 10–60/mL or *Artemia* nauplii over the range of 0.5–10/mL had little apparent effect on growth and survival of vigorous healthy larvae. System design, container volumes, larval stocking density and economic considerations appear to be critical in the choice of appropriate feeding rates.

Table 1. Larval food regimes used to rear *Scylla* larvae in various laboratories.

Researcher	Larvae/ L	Rotifers/ mL	Rotifers/ larva	*Artemia*/ mL	*Artemia*/ larva
Zeng	150	60	400	10	67
Dat	60	25	417	5	83
	80*	25	313	5	63
	150	25	167	5	33
Williams	10	10	1000	0.5	50
Mann	10	10	1000	1	100
	10	10	1000	3	300
Baylon	10	12	1250	5	50
	50*	12	2 50	5	10

* = Optimum reported for laboratory

- As with feeding rates, larval stocking rates within the wide range of 10–150/L do not appear to have a critical effect on larval growth and survival. All other things being equal, system design, container volumes and operational considerations are the most important constraints in choice of appropriate larval stocking rates.
- Use of premium quality — high concentration highly unsaturated fatty acid (HUFA) *Artemia* nauplii eliminates the need for HUFA boosting of live foods.
- Chilled storage of *Artemia* nauplii until the point of feeding to aid consumption by early zoeal larvae (especially Z_1) had the dual benefit of preserving the nutritional value of the nauplii and increasing the feeding success of the crab larvae.

2. Physico-chemical conditions

a. *Salinity.* Results (Table 2) of a systematic investigation of the effects of salinity on growth and survival of zoeal stages by SEAFDEC (Parado-Estepa and Quinitio, these Proceedings) indicated some variation in optimum salinity with stage. However, several other researchers expressed some scepticism as to the relevance of these results across different strains and species of *Scylla* based on their own studies and experience. Points of disagreement were: (a) that survival of early Z_1 and Z_2 was just as good if not better at full oceanic salinity (35 ppt) than at the lower range of 20–32 ppt indicated by the SEAFDEC research; (b) that a compromise 'constant' salinity of 28 ppt may yield better results than successive adjustments and associated stress at each larval stage. Near concensus was reached on the need for reduced salinity at the Z_5 and megalopal stages and a need throughout hatchery rearing to maintain salinities above 25 ppt.

b. *Temperature.* One researcher (Zeng) asserted that survival was enhanced if temperature was raised gradually from 25 °C (Z_1) to ≅ 30 °C (Z_5) and 30–35 °C for Meg. However, the weight of opinion was that satisfactory growth and survival are maintained at constant temperature within the range 27–30 °C and that sudden fluctuation in temperature of ≅1 ºC had adverse effects and could even cause death.

c. *pH.* Unpublished experimental evidence (Sugama) was cited that survival from Z_1 to Meg is increased by raising pH from 7.9–8.1 (normal situation control) to 8.5–9.0 and 9.1–9.5. Dr Sugama reported that this effect had been demonstrated in a range of tank volumes of 30, 500 and 2500 L in which pH of 9.1 and 9.5 supported excellent survival rates in the range 29% to 40% (Z_1 to Meg). It was generally agreed that these results should be validated by other researchers.

Table 2. Salinity tolerance of mud crab zoea larvae recorded at SEAFDEC.

	20	21	22	23	24	25	26	27	28	29	30	31	32
Z_1	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Z_2	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Z_3					-------	-------	-------	-------	opt				
Z_4					-------	-------	-------	-------	opt	-------	-------	-------	-------
Z_5									opt				

d. *Turbulence.* Near total concensus was reached that turbulence should be minimised in order to promote feeding success and minimise physical damage especially fracture and infection of fragile dorsal spines of zoea larvae.
e. *Light*. One researcher (Baylon) reported apparently improved feeding efficiency by zoea larvae exposed to natural lighting conditions encountered in outdoor culture.

3. Provision of substrates

Provision of suspended plastic mesh or filamentous (Xmas tree) substrates providing attachment sites and/or shelter for resting and/or moulting appear to be critical for high density rearing beyond the pelagic zoeal stages.

These findings which have been made independently by several current researchers corroborate those of earlier studies by Heasman et al. (1985) working with *S. serrata* and other brachyuran crabs. They are also consistent with the finding of MacIntosh et al. that *Scylla* megalopae make use of fallen mangrove leaves for the same purposes and for facilitated transport from lower to upper regions of mangrove estuaries.

4. Hygiene and quarantine protocols

Consensus was reached that:

a. Luminescent *Vibrio* bacteria including virulent strains of *Vibrio harveyi* is the most probable and universal cause of larval mortality.
b. Hygiene and quarantine practices must be targeted primarily at breaking the 'vibriosis cycle'.
c. As in the case of *P. monodon* hatcheries, incidence and severity of larval vibriosis appears to be related to number of successive hatchery cycles completed without intermediate dry-out and disinfection.
d. Regular (alternate day) changing of rearing vessels and associated equipment, as practised when small scale (1–10 L) experimental rearing vessels are used, combined with other best practices identified to date enables regular, high survival to be achieved.

The principal challenge is thus to extend the success of experimental scale culture to large scale commercial culture. Hazard Analysis Critical Control Point (HACCP) analysis was suggested as a necessary tool to identify and combat portals of entry and mechanisms of propagation of virulent strains of *V. harveyi* and other potential pathogens (see Table 3). Entry portals for disease causing organisms are via:

- Vertical transmission from broodstock to eggs to larvae;
- Seawater;
- Food (rotifers and *Artemia*);
- Contaminated utensils and vessels and associated equipment;
- Aerosols from the atmosphere or from other contaminating areas.

Table 3. Entry portals for disease causing organisms and methods for combating such entry in mud crab larvae.

Entry portal	Combat method	
	Oceanic quality	Non-oceanic quality
Seawater	1 µm filtration	Settle and age 9–12 days + 1 µm filtration OR 1 µm filtration + chlorination/dechlorination OR 1 µm filtration + UV
Food • Rotifers	Thorough rinsing in clean seawater following harvest and prior to feeding + regular testing for luminescent *Vibrio.*	
• *Artemia*	Decapsulation of cysts and secondary disinfection of nauplii immediately prior to feeding.	
Contaminated utensils and operators	• Disinfection of all equipment between successive production runs and quarantine of nearby operators. • Use of dedicated equipment for each area of operation. • Regular alternation of rearing vessels. • Regular dryout and disinfection of entire larval rearing areas and/or alternate use of isolated rearing units.	
Aerosol	Prefiltration of air to 0.2 µm and separation of air spaces via plastic film tents.	

5. Rearing systems

Two rearing systems which may have high potential for successful up-scaling of rearing practices to commercial hatcheries were described and discussed.

a. Recirculation in a 'clean' system. This system shown in Figure 1 relies on total or near total exclusion of pathogenic bacteria and use of a companion or jockey vessel to facilitate:
   - Removal of uneaten food;
   - Heating of water outside of the larval rearer;
   - Continuous or periodic exchange of sterile (chlorinated) water;
   - Integration of biofiltration.

b. Flow-through system. This system shown in Figure 2 is based on the 'Bayes' system (Holliday 1986) developed for rearing oyster larvae. It allows for very high stocking density based on continuous slow exchange of temperature matched seawater and food so that optimal food densities are maintained. This system also incorporates alternate day changes of rearing vessels, hence wet sieve harvesting, rinsing and restocking of larvae into new clean vessels free of bacterial films and solid wastes including shed, exuviae which are shed every 3 days, is possible.

NB. Economic considerations will probably dictate that the 'Bayes' system will require stocking densities in the order of >100–1000/L using volumes in the order of 200–1000 L due to mechanical, operating, and economics of scale constraints. The 'Bayes' system thus appears to offer an advantage in combating compounding increases in pathogenic bacteria such as *Vibrio* spp. which are highly associated with specific surfaces/substrates. In the case of *V. harveyi*, surface association is with the chitinous cuticle and exuviae of larval crustaceans (Chen and Hanna 1994).

Recommended best practices

1. Seawater:
   - Deep oceanic or near oceanic–1 µm filtered, aged 9–12 days
   - or filtered 1 µm and chlorinated/dechlorinated
   - >30 ppt for broodstock
   - Salinity regime options – 30–35 ppt, Z_1–Z_5 reducing to 28 at Meg, or constant 28 ppt.

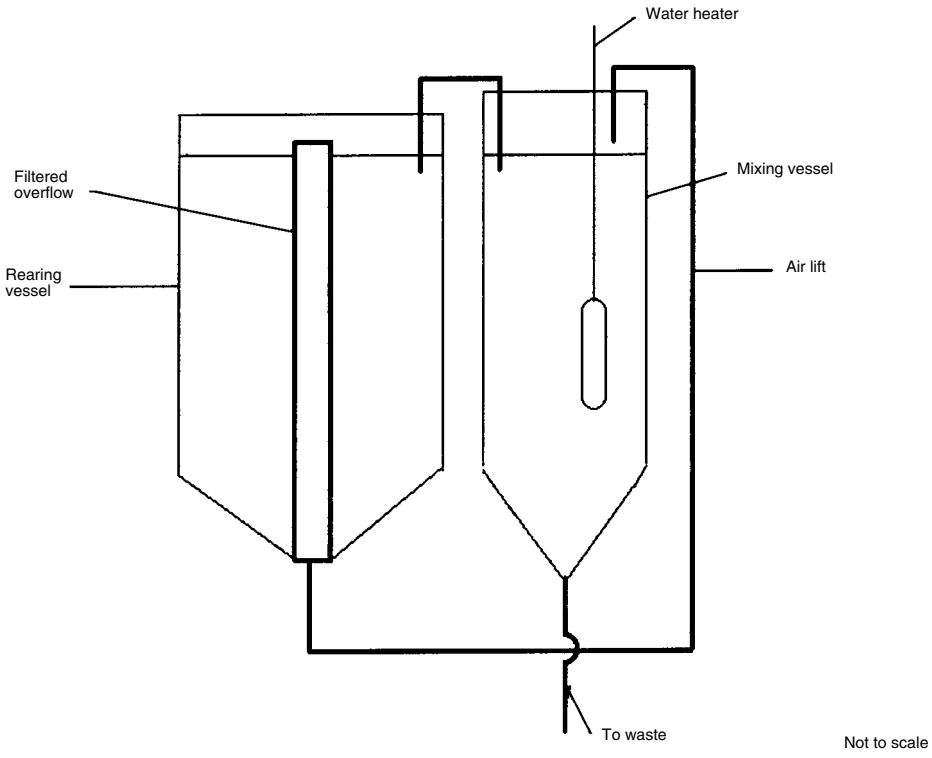

Figure 1. 'Clean' recirculation system for rearing mud crab larvae (after Mann et al., these Proceedings).

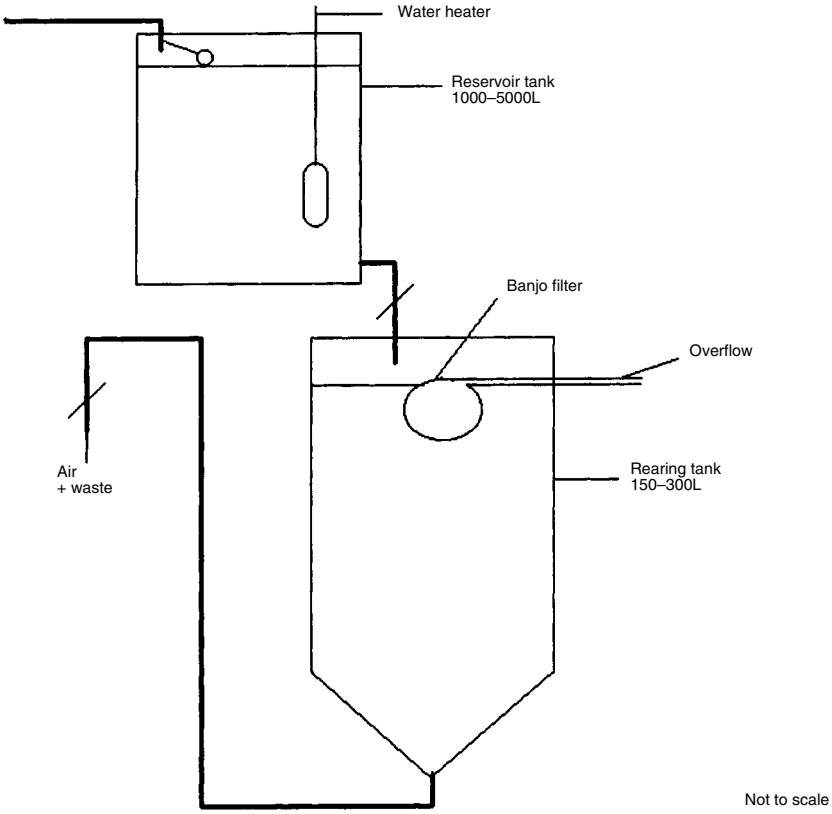

Figure 2. Flow-through system for rearing mud crab larvae (modified 'Bayes' system after Holliday 1986).

2. Broodstock:
   - Females should be free of lesions and have a full complement of limbs.
   - Diet — Mixed diet or special formulated diet (SEAFDEC) or mixed diet with high mollusc component.
   - Spawning induction — Avoid bilateral ablation.
   - Disinfection of broodstock — 10 ppm formaldehyde for 12 h.
   - Shelter — All females provided with individual shelters and stocked at low density.
   - Substrate — Aerated sand substrate in (0.8 m^2) spawning tray in otherwise bare tank which is cleaned daily.
   - Incubation. Isolate berried females in separate incubation tanks equipped with aerated sand tray and recirculated, UV treated seawater. Do not feed during 8–12 days incubation period.
   - Hatching. Transfer berried females to floating chamber in separate spawning tank with open exchange of 1 µm filtered seawater 1–2 days before spawning.

3. Larvae:
   - Collect only vigorous positively phototropic larvae and immediately flush with 1 µm filtered, UV disinfected seawater. Transfer larvae to culture tank in 5 L dish allowing 45 minutes of water blending and acclimation.
   - Salinity — see Table 2.
   - Feeding regime — see 4 below.
   - Change rearing vessels for fresh disinfected units regularly.

4. Feeding regime:

	Rotifers	*Artemia* nauplii*
Zoea 1	10/mL and 500–1000/larva	
Zoea 2	"	1–5/mL and 50/larva
Zoea 3	"	"
Zoea 4		"
Zoea 5		"
Megalopa		"
Crab 1		

*certified high HUFA

Rotifers should be harvested and rinsed before feeding. 'Green water' requirements are not known and need further investigation. *Artemia* cysts should be fully decapsulated. Nauplii should be chilled and disinfected immediately prior to feeding.

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Participants

Dr Juliana Baylon
College of Arts and Sciences
University of the Philippines in the Visayas
Miagao 5023
Philippines
Tel: +63 33 315 8142
Fax: +63 33 338 1534
ccbaylon@iloilo.net

Dr Alan Blackshaw
13 Jenkinson Street
Indooroopilly QLD 4068
Australia
Tel: +61 7 3378 2395
Fax: +61 7 3365 1766
A.Blackshaw@mailbox.uq.oz.au

Mr Brian Cann
Senior Economist
NTDPIF
Berimah Agricultural Research Centre
GPO Box 990
Darwin NT 0801
Australia
Tel: +61 8 8999 2028
Fax: +61 8 8999 2200

Dr Fuad Cholik
Director
Central Research Institute for Fisheries
Agency for Agricultural Research and Development
PO Box 6650 Slipi
Jakarta 11410A
Indonesia
Tel: +62 21 5709160
Fax: +62 21 5709159

Mr Brian Dalliston
Darwin Aquaculture Centre
GPO Box 990
Darwin NT 0801
Australia
Tel: +61 8 8999 4362
Fax: +61 8 8999 4193

Dr Hoang Duc Dat
Head Department of Ecology and Development
Institute of Tropical Biology
85 Tran Quoc Toan St.
Dist. 3, Ho Chi Minh City
Viet Nam
Tel: +84 8 8294243
hddat@hcm.vnn.vn

Dr Don Fielder
Associate Professor
Zoology Department
University of Queensland Qld 4072
Australia
Tel: +61 7 3365 2455
Fax: +61 7 3365 1655
dfielder@zoology.uq.edu.au

Dr Romeo Fortes
Professor of Aquaculture
Institute of Aquaculture, College of Fisheries
University of the Philippines in the Visayas
Miagao 5023
Philippines
Tel: +63 33 315 8090 (Office)
+63 33 329 6638 (Home)
Fax: +63 33 338 1534 (Miagao)
0912-520-0626 (BAC Leganes)

Mr Jerome Genodepa
Institute of Aquaculture, College of Fisheries
University of the Philippines in the Visayas
Miagao, Iloilo 5023
Philippines
Tel: +63 33 315 8090
Fax: +63 33 338 1534

Dr Mike Heasman
NSW Fisheries Aquaculture Centre
Taylors Beach Road
Taylors Beach NSW 2316
Australia
Tel: +61 49 82 1232
Fax: +61 49 82 1107
mheasman@ozemail.com.au

Dr Johannes Hutabarat
Director
Research Centre for Development Technology
Universitas Diponegoro
Jalan Imam Bardjo, SH No.5
Semarang 50241
Indonesia
Tel: +62 24 411450
Fax: +62 24 442703
jhutabarat@usa.net

Mr Abdullah Mhd Ikhwanuddin
Fisheries Officer In-Charge
Inland Fisheries Semongok
Sarawak Agriculture Department
Kuching
Sarawak 93250
Malaysia
Tel: +60 82 613630
+60 82 320105
Fax: +60 82 611871
amikhwan@mailhost.unimas.my

Dr Danielle Johnston
Project Biologist PN 9412
Australian Institute of Marine Science
PMB 3, Townsville, Qld, 4810
Australia
Tel: +61 77 534 333
Fax: +61 77 725 852
d.johnston@aims.gov.au

Dr Clive Keenan
Principal Biologist
Bribie Island Aquaculture Research Centre
PO Box 2066
Bribie Island Qld 4507
Australia
Tel: +61 7 3400 2016
Fax: +61 7 3408 3535
keenanc@dpi.qld.gov.au

Dr Chan Lee
Senior Lecturer
Faculty of Science
Northern Territory University
Casuarina Campus
Darwin NT 0909
Australia
Tel: +61 8 8946 6358
Fax: +61 8 8946 6690
CLEE@darwin.ntu.edu.au

Dr Don Macintosh
Research Council Professor
Centre for Tropical Ecosystems Research
Dept of Ecology and Genetics
Building 540, Aarhus University
Aarhus C. DK8000
Denmark
Tel: +45 8942 3302
Fax: +45 8942 3350
don.macintosh@biology.aau.dk

Mr David Mann
Project Biologist PN 9217
Bribie Island Aquaculture Research Centre
PO Box 2066
Bribie Island Qld 4507
Australia
Tel: +61 7 3400 2023
Fax: +61 7 3408 3535
mannd@dpi.qld.gov.au

Mrs Evelyn Marasigan
Institute of Aquaculture, College of Fisheries
University of the Philippines in the Visayas
Miagao, Iloilo 5023
Philippines
Tel: +63 33 315 8090
Fax: +63 33 338 1534

Mrs Oseni Millamena
Aquaculture Department
SEAFDEC
PO Box 256
Iloilo City 5000
Philippines
Tel: +63 33 335 1009
+ 63 33 336 2937 / 65
Fax: +63 33 335 1008
oseni@aqd.seafdec.org.ph

Ms Julia Lynne Overton
Research Assistant
Centre for Tropical Ecosystems Research
Dept of Ecology and Genetics
Building 540, Aarhus University
Aarhus C. DK8000
Denmark
Tel: +45 8942 3349
Fax: +45 8942 3350
JULIALYNNE@compuserve.com

Dr Emilia Quinitio
Aquaculture Department, SEAFDEC
PO Box 256 Iloilo City 5000
Philippines
Tel: +63 33 335 1009
+63 33 336 2937 / 65
Fax: +63 33 335 1008
etquinit@aqd.seafdec.org.ph

Dr Colin Shelley
Assistant Director Aquaculture
Darwin Aquaculture Centre, DPIF
GPO Box 990 Darwin NT 0801
Australia
Tel: +61 8 8999 4363
Fax: +61 8 8999 4193
shelleyc@ozemail.com.au

Mr Barney Smith
ACIAR Fisheries Program Coordinator
C/- NSW Fisheries Research Institute
PO Box 21, Cronulla NSW 2230
Australia
Tel: +61 2 9527 8462 / 3
Fax: +61 2 9523 5966
smithb@fisheries.nsw.gov.au

Dr Ketut Sugama
Director
Gondol Research Station for Coastal Fisheries
Agency for Agriculture Research and Development
PO Box 140, Singaraja
Bali 81101
Indonesia
Tel: +62 362 92278
Fax: +62 362 92272
+62 362 23109
sugama@singaraja.wasantara.net.id

Dr Eddy S.P. Tan
Associate Professor
School of Biological Sciences
Universiti Sains Malaysia
Penang 11800
Malaysia
Tel: +604 6575150
Fax: +604 6565125
espt@usm.my

Mr Avelino Triño
Aquaculture Department, SEAFDEC
PO Box 256, Iloilo City 5000
Philippines
Tel: +63 33 335 1009
+63 33 336 7762
Fax: +63 33 335 1008
attrino@aqd.seafdec.org.ph

Mr Nguyen Van Trong
Head
Division of Environment and Fishery Resources
Research Institute for Aquaculture No. 2
116 Nguyen Dinh Chieu St.
Dist. 1, Ho Chi Minh City
Viet Nam
Tel: +84 8 8226806
Fax: +84 8 8226807
system@ria2.ac.vn

Mr Graham Williams
Darwin Aquaculture Centre
GPO Box 990, Darwin NT 0801
Australia
Tel: +61 8 8999 4362
Fax: +61 8 8999 4193

Mr John Wood
Darwin Aquaculture Centre
GPO Box 990, Darwin NT 0801
Australia
Tel: +61 8 8999 4362
Fax: +61 8 8999 4193

Dr Chaoshu Zeng
c/- Fisheries Research Station,
Kyoto University,
Maizuru, Kyoto 625
AND
Kyoto University
Faculty of Agriculture
Department of Fishery
Kyoto 606
Japan
Tel: +773 62-5512
Fax: +773 62-5513
cszeng@kais.kais.kyoto-u.ac.jp


ACIAR
The Australian Centre for International Agricultural Research