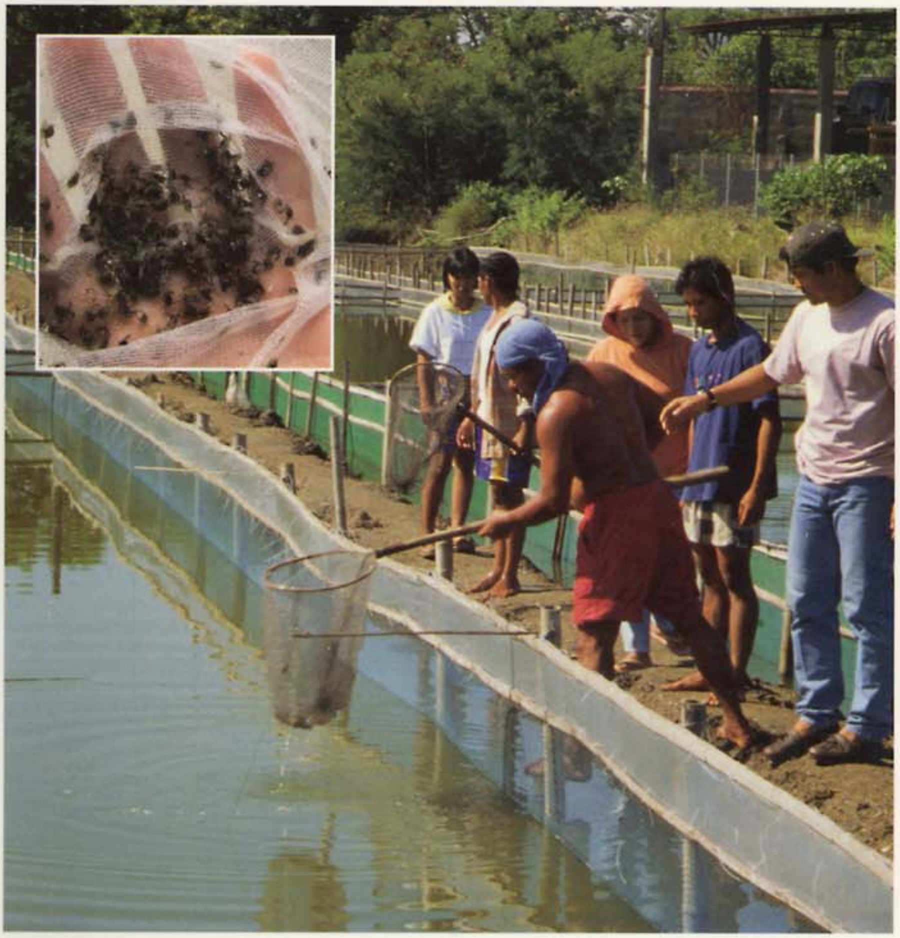
MUD CRAB AQUACULTURE AND BIOLOGY



ACIAR PROCEEDINGS No. 78

Cover page: Sampling of Mr Trino's experimental ponds at Molo, on the Iloilo River, Panay, Philippines. The results of this experiment are detailed in these Proceedings.

Inset: Hatchery-reared *S. serrata* crablets (C1 and C2) reared at the Bribie Island Aquaculture Research Centre.

Photos: **Clive Keenan.**

Mud Crab Aquaculture and Biology

Proceedings of an international scientific forum held in Darwin, Australia, 21–24 April 1997

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Contents

Foreword **5**

Setting the Scene 7

Aquaculture of the Mud Crab, Genus *Scylla* — Past, Present and Future
Clive P. Keenan **9**

Review of Mud Crab Culture Research in Indonesia
Fuad Cholik **14**

Mixed Shrimp Farming—Mangrove Forestry Models in the Mekong Delta: ACIAR PN 9412
Nguyen Van Trong **21**

Malaysian Crab Research
Eddy S.P. Tan **25**

Mud Crab Research and Development in the Philippines: An Overview
Romeo Diño Fortes **27**

Genetics and Ecology 33

Morphometrics and Ecology of the Mud Crab (*Scylla* spp.) from Southeast Asia
Julia Lynne Overton **35**

Genetic Characterisation in the Mud Crab *Scylla* (Brachyura: Portunidae)
Ketut Sugama and Jhon H. Hutapea **43**

The Fourth Species of *Scylla*
Clive P. Keenan **48**

Growout in Ponds 59

Monosex Culture of the Mud Crab *Scylla serrata* at Three Stocking Densities with *Gracilaria* as Crab Shelter
Avelino T. Triño, Oseni M. Millamena and Clive P. Keenan **61**

Description of Mud Crab (*Scylla* spp.) Culture Methods in Vietnam
Hoang Duc Dat **67**

Preliminary Results of the Rearing of Mud Crab *Scylla olivacea* in Brackishwater Earthen Ponds
Romeo Diño Fortes **72**

Preliminary Economic Analysis of Mud Crab (*Scylla serrata*) Aquaculture in the Northern Territory of Australia
Brian Cann and Colin C. Shelley **76**

Growout in Mangroves 81

Pen Culture of Mud Crabs, Genus *Scylla,* in the Mangrove Ecosystems of Sarawak, East Malaysia
William Chang Wei Say and Abdullah Mhd Ikhwanuddin **83**

Pen Culture Experiments of the Mud Crab *Scylla serrata* in Mangrove Areas
Jerome G. Genodepa **89**

Mud Crab Culture in the Minh Hai Province, South Vietnam
Danielle Johnston and Clive P. Keenan **95**

Broodstock 99

Performance of Mud Crab *Scylla serrata* Broodstock Held at Bribie Island Aquaculture Research Centre
David Mann, Tom Asakawa and Alan Blackshaw **101**

Diets 107

Suitability of Local Raw Materials for Mud Crab Feed Development
Johannes Hutabarat **109**

Reproductive Performance of Pond-sourced *Scylla serrata* Fed Various Broodstock Diets
Oseni M. Millamena and Emilia T. Quinitio **114**

Larval Rearing 119

Investigations into the Reproductive and Larval Culture Biology of the Mud Crab, *Scylla paramamosain*: A Research Overview
Shaojing Li, Chaoshu Zeng, Hong Tang, Guizhong Wang and Qiongwu Lin **121**

Development of Hatchery Techniques for the Mud Crab *Scylla serrata*: 1. Comparison of Feeding Schemes
Emilia T. Quinitio, Fe Parado-Estepa and Veronica Alava **125**

Mud Crab (*Scylla serrata*) Megalopa Larvae Exhibit High Survival Rates on *Artemia*-based Diets
Graham R. Williams, John Wood, Brian Dalliston, Colin C. Shelley and Chris Kuo **131**

Larval Rearing of the Mud Crab *Scylla serrata* in the Philippines
Juliana C. Baylon and Alan N. Failaman **141**

Preliminary Studies on Rearing the Larvae of the Mud Crab (*Scylla paramamosain*) in South Vietnam
Hoang Duc Dat **147**

Development of a Hatchery System for Larvae of the Mud Crab *Scylla serrata* at the Bribie Island Aquaculture Research Centre
David Mann, Tom Asakawa and Morris Pizzutto **153**

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 and Shaojing Li **159**

Larval Ecology and Nursery 167

Quality Control Using Hazard Analysis Principles for Mud Crab Culture
Alan Blackshaw, David Mann and Clive P. Keenan **169**

Larval Survival and Megalopa Production of *Scylla* sp. at Different Salinities
Fe Parado-Estepa and Emilia T. Quinitio **174**

Transport Mechanisms of Crab Megalopae in Mangrove Ecosystems, with Special Reference to a Mangrove Estuary in Ranong, Thailand
D.J. Macintosh, F. Gonçalves, A. Soares, S. Moser and N. Paphavisit **178**

Development of Practical Diet for Grow-out of Mud Crab Species *Scylla serrata* and *S. tranquebarica*
Evelyn T. Marasigan **187**

Workshops 197

Workshop 1: Farming systems
Donald J. Macintosh and Eddy S.P. Tan **199**

Workshop 2: Larval Rearing and Nursery Production
Don R. Fielder and Mike P. Heasman **209**

Participants 215

Foreword

Until recently, mud crabs have only been reared from stock captured from the natural environment, in those countries where they are being farmed. This practice may threaten the viability of natural stocks, and contribute to concerns about the sustainability of mud crab aquaculture. Now, as reported in these Proceedings, larval production of mud crabs (*Scylla serrata*) can be reliably achieved, the rapid growth of mud crabs identified and their taxonomy clarified.

These significant events were the result of an international collaborative research effort, involving scientists from the Bribie Island Aquaculture Research Centre and Darwin Aquaculture Centre and their counterparts at two institutions in the Philippines, the Aquaculture Department of SEAFDEC and the University of the Philippines in the Visayas.

The commercial implications of this successful research are enormous, as are the implications for preserving the natural resources of the world's fisheries.

The Queensland Government, through the Department of Primary Industries, has contributed to this outstanding advance through its commitment to the facilities and the research being undertaken at the Bribie Island Aquaculture Research Centre. The Australian Government has also contributed to this work through the funding provided by its agency, the Australian Centre for International Agricultural Research, which must be congratulated for its long-term commitment to development through scientific discovery and application.

I recommend "Mud crab aquaculture and biology: proceedings of an international scientific forum held in Darwin", to all interested in aquaculture, and congratulate those associated with this research.

Dr Warren Hoey
Director-General, Queensland Department of Primary Industries

SETTING THE SCENE

Aquaculture of the Mud Crab, Genus *Scylla* — Past, Present and Future

Clive P. Keenan[1]

Abstract

Crabs of the genus *Scylla* are strongly associated with mangrove areas throughout the Pacific and Indian oceans and form the basis of substantial fishery and aquaculture operations. Aquaculture production currently relies on wild-caught seed for stocking ponds, as larval rearing at a commercial scale is still difficult. One of the major problems for effective mud crab management and aquaculture is the likelihood that there are a number of genetically distinct species. Research has demonstrated the presence of at least four distinct species. Laboratory experiments of the larval stages of each species should provide valuable information on each species' biological and ecological requirements. There are two basic forms of land-based mud crab aquaculture: fattening of crabs with a low flesh content, and growout of juveniles to market size. Fattening is a very profitable activity, employing high densities of crabs and low costs. However, total production is low because of mortalities due to cannibalism. Growout systems for mud crabs show much more variety and production can be very high. Growout systems are usually pond-based, with or without mangroves, although intertidal pens can also be used. Without mangroves, lower stocking rates provide the best return. In shallow mangrove ponds, there are two distinct forms of aquaculture: (i) intensive, with higher stocking rates and supplemental feeding; and (ii) extensive, in large mangrove silviculture ponds where the stocking rate is very low, and no supplemental feeding is involved. Growth rates under all systems are comparable, with production of commercial-sized crabs three to four months after stocking with seed crabs. Further research is required into the habitat preferences of each species so that production techniques can be modified to suit their respective requirements. With advances in the hatchery production of mud crab juveniles for stocking into ponds and enclosures, the future of mud crab aquaculture looks promising.

VARIOUS species of mud crab, *Scylla* spp., occur throughout tropical to warm temperate zones where they form the basis of small but important inshore fisheries. Also known as mangrove crabs, they are commonly associated with mangrove swamps and nearby intertidal and subtidal muddy habitat. Their size, high meat yield and delicate flavour mean that everywhere they occur, mud crabs are sought after as a quality food item (Rattanachote and Dangwatanakul 1992). As they are easily caught using very simple traps or nets, remain alive for considerable periods after capture (Gillespie and Burke 1992) and are of high value, the animal is an important source of income for small-scale fishers throughout the Asia-Pacific region.

Aquaculture of the mud crab has been conducted for at least the past 100 years in China (Yalin and Qingsheng 1994) and for the past 30 years throughout Asia. In Japan, sea-ranching of hatchery-reared mud crab seed has been employed but seed production has not proved reliable (Shokita et al. 1991). Almost all crab aquaculture production relies on wild-caught stock, as larval rearing has not yet reached a commercially viable level for stocking into aquaculture farms.

The major constraint restricting further expansion of mud crab culture is the limited supply of crab 'seed' for stocking enclosures. Even at the current size of the mud crab culture industry, quantities of crab 'seeds' caught by fishermen are not sufficient to meet demand (Cowan 1984; Liong 1992). Contributing to this is the loss of mangrove forest, over-exploitation of wild stocks and recent growth in crab

[1]Bribie Island Aquaculture Research Centre, PO Box 2066, Bribie Island, Qld 4507 Australia

culture operations. The seasonal nature of availability of 'seed' crabs compounds the supply problem. In general, supplies of juvenile crabs for culture are insufficient to allow any further growth in the scope of present culture operations.

These problems were recognised at a Regional Seminar on Mud Crab Culture and Trade in the Bay of Bengal Region held in Surat Thani, Thailand in November 1991 (Angel 1992). This meeting was sponsored by the FAO-supported Bay of Bengal Program for Fisheries Development in an attempt to improve conditions for small-scale fishing communities through mud crab fattening and culture. Interest in this seminar was very high with 35 papers being presented from Australia, Bangladesh, India, Indonesia, Malaysia, Myanmar, Philippines, Sri Lanka and Thailand (INFOFISH 1992). Many of the papers presented at the meeting were experiential and while informative were not based on rigorous scientific experimental disciplines. There was, in general, a need to collect hard, science-based data on many aspects of crab culture. Recommendations of the seminar responded to what were seen as key issues. It was recommended that:

1. **More intensive research be carried out on larval rearing techniques, including water quality and nutritional requirements of larvae, as well as broodstock maturation and spawning.** This was in response to the observation that mud crab culture development was being restricted by limitations of seed supply. It was also thought that progress in larval rearing could benefit natural stocks through seeding programs.

2. **Studies on nutrition, cannibalism, water quality, pond management and disease need to be undertaken to improve growout survival.** Included with this major recommendation were comments on the identification of nutritional requirements of crabs, so that prepared feeds could replace the trash fish which constitute the main supplemented feeds used at present.

3. **The genetic or systematic basis of mud crab populations in Southeast Asia needs to be defined.** This arises from the experience throughout Southeast Asia with different 'races' or species of crabs which grow with different growth rates and appearance – and differential market value.

4. **Technical support be provided for the mud crab trade, including improving packaging technology, market intelligence as well as extension and training programs to popularise mud crab culture and fattening.**

In 1995, an Australian Centre for International Agriculture Research (ACIAR) funded research project No. 9217 'Development of improved mud crab culture systems in the Philippines and Australia' began to examine these important facets of mud crab aquaculture. Many of the results presented within these Proceedings are the results of research arising directly from this project. Further, as the mud crab is a priority species throughout many Asian countries and each country has scientists working on solving problems related to crab aquaculture, their attendance and contribution to the Proceedings has expanded considerably the mud crab information network.

The future of crab aquaculture looks exceedingly bright. Rigorous scientific information to be presented at this meeting (Triño et al., these Proceedings) provides the first cogent evidence of the commercial benefits of crab aquaculture and the tremendous growth rates that can be achieved. In addition, the possibility of 'environment friendly' farms (Chang and Ikhwannddin; Johnson and Keenan, these Proceedings) suggest that the integration of crab aquaculture with mangrove silviculture is a distinct possibility providing both immediate and long-term commercial and environmental benefits. Apart from the work presented in these Proceedings, there are obviously many areas of mud crab aquaculture research that require further investigation and the topics of disease, selective breeding and growout diet development immediately come to mind.

The depth of knowledge in all aspects of mud crab aquaculture has significantly increased since the Bay of Bengal Meeting.

Species

- A solid taxonomic base has now been established (Keenan et al. 1998) so that, for the first time, correct species names can be applied to research animals from east Africa through to the western Pacific islands.

Broodstock

- Broodstock holding and maturation methods have been improved and several papers in these Proceedings discuss these developments.

Larval rearing

- Larval rearing improvements have been achieved but further development is still required to achieve high survival and commercially viable production. There is an increased understanding of the nature of problems faced with rearing mud crab larvae. There are many papers in these Proceedings that examine these factors.

Nursery

- Crab seed are usually stocked into ponds at a minimum size of 10 g. Therefore, there appears to be a requirement for a long nursery phase, where megalopa and C1 crabs of about 25 mg are raised, under ideal conditions, to a size suitable for stocking. There have been some developments in this area, but more work will need to be done to achieve high survival and reduced costs.

Growout

- As mentioned above, several research studies have now been conducted on growout and results are presented in these Proceedings. As well, during travel to many mud crab growing areas, a huge diversity of methods has been observed. There are two aspects of growout that require independent examination: (I) fattening of empty crabs; and (II) rearing or growout of seed crabs. Information obtained through discussions with farmers is presented below to highlight production differences between some of these different systems.

Marketing and profitability

- Finally, to become a significant commercial activity, there needs to be sufficient profit and large markets to sustain increased production and business investment. Several recent studies have examined crab markets worldwide (Brienl and Miles 1994; GLOBEFISH 1995; AUSTRADE 1996) and suggest the market is very large and increasing. Papers in these Proceedings examine the profitability of crab farming under simulated conditions in Australia (Cann and Shelley) and actual costs in the Philippines (Triño et al.).

Crab Aquaculture Production Systems

Land-based aquaculture of the mud crab is conducted using a variety of approaches. Fattening is primarily conducted in small bamboo enclosures in ponds or rivers, although more extensive pond-based systems can be successfully used (Table 1). The density of crabs for fattening can be very high (>15/m^2) and supplemental feeding rates are also high. To be successful, fattening must be completed prior to moulting, otherwise, mortality reduces production (Rattanachote and Dangwattanakul 1992).

Pond-based aquaculture of crabs is usually a very profitable operation (Triño et al. these Proceedings). Stocking rates in ponds vary between 0.05/m^2 for extensive stocking, 1.5/m^2 for pond aquaculture, and up to 5/m^2 for enclosures. Growth rates under all systems are comparable and surprisingly fast, with production of commercial sized crabs of 400 g in three to five months, dependent on the size at stocking.

Crab fattening

The results of a study conducted in 1996 by Dinas Perikanan Dati II Kab. Demak of crab fattening in cages placed in Indonesian tambaks are presented in Figures 1 and 2 and Table 1. A feeding rate of 10% wet weight was employed, with the food items consisting of dried fish and small crabs caught from the tambaks. Survival over the 20-day growth period was 80% to 85%. The male crabs added 110 g on average and females added 90 g body weight over this period.

While the profit of this operation was good, because of the price differential of ovigerous female

Table 1. Summary of production parameters for several different types of crab production methods in Southeast Asia.

Method	Fattening		Rearing		
	Pond	Cage	Mangrove enclosure	Mangrove pond	Open pond
(Location)	(Sarawak)	(Semarang)	(Sarawak)	(Mekong)	(SEAFDEC)
Species	*S. olivacea* *S. tranquebarica*	*S. paramamosain*	*S. olivacea* *S. tranquebarica*	*S. paramamosain*	*S. serrata*
Size of pond (m^2)	8000	9	200	100 000	150 (experimental)
Stocking rate/m^2	10	10	3	0.05	1.5
Size of seed (g)	250	350	85	10–100	10
Sex	Mixed	Mixed	mixed	mixed	Single
Feeding rate	2.5%	5%	5%	—	8%
Food items	trash & offal	dried fish & crabs	trash fish	natural production	25% fish 75% mussel
Cover	Vegetated centre mound	small branches	mangroves	mangroves	*Gracilaria*
Rearing period	30 days	20 days	120 days	90–120 days	120 days
Survival	70%–90%	85%	85%	50%–60%	54%
Production (kg)/ stocked weight (kg)	1.5	1.07	2.5	20.9	20

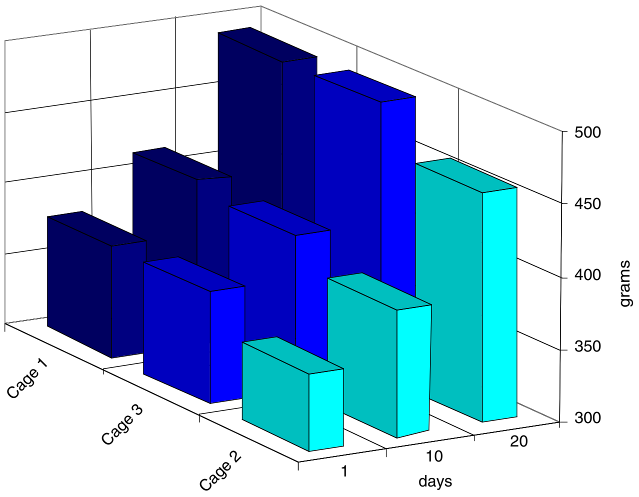

Figure 1. Male crab fattening in tambak cages.

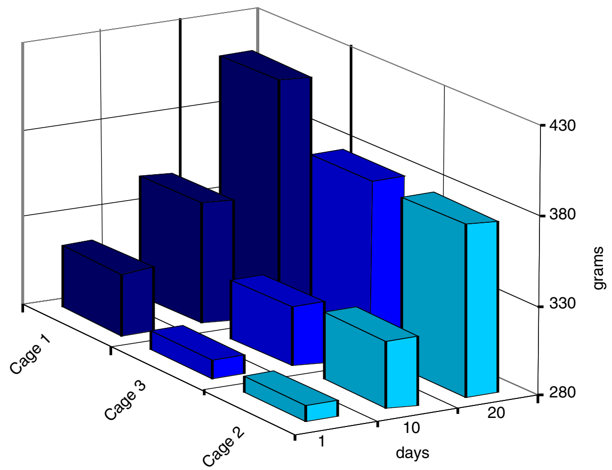

Figure 2. Female crab fattening in tambak cages.

crabs and the higher price of 'full' crabs compared to 'water' crabs, total production was very low because of the mortality. The total weight increase was only 7%. If survival was 100% then the weight increase would have been much greater.

Growout

Growout systems for mud crabs show much more variety. Juvenile seed crabs (crablets), from 10–100 g are purchased from suppliers for stocking. Growout systems are usually pond based, with or without mangroves. In prawn-type ponds without mangroves, stocking rates are commonly 1–3 crabs/m^2, although some farmers try stocking at 5/m^2, and supplemental feeding is always used. These ponds are usually not aerated, and often have concrete walls. In shallow, mangrove ponds, there are two distinct forms of aquaculture: (i) intensive in pens; and (ii) extensive, combined with mangrove silviculture.

In the intensive mangrove pen culture practised in Sarawak (Chang and Ikhwannddin, these Proceedings) high stocking rates of up to 5–7 crabs/m^2 are used and there is supplemental feeding of trash fish. Survival of the crabs in enclosures is between 50–90%, dependent on the stocking rate. The wet-weight feeding rate is 5% per day and cost is about one half of the income derived from the sale of crabs. There is some natural food production within the mangrove enclosures.

In extensive crab culture in large, up to 10 ha, mangrove silviculture ponds of the Mekong Delta, a low crab stocking rate of about 0.05 crabs/m^2 is used. No supplemental feed is added, the crabs forage across the forest floor for natural food. The profit from such operations is high and production, as a ratio of outputs to inputs, is very high (Table 1). The cost of crab seed is a major input cost, about a third of the gross income. There are little to no feed costs and the substantial and regular income derived from crabs is a bonus to the income derived from the mangrove timber, which is harvested after 15–25 years.

The different crab aquaculture techniques employed in the various regions of Southeast Asia may not be suitable for all four of the mud crab species. Further research is required into the habitat preferences of each species so that production techniques can be modified to suit their respective requirements. Given the progress in hatchery production of seed crabs, the development of improved and sustainable growout technology, the high growth rates achieved in low technology aquaculture ponds, and the high demand for the product, crab aquaculture has a promising future.

References

Angell, C.A. ed. 1992. Report of the Seminar on the Mud Crab Culture and Trade, held at Surat Thani, Thailand, November 5–8 1991. Bay of Bengal Program, BOBP/REP/51, Madras, India, 246 p.

AUSTRADE 1996. Mud Crab Market Research in Selected Overseas Markets. Northern Territory Department of Primary Industries and Fisheries, Technical Bulletin No. 228, Darwin, NT. 30 p.

Brienl, J. and Miles, K. 1994. A Preliminary Analysis of the World Market for Mud Crabs. QDPI, Agribusiness Marketing Services, Brisbane, Queensland. 19 p.

Cowan, L. 1984. Crab Farming in Japan, Taiwan and the Philippines. Information Series, QI84009, Queensland Department of Primary Industries, Brisbane, Queensland, 85 p.

Gillespie, N.C. and Burke, J.B. 1992. Mud crab storage and transport in Australian commerce. In: Angell, C.A. ed. Report of the Seminar on the Mud Crab Culture and Trade, held at Surat Thani, Thailand, November 5–8 1991. Bay of Bengal Program, BOBP/REP/51, Madras, India, 207–209.

GLOBEFISH 1995. The World Market for Crab. FAO/GLOBEFISH Research Programme, Vol. 37. FAO, Rome, Italy. 59 p.

INFOFISH 1992. Mud crab seminar. Infofish International, 1/92,16–17.

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.

Liong, P.C. 1992. The fattening and culture of the mud crab (*Scylla serrata*) in Malaysia. In: Angell, C.A. ed. Report of the Seminar on the Mud Crab Culture and Trade, held at Surat Thani, Thailand, November 5–8 1991. Bay of Bengal Program, BOBP/REP/51, Madras, India, 185–190.

Rattanachote, A. and Dangwattanakul R. 1992. Mud crab (*Scylla serrata* Forskål) fattening in Surat Thani Province. In: Angell, C.A. ed. Report of the Seminar on the Mud Crab Culture and Trade, held at Surat Thani, Thailand, November 5–8 1991. Bay of Bengal Program, BOBP/REP/51, Madras, India, 171–177.

Shokita, S., Kakazu, K., Tomori, A. and Toma, T. 1991. Aquaculture in Tropical Areas. (English edition ed. Yamaguchi, M). Midori Shobo Co., Ltd., Tokyo, Japan.

Yalin, S. and Qingsheng, L. 1994. Present status of mangrove crab (*Scylla serrata* (Forskål)) culture in China. NAGA, The ICLARM Quarterly, 17 (1), 28–29.

Review of Mud Crab Culture Research in Indonesia

Fuad Cholik[1]

Abstract

Mud crab fisheries in Indonesia entail the capture of wild stock in coastal waters, especially in mangrove areas and estuaries, and culture in brackish-water ponds. The latter, however, is limited to certain areas only. Due to its economic incentive, the mud crab capture fishery has been growing. Several provinces have reported that market demand for mud crabs has exceeded supply in recent years. Such a situation has stimulated government institutions and the private sector to initiate mud crab culture, but success in the endeavour, however, is still far behind expectations. Lack of production inputs, especially crablets and crab feed, and the absence of a culture technology, have constrained the development of mud crab culture in Indonesia. Research on various aspects of mud crab culture has been conducted in Indonesia for at least a decade. The research efforts have not been able to solve the most critical problem in the development of the mud crab culture industry, i.e., ensuring a sufficient and timely supply of crablets. Research to generate crab hatchery technology is on-going. It seems that the research requires new approaches, specifically in the selection of the right mud crab species suitable for pond culture, and the size of seed for stock enhancement.

IN INDONESIA, the mud crab has been an important fisheries commodity since the early 1980s. During the decade (1985–1994), its production increased by 14.3% per year. In 1994, mud crab production reached 8756 tonnes, with 66.7% derived from capture and the remainder from culture (Direktorat Jenderal Perikanan 1985–1994).

Major mud crab producers are located in North Sumatra, East Kalimantan, West Kalimantan and East Java provinces. In addition, since 1991, East Nusa Tenggara province and in 1992, Riau province, have become important producers of mud crabs. In 1994, mud crab production from all these provinces accounted for 67.6% of total Indonesian mud crab production. However, the rate of production growth in these provinces has indicated slower or even declining trends during the last few years.

This alarming condition should be given urgent attention by all parties concerned for the sustainability of the resource. The pressure on the resource will increase, because the economic incentives to tap them are really remarkable. This is indicated by the ever-increasing export volume and value annually. During the past decade the value of mud crab exports from Indonesia has increased by 11.79% per year. In 1985, the export value amounted to US$0.77 million and had increased to US$21.03 million by 1994. During this time, the price has increased from US$0.44/kg to US$3.05/kg (Direktorat Jenderal Perikanan 1994).

The two types of mud crab fishery, i.e. capture and culture, should be maintained to provide employment and income to local fishers and fishfarmers. Both activities, however, should be implemented in a responsible manner, based on precautionary principles aimed at the sustainability of the fishery and its resources.

The mud crab capture fishery can be improved through stock enhancement such as habitat improvement and restocking. However, the dynamics of the environment should be carefully studied prior to the enhancement program. The program must supply sufficient seed and the only reliable source would be from hatcheries. Meanwhile, no technology on seed production has been established. In this regard, research on seed crab production technology should be further intensified.

[1]Central Research Institute for Fisheries, PO Box 6650 Slipi, Jakarta 11410A, Indonesia

Sustainable development of the mud crab fishery may be obtained through a viable and environmentally friendly culture industry. Viable mud crab culture requires productive, efficient and cost effective culture technology. It also needs sufficient and timely supply of production inputs, especially crablets, feed and feeding technology, sound health and water quality management protocols. Lastly, the industry should be supported by the availability of financial facilities and secured by supportive legal aspects. Most of these requirements can be made available through research.

Seed Production Research

During the past decade, there has been much mud crab culture research, comprising both hatchery and grow-out aspects conducted in Indonesia. The hatchery research has been focused on gonadal maturation, spawning and hatching, larval rearing, pathogens and diseases.

Gonadal maturation, spawning and hatching

The results of research indicate that gonadal maturation of the mud crab may be easily conducted in ponds and tanks, with or without eyestalk ablation. The process is so easy that culture techniques to produce specially egg bearing females has been introduced to brackish-water ponds by farmers in some provinces in Indonesia, such as in South Sulawesi and West Kalimantan. Mud crab females of 200–250 g individual weight, stocked in bamboo cages or pens placed in ponds and fed with trash fish, have been found fully matured within 10 to 14 days after stocking (Sulaeman et al. 1993). Matured females of various stages of gonadal maturity have been collected by Kasprijo and Sutarmat (in press) from brackish-water ponds in East Java. They found that 70% of the samples were showing immature, maturing and ripe gonads.

Experiments on gonadal maturation by eyestalk ablation have also been reported by Sulaeman and Hanafi (1992). They concluded that there was no difference in gonadal maturation between the ablated matured (stage I) females and the unablated matured (stage I) females. Both females reached stage III maturity after three weeks. However, immature females responded significantly to eyestalk ablation. The average individual size of the females used for the experiment was 225 g.

Mass production of ripe females of the mud crab in concrete tanks has also been reported by Suwoyo and Suryanto (1994). They suggested that the optimum depth of water in a tank should be 1 m. The average individual weight of females used in their experiment was 227 g.

Other experiments on gonadal maturation dealt with the effects of substrates (Rusdi et al. 1994a, Mardjono and Suryanto 1996), feeds (Kasprijo et al. in press) and nutrition (Kasprijo et al. 1995). In those experiments, types of bottom substrate affected differently the maturation as well as the spawning of crabs. According to Rusdi et al. (1994a) white sand is required to enhance maturation and spawning of mud crab, while Mardjono and Suryanto (1996) noted that a muddy bottom is more suitable than sandy mud. Regarding types of feed, Kasprijo et al. (in press) noted that 38% of matured crabs fed with pelletised feed spawned, while the percentages of matured crabs fed with crumble feed and trash fish spawned were 27% and 8%, respectively. There were no significant differences of the effects of the three feed types on gonadal maturation of the crabs. From a separate experiment, Kasprijo et al. (1995) reported that provision of artificial feed containing animal and plant origin fat in a proportion of 3 to 1 affected both maturation and spawning of mud crab.

Other important aspects of mud crab reproduction for the establishment of hatchery technology, which have been researched by Indonesian workers, are fecundity and incubation period. Rusdi et al. (1994b) and Suwoyo and Suryanto (1994) reported a matured female crab may produce 400 000 to 2 000 000 eggs depending on the size. Rusdi et al. (1994b) claimed that females weighing between 170 g to 208 g can produce 900 000 to 2 000 000 eggs. Incubation periods according to these reports were between 10 to 12 days.

Hatching rates of mud crab eggs depend on salinity of the medium. At water temperatures of 29–30 °C, a higher hatching rate (93.6%) was attained at a salinity of 35 ppt (Rusdi et al. 1994c). This report also indicated that at lower salinities (20–30 ppt) the hatching rate dropped to 65.9–69.6%. At 15 ppt, hatching rate was only 15.2% and the larvae died within 4 hours after hatching.

Larval rearing

Attempts to develop techniques for mass production of mud crab seed have been made in Indonesia for more than 10 years. However, success is still far from expectations. Most research on various aspects of larval rearing, such as stocking density, feed and feeding protocol, water quality management and disease control were forcefully terminated due to mass mortality during zoea and megalopa stages.

The highest survival rate at the stage of crab instar 1 (C1), to date, was attained by Marjono and Arifin (1993). Using stocking densities of 100 Z1 and

200 Z1 per litre of water as treatment in 62 000 L larval rearing tanks, the researchers successfully harvested 3200–6481 C1/tank and 185–4225 C1/tank, respectively. In terms of percentage, the highest survival rate was 3.2% and the minimum was 0.5%. They recommended 100 Z1/L as the maximum stocking density for larval rearing.

A lower survival rate at C1 stage (0.07% to 0.19%) was reported by Basyar (1994). Using a stocking density of 100 Z1/L water and *Tetraselmis chuii* at 10 000 cells/mL and *Brachionus* sp. at 15–30 pieces/mL as larval feed during Z1 and Z2, Basyar noted survival rate at Z2 ranged between 30.3% to 34.6%. Providing the larvae of the proceeding stages, megalopa and C1, with various densities of *Artemia* nauplii (30 to 50/larva) and *Artemia* flake at 0.5 to 2 ppm did not improve the survival at C1.

Yunus et al. (1994a, b) tested four stocking densities (25, 50, 75 and 100 Z1/L), each in triplicate, for 12 days. They concluded that survival rate decreased with increasing stocking density. At 100 Z1/L, the average survival rate of the larva was only 8.9% and at the stocking densities of 75, 50 and 25 Z1/L the average survival rates were 9.7%, 13.7% and 18.9%, respectively. In all treatments, high mortality had occurred during the first 6 days of the experiment or while the larvae were still at stage Z1 to stage Z2. The only reasonable explanation available from the report was the low water temperature (24–26 °C). It seems that other factors such as feeding, pathogens and parasites, and cannibalism may contribute to the high mortality during the early larval stages.

Effects of feed and feeding on growth and survival of mud crab larvae have been tested by several researchers in Indonesia. From his experiment, Yunus (1992) found that a higher density of rotifer (60 pieces/mL) is required to attain higher survival rates (55%) of Z1 and Z2. Compared to survival rates after six days of the larval rearing experiment reported by Yunus et al. (1994b), the survival rate of Yunus (1992) was much higher. The logic behind it was that the early larval stage was still too physically weak to search for food. However, rotifers are slow moving zooplankton and are suitable for Z1 and Z2 (Mardjono and Arifin 1993). At Z3 and afterward, the larvae are actively searching for food and they can be fed with *Artemia* nauplii. Even at the megalopa stage they can eat 2-day-old *Artemia* (Basyar 1994). Aside from their density and movement, the size of zooplankton also contributes to the survival of the early stage zoea. According to Setyadi et al. (in press), the size of the mouth opening of Z1 was approximately 100 µm, or smaller than the size of a rotifer, even compared to S type rotifers whose size is around 150 µm. This may explain the high mortality at Z1 and Z2.

Other research to improve survival rate of mud crab larvae, especially at Z1 and Z2, through enrichment of rotifers has been reported by Yunus et al. (in press). Survival of Z1 (5 days after stocking) of 74.1% has been obtained through provision of S type rotifers at densities of 15–20 pieces/mL, previously enriched with a mixture of 10 g cod oil, 20 g egg yolk and 5 g yeast dissolved in 100 L water. The rotifers were incubated in the medium for 2 hours.

Pathogens and disease

Mass mortality of larvae may occur due to pathogens and disease. Incubated eggs of berried females harvested from brackish-water ponds are usually infested with ectoparasites such as *Zoothamnium, Epistylis* and *Lagenidium*. Madeali et al. (unpublished) identified four kinds of parasites, namely *Lagennophrys* sp., *Epistylis* sp., *Zoothamnium* sp., and *Vorticella* sp., on infested eggs of berried females collected from brackish-water ponds. Prastowo and Wagimsan (1996) found that in tank-reared broodstock, after hatching, the parasites may infest the recently hatched zoea ending in mass mortality. Zafran et al. (1993) isolated a fungus identified as *Lagenidium* from zoea used in larval rearing experiments. They reported that within 24 hours the fungus may produce 20 to 40 zoospores which will be released after one hour. The fungus grew best at 35 °C and tolerated temperatures from 20–40 °C and pH from 4 to 11. It was killed by 24 hours exposure to 10 ppm formalin or 5 hours exposure to 20 ppm formalin. Ten ppm formalin was safe for zoea, but the larvae died if exposed to 20 ppm formalin for 3 hours. Zafran et al. (1993) suggested the use of formalin to prevent infestation by the fungus of mud crab zoea.

Other research to control *Lagenidium* in mud crab larval rearing has been conducted by Zafran and Taufik (in press). Effectiveness of four kinds of fungicide (treflan, malachite green, formalin and potassium permanganate) in controlling the fungus and their toxicity to mud crab larvae was tested. The results indicated that the minimum effective concentrations (MEC) of treflan and malachite green to inhibit vesicle formation were equal (0.1 ppm); MEC to inhibit zoospore production were 0.1 ppm and 0.2 ppm, respectively. The MEC of formalin for inhibiting vesicle formation was 16 ppm, and permanganate 20 ppm. To inhibit zoospore production the MEC of formalin and permanganate were 14 ppm and 9 ppm, respectively. Results of toxicity tests of the four fungicides to zoea 1 of the mud crab concluded that except for permanganate, the other three fungicides at certain concentrations are safe for the zoea. Zafran and Taufik (in press) suggested the use of treflan or malachite green at 0.1 ppm or 14 ppm formalin to control *Lagenidium* infection in zoea of mud crabs.

To improve hatching rate and survival of zoea, Prastowo and Wagiman (1996) tested caltrocyn and treflan in combination with the rates of water exchange. They suggested a mixture of caltrocyn (1.3 ppm) and treflan (0.02 ppm) in combination with water exchange at the rate of 50%, conducted once every 3 days. They claimed very healthy zoea were produced by such treatment. Kasry (1986) used two antibiotics (Penicillin G and Polymixin-B) in combination with larval feeding treatments (rotifers and *Artemia* nauplii) and two salinity ranges (25–27 and 31–33 ppt). He found that a combination of antibiotics (35 ppt Penicillin-G and 7 ppm Polymixin-B) with rotifers and *Artemia* each at a density of 15 pieces/mL was found to give a high survival rate of larvae (52.1%) at zoea 5.

Pathogenicity of some vibrios to zoea of the mud crab was tested by Parenrengi et al (1993). They isolated three species of vibrio, namely *V. carcharie*, *V. alginolyticus* and *V. parahaemolyticus* from zoea used in larval rearing experiments. The test concluded that the three species of vibrio are pathogenic to zoea, but considered as moderate compared to *V. harveyii*. According to Boer et al. (1993) mud crab zoea are very sensitive to luminous bacteria such as *V. harveyii*.

Pond Culture Research

Based on the end product, there are three types of culture of mud crab in ponds, namely: (1) grow-out from juvenile to consumption size; (2) fattening; and (3) production of egg bearing (gravid) females. Recently, soft-shelled mud crab has also been introduced to the market. Among the three culture types, fattening and the production of gravid females are more attractive than grow-out due to economic incentive and high turnover.

The research related to the above mentioned culture types are reviewed as follows:

Grow-out

Cholik and Hanafi (1991) described grow-out of mud crabs as practiced by farmers. Problems such as low survival of the cultured crab, shortage of seed supply and feed were noticed in the field.

Experiments to obtain data on the optimum stocking density of crablets were reported by Gunarto and Rusdi (1993). They tested three levels of stocking density (1, 3, and 5 crablets/m^2) duplicated in six 12 × 8 m earthen ponds. Survival rates decreased with increasing stocking density. The highest average survival rate (81.2%) was attained at a stocking density of one crablet/m^2, followed by 3 pieces/m^2 (43.1%) with the lowest survival (32.9%) at a stocking density of 5 pieces/m^2.

The effects of stocking densities on growth of the cultured crab were not significant among the treatments. After 90 days the average weight gained by the crabs at 1, 3 and 5 pieces/m^2 stocking density were 146 g, 159 g and 148 g, respectively. Manganpa et al. (1987) concluded that male crabs grew faster than females. The males grew at an average growth rate of 1.3 g/day, while the females grew only 0.9 g/day. The crabs raised as mixed sex groups grew slower than males or females kept separate (0.8 g/day).

Cannibalism is reported as a serious problem in the grow-out of mud crab in ponds. The decrease of survival rates with increased stocking density mentioned above is believed to be due to greater cannibalism at the higher stocking density. Another factor that causes high apparent mortality is the ability of crabs to escape from the pond through hole digging or climbing out over the dykes or fences (Sulaeman et al. 1993). Further, Gunarto and Rusdi (1993) stated that behaviours such as mating and migration also contributed to the high ‘mortality’ of cultured crab. To overcome these problems, Sulaeman et al. (1993) tested three types of pond design, namely ponds with concrete banks, ponds with bamboo fences on the top (crown) of pond dykes and ponds with bamboo fences posted throughout the inner foot of dykes. The lowest survival rate was found in ponds with a bamboo fence on the crown of dykes (29.2%). In terms of growth, the crabs in the concrete ponds grew slower (0.97 g/day) compared to the other treatments. The highest growth rate at 1.3 g/day was shown by crabs cultured in the ponds with bamboo fences posted in the inner foot (edge) of dykes.

Research and observation on feed and feeding habits of mud crabs in ponds have been reported by Wedjatmiko and Yukarsono (1991), Sulaeman and Hanafi (1992) and Wedjatmiko and Dharmadi (1994). The crab will eat any kind of trash fish. However, attention must be paid to economic considerations. Moreover, the use of trash fish directly competes with human consumption. The other main constraint on the use of trash fish as crab feed is its seasonal availability. The development of artificial feed, therefore, is urgently needed.

The crab also responds well to fish balls. However, they should reach a certain elasticity to minimise waste (Sulaeman and Hanafi 1992). Regarding feeding frequency, Wedjatmiko and Dharmadi (1994) stated that feeding once per day is sufficient in crab grow-out. The ration should be 6–8% bodyweight per day.

Fattening, production of egg-bearing females and soft-shelled crabs

These types of crab culture have been adopted by farmers in several provinces in Indonesia such as South Sulawesi, Southeast Sulawesi, North Sumatra and West Kalimantan. Many farmers are enthusiastic to adopt the technology due to its simplicity and ease of operation, as well as the economic attractiveness.

Constraints to the development of this industry are insufficient seed supply and feed. Research to solve these problems should be intensified. Furthermore, problems of harvest and handling at post-harvest must be anticipated.

The culture of soft-shelled mud crab has just started. Experiments conducted by Ariawan and Sulistyono (1996) resulted in inconclusive results. However, demand on this commodity seems to be increasing.

Future Research

In the near future, mud crab research should be focused on topics to establish the mass production technology of crablets. It is clear from various reports that a critical period of larval rearing of mud crab is during the zoeal stage, especially Z1 and Z2. Improvements in increasing accessibility of larva to nutritious feed, through increased stocking density, size and movement suitability of natural food for the larvae, are required. Health management of seed production systems is also important. Design and construction of hatchery facilities should also be considered. Success of the establishment of hatchery technology not only will support culture development but also will reduce pressure on the wild resources from capture. Similarly, it is important for stock enhancement.

In mud crab culture, research is urgently needed on the development of artificial feeds and reduction of cannibalism behaviour. Efforts to improve survival rate from the present level should be given a high priority to make mud crab culture more competitive. Proper design and construction of culture facilities, harvest, post-harvest handling and culture based mud crab fisheries are research topics worth consideration.

References

Ariawan, K. and Sulistyono, D.J. 1996. Rekayasa pemeliharaan kepiting bakau berkulit lunak. (The culture engineering of soft shell mud crab). Laporan Tahunan BBAP Jepara. (Annual Report of the Brackishwater Aquaculture Development Center of DGF, Jepara).

Basyar, A.H. 1994. Penggunaan *Artemia* sebagai makanan hidup bagi larva kepiting (*Scylla serrata*). (The use of *Artemia* as live food for the larvae of mud crab, *Scylla serrata*). Laporan Tahunan BBAP Jepara. (Annual Report of the Brackishwater Aquaculture Development Center of DGF, Jepara).

Boer, D.R., Zafran, A., Parenrengi and Abmad, T. 1993. Studi pendabuluan penyakit kunang-kunang pada larva kepiting bakau (*Scylla serrata*). (Preliminary study of luminescent vibrio infection on mangrove crab, *Scylla serrata* larvae). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 9(3), 119–123.

Cholik, F. and Hanafi, A. 1991. Status of mud crab (*Scylla serrata*) fishery and culture in Indonesia. Presented at the Seminar on the Mud Crab Culture and Trade, Bay of Bengal Programme. 5–8 November 1991, Surathani, Thailand.

Direktorat Jenderal Perikanan 1985–1994. Statistik Perikanan Indonesia. (Fisheries Statistics of Indonesia). Deptan, Jakarta (Ministry of Agriculture, Indonesia).

Direktorat Jenderal Perikanan 1994. Statistik Ekspor Hasil Perikanan 1994 (The International Trade (Export) of Fisheries Commodities). Deptan, Jakarta (Ministry of Agriculture, Indonesia).

Gunarto and Rusdi, I. 1993. Budidaya kepiting bakau (*Scylla serrata*) di tambak pada padat penebaran berbeda. (The survival and growth rate of mud crab, *Scylla serrata* cultured at various stocking densities). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 9 (3), 7–11.

Kasprijo and Sutarmat, T. (in press). Pematangan gonad kepiting bakau (*Scylla serrata*) di tambak kabupaten Pasuruan, Jawa Timur. (Gonadal maturation of the mangrove crab, *Scylla serrata,* in brackishwater ponds in Pasuruan, East Java). Jurnal Penelitian Perikanan Indonesia (Indonesian Fisheries Research Joumal), CRIFI, Jakarta, Indonesia.

Kasprijo, Yunus, Marzuqi, M. Sutarmat, T. and Setyadi, I. 1995. Pengaruh pakan buatan dengan kombinasi kandungan asam lemak omega-3 dan omega-6 yang berbeda terhadap pematangan gonad kepiting bakau (*Scylla serrata*). (The effect of artificial feed with different ratios of essential fatty acid omega-3 and omega-6 on the gonadal maturation and spawning of the mangrove crab, *Scylla serrata*). Jurnal Penelitian Perikanan Indonesia (Indonesian Fisheries Research Journal), CRIFI, Jakarta, Indonesia.

Kasprijo, Yunus, Setyadi, I., Marzuqi, M. and Sutarmat, T. (in press). Pengaruh formulasi pakan dengan bentuk uang berbeda teradap pematangan gonad kepiting bakau (*Scylla serrata*). (The effect of different feed formulation and types on gonad maturation of the mud crab, *Scylla serrata*). Jurnal Penelitian Perikanan Indonesia (Indonesian Fisheries Research Journal), CRIFI, Jakarta, Indonesia.

Kasry, A. 1986. Pengaruh antibiotik dan makanan terhadap kelulus hidupan dan perkembangan larva kepiting (*Scylla serrata* Forskål). (Effect of antibiotics and food on survival and development on larvae mangrove crab (*Scylla serrata* Forskål). Jurnal Penelitian Perikanan Laut (Research Journal on Marine Fisheries), 36, 1–5.

Madeali, M.I., Muliani and Nurjanna. (unpublished). Identifikasi dan penanggulangan penyakit parasiter pada telur kepiting bakau (*Scylla serrata*). (Identification and prevention of parasitic infection on mud crab eggs). Risalah Serninar Hasil Penelitian. (Monthly Report). RICA, Maros, Indonesia.

Mangampa, M., Ahmad, T., Wedjatmiko, Utojo and Mustafa, A. 1987. Pertumbuhan kepiting (*Scylla serrata* Forskål) jantan dan betina dalam tambak. (The growth of female and male mud crab, *Scylla serrata* Forskål raised in brackish-water ponds). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 3 (2), 94–101.

Mardjono, M. and Arifin, M. 1993. Pemeliharaan larva kepiting dengan tingkat kepadatan yang berbeda. (Rearing experiments of the mud crab using different stocking densities). Laporan Tahunan BBAP Jepara (Annual Report of the Brackishwater Aquaculture Development Center of DGF, Jepara).

Mardjono, M. and Suryanto. 1996. Pematangan gonad onduk kepiting dengan berbagai substrat dasar dan kedalaman air. (Gonad maturation of the mud crab using different substrates). Laporan Tahunan BBAP Jepara (Annual Report of the Brackishwater Aquaculture Development Center of DGF, Jepara).

Parenrengi, A., Zafran, Boer, D.R. and Rusdi, I. 1993. Identifikasi dan patogenisitas beberapa bakteri *Vibrio* pada larva kepiting bakau (*Scylla serrata*). (Identification and pathogenicity of various vibrios on the mangrove crab, *Scylla serrata,* larvae). Jurnal Penelitian Budidaya Pantai (Research Journal of Coastal Aquaculture), 9 (3), 125–129.

Prastowo, R. and Wagiman, H. 1996. Penggunaan obat-obatan dan penggantian air selama masa inkubasi untuk meningkatkan kualitas telur induk kepiting. (The use of drugs and water change during egg incubation to improve hatching rates). Laporan Tahunan BBAP Jepara (Annual Report of the Brackishwater Aquaculture Development Center of DGF, Jepara).

Rusdi, I., Ahmad, T. and Makatutu, D. 1994a. Studi pendahuluan tingkat keberhasilan pemijahan kepiting bakau (*Scylla serrata*) pada substrat yang berbeda. (Preliminary study on spawning rate of the mangrove crab, *Scylla serrata,* on different substrates). Jurnal Penelitian Budidaya Pantai (Research Joumal on Coastal Aquaculture), 10 (3), 25–30.

Rusdi, I., Gunarto and Prarnana, H. 1994b. Penggemukan kepiting bakau (*Scylla serrata*). (Fattening of the mangrove crab, *Scylla serrata*). Warta Balitdita (Coastal Aquaculture Newsletter), 2 (1), 11–12.

Rusdi, I., Parenrengi, A. and Makatutu, D.1994c. Pengaruh perbedaan salinitas terhadap penetasan dan kelangsungan hidup zoea awal kepiting bakau (*Scylla serrata*). (Effect of different salinities on hatching and survival rates of the mangrove crab, *Scylla serrata*). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 10 (1), 141–144.

Setyadi, I., Yunus, Prijono, A. and Kasprijo. (in press). Pengaruh penggunaan tipe rotifera (*Brachionus plicatilis*) yang berbeda terhadap laju sintasan dan perkembangan larva kepiting bakau (*Scylla serrata*). (Effect of utilization of different rotifer types on the survival and growth rates of the mangrove crab, *Scylla serrata,* larvae). Jurnal Penelitian Perikanan Indonesia (Indonesian Fisheries Research Journal), CRIFI, Jakarta, Indonesia.

Sulaeman and Hanafi, A. 1992. Pengaruh pemotongan tangkai mata terhadap kematangan gonad dan pertumbuhan kepiting bakau (*Scylla serrata*). (Effect of eyestalk ablation on gonadal maturation and growth of the mangrove crab, *Scylla serrata*). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 8 (4), 55–62.

Sulaeman, Tjaronge, M. and Hanafi, A. 1993. Pembesaran kepiting bakau (*Scylla serrata)* dengan konstruksi tambak yang berbeda. (Grow-out of the mangrove crab, *Scylla serrata* in different pond constructions). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 9 (4), 41–50.

Suwoyo, D. and Suryanto. 1994. Pengamatan pematangan telur induk kepiting bakau (*Scylla serrata*) pada berbagai kedalaman air media. (Gonadal maturation of the mud crab at various water depths). Laporan Tahunan BBAP Jepara (Annual Report of the Brackishwater Aquaculture Development Center of DGF, Jepara).

Wedjatmiko and Dharmadi. 1994. Pengaruh frekuensi pemberian pakan terhadap pertumbuhan kepiting bakau (*Scylla serrata*). (The effect of feeding frequency on mud crab (*Scylla serrata*) growth). Warta Balitdita (Coastal Aquaculture Newsletter), 6 (3), 37–39.

Wedjatmiko and Yukarsono, D. 1991. Pola kebiasaan waktu makan kepiting bakau (*Scylla serrata*) di tambak Kamal Jakarta. (Feeding pattern of mud crab, *Scylla serrata* in ponds in Kamal, Jakarta). Warta Balitdita (Coastal Aquaculture Newsletter), 3 (1), 1–4.

Yunus. 1992. Pemeliharaan larva kepiting bakau (*Scylla serrata*) dengan beda kepadatan rotifera, (*Brachionus plicatilus*). (Larval rearing of the mangrove crab, *Scylla serrata* with different densities of rotifers, *Brachionus plicatilus*). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 8 (2), 9–14.

Yunus, Ahmad, T., Rusdi, I. and Makatutu, D. 1994a. Percobaan pemeliharaan larva kepiting bakau (*Scylla serrata*) pada berbagai tingkat salinitas. (Experiments on larval rearing of the mangrove crab *Scylla serrata* at different salinities). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 10 (3), 31–38.

Yunus, Rusdi, I., Mahasetiawati, K. and Ahmad, T. 1994b. Percobaan pemeliharaan larva kepiting bakau (*Scylla serrata*) pada berbagai padat penebaran. (Experiments on larval rearing of mangrove crab, *Scylla serrata,* with different stocking densities). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 10 (1), 19–24.

Yunus, Suwirya, K., Kasprijo and Setyadi, I. (in press). Pengaruh pengkayaan rotifera (*Brachionus plicatilis*) dengan menggunakan minyak hati ikan cod terhadap kelangsungan hidup larva kepiting bakau (*Scylla serrata*). (The effect of cod liver oil enriched rotifers, *Brachionus plicatilis,* on the survival of mud crab larvae). Jurnal Penelitian Perikanan Indonesia (Indonesian Fisheries Research Journal), CRIFI, Jakarta, Indonesia.

Zafran, Boer, D.R. and Parenrengi, A. 1993. Karakteristik dan penanggulangan penyakit jamur (*Lagenidium* sp.) pada larva kepitmg bakau (*Scylla serrata*). (Characteristics and prevention of *Lagenidium* sp. on mud crab, *Scylla serrata* larvae). Jurnal Penelitian Budidaya Pantai (Research Journal on Coastal Aquaculture), 9 (4), 29–39.

Zafran and Taufik, I. (in press). Efektivitas berbagai fungisida (Treflan, malachite green, formalin dan kalium permanganat) dalam menghindarkan infeksi *Lagenidium* sp. pada larva kepiting bakau (*Scylla serrata*). (Effectiviness of fungicides (Treflan, malachite green, formalin and potassium permanganate) against *Lagenidium* sp. infection in the mangrove crab, *Scylla serrata*). Jurnal Penelitian Perikanan Indonesia (Indonesian Fisheries Research Journal), CRIFI, Jakarta, Indonesia.

Mixed Shrimp Farming–Mangrove Forestry Models in the Mekong Delta: ACIAR PN 9412

Nguyen Van Trong[1]

Abstract

In 1991, 50% of the total fisheries exports from Vietnam of US$120 million came from the Mekong Delta region and one third of the 500 000 tonnes of fisheries production in the region presently comes from aquaculture (Mekong Delta Master Plan 1993). Approximately 10% of this aquaculture production is derived from intertidal mangrove habitats in Minh Hai and Tra Vinh provinces, although this percentage will increase markedly if current trends continue (Table 1). The rapid expansion of all forms of shrimp culture in the coastal regions of the Mekong Delta has had a disastrous effect on mangrove forests. After the end of the war in 1975, much of the mangrove forest in southern Vietnam that had been killed by defoliants was replanted or naturally revegetated. However, during the 1980s, migration of people into the region, and expansion of the shrimp culture industry, destroyed much of the mangrove areas at a rate of 5000 hectares per year. Much of the intertidal land that has been given over entirely to extensive shrimp culture in Minh Hai province has now had nearly all mangrove vegetation removed. The yields of ponds in these areas have dropped in recent years, mainly due to a low supply of naturally occurring shrimp larvae and environmental problems. The provincial managers have reacted to this situation by establishing 22 mixed shrimp farming-mangrove forestry enterprises, where both shrimp and mangroves are produced by individual farmers on small plots. These enterprises offer the best potential solution to the problem of conflicting land use. However, current management practices of both shrimp ponds and mangrove forests have led to decreasing yields. This ACIAR project will investigate the likely causes of decreasing yields from shrimp ponds and mangrove forestry, and evaluate alternative management practices to provide a scientific basis for maximising yields from these systems in a sustainable way.

THE GOAL of the project is to optimise the economic yield from mixed shrimp aquaculture-mangrove forestry farming systems in Minh Hai province in a suitable manner.

Table 1. Changes in the area of shrimp ponds, shrimp production and mangrove area in the brackish water regions of Minh Hai province during the period 1982–1992.

	Year	
	1982	1991
Area of shrimp ponds (ha)	12 000	100 000
Shrimp production (tonnes)	4 000	32 000
Area of mangrove forest (ha)	98 044	<50 000

[1]Division of Environment and Fishery Resources, Research Institute for Aquaculture No. 2, 116 Nguyen Dinh Chieu St. Dist. 1, Ho Chi Minh City, Vietnam

Project objectives

The objectives of the project are:

- to investigate factors controlling the yields of shrimp and wood from existing shrimp farming-mangrove forestry systems in Minh Hai province of Vietnam;
- in co-operation with selected farmers and appropriate managers, to experiment with shrimp pond and mangrove forest management to evaluate different culture options;
- to identify improved culture methodologies for these systems and to quantify where possible expected yields and costs;
- assist national and provincial authorities to transfer results of the project to wider coastal farming communities in the Mekong Delta.

Expected project outputs

1. Better knowledge of food chains and nutrient cycles in shrimp ponds and factors controlling yields in mangrove plantations.
2. Models for shrimp farming-mangrove forestry systems that have improved yields relative to existing models, but which are sustainable.
3. Management advice to farmers and officials on methods to optimise yields from mixed shrimp farming-mangrove forestry systems. This will be ongoing throughout the project.
4. Scientific publications on all aspects of these mixed farming systems.

Research contents

1. Shrimp pond ecology.
2. Mangrove plantation forestry.
3. Hydrodynamics
4. Sociology.

Project sites

The project area is located in Tam Giang Commune, Ngoc Hien district, Minh Hai province, including two Fisheries-Forestry Enterprises (Tam Giang 3 Enterprise and 184 Enterprise).

1. TG 3 Enterprise

Total area: 3300 hectares. Land use of the area is shown in Table 2.

Forestry activities:

Annually, the enterprise has to replant new forest on the harvested area and protection forest belts along river sides. The species replanted is *Rhizophora apiculata.* The area of replanted forest is shown in Table 3.

Aquaculture

In TG 3, two shrimp farming systems exist:

I: 'Mixed Shrimp–Forest' farming system, in which internal canals in forestry plots are used for shrimp culture. During the early years of replanted forests, farmers who were assigned to manage this forestry plot had the right to use the internal canals for shrimp culture. The forest area mostly accounts for 80% of the plot, canals and dykes 20%. The density of Rhizophora is 10 000–20 000 trees per hectare. This system is often applied in depressed zones that lie in the centre of enterprise areas.

Table 4 shows details of the development of mixed shrimp–forest farming systems in the TG 3 enterprise.

Table 4. Size (hectares), density and production of shrimp of replanted areas in TG 3 enterprise.

Year	Newly replanted area (ha)	Density (trees/ha)	Production of shrimp (kg/ha of forest area/year)
1987	107.0	20 000	250
1990	108.0	20 000	250
1991	49.6	10 000	180–210
1992	252.7	10 000	180–210
1993	154.9	10 000	150
1994	11.2	10 000	—
Total	638.4		

Table 2. Existing land use in TG 3 enterprise.

Land use	Forest (ha)	Canals & dykes (ha)	Fallow land (ha)	Homestead (ha)	Rivers (ha)	Total (ha)
Mixed shrimp-forest farming	456.2	179.2		56.5		691.9
Separate shrimp-forest farming	477.2	206.2				683.4
Breeding forest	87.8	33.2				121.0
Production forest	1471.7		25.2			1496.9
Other uses				102.2		102.2
Natural rivers					204.6	204.6
Total	2,492.9	418.6	25.2	158.7	204.6	3,300

Table 3. The area of replanted forest in TG 3 enterprise.

Year	1987	1988	1989	1990	1991	1992	1993	1994	1995
Area (ha)	114	33	70.5	258.2	242.5	138.4	107.2	167.8	52.9

In this system, extensive shrimp culture has been applied; farmers recruit natural shrimp seeds through sluices and do not feed them. Marketable shrimp is harvested monthly during spring tide periods of the lunar cycle.

II: 'Separate forest–shrimp' farming system that is applied in a surrounding belt of the enterprise. Each plot covers an area of about 10 hectares, of which 2 hectares adjacent to a river or main canal are used for building shrimp ponds that comprises about 60% of the water surface and 40% of dykes; the 8 hectares remaining is replanted with mangrove trees. There are about 691.9 hectares of this system applied in the TG 3 enterprise. The shrimp production of this farming system in recent years is shown in Table 5.

Table 5. Shrimp production in the TG 3 enterprise.

Year	Production (kg/ha/year)	Remarks
1990	30	
1991	400–500	With supplementary stock of PL of *P. merguiensis*, no feed supply
1992	600	With supplementary stock of PL of *P. merguiensis*, and trash fish supply
1993	600	With supplementary stock of PL of *P. merguiensis*, and trash fish supply
1994	0	Shrimp disease outbreak, first crabs stocked
1995	0	Shrimp disease outbreak, more crabs stocked

The native species white shrimp *Penaeus merguiensis* is popularly reared in these enterprises but *P. monodon* rarely.

2. '184' Enterprise

Total area: 4150 hectares. Existing land use is shown in Table 6.

Table 6. Existing land use of the '184' enterprise.

Land use	Total area (ha)	Forest (ha)	Canals and dykes (ha)
Natural Forest	20	20	
'Forest-shrimp' farming	970	388	582
'Shrimp-Forest' farming	3049	2202	847
Homestead, rivers and canals	111		
TOTAL	4150	2610	1429

Forestry activities

Annually, the enterprise replants an average area of 300 hectares of new forest on barren land on which shrimp farming mixed with forest replantation is applied. Replanted species is *Rhizophora apiculata* and the density is 10 000 trees per hectare.

Aquaculture

In the '184' enterprise, there is no exploitable forest, most of the trees are young or newly replanted and allocated to farm households. While doing shrimp culture in internal canals, farmers must be responsible for managing and replanting forest in their plots. Being different from the TG 3 enterprise, there is no 'Separate forest-shrimp' farming system here. There are now two systems of shrimp farming in combination with forest replanting applied in the enterprise.

1 — 'Shrimp–Forest' farming system, in which 30% and 70% of plot area are used for internal canals and forest, respectively.

2 — 'Forest–shrimp' farming system, in which 60% and 40% of plot area are used for internal canals and forest, respectively.

Extensive shrimp culture has been mainly applied in these systems. Postlarvae (PL) of shrimp are naturally recruited through sluices but sometimes PLs from hatcheries are also used for supplementary stock in ponds. Marketable sizes are harvested every 15 days dependent on spring tide periods of lunar cycle. The average yield of shrimp was 150 kg/hectare of forestry land area during 1987–1993 but dramatically declined in 1994–1995 because of shrimp disease outbreak.

Project Activities

1. Monitoring of aquaculture.

Ecology/biology of shrimp ponds, main rivers and canals: water quality, plankton, zoobenthos, primary production, carried out by AIMS experts and RIA 2 staff in April, June, July and October 1996 at 12 households.

Shrimp surveys, carried out in July, August, September, October, November 1996 and February 1997 at two fixed households:

Juvenile shrimp survey: species composition, length distribution, density.

Shrimp harvest survey: species composition, production, length distribution.

2. Monitoring of forestry.

3. Socio economic survey, carried out by NACA and Can Tho University.

4. Some preliminary results of the survey on aquaculture.

An analysis of results obtained so far indicates a number of problems with pond water quality and pond management.

High levels of suspended solids due to high sediment loading in the source waters (>1 g/L). The pH of ponds and canals is 6–7, but the pH of water near the bottom of the pond is acid (pH<6). Very low oxygen concentrations, especially near the bottom of the pond (<1 mg/L). Low chlorophyll concentration and low phytoplankton production (<0.2 mg/L). This is probably caused mainly by the high turbidity of the water in the pond and canal. The existing management practice of harvesting and recruitment on the same tidal cycle every 15 days appears to be unsatisfactory. A significant proportion of post larvae and juveniles recruited on the incoming tide area is lost from the pond while harvesting on the outgoing tide. Furthermore, harvested shrimp are mostly of small size, owing partly to the short growout period.

The water level in ponds is too low, exposing the sides of the levees to oxidation and reducing the opportunity for shrimp to utilise the sides for feeding. In addition, the shallow water facilitates the development of bottom algae whose death creates water pollution, exposing shrimp to lower oxygen concentrations near the bottom of the pond and perhaps to higher temperatures, particularly if the pond is drained during the daytime. In general, these conditions are not favourable for shrimp culture and health.

5. Manipulation experiment.

Based on the above analysis, it is thought that a management approach combining a lengthening of the grow-out phase, the application of lime, increasing the depth of water in the pond and minimal water exchange may be an effective way of improving water quality and pond yields in the short term.

Objectives

To assess the effect of the addition of lime and the length of the growout phase on pond water quality and pond yield. The purpose of these trials was to provide a rapid assessment of whether or not the experimental treatments showed promise of improving water quality and pond yield.

Expected outcomes

- An increase in pH and alkalinity as a result of liming.
- Reduced turbidity owing to enhanced flocculation of suspended solids after liming, to longer residence time of water in the pond, and a reduction in sediment input through minimal water exchange.
- Higher phytoplankton densities and growth as result of lower water turbidity.
- Higher dissolved oxygen concentrations as result of higher plankton densities.
- Larger, more valuable shrimp in the 60 day growout treatment.

On the other hand, attention has been paid to the diversification of species cultured. The culture of the mud crab seems to be potentially feasible and at present, some farmers have been successful in crab culture in mangrove areas. To alleviate problems with shrimp disease, a survey program has been established to assess the risk and incidence of shrimp diseases in the project area and to suggest possible remedies.

Reference

Mekong Delta Master Plan, 1993. State Planning Committee of Vietnam. Based on interview with Dr Dang Van Phan, General Secretary MDMP, Ho Chi Minh City, August 1993.

Malaysian Crab Research

Eddy S.P. Tan[1]

Abstract

Farming of mud crabs in the coastal waters of Malaysia can be developed as an alternative employment option for many inshore fishing communities which are experiencing declining fish catches in coastal waters, provided cost-effective solutions can be obtained through research and pilot culture trials to ensure that the needs of crabs to grow and reproduce are fully understood. While the results of fattening crabs in floating cages and the growth of crabs in pen enclosures under the canopy of mangrove trees are very encouraging, intensified research efforts should be focused to minimise the dependence of crab seed from natural sources and to improve the management techniques for increasing the yield of the culture systems. They relate to differences in the behavioural ecology and preferred diet of the crab at various phases of its life cycle and to a lack of appreciation of what induces stress in crabs and how crabs can be stimulated to moult and grow faster.

THE increasing price of mud crabs in Malaysia has encouraged many coastal fishing communities to initiate culture trials in floating cages, in specially designed earthen ponds and more recently in pen enclosures in mangrove forests.

Basically, there are two types of farming activities in crab farming in Malaysia. Firstly, the grow-out of juvenile crabs in ponds or pens involves the crabs having to moult several times before they reach marketing size. The optimal conditions for such activities with minimal mortality, obviously found in the natural habitat of the crab, the mangrove swamp, have prompted the farmers in Sarawak to grow crabs in pens under the canopy of mangrove trees, where the leaf litter can provide the organic base needed to enhance the natural productivity of the culture site.

These recent attempts provide very encouraging results and have raised many interesting research questions. In contrast, the transient culture of 'water crabs' in floating cages for a short duration of 10–20 days is intended to fatten the crab. However, such crabs do not need to moult as they are already of marketable size (exceeding 150 g), but are only maintained to allow the crab to develop a firmer flesh and in some cases to harden its shell.

The duration of fattening is short to minimise problems of cannibalism that can arise as the crabs become territorial and increasingly aggressive. This traditional method of fattening marketable size crabs is widely practised to improve the quality of the crabs. Crab production is still relatively small in Malaysia with an estimated annual figure of about 650 tonnes (Liong 1992).

It is the intention of this paper to summarise the current status of research projects related to the farming of mud crabs and to highlight future areas of research that would be useful to promote the farming of mud crabs in Malaysia.

Research Status and Institutional Involvements

Experimental studies on the larval biology of the mud crab were initiated in the early 1960s at the Fisheries Research Institute, Penang, where the different larval stages were described (Ong 1964, 1966). Subsequently in the 1980s, there were attempts to mass produce crab seed at the National Prawn Fry Research and Production Centre (NAPFRE) at Pulau Sayak, Kedah. High mortality of the megalopa and young crab stages due to cannibalism even when 'enough food was provided' was reported (Jamari 1992). At the Sematan Crab Research Station operated by the Inland Fisheries

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Branch, Department of Agriculture, Sarawak, a similar problem was experienced when experiments to produce crab seed were initiated in 1995. The low survival rate of the megalopae under laboratory conditions is a major problem that has to be further investigated using alternative approaches. Universiti Sains Malaysia (USM) is presently providing technical support to assist the Station at Sematan, Sarawak. Success has yet to be achieved in the mass production of crab seed in Malaysia.

Studies on the production of crabs in various culture systems are attempting to improve the management techniques by manipulating initial stocking density complemented with staggered harvesting and restocking. Alternative formulated diets other than trash fish are presently being tested. The grow-out system of crabs in pen enclosures under the canopy of mangrove trees is presently the most successful as such a system is not only environmentally friendly but appears to be highly productive, as will be presented by Ikhwanuddin at this meeting. This system, as practised in Sematan, Sarawak, also provides a continuous supply of berried females for seed production experiments.

Monitoring of the biological productivity of the mangrove ecosystem has been the research focus of several projects in Malaysia, which are funded by the Malaysian government or by the Japanese International Research Centre for Agricultural Science (JIRCAS). Currently, the Fisheries Research Institute at Penang, USM and the University of Malaya are conducting research on various aspects of the mangrove ecosystem.

Future Approach

The failure of mass production of crab seed in Malaysia is no fault of the crabs but largely due to a lack of appreciation by scientists of what crabs really need and prefer. There is a need to define what are the preferences of the crab at different stages of its life cycle under natural conditions. A different research approach has to be developed where scientists must provide the appropriate environmental conditions, either in terms of water conditions (salinity, turbidity) food and hiding places so that the crab can interact socially without becoming excessively aggressive.

While a multi-disciplinary collaborative approach is recommended, more research emphasis on the behavioural tendencies of the crab, especially the megalopa and young crab stages, under different sets of culture conditions could be very rewarding. It would not be surprising that different species or subspecies of mud crabs (Sivasubramaniam and Angell 1992) may show varying behavioural characteristics, some of which may provide the clues leading to the future successful mass production of crab seed. The crab farmers at Sematan, Sarawak, have reported increasing numbers of wild juvenile crabs since the pen culture of crabs was started.

In conclusion, the following quotation will hopefully set the stage for this meeting in Darwin:

> *'Imagination is more important than knowledge, for knowledge is limited to all we now know and understand, while imagination embraces the entire world and all there ever will be to know and understand'*
>
> Albert Einstein

Acknowledgement

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References

Jamari, Z. 1992. Preliminary studies on rearing the larvae of the mud crab (*Scylla serrata*) in Malaysia. In: Angell, C.A. ed., Report of the seminar on the mud crab culture and trade held at Surat Thani, Thailand, November 5–8. 1991. BOBP/REP/51, 143–147.

Liong, P.C. 1992. The fattening and culture of mud crab (*Scylla serrata*) in Malaysia. In: Angell, C.A. ed., Report of the seminar on the mud crab culture and trade held at Surat Thani, Thailand, November 5–8. 1991. BOBP/REP/51, 185–190.

Ong, K.S. 1964. The early developmental stages of *Scylla serrata* Forskål (Crustacea: Portunidae) reared in the laboratory. Proc. Indo-Pacific Fish. Council, 11(2), 135–146.

Ong, K.S. 1966. Observations of the postlarval life history of *Scylla serrata* Forskål reared in the laboratory. Mal. Agri. J., 45(4), 429–443.

Sivasubramaniam, K. and Angell, C.A. 1992. A review of the culture, marketing and resources of the mud crab (*Scylla serrata*) in the Bay of Bengal region. In: Angell, C.A. ed., Report of the seminar on the mud crab culture and trade held at Surat Thani, Thailand, November 5–8. 1991. BOBP/REP/51, 5–12.

Mud Crab Research and Development in the Philippines: An Overview

Romeo Diño Fortes[1]

Abstract

In 1995, total mud crab production (*Scylla* spp.) in the Philippines reached 2782 tonnes with an average yield of 920 kg/ha. In the same year, the total mud crab production from the top 10 mud crab producing provinces in the country was 2731 tonnes (BFAR 1996). These provinces were: Bulacan, Camarines Sur, Capiz, Masbate, Metro Manila, Pampanga, Pangasinan, Sorsogon, Surigao del Sur and Zamboanga del Sur. This reported production is still very small compared to the potential of mud crab aquaculture to produce what is needed by the industry especially if the identified issues and problems are given priority and proper attention. This will turn a potential into reality and elevate mud crab aquaculture to a level similar to those of other aquaculture species that have significantly contributed to the economy of many nations. The issues and problems confronting the Philippine crab industry as identified by PCAMRD (1993) include the following: (1) lack of information on the natural wild stock; (2) lack of seeds; (3) limited technology; and (4) poor production and low value-added products for export.

MUD crab farming has been going on for at least three decades but mud crab aquaculture has not reached even its optimum potential. Significant interest has been observed in the desire to increase production but the seeds are limited and aquaculture technology has yet to be fully developed.

One major constraint to the full development of mud crab aquaculture is the supply of seeds — the industry still depends on wild-caught crablets, the sources of which are dwindling. Collection, transport, handling and holding methods for the crablets need to be improved and hatchery techniques developed.

Currently, crablets are collected by several means (use of fine scissors and push nets, from introduced shelters of various materials, or by collecting bivalves that are associated with crablets of *Scylla* spp.). These are then stored in boxes and transported in the same boxes to a dealer where these are transferred to cages installed in brackishwater ponds. These are finally packed in layers in boxes separated only by newspaper and transported to the user by land, boat or aircraft for up to 48 hours. They are then stocked in ponds, cages and pens in mangrove areas, nipa swamps or in the estuaries (PCAMRD 1996). This source of seedstocks is very unstable, making the industry equally unstable.

Another constraint is the species used. There are several species of mud crab in the Philippines and not all of them are ideal for aquaculture. Estampador (1949a) identified three species and a fourth subspecies of mud crab in the Philippines, and all four species are farmed. Despite Estampador's classification, however, all four species have been known for many years as *Scylla serrata* in the aquaculture industry and in several scientific publications. Therefore, very little is known about the characteristics of each species and their suitability for culture. Recently, Keenan et al. (1998) revised the genus and presented this classification:

Estampador (1949a)	**Keenan et al. (1998)**
S. serrata	*S. olivacea*
S. oceanica	*S. serrata*
S. serrata var. *paramamosain*	*S. paramamosain*
S. tranquebarica	*S. tranquebarica*

[1]Institute of Aquaculture, College of Fisheries, University of the Philippines in the Visayas, Miagao, Iloilo, Philippines

In this report, the classification of mud crabs follows that of Keenan et al. (1998). The crab industry in the Philippines uses the term ***King Crab*** as the local name for *S. serrata,* considered as the biggest and fastest growing mud crab. *S. tranquebarica* and *S. olivacea* are two important species and can be distinguished from the other species because of the outstanding green to grayish-green colour, and purplish-brown colour, respectively.

While the King Crab is the most sought-after species because of its faster growth, the other species are also acceptable in both the domestic and international markets.

To emphasise its importance in the Philippines, the Department of Science and Technology classified the mud crab in the list of Export Winners in aquaculture under STAND 2000 (Science and Technology Agenda for National Development for the year 2000).

Brief Status of the Industry

Scylla species have been farmed in ponds, cages and pens but production is erratic. Production data for the period 1979 to 1991 showed increasing production from brackishwater ponds from 1983 to 1989 but this declined significantly in 1991. Catch from municipal waters for the same period ranged from 135 tonnes to 374 tonnes (Table 1). In 1991 the estimated value of brackishwater pond production was placed at P72 million. These were exported in the international market as frozen, prepared and preserved forms. Major markets include the United States of America, Hong Kong, Trust Territories of the Pacific Islands and Japan (Table 2).

Table 1. Mud crab production, in metric tonnes, from brackishwater ponds and municipal waters in the Philippines.

Year	Brackishwater ponds	Municipal waters	Total
1979		65	65
1980		16	16
1981		28	28
1982		82	82
1983	924	135	1059
1984	833	374	1207
1985	833	244	1077
1986	1034	301	1335
1987	1122	224	1346
1988	136	62	1198
1989	1442	168	1610
1990		179	179
1991	597	158	755

Table 2. Philippine crab exports. (Source: Philippine Foreign Trade Statistics, cited by PCAMRD 1993).

	Frozen		Other		Prepared/ preserved	
Year	Kg	US$	Kg	US$	Kg	US$
1991	**750 083**	2 932 755	**39 265**	112 714	**115 813**	1 112 353
1992	**297 585**	3 735 529	**250**	000	**175 068**	1 710 112

As early as the 1960s, mud crab culture was practiced in Northern Samar, Sorsogon, Iloilo, Cotabato and other parts of the Philippines. The crablets are collected from the wild and grown in ponds that cannot be drained. After about 4 to 6 months, depending upon the size of crablets that were stocked, they are harvested by means of a *bintol* or lift net (a rectangular to square trap net with bait, usually animal meat or fish, set in the pond and lifted after a certain period of time). This method is still practiced in the country.

Many enterprising aquafarmers ventured into mud crab fattening and a few into farming, both in ponds, cages and pens. The techniques used are basically the same as those practiced for 30 years but some farmers are now trying cages and pens inside the ponds. This indicates the need for new techniques that would improve production and at the same time sustain the industry by developing environment-friendly technologies.

There is therefore a need to develop new techniques and to identify the best species of mud crab for farming so that hatchery techniques can be developed and the source of seeds can be sustained. The Southeast Asian Fisheries Development Center/ Aquaculture Department (SEAFDEC/AQD 1989) published mud crab Abstracts which included most of the work on mud crabs in the Philippines.

Some other work on fattening methods, culture and breeding techniques of the mud crab in captivity had been done but this was sporadic and was not sustained; therefore, research and development work on *Scylla* spp. needs to be pursued systematically in order to give the necessary research and development (R&D) support to this industry of such high potential.

Research and Development

There had been sporadic R&D work on mud crabs in the Philippines which started with the work of Arriola (1940) on the life history of *Scylla serrata* and that of Estampador (1949b) on the comparative studies on the spermatogenesis and oogenesis in *Scylla* and on the description of the species belonging to the genus *Scylla* (Estampador 1949a). Other early work was done by Escritor (1970, 1972) on the monoculture of *Scylla serrata*; Laviña (1980) on the biology and aquaculture of *S. serrata* and the polyculture of *S. serrata* and milkfish (*Chanos chanos* Forskål).

Experiments to establish stocking density of mud crabs raised in brackishwater ponds were set up by Baliao et al. (1981). The feasibility of mud crab culture in brackishwater ponds in combination with milkfish was also tested (Baliao 1983, 1984). In an earlier experiment, Lijauco et al. (1980) reported a survival rate of 56% in a trial using 2500/ha of milkfish and 5000/ha of mud crab in combination, reared in brackishwater ponds. In 1992, Cerezo (unpublished) attempted to determine the effects of different materials as substrates on the culture of mud crabs in tanks. Cajilig (unpublished) on the other hand worked on feeds and rates of feeding on the fattening of mud crabs in cages installed in a tidal river.

More and more attention is now given to mud crab aquaculture research and development. Development of appropriate technologies is a preoccupation of most R&D institutions in the country. Some of these institutions are: the University of Eastern Philippines in Catarman, Northern Samar; Eastern Samar State University in Borongan, Eastern Samar; Pangasinan State University in Binmaley, Pangasinan; Bicol University College of Fisheries in Tabaco, Albay; and the Aquaculture Division of the Bureau of Fisheries and Aquatic Resources, Department of Agriculture.

The work done in these institutions is mostly on the monoculture and polyculture of mud crabs with finfish (*Chanos chanos*), other crustaceans (*Penaeus* spp.) and seaweeds (*Gracilaria* sp.) as the secondary species; and tests of various materials as shelters in mud crab farming in different culture systems. Preliminary work on marketing strategies for mud crabs in the Philippines is on-going at the University of Eastern Philippines.

While several institutions have worked and have shown interest on mud crab aquaculture, two research institutions in the country implemented a comprehensive project on mud crab aquaculture, focusing on *Scylla serrata*. This was in response to a call to sustain the production of mud crabs during the Regional Seminar on Mud Crab Culture, in Thailand in 1991.

The Institute of Aquaculture, College of Fisheries (UPVCF/IA) and the Division of Biological Sciences, College of Arts and Sciences of the University of the Philippines in the Visayas embarked on the development of hatchery techniques for this mud crab, initially funded by the Philippine Council for Aquatic and Marine Research and Development (PCAMRD) in 1993–94. In 1995, the work on mud crab research at UPV was expanded when the Australian Centre for International Agricultural Research (ACIAR) approved and funded the project proposal initiated by scientists of Bribie Island Aquaculture Centre (BIARC) in Queensland, Australia. This also involved collaboration with the Darwin Aquaculture Centre (DAC) in the Northern Territory, Australia and another counterpart Philippine institution, the Southeast Asian Fisheries Development Center/ Aquaculture Department (SEAFDEC/AQD) in Tigbauan. Additional support to UPVCF/IA as counterpart funds of the government of the Philippines from PCAMRD of the Department of Science and Technology (DOST) was also made available. The work pursued under this project includes: broodstock development, larval rearing, nursery techniques, feeds and feeding (for larvae, juveniles and grow-out), culture systems (ponds, pens, cages in mangrove areas) and biological and ecological studies. In this report, some accomplishments of the ACIAR/PCAMRD supported mud crab project, implemented by UPV and SEAFDEC/AQD, are highlighted to emphasise the present status of mud crab aquaculture. Details of these are included in project reports.

Broodstock and larval rearing

The work of UPV and SEAFDEC/AQD has focused on *Scylla serrata* (the King crab). Several trials on the development of a broodstock diet for this species have been completed and the reproductive performance of the broodstock fed these diets evaluated in terms of maturation rates, percent spawning, number of eggs, body weight of females and hatching rates. The diets tested were natural food, artificial diet and combination of the two. In general, the combination diet gave better performance than natural food and the artificial diet. Refinement of this diet is being continued.

Attempts to breed mud crabs in captivity have been made in the past but until now, the hatchery of mud crabs has not been fully developed. Initial tests to determine food preferences of mud crab larvae had been done as early as 1975 at the Mindanao State University, Naawan, including preliminary studies of the spawning and development of *Scylla olivacea* (Anon. 1975a, 1975b). Several attempts were also made in developing broodstock of the mud crab and some success was attained and enabled practitioners to learn more about mud crab spawning.

The most common practice in the production of mud crab larvae is to obtain berried females, allow them to release their eggs then hatch the eggs in the laboratory. Larvae have been raised to the zoea, megalopa and crablet stages and valuable information obtained on the mass larval rearing of the mud crab. Now that mud crab larvae and juveniles of the King Crab are produced in the hatcheries of UPV and SEAFDEC/AQD, it is only a matter of time before the supply of mud crab juveniles from hatcheries can fully provide for the needs of the industry.

Significant advances in broodstock development and larval rearing of the King Crab have been attained. Ovigerous females collected from ponds where they were raised have been spawned in the laboratories and, furthermore, spawners have been produced from these larvae hatched in the experimental hatcheries of UPVCF/IA and SEAFDEC/AQD. Although the survival of the larvae from the laboratories is not yet very significant (between 1% to 5%), hatchery techniques are slowly being developed and soon an acceptable survival rate of the larvae to the crablet stage should be attained that will make hatchery operations technically feasible and economically viable. Lately, 16% to 80% survival from megalopa to crablet stage has been reported by both UPV and SEAFDEC/AQD.

Testing of different types of artificial diets, feeding levels and feeding schemes has been done and significant improvement in survival and growth has been attained. It was observed that larvae of mud crab cannot survive on artificial diets alone. Two larval rearing experiments were conducted where observations on the collapse of *Tetraselmis* sp. occurred. Water quality was identified as a very important factor that needs to be monitored because this causes food inadequacy as a result of the collapse of food organisms, mainly by poor water quality. One must for a hatchery is *chlorination,* used as a disinfecting agent for all culture media and hatchery facilities to avoid contamination that eventually leads to collapse of the culture. The use of commercially available enrichment media (*Chlorella* paste) is also suggested to ensure a good quality of rotifers. Other microalgae such as *Nannochloropsis* sp., *Chlorella* sp., and *Pavlova* sp. need to be tested as feed for rotifers and their effects on growth performance.

Nursery

Experimental runs to determine the appropriate food for the larvae reared in nurseries up to the crablet stage are being tested. Development of nutritious feeds from locally available feed materials indicated good growth of megalopa to the juvenile stage using squid and mussel meat. Several other materials have been analysed in laboratories in Japan to see if they can approach the positive effect of squid and mussel meat on mud crab growth. Preliminary trials showed the feasibility of rearing megalopa in canvas-lined ponds to crablet or juvenile stage. At least 16% of the megalopa stocked directly in the pond survived and reached the crablet stage. This indicates the possibility of crablet production through direct stocking of zoea 5 and megalopae into the ponds and rearing them up to the juvenile stage.

Evaluations of the performance of experimental diets on the growth of mud crabs in an indoor flow-through system have been made. Crabs were fed diets containing three levels of fishmeal substituted with soybean meal at 0%, 25% and 50%, with mussel meat as a control diet for 60 days. A decreasing trend in specific growth rate was observed in crabs fed increasing levels of soybean. Better rates of growth were observed in crabs fed mussel meat compared to crabs fed formulated diets. A significantly higher number of moults were observed in crabs fed mussel meat compared to crabs fed formulated diets, and a decreasing trend was observed in the number of moults of crabs fed increasing levels of soybean meal.

Mud Crab Culture Systems

Mud crab farming in ponds

Trials to determine the advantage of monosex culture of the King Crab in brackishwater earthen ponds were conducted by SEAFDEC/AQD with encouraging results. Production of mud crabs from all-male stockings were higher than those in all-female; the sizes of the male crabs were larger at a lower stocking density ($0.5/m^2$) than at higher stocking densities ($1.5/m^2$ and $3.0/m^2$). In this trial, there was no interaction of survival between sex and stocking density levels. Survival was significantly higher at lower stocking densities ($0.5/m^2$) but total production was lower.

At UPVCF/IA, attempts to raise mixed-sex mud crabs (*S. olivacea* and *S. serrata*) were made in separate trials. Several problems were identified in the use of *S. olivacea*. This species burrows in the mud, wants to escape from the ponds when it reaches the spawning stage and appears to need shallow areas periodically during the culture period, which indicates a need for an engineering design for mud crab ponds. These tendencies, however, were not observed in *S. serrata*, indicating the desirable characteristics of this species in pond culture. However, it needs shelters to protect it from predators during moulting. The best ratio of the number of shelters to the number of mud crabs in a pond is being determined, including the establishment of an appropriate density for mud crabs raised in ponds. Some of the research results are now being trialled in private fish farms using hatchery produced crablets, grown to juveniles through direct stocking of the megalopae into canvas-lined nursery ponds.

Mud crab farming in pens in mangrove areas

Evaluation of the effect of stocking densities (2.5/m^2 and 5/m^2) and feeding on the growth and production of mud crabs grown in pens in mangrove areas was made after 5 months of culture. Survival was significantly lower in treatments with no feeding compared to treatments fed at 3% body weight daily regardless of the stocking density. The average body weight at harvest was inversely proportional to survival, indicating the high influence of cannibalism on growth. In the absence of added animal food, the mud crabs resorted to cannibalism rather than feeding on available plant sources.

Source of King Crab (*S. serrata*) juveniles

While hatcheries for mud crabs are being developed, farming of mud crabs in ponds, pens, cages and other culture systems continues. The major constraint is seedstock due to its high cost, which is related to the system of collection and distribution of the mud crab juveniles.

Initially, it was thought that the main source of the crablets of the King Crab was Pontevedra, Capiz on the island of Panay. Preliminary information, however, indicates that it is the source of juveniles of *S. olivacea* but not of *S. serrata*. In one of the earlier attempts to procure mud crab juveniles for the pond culture projects of both SEAFDEC/AQD and UPV, the source of the juveniles was Camarines Norte in Southern Luzon. The source of the King Crab (*S. serrata*) juveniles used in later trials in both UPV and SEAFDEC/AQD, came from Northern Samar. Based on the records of the dealer in San Jose, Northern Samar, who engages collectors from all the municipalities in Northern Samar where the juveniles are collected, the King Crabs are distributed to Bulacan, Pampanga, Quezon, Sorsogon, Capiz, Iloilo, Negros Occidental and Masbate.

In several visits to the various municipalities in this province, it was observed that the actual cost of juveniles is very much lower than the dealer's sale price. The dealer's price per piece already includes the cost of mortality during collection, transport, handling and holding which is placed at 25% in each stage of activity (collection, transport etc.). This means that if the cost of the crablet at the collection site is P1.00/piece, it would cost P5 at the dealer's place because another P1.00 is included for profit. When delivered from Northern Samar to Capiz in Panay, the price per piece shoots up more than 100% and the estimated mortality is quite high. On this basis, there is a need to develop better collection methods, transport, handling and holding techniques in order to significantly reduce mortality and thus the cost of the seedstock.

Due to the availability of several species of mud crab in the Philippines, there is a need to determine the geographical areas where each of the mud crab species is dominant so that the fish farmers can determine, more or less, the kind of mud crab stocked in their aquaculture facilities. On this basis, there is a need to conduct ecological studies and investigations of the natural habitat of the mud crab in its area of origin.

Mud crab aquaculture is progressing and it is only a matter of time before it will approach the level of aquaculture of other important cultivable aquatic organisms, especially if the issues and problems identified are sincerely addressed, systematically and vigorously pursued and generously supported. It is high time that the Philippines gave its focused attention to a potentially high export winner — aquaculture.

References

Anon. 1975a. Notes on the food preferences of *Scylla serrata* Forskål. Annual Report. Mindanao State University. Institute of Fisheries Research and Development, 121–122.

Anon. 1975b. Preliminary study on the spawning and development of *Scylla serrata* Forskål. Annual Report. Mindanao State University. Institute of Fisheries Research and Development, 123–126.

Baliao, D.D., Rodriguez, E.M. and Gerochi, D.D. 1981. Culture of the mud crab, *Scylla serrata* (Forskål) at different stocking densities in brackishwater ponds Quarterly Research Report. SEAFDEC Aquaculture Department, 5(1), 10–14.

Baliao, D.D. 1983. Culture of *alimango* and *bangus* in brackishwater ponds. In: Modern Fish Farming (Special issue). 7 p.

Baliao, D.D. 1984. Mud crab, *alimango* production in brackishwater pond with milkfish. In: Aquabusiness Project Development and Management VII, Tigbauan, Iloilo, 8–28 Feb. 1984. Technical Papers. Tigbauan, Iloilo, Aquaculture Department, Southeast Asian Fisheries Development Center. 15 p.

Bureau of Fisheries and Aquatic Resources (BFAR) 1996. 1995 Philippine fisheries profile. 860 Quezon Ave. Quezon City, Metro Manila.

Escritor, G.L. 1970. Report on the experiments in the culture of the mud crab (*Scylla serrata)*. Proceedings Indo-Pacific Fisheries Council, 14 (IPFC/C70/Sym 46).

Escritor, G.L. 1972. Observations on the culture of the mud crab, *Scylla serrata*. In: Pillay, T.V.R. ed. Coastal Aquaculture in the Indo-Pacific Region. West Byfleet, Fishing News (Books), 355–361.

Estampador, E.P. 1949a. Studies on *Scylla* (Crustacea: Portunidae), I. Revision of the genus. Philippine Journal of Science, 78 (1), 95–109.

Estampador, E.P. 1949b. *Scylla* (Crustacea: Portunidae) II. Comparative studies on spermatogenesis and oogenesis. Philippine Journal of Science, 78 (3), 301–353.

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.

Lijauco, M. M., Prospero, O.Q. and Rodriguez, E.M. 1980. Polyculture of milkfish (*Chanos chanos*) and mud crab (*Scylla serrata*) at two stocking densities. Quarterly Research Report. SEAFDEC Aquaculture Department, 4(4), 19–23.

PCAMRD 1993. Philippine shrimp and crab industry profile and action research and development program. Science and Technology Agenda for National Development (STAND). PCAMRD-DOST Primer STAND 2000. No. 20. Philippine Council for Aquatic and Marine Research and Development, Department of Science and Technology, Los Baños, Laguna, Philippines.

PCAMRD 1996. The crab industry in the Philippines. PCAMRD Currents, Vol. 1, No. 3. Philippine Council for Aquatic and Marine Research and Development, Department of Science and Technology, Los Baños, Laguna, Philippines.

SEAFDEC/AQD 1989. Mud crab Abstracts. Brackishwater Aquaculture Information System, Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines.

GENETICS AND ECOLOGY

Morphometrics and Ecology of the Mud Crab (*Scylla* spp.) from Southeast Asia

Julia Lynne Overton[1]

Abstract

Traditional taxonomic studies of the mud crab, *Scylla*, have created much confusion as to whether there is more than one species. This paper describes two studies that applied multivariate techniques to discriminate between phenotypes of *Scylla* in a wide geographic context. Twenty-two morphometric characters were measured on male crabs from seven locations from four countries in Southeast Asia. In both studies, canonical variate analysis (CVA) revealed that the crabs could be discriminated into three discrete clusters. In Study 1, it was shown, by using multiple-group principal-components analysis, that 'size' was not having an effect on the results. In Study 2, one of the three clusters exhibited strong evidence of a cline which correlates with the relative geographical position of these sites along the coast of Vietnam and the Gulf of Thailand. Other research in progress is looking for supporting evidence to explain the presence of separate morphs (species?) related to the biology and ecology of *Scylla*. This includes studies on habitat preference of two morphs from Surat Thani, their reproductive seasonality and morphological barriers to inter-breeding.

PREVIOUSLY, studies on the taxonomy of portunid crabs of the genus *Scylla* have been based on traditional descriptive methods involving relatively few specimens and/or samples from a restricted area. The discrepancy between published descriptions has created much confusion regarding the taxonomic status of *Scylla*, i.e., whether there is more than one species.

The original descriptions identify one species, but use different species names (Forskål 1775, Fabricius 1798, Dana 1852; cited by Alcock 1899). Estampador (1949) revised the taxonomy, recognising three species and one variant of *Scylla* from the Philippines; this view was supported by Serene (1952) based on a similar study which examined spination and colour of *Scylla* populations in Na Trang, southern Vietnam. Stephenson and Campbell (1960), Stephenson (1972) and Holthius (1978) all suggested that the racial variation seen in *Scylla* is not substantial enough to establish separate species, whereas Radhakrishran and Samuel (1982) and Joel and Raj (1983) recognised two species in Indian waters.

[1]Centre for Tropical Ecosystems Research, Department of Ecology and Genetics, Building 540 University of Aarhus, DK-8000 Aarhus C, Denmark.

The genus *Scylla* has an extremely wide range, from east Africa to the Pacific. By looking at the phenotype/genotype in the larger geographical context than earlier studies, one is able to gain a better insight into the taxonomic status of *Scylla*. By assessing the genetic and/or phenetic similarity between spatially segregated populations of *Scylla*, one can tackle issues such as evolutionary events and the possible selection pressures (e.g., environmentally induced selection) creating the variability seen in the phenology of *Scylla* today.

Previously, morphometric studies on *Scylla* have been based on bivariate analysis of regression, using the internal carapace width as the independent variable and frontal length, or claw measurements, as dependent variables to discriminate phenotypes in the genus. However, bivariate analysis has the disadvantage that only two variables may be used at any one time. Thus, the choice of character to carry out the analysis may affect the result obtained and therefore the interpretation. Where different populations of a species are under investigation, one set of characters may result in a significant difference between populations. This may not be present if a different set of characters from the same populations is used (Thorpe 1976). With multivariate analysis, several quantitative characters can be analysed simultaneously using different types of data (binary, continuous, etc.) resulting in a more thorough investigation into the similarity between populations, provided the data are standardised beforehand.

Other advantages associated with the use of multivariate morphometrics to analyse populations are:

- it is a relatively simple technique that is easy to apply in the field;
- it can generate results very rapidly, working from dead and/or preserved materials;
- it is relatively inexpensive and no specific laboratory facilities are required; and
- specimens do not have to be sacrificed in order to obtain the necessary data.

This paper is part of an ongoing study that is addressing two main objectives. These are:

1. To understand the biological basis for the presence of more than one phenotype (species?) of *Scylla* in Southeast Asia by using genetics, morphometrics and ecological techniques to study crabs from several sites within this region.
2. To review, based on these studies plus information obtained from questionnaires conducted with crab fishermen, farmers and dealers, the condition of *Scylla* fisheries and aquaculture in the locations and suggest more sustainable forms of exploitation of mud crab in Southeast Asia.

Only the first of these two objectives will be addressed in this paper. Its focus is on the use of morphometrics to segregate different populations of *Scylla* with respect to their selected morphological parameters. Two morphometric studies were carried out based on crab samples obtained from a total of six locations.

Study 1

Methodology

The first investigation was undertaken to investigate the morphological differences between populations of *Scylla* collected from four locations in Southeast Asia that were separate enough to be seen as discrete populations. The four sites chosen were Klong Ngao, Ranong Province, southwest Thailand; Ban Don Bay, Surat Thani Province; Can Gio district in the Mekong Delta, southern Vietnam; and Sematan, in Sarawak, East Malaysia. These locations are illustrated in Figure 1.

Coastal mangrove is a primary feature of the habitat in all the sites chosen, although Surat Thani Province has been subjected to more coastal development than the other three sites. All four sites also support poor coastal communities where crab fishing is a vital means of income generation.

Thirty crabs were collected from each site except for Surat Thani where two morphs of *Scylla* coexist. Here 20 extra crabs were collected of the second morph. This resulted in five groups of *Scylla* for measurement. A selection process was used in order to collect samples of crabs that would provide data that would not violate the multivariate statistics applied. This meant that only male crabs of about 200 g size, with all limbs attached, were used (thereby lowering the variance attributable to sexual dimorphism and ontogenic influences including size).

In total, 22 characters were measured on each crab as illustrated in Figure 2. Any individuals subsequently found with broken or damaged limbs during the measuring process were removed from the analysis so that a complete data set could be obtained.

A stepwise discriminant analysis program, BMDP-7M, (BMDP Statistical Software Inc. Cork, Ireland) was used to analyse the data. This discriminant function analysis (also known as canonical variate analysis, CVA) is an ordination technique which aims to express as much of the between group variation as possible in a reduced number of dimensions (usually two or three dimensions). Canonical variate analysis is related to the Mahalanobis D^2 statistic. Mahalanobis is one type of similarity coefficient that uses covariance matrices to calculate the similarity between populations. It also takes into account within-group correlation which other similarity coefficients do not (Manly 1990).

Multiple-group principal-components analysis (MGPCA) was used to discover if size was having an effect on the result by identifying the size vector (in this case the first vector) and removing it from the subsequent analysis. This 'size-out' analysis was compared to the previous analysis to show if 'size' influenced the relationships between the groups analysed. MGPCA also allows the assessment of the relative contribution of within-group components to the overall between-group discrimination (Thorpe 1988).

Results of Study 1

The first two canonical variates account for 87% of the between group variance. When these are plotted, the five crab groups analysed form three main clusters with no evidence of chain-linking (Figure 3). The individuals from Ranong and Sarawak which form one of these clusters, also share a similar phenology (typically dark, heavy body structures with the frontal

Figure 1. Location and collection sites for morphometric analysis of the mud crab, *Scylla*, in Southeast Asia.

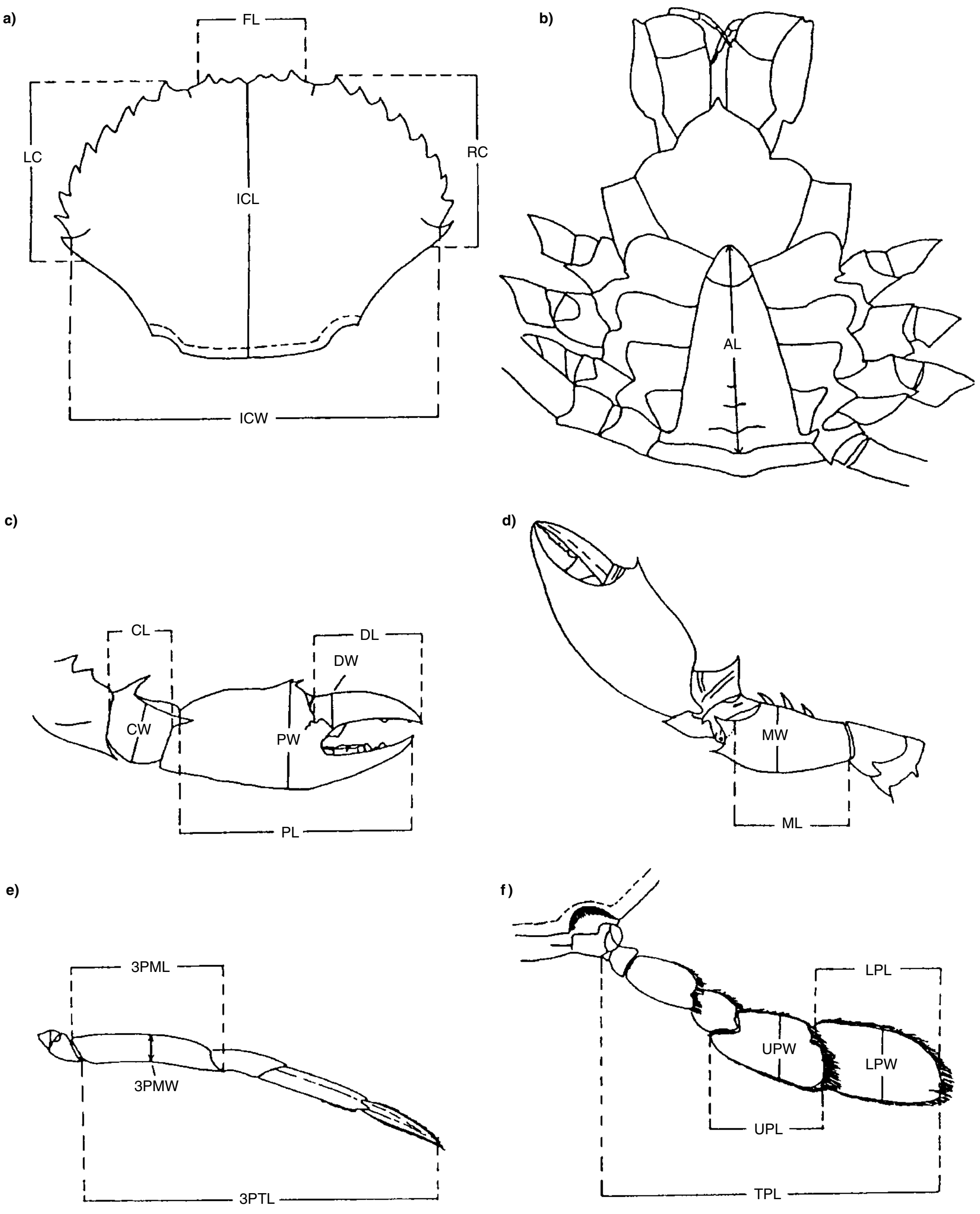

Figure 2. Illustration of 22 characters forming the data for the multivariate analysis. a) carapace, b) abdomen, c) outer cheliped, d) inner cheliped (both chelipeds measured), e) third right cheliped and f) fifth right pereiopod (taken from Overton et al. 1997). AL abdominal length; CL carpus length; CW carpus width; DL dactylus length; DW dactylus width; FL frontal length; ICL internal carapace length; ICW internal carapace width; LC left anterolateral length of carapace; LPL lower paddle length; LPW lower paddle width; ML merus length; MW merus width; PL propodus length; 3PML third pereiopod merus length; 3PMW third pereiopod merus width; 3PTL third pereiopod total length; PW propodus width; RC right anterolateral length of carapace; TPL total length of swimming leg; UPL upper paddle length; UPW upper paddle width.

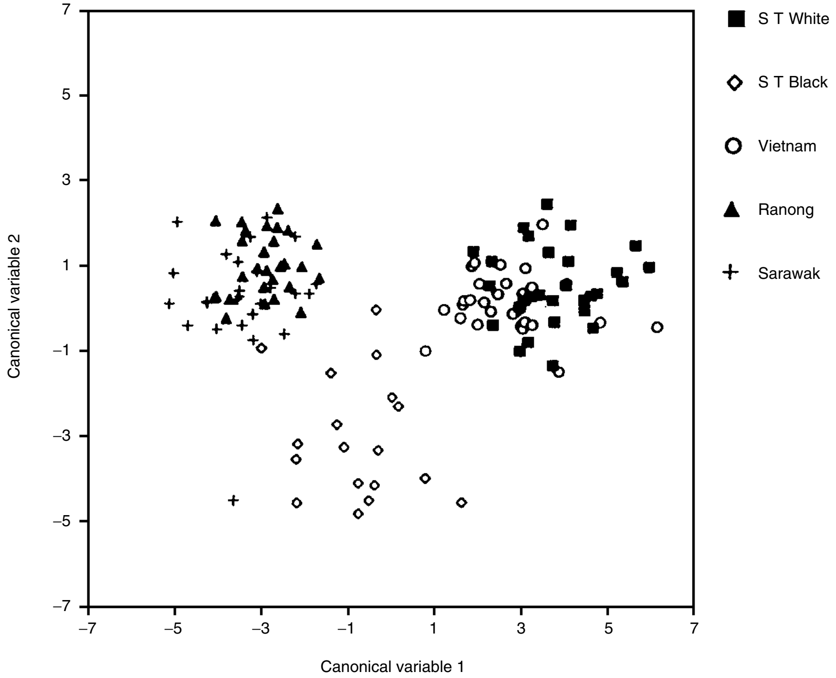

Figure 3. Canonical variate analysis (CVA) of five groups of *Scylla* collected from four sites in Southeast Asia after applying multiple-group principle-components analysis.

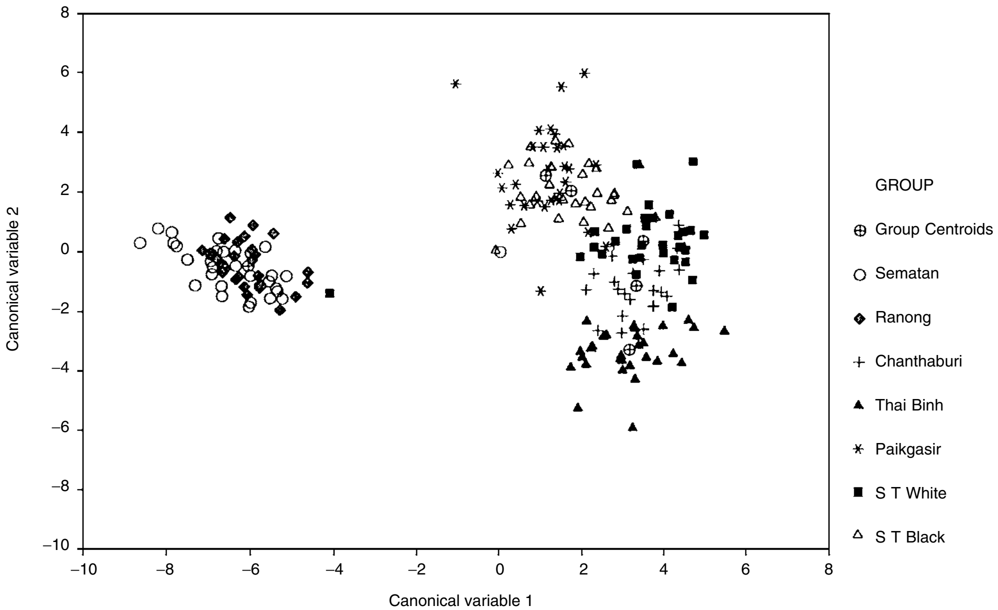

Figure 4. Canonical variate analysis (CVA) of seven groups of *Scylla* collected from six sites in Southeast Asia.

lobe expressing smooth spines). These were designated as the 'black' type. One morph from Surat Thani, and the crabs collected from southern Vietnam form a discrete cluster and were labelled as the 'white' type (typically exhibiting pale body colouration with spots on outer chelae and a frontal lobe expressing sharp, v-shaped spines). The second morph from Surat Thani formed a third cluster by itself, with a group mean which lies equidistant between the other two clusters. The presence of a third cluster is surprising because this group expresses all the phenotypic features of the 'black' morph represented by the Ranong and Sarawak crabs. This third group also shows a wider scatter of individuals in the plot, indicating more within group variance.

Examination of the results of the MGPCA scores revealed that frontal length, right and left antero-lateral lengths of the carapace, right dactylus width, right propodus length, right and left carpus lengths and right merus width contributed most to the discrimination between groups. The 'size-out' analysis revealed that growth-dependant size was not having an effect on the outcome of the analysis.

Study 2

The unexpected result from Study 1, showing that the 'black' crabs from Surat Thani form their own, discrete group in the CVA, raised several concerns and possible interpretations:

a) There were fewer individual samples from this group compared to the other four *Scylla* groups, thus raising the relative group error;
b) These 'black' individuals may be part of a cline that was not revealed due to the choice of sampling sites;
c) These individuals are evidence of a hybrid group that share morphological characters with the other two 'groups' thus forming a third cluster in CVA; or

These individuals are in fact a third morph (species?).

In view of these uncertainties, a second study was conducted to provide data from crabs sampled in three additional sites.

Methodology for Study 2

The additional sites selected for Study 2, were Chantaburi, northern Gulf of Thailand; Thai Binh Province in Vietnam, where the Red River Delta meets the Gulf of Tonkin; and Paikgasir, on the edge of the vast Sunderbans mangrove forest, southern Bangladesh. A second sample of crabs from Surat Thani was also measured to confirm the result obtained for the two morphs in study one and to increase the number of individuals for the 'black' morph. The same criteria for sampling and data analysis were used as described in Study 1. Samples from Chantaburi were representative of the 'white' morph, although three morphs are actually recognised in this location.

Results of Study 2

The results of the analysis from Study 2 are shown in Figure 4. Ninety percent of the between group variation was accounted for by the first two canonical variates. Again the three population theory is supported by the data. The 'black' morph from Surat Thani forms a separate group again, this time together with the crabs from Paikgasir, Bangladesh indicating that they are valid as a third cluster as suggested by Study 1. The Sematan and Ranong groups form their own discrete group, as before. The 'white' crabs from Chantaburi, Thai Binh and Surat Thani exhibit strong evidence of clinal variation where their relative position in their cluster agrees broadly with their geographical position along the eastern seaboard of Vietnam/Thailand. When looking more closely at the first canonical variate (representing 76% of the between group discrimination) the Surat Thani 'black'/Paikgasir cluster seems to be more closely related to the 'white' groups described above, than to the Ranong/Sarawak 'black' cluster.

General Discussion

What is most striking about the results of Study 1 and Study 2 is the formation of three clusters, suggesting three phenotypic groups of *Scylla* from seven locations sampled in Southeast Asia. In both studies, the frontal width was one of the most significant characters contributing to the between-group discrimination. This three 'species' theory for *Scylla* was also proposed by Chayarat and Kaew-ridh (1984) who demonstrated (using regression analysis) that the width of the frontal lobe was wider in the 'white morph' than the other two recognised morphs from Chantaburi Province labelled as 'red' and 'green' morphs.

Multivariate analysis of morphometric data has been shown to separate other crustacean species. Examples of this include *Procambarus* crayfish from Mexico (Allegrucci et al. 1992). It is hard to know how much of the expressed variation in *Scylla* is genetically controlled and how much is due to environmental induction either through selective pressures or ontogenic influences.

Like many other mangrove crustaceans, *Scylla* has a marine pelagic larval phase. Larval dispersion can be expected to result in high gene flow between populations of *Scylla*. Therefore, it would be expected that discrimination between populations would not be so clear if they were all variants of the same species. However, a combination of presettlement predation and higher retention rates of locally spawned larvae than was first thought may result in fairly well structured populations. This does not necessarily explain the separate clusters, but it would explain the clinal variation exhibited in Study 2 for the 'white' morphs of *Scylla* from Thai Binh, Chantaburi and Surat Thani.

Similar results were obtained for populations of the blue crab, *Callinectes sapidus*, from the eastern seaboard of the United States using allozyme electrophoresis where both a cline and patchiness between populations were believed to be due to heterogeneous patches of larvae, created by currents and other isolating factors, that were then modified by ontogenic or local selective processes at the post-settlement stage (McMillan-Jackson et al. 1994). Larval ecology is one area of mud crab research which needs to be addressed if there is to be some understanding of population structuring within *Scylla* species.

In addition to the effects on larval recruitment, it is known that adult crabs do not travel far outside their immediate habitat (Hyland et al. 1984) except when females migrate offshore to spawn (Arriola 1940; Hill 1975). Therefore, there may be some structuring within the effective population among those that have potential to breed.

In general, heterogeneous coastal environments can be expected to have a significant influence on phenotypic expression. The two sympatric morphs of *Scylla* located in Surat Thani suggest that there is more than environmental induction that is resulting in the phenotypic variation found within the genus.

It has been recognised by crab fishermen that different morphs of *Scylla* which are called 'Banhawin' and 'Mamosain' have different behaviours and inhabit different parts of the mangrove zone. The former is described as being subtidal and less likely to burrow, in contrast to the latter which lives in deep burrows within the intertidal areas. This also describes the behaviours of the white and black morphs of Surat Thani respectively.

One way to confirm this believed habitat preference is to look at the dietary preference over an extended period of time. The mud crab is an opportunistic feeder, feeding primarily on slow moving or stationary food items. An in-depth study on prey items of *Scylla* by looking at the gut contents was carried out by Prasad and Neelakantan (1988). They found a whole range of food items where 'detritus' (of which 61.25% was inorganic sediment) was the main food for juveniles whereas adult *Scylla* had a much higher protein diet.

Many of the food items are mangrove related and can be identified to certain parts of the mangrove/estuarine zone. This study hopes to be able to link the gut contents to the seasonal movement of female crabs over a prolonged period of time. Females are chosen as they are known to travel the furthest out of the two sexes.

Whether the behaviour and habitat preferences of the different forms of *Scylla* are genetically controlled has not been established. An example of habitat preference that is polygenically controlled is illustrated in an estuarine amphipod studied in the Squamish estuary in Canada (Stanhope et al. 1992).

Other experimental work carried out on groups of amphipods has shown considerable sympatric population divergence (progressing towards sympatric speciation) can occur if mate choice is closely coupled with habitat preference. In other words, there has also to be some assortative mating taking place. The apparent absence of intermediates between the two morphs of *Scylla* in Surat Thani suggests that assortative mating may be occurring here (Overton et al. 1997).

One of the specific objectives of the current study is to ascertain whether there is any physical reason why there is no cross-mating taking place between the two morphs (species?) that are morphologically so similar. This includes:

a) looking at the male genital morphology; and
b) whether there is any difference in reproductive seasonality between the two morphs found in Surat Thani Province.

Evidence from other crustacean groups point to the possible significance of these factors. For example, male genital structures can show great morphological difference even between closely related brachyuran species such as the fiddler crabs, *Uca* spp. (Crane 1975) while it has been shown that gammarid amphipod species found living sympatrically have distinct and displaced reproductive periods (Kolding and Fenchel 1979).

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References

Alcock, A. 1899. Materials for a carcinological fauna of India, No. 4. The Brachyura of Cyclomatopa. Part II. A revision of the Cyclometopa with an account of the families Portunidae, Canridae and Corystidae. Journal of the Asiatic Society of Bengal, 68, 1–111.

Allegrucci, G., Baldari, F., Caesaroni, D., Thorpe, R.S. and Sbordoni, V. 1992. Morphometric analysis, interspecific and microgeographic variation of crayfish from a Mexican cave. Biological Journal of the Linnean Society, 47, 455–468.

Arriola, F.J. 1940. A preliminary study of the life history of *Scylla serrata* (Forskål). Philippine Journal of Science, 73, 4, 455–468.

Chayarat, C. and Kaew-ridh, B. 1984. Some possibilities of the mud crab *Scylla* spp. (Crustacea: Portunidae), identification in the vicinity of Chantaburi Fisheries Station. Department of Fisheries Technical Paper, 18 p.

Crane, J. 1975. Fiddler crabs of the world (Ocypodidae: genus *Uca*). Princeton University Press, New Jersey, 736 p.

Estampador, E.P. 1949. Studies on *Scylla* (Crustacea: Portunidae), 1. Revision of the genus. Philippine Journal of Science, 78, 95–108.

Hill, B.J. 1975. Abundance, breeding and growth of the crab *Scylla serrata* in two South African estuaries. Marine Biology, 80, 57–61.

Holthius, L.B. 1978. A collection of decapod Crustacea from Sumba, lesser Sunda Islands, Indonesia. Zoologische Verhandelingen uigegeven door het rijksmuseum van natuurlijks historiete Leiden. no162, Leiden.

Hyland, S.J., Hill, B.J. and Lee, C.P. 1984. Movement within and between different habitats by the portunid crab *Scylla serrata*. Marine Biology, 80, 57–61.

Joel, D.R. and Raj, P.J.S. 1983. Taxonomic remarks on two species of the genus *Scylla* de Haan (Portunidae: Brachyura) from Pulicat Lake. Indian Journal of Fisheries, 30, 13–26.

Kolding, S. and Fenchel, T.M. 1979. Coexistence and life cycle characteristics of five species of the amphipod genus *Gammarus* in the Baltic. Oikos, 33, 323–327.

Manly, B.F.J. 1990. Multivariate Statistical Methods: A Primer. Chapman and Hall, London. 159 p.

McMillan-Jackson, A.L., Bert, T.M. and Steele, P. 1994. Population genetics of the blue crab *Callinectes sapidus*: modest population structuring in a background of high gene flow. Marine Biology, 118, 53–65.

Overton, J.L., Macintosh, D.J. and Thorpe, R.S. 1997. Multivariate analysis of the mud crab *Scylla serrata* (Brachyura: Portunidae) from four locations in Southeast Asia. Marine Biology, 128, 55–62.

Prasad, P.N. and Neelakantan, B. 1988. Food and feeding of the mud crab *Scylla serrata* Forskål (Decapoda: Portunidae) from Karwar waters. Indian Journal of Fisheries, 35, 3, 164–170.

Radhakrishnan, C.K. and Samuel, C.T. 1982. Report on the occurrence of one subspecies of *Scylla serrata* (Forskål) in Cochin Backwaters. Fisheries Technology, 19, 5–7.

Serene, R. 1952. Les espèces du genre *Scylla* à Natrang (Vietnam). Proceedings of the Indo-Pacific Fisheries Council, 113–137.

Stanhope, M.J., Leighton, B.J. and Hartwick, B. 1992. Polygenic control of habitat preference and its possible role in sympatric population subdivision in an estuarine crustacean. Heredity, 69, 279–288.

Stephenson, W. 1972. An annotated checklist and key to the Indo-West-Pacific swimming crabs (Crustacea: Decapoda: Portunidae). Royal Society of New Zealand, Bulletin No. 10. Royal Society of New Zealand. 1–64.

Stephenson, W. and Campbell, B. 1960. The Australian portunids (Crustacea: Portunidae) IV. Remaining genera. Australian Journal of Marine and Freshwater Resources, 11, 73–122.

Thorpe, R.S. 1976. Biometric analysis of geographic variation and racial affinities. Biological Reviews, 51, 407–452.

Thorpe, R.S. 1988. Multiple group principal components analysis and population differentiation. Journal of the Zoological Society of London, 216, 37–40.

Genetic Charaterisation in the Mud Crab *Scylla* (Brachyura : Portunidae)

Ketut Sugama[1] and Jhon H. Hutapea[1]

Abstract

In order to examine the status of mud crab, *Scylla*, from Indonesia, biochemical genetic variation within the genus and among populations sampled from East Java, Lombok and South Sulawesi were assessed by allozyme electrophoresis and principal component analysis. Three proposed species, *S. olivacea, S. tranquebarica* and *S. paramamosain* were analysed electrophoretically for genetic variation at 14 loci. Three loci were polymorphic in *S. olivacea,* two in *S. tranquebarica* and one in *S. paramamosain*. Average heterozygosity ranged from 0.001 to 0.036. Allele frequencies of 14 loci were used to estimate Nei's genetic distance (*D*). The *D* value ranged from 0.078–0.199. Four loci (EST*, MPI*, PGM* and SOD*) were found to be the most reliable species-specific markers for identification.

TWO groups of *Scylla* are identified in Indonesia, one reddish or brownish green, and the other greyish green. The former are *Scylla olivacea* while the latter are *S. tranquebarica* and *S. paramamosain* (Keenan et al. 1998). *S. olivacea* is the dominant species in Indonesia, about 80% of the total anual landings of mud crab consist of this species (Cholik and Hanafi 1991).

It has been assumed that the genus *Scylla* had only one species. However, colour, morphological and biological characteristics of the genus *Scylla* reported from the Philippines, Vietnam, India and Japan have established the existence of more than one species (Estampador 1949; Kathirvel and Srinivasagam 1991; Keenan et al. 1995; Fuseya and Watanabe 1996).

By observing colour and morphological features (colour in carapace, polygonal pigmented area, anterolateral teeth of carapace, 'H' mark on carapace, length of cheliped size attained), the mud crab was classified into three species and one variety, i.e., *S. olivacea*, *S. tranquebarica, S. serrata* and *S. serrata* var. *paramamosain* (Estampador 1949). Kathirvel and Srinivasagam (1991) classified two distinct species, namely *S. olivacea* and *S. tranquebarica*, and furthermore, said *S. serrata* was a synonym of *S. tranquebarica*, this finding characterised by differences in size, spines on the outer border of the carpus of the cheliped and habitat preferences.

In recent years, mud crab capture and culture have been expanding in Indonesia because of the high economic value of the species and its potential as an export commodity. The principal constraint in the expansion of aquaculture is lack of seed. An attempt at seed production of *Scylla* was performed without considering species differentiation. Since four species have now been identified and are morphologically and genetically different (Keenan et al. 1998) it is necessary to evaluate and understand the genetics of each species.

Allozyme electrophoresis is considered to be an extremely useful technique in population genetics and is particularly powerful in identify cryptic species which are difficult to distinguish morphologically (Allendorf and Utter 1979; Lavery and Shaklee 1991). This technique is used in the present study to identify diagnostic loci for the genus *Scylla* for specimens from Indonesia.

Materials and Methods

A total of 227 mud crab samples were collected from three localities (Table 1). The species classifications

[1]Gondol Research Station for Coastal Fisheries, PO Box. 140, Singaraja, Bali 81101, Indonesia

listed in Table 1 are based on colour and polygonal pigmentation on chelipeds and walking legs. The samples were brought alive from each site to the Gondol Research Station for Coastal Fisheries, Bali. Muscle tissue was taken from each individual and kept in a deep freeze at −25 °C until used for electrophoresis. The methods of starch gel electrophoresis were the same as those described previously (Sugama et al. 1988, Taniguchi and Sugama 1990). Detection of allozymes and nomenclature of locus designation follows Shaklee et al. (1990).

Table 1. Samples used for electrophoretic analysis in the genus Scylla.

Location	Species	Number of samples
East Java (Pasuruan,	*S. olivacea*	42
50 kms SSE of Surabaya)	*S. tranquebarica*	32
	S. paramamosain	26
Lombok Island	*S. olivacea*	36
(Sekotong near Mataram)	*S. tranquebarica*	24
	S. paramamosain	8
South Sulawesi	*S. olivacea*	30
(Bone)	*S. tranquebarica*	16
	S. paramamosain	13

The experimental protocol used to separate and resolve the 12 enzymes systems, encoding a total 14 loci, is summarised in Table 2. The allele frequencies, proportion of polymorphic loci, number of alleles per locus and heterozygosities were calculated as measures of genetic variability.

Allelic variants were designated according to their relative mobility. The most common allele in *S. olivacea* was designated 100 and other alleles were given numbers indicating their mobility relative to that of the common allele. Cathodal systems were designated in a similar way but were given a negative sign. The differences between alleles at the same locus were decided by the position of allozymes on the same gel. Genetic distance was calculated from the formula proposed by Nei (1972). Average heterozygosity was determined by totalling the number of observed heterozygosities for each locus, dividing this by the total number of individuals with data, and then averaging over all loci.

Results

The list of enzymes, buffer specificity and loci detected are given in Table 2. Twelve enzyme coded by 14 loci were clearly resolved in all samples and three loci AAT*-2, GPI* and MDH*-2 were polymorphic in at least one of the samples (Table 2).

Allele frequencies of polymorphic loci are given in Table 3. The genotypic distribution observed at each polymorphic locus in all of the samples was found to be in agreement with that expected from the Hardy-Weinberg equilibrium.

The electropherograms of allozymes were examined for proposed species of *Scylla*. Individuals were readily identifiable to species from the combination of EST*, MPI*, PGM* and SOD* loci. It can be seen that no three species have the

Table 2. Electrophoretic protocols used to reveal allozyme, enzyme polymorphism and alleles at loci showing fixed differences among samples of mud crab, genus Scylla.

Enzymes (Abbreviations)	E.C. No.	Buffer[1]	No. of loci	Polymorphism[2]	Fixed[3] difference
Aspartate aminotransferase (AAT)	2.6.1.1	TC-8	AAT-1*	M	ND
			AAT-2*	P	ND
Alcohol dehydrogenase (ADH)	2.6.1.1	CAPM-7	ADH*	M	ND
Esterase (EST)	3.1.1	CAPM-6	EST-2*	M	D
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	CAPM-6	GPI*	P	ND
Isocitrate dehydrogenase (IDH)	1.1.1.42	CAPM-6	IDH*	M	ND
Lactate dehydrogenase (LDH)	1.1.1.27	CAPM-7	LDH*	M	ND
Malate dehydrogenase (MDH)	1.1.1.37	CAPM-6	MDH-1*	M	ND
			MDH-2*	P	ND
Mannose phosphate isomerase (MPI)	5.3.1.8	CAPM-6	MPI*	M	D
6-Phosphogluconate dehydrogenase (6-PGD)	1.1.1.44	CAPM-6	6-PGD*	M	ND
Phosphoglucomutase (PGM)	5.4.2.2	CAPM-6	PGM*	M	D
Superoxide dismutase (SOD)	1.15.1.1	TC-8	SOD*	M	D
Sorbitol dehydrogenase (SDH)	1.1.1.22	TC-8	SDH*	M	ND

[1]CAPM-6,7 : Citric acid aminoprophylmorpholine pH 6 and 7; TC-8 : Tris-citric acid pH 8
[2]M : monomorphic; P : Polymorphic
[3]ND = no divergence; D = divergence

Table 3. Allele frequencies at 14 loci in the Scylla species.

Locality locus	Allele	*S. olivacea*			*S. tranquebarica*			*S. paramamosain*		
		Pasuruan (42)	Sekotong (36)	Bone (30)	Pasuruan (32)	Sekotong (24)	Bone (16)	Pasuruan (26)	Sekotong (8)	Bone (13)
AAT-1*	100	1	1	1	1	1	1	1	1	1
AAT-2*	120	0.107	0.042	0.017	0.016	0	0	0	0	0
	100	0.893	0.958	0.983	0.984	1	1	1	1	1
ADH*	−100	1	1	1	1	1	1	1	1	1
EST*	100	1	1	1	0	0	0	0	0	0
	80	0	0	0	1	1	1	1	1	1
GPI*	150	0.024	0	0	0.078	0.042	0.063	0.019	0	0
	100	0.881	0.903	0.933	0.906	0.958	0.938	0.981	1	1
	80	0.095	0.097	0.067	0.016	0	0	0	0	0
IDH*	100	1	1	1	1	1	1	1	1	1
LDH*	100	1	1	1	1	1	1	1	1	1
MDH-1*	100	1	1	1	1	1	1	1	1	1
MDH-2*	125	0.024	0	0	0	0	0	0	0	0
	100	0.976	1	1	1	1	1	1	1	1
MPI*	100	1	1	1	1	1	1	0	0	0
	90	0	0	0	0	0	0	1	1	1
6-PGD*	100	1	1	1	1	1	1	1	1	1
PGM*	100	1	1	1	1	1	1	0	0	0
	85	0	0	0	0	0	0	1	1	1
SOD*	150	0	0	0	0	0	0	1	1	1
	100	1	1	1	1	1	1	0	0	0
SDH*	−100	1	1	1	1	1	1	1	1	1

same common alleles at all of these loci (Figure 1 and Table 3). Alleles MPI-90* and PGM-85* was found exclusively in *S. paramamosain.* Although *S. olivacea* and *S. tranquebarica* show the same common allele MPI-100* and PGM-100* at these loci, they can be easily separated at EST* locus. At the EST* locus, allele EST*-100 is specific to *S. olivacea*.

Table 4 summarises the genetic variation for the three species of mud crab. Proportion of polymorphic loci per species range from 7.14% (*S. paramamosain)* to 21.43% (*S. olivacea*). The average number of alleles per locus per species ranges from 1.07 (*S. paramamosain*) to 1.21 (*S. olivacea*). The average observed heterozygosity ranges from 0.001 (*S. paramamosain*) to 0.036 (*S. olivacea*).

In order to estimate the degree of genetic difference among the three species, the genetic distance (*D*) was calculated between every pair of species using the allele data shown in Table 3. The average genetic distances between *S. olivacea* vs. *S. tranquebarica, S. tranquebarica* vs. *S. paramamosain* and *S. olivacea* vs. *S. paramamosain* were 0.078, 0.117 and 0.199 respectively. The average genetic distance was greatest between *S. olivacea* vs. *S. paramamosain* and lowest between *S. olivacea* vs. *S. tranquebarica.*

Table 4. Summary of genetic variation at 14 loci in the genus Scylla.

	Species		
	S. olivacea	*S. tran–quebarica*	*S. para–mamosain*
No. of individuals examined	108	72	47
No. of loci examined	14	14	14
No. of polymorphic loci	3	2	1
Proportion of polymorphic loci (%)	21.43	14.28	7.14
Number of alleles per locus	1.21	1.14	1.07
Heterozygosity: Observed	0.036	0.011	0.001
Expected	0.033	0.010	0.001

Discussion

The genetic data clearly showed similarities and differences within the genus of *Scylla* in mobility of the common band for the various loci. These diagnostic

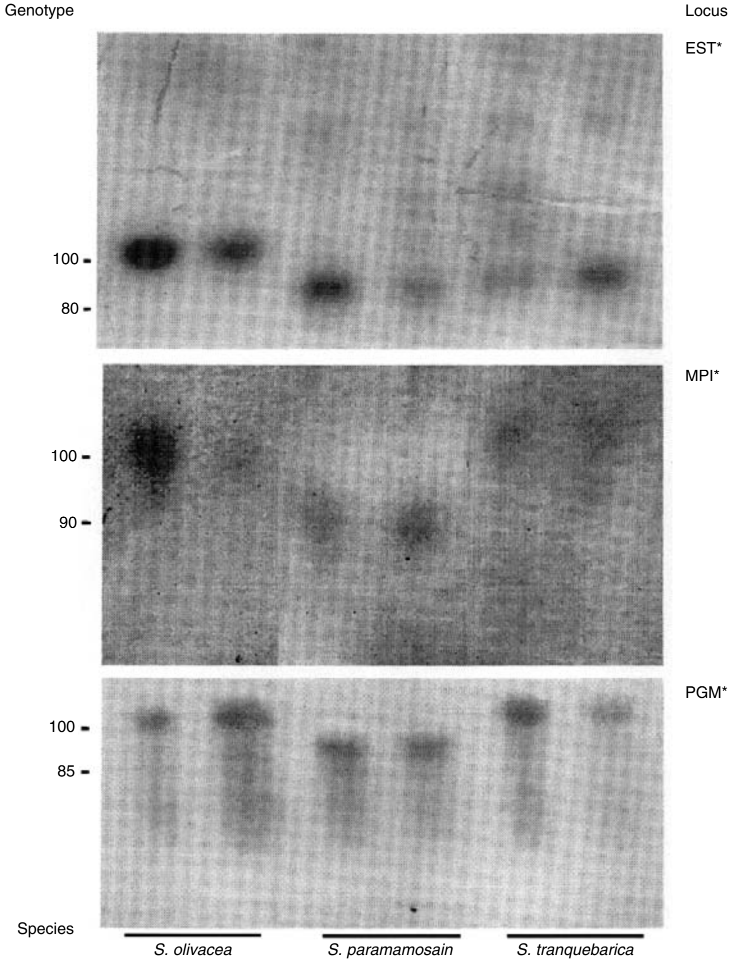

Figure 1. Electropherograms of EST*, MPI* and PGM* showing fixed allele differences in the genus *Scylla*.

loci can thus be used as reliable markers to identify these three species of *Scylla*.

Using common allele differences at the EST*, GPI*, PGM* and SOD* loci (Figure 1 and Table 3), it is easy to distinguish the three species of *Scylla*. The three species morphometrically classified by Estampador (1949) and genetically classified by Keenan et al. (1995) and Fuseya and Watanabe (1996) agree well with the present results.

Electrophoresis can give an independent estimate of the level of variation within a population without an extensive survey of morphology and other quantitative traits. The average heterozygosities calculated here for *S. olivacea* (0.036) and *S. paramamosain* (0.001) are relatively low. However, such estimates are particularly dependent on the type and number of loci analysed (Allendorf and Utter 1979). Based on 17 loci detected from 11 enzymes, Fuseya and Watanabe (1996) found similar levels of average heterozygosities for three *Scylla* species, 0.004 to 0.0049. It is probably a reasonable assumption that the amount of isozyme variation reflects the relative amount of genetic variation found at other loci in the genome (McAndrew and Majumdar 1983).

Genetic differences between species have been observed in many fishes using biochemical markers (Ayala 1983). Higher categories are on the average more different than lower ones. In the family Pleuronectidae, the average genetic distance was reported as being 0.01 between species and 1.11 between genera (Ward and Galleguillos 1983). In the genus *Scylla*, Fuseya and Watanabe (1996) reported the genetic distance (*D*) among populations ranged from 0–0.003, and between species from 0.059–0.187, both much lower than fish in the family Pleuronectidae.

In the present study, the average *D* values between species were similar to those reported by Fuseya and Watanabe (1996) and ranged from 0.078 to 0.199. Typically, closely related species have *D* values around 0.5 (Ayala 1983). It is possible that larger differences among these species may be found by increasing the number of loci surveyed but unless the loci examined here are entirely unrepresentative, it must be concluded that there is little genetical difference among these *Scylla* species.

References

Allendorf, F.W. and Utter, F.M. 1979. Population genetics. In: Hoar, W.S., Randall, D.J. and Brett, J.R., eds. Fish Physiology, Vol. 8, 407–454. Academic Press, New York.

Ayala, F.J. 1983. Enzymes as taxonomic characters. In: Oxford, G.S. and Rollinson, D. eds. Protein Polymorphism: Adaptive and Taxonomic Significance, 3–36. Academic Press, New York.

Cholik, F. and Hanafi, A. 1991. A. review of the status of mud crab (*Scylla* sp.) fishery culture in Indonesia. In: Angell, C.A. ed. Report of the Seminar on the Mud Crab Culture and Trade, held at Surat Thani, Thailand, November 5–8, 1991. Bay of Bengal Program, BOBP/REP/51, Madras, India.

Estampador, E.P. 1949. Studies on *Scylla* (Crustacea: Portunidae), I. Revision of the genus. Philippine Journal of Science 78 (1), 95–109.

Fuseya, R. and Watanabe, S. 1996. Genetic variability in the mud crab Genus *Scylla* (Brachyura: Portunidae). Fisheries Science 62 (5), 705–709.

Kathirvel, M. and Srinivasagam, S. 1991. Taxonomy of the mud crab, *Scylla serrata* (Forskål), from India. In: Angell, C.A. ed., Report of the Seminar on the Mud Crab Culture and Trade, held at Surat Thani, Thailand, November 5–8, 1991. Bay of Bengal Program, BOBP/REP/51, Madras, India. 246 p.

Keenan, C.P., Mann, D., Lavery, S. and Davie, P. 1995. Genetic and morphological relationships of mud crab genus *Scylla* from throughout the Indo-Pacific. ACIAR Project Report, Southern Fisheries Centre, Deception Bay, Australia, 80 p.

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.

Lavery, S. and Shaklee, J.B. 1991. Genetic evidence for separation of two sharks, *Carcharhinus limbatus* and *C. tilstoni*, from Northern Australia. Marine Biology, 108, 1–4.

McAndrew, B.J. and Majumdar, K.C. 1983. Tilapia stock identification using electrophoretic markers. Aquaculture 30, 249–261.

Nei, M. 1972. Genetic distance between populations. American Naturalist, 106, 283–292.

Shaklee, J.B., Allendorf, F.W., Morizot, D.C. and Whitt, G.S. 1990. Gene nomenclature for protein coding loci in fish. Transactions of the American Fisheries Society, 119, 2–15.

Sugama, K., Taniguchi, N. and Umeda, S. 1988. An experimental study on genetic drift in hatchery population of red sea bream. Nippon Suisan Gakkaishi, 54 (15), 739–744.

Taniguchi, N. and Sugama, K. 1990. Genetic variation and population structure of red sea bream in the coastal waters of Japan and the East China Sea. Nippon Suisan Gakkaishi, 56 (7), 1069–1077.

Ward, R.D. and Galleguillos, R.A. 1983. Biochemical systematics and genetic variation in flatfish of the family Pleuronectidae. In: Oxford, G.S. and Rollinson, D., eds. Protein Polymorphism: Adaptive and Taxonomic Significance, 165–178. Academic Press, New York.

The Fourth Species of *Scylla*

Clive P. Keenan[1]

Abstract

Previous genetic research has shown three genetically distinct *Scylla* species. Mud crabs, morphologically different from these three species, were obtained from near Hong Kong, the Mekong Delta, Vietnam and near Semarang, Central Java, Indonesia. Allozyme electrophoresis provided a simple and direct method of determining fixed genetic differences between all of these new samples and the other three identified species. To confirm the distinctiveness of the new samples, sequencing of two mitochondrial DNA genes, 16s and COI, was completed for one sample from each area. All new samples were closely related and distinctly different from the other three species, indicating they all belonged to a fourth species of *Scylla*, *S. paramamosain*.

THE UNCERTAINTY of genetic relationships and taxonomic details of the genus *Scylla* de Haan is a primary constraint to the management of the wild fishery and development of aquaculture (BOBP 1992; Brown 1994). While it is widely recognised that the mud crabs of the Indo-west Pacific region belong to more than one morph of the genus *Scylla* (BOBP 1992) there is considerable confusion of the taxonomic nomenclature (Joel and Raj 1980) and the identification of species. Some authorities have not accepted the justification of Estampador (1949) for the classification of members of the genus *Scylla* into different species and varieties. All morphs were placed in synonymy by Stephenson and Campbell (1960), a move supported by Ong (1964). Recently, several genetic studies to determine relationships between these different forms have been completed (Keenan et al. 1995; Keenan 1996; Fuseya and Watanabe 1996; Sugama and Hutapea these Proceedings) and Keenan et al. (1998) have examined and revised the taxonomy of species within the genus. Dorsal and frontal photographs of the species described by Keenan et al. (1998) are presented in Figures 1–4.

Knowledge of the morphology and distribution of any species and its population structure are important for the development of sustainable culture and the implementation of fisheries management regulations. Allozyme electrophoresis is a very powerful method for the determination of biochemical genetic variation and provides a simple and direct method of determining the genetic relationships and the extent of species and population differentiation (Sarich 1977; Keenan and Shaklee 1985; Richardson et al. 1986). The advantage of genetic-based methods over morphological taxonomy is that breeding relationships and the absence of gene flow can be quantified. Therefore, conclusions as to the breeding structure of a species, and the ability of isolated populations to interbreed in nature are more specific than those based on morphology. In addition, such conclusions can be used to provide morphological information, based on the known 'biological' species, to identify clearly the different morphs.

From the definition for species (Holmes 1979), "***a group of interbreeding individuals not interbreeding with another such group****, being a taxonomic unit including geographical races and varieties and having 2 names in binomial nomenclature, the generic and specific epithet, similar and related species being grouped into a genus*", the criterion for defining a species can be tested by simple genetic methods. This definition implies that identification of a species can be based upon the presence of shared fixed genetic differences between two different groups, which indicates a lack of gene exchange. These characters can be used as diagnostic characters and applied as a reference point to assist

[1]Bribie Island Aquaculture Research Centre, PO Box 2066, Bribie Island, Qld 4507 Australia

Figure 1. Photographs of adult female *Scylla serrata* showing diagnostic features: high, bluntly pointed frontal lobe spines; pairs of large spines obvious on carpus and propodus; polygonal patterning clearly present on all appendages. A – dorsal, B – frontal. Photo: Queensland Museum.

Figure 2. Photographs of adult male *Scylla tranquebarica* showing diagnostic features: moderate, blunted frontal lobe spines; pairs of large spines obvious on carpus and propodus; polygonal patterning present on last two pairs of legs, weak or absent on other appendages. A – dorsal, B – frontal. Photo: Queensland Museum.

Figure 3. Photographs of adult male *Scylla paramamosain* showing diagnostic features: moderately high, pointed and triangular frontal lobe spines usual; pair of large spines obvious on propodus, on carpus inner spine absent and outer spine reduced; polygonal patterning present on last two pairs of legs, weak or absent on other appendages. A – dorsal, B – frontal. Photo: Queensland Museum.

Figure 4. Photographs of adult male *Scylla olivacea* showing diagnostic features: low and rounded frontal lobe spines; pair of reduced spines obvious on propodus, on carpus inner spine absent and outer spine reduced; polygonal patterning absent from all appendages. A – dorsal, B – frontal. Photo: Queensland Museum.

with the identification of physical characteristics useful for species diagnostics (Keenan et al. 1998).

When testing the new samples (from a suspected species against known species), the null hypothesis was: Are the new mud crabs from the same species, i.e., possess no fixed genetic differences from the other identified species? This hypothesis is falsified if fixed differences are observed, usually at two or more loci (Richardson et al. 1986). Further, if a reasonable number of samples are examined, the presence or absence of rare heterozygotes (i.e., hybrids) can be determined. If heterozygotes are absent between the suspected new species and other sympatric (co-occurring) species, for loci where fixed differences were observed, this provides evidence that speciation has developed to a stage where hybridisation can no longer occur and that they constitute 'biological species' as defined above.

Examination of mitochondrial (mt) DNA also can provide additional evidence of speciation. However, because mtDNA is haploid and inherited maternally, the presence of fixed differences cannot be used as a basis for species determination as hybrids between species cannot be determined. Mitochondrial DNA segments can be sequenced using specific primers and the polymerase chain reaction (PCR) (Mullis et al. 1986). Genetic distance between samples, based on the number of nucleotide differences can be calculated. Sufficient knowledge has been accumulated on the genetic distance between isolated populations and between species, that comparisons can be drawn. Further, if crabs from widely separated geographic locations have almost identical mtDNA sequences, which are distinctly different from sympatric samples of other species, then one can be confident that they have a common ancestor and are from the same species.

Materials and Methods

Collection of samples

For this study, additional samples of mud crab were obtained from Hong Kong, Timbulsloko near Semarang, Central Java and TGIII, Bac Lieu Province, Vietnam (Table 1). These were compared to crabs from the three known species obtained from locations throughout the Indo-Pacific; including Australia, the Philippines, Malaysia, Thailand, Vietnam, India, Pacific Island countries, west to the east African coast and north to Okinawa (Keenan et al. 1995). Leg muscle and hepatopancreas were dissected and prepared for electrophoresis by placing into cold 1.5 mL microcentrifuge tubes with a small amount (3–5 drops) of invertebrate homogenising buffer (Siciliano and Shaw 1976).

Table 1. Collection sites and sample sizes for samples of mud crabs examined for this study.

Date	Location	Number (male/female)	Collector
May 96	Jepara, Central Java	4 (4/0)	J. Hutabarat
Dec. 96	Near Hong Kong	9 (7/2)	K.H. Chu
Jan. 97	TGIII and 184 Enterprises, Lower Mekong Delta, Vietnam	13 (7/6)	C. Keenan and Mr Xuan
Feb. 97	Timbulsloko, Sagang, near Semarang, Central Java, Indonesia	6 (3/3)	C. Keenan and J. Hutabarat

Electrophoresis

Allozyme genetic data were collected using techniques described by Keenan (1996). These data were examined for the presence of fixed genetic differences, congruent between specimens to determine major taxonomic groupings.

mtDNA

DNA was extracted from frozen leg muscle using techniques described in detail by Keenan et al. (1995). The PCR amplification used 1 µL of 1/10 dilution of template in a 50 µL reaction. The primers used for both cytochrome oxidase I (COI) and 16s RNA (16s) genes were from Simon et al. (1991):

COIa (21mer) 5' – AGTATAAGCGTCTGGGTAGTC –3'
COIf (20mer) 5' – CCTGCAGGAGGAGGAGAYCC –3' (Y – C or T)
16sar (20mer) 5' – CGCCTGTTTAACAAAAACAT –3'
16sbr (22mer) 5' – CCGGTCTGAACTCAGATCACGT –3'

The PCR reaction involved initial denaturation at 94 °C for 90 secs, followed by a reaction cycle (94 °C for 5 secs, 45 °C for 20 secs, 72 °C for 20 secs) repeated 35 times with a final extension step of 72 °C for 5 minutes.

The PCR products were purified from primers, dNTPs and buffer. Approximately 200 ng of PCR product was used as the template in a cycle-sequencing reaction with fluorescently labelled dideoxy nucleotides (using the ABI PRISM kit and protocols). Each cycle-sequencing reaction used one of the same primers as those in the initial amplification. After phenol/chloroform extraction to remove excess fluorescent nucleotides and ethanol precipitation, the single-stranded extension products were electrophoresed and analysed on an ABI 373A automated sequencer. Approximately 400–500 bases were routinely sequenced in each direction for both mtDNA gene fragments in each individual.

The sequences were aligned manually using the ABI sequence alignment editor SeqEd. The sequences were manipulated and analysed using MEGA (Kumar et al. 1993) to provide sequence divergences and diversities, and UPGMA dendrograms of Tamura (1992) genetic distances.

Results

The additional crabs from Hong Kong, Semarang and the Mekong Delta could be divided into two species based on the sharing of congruent fixed differences. One of these species expressed a similar pattern of fixed differences to those observed previously (Keenan 1996). The other samples expressed a new pattern of fixed differences. Variation, either within or between all four species, was observed in the mobility of alleles at 22 of the 36 enzymatic loci screened for all four species (Keenan 1996).

At 11 loci, fixed genetic differences **between** these species were observed. The loci useful for identifying species, through the fixed genetic differences between pairs of species, are listed in Table 2. *GPI*, while showing significant differences in allele frequency between species, did not demonstrate fixed differences as the 100 allele was observed in all species. Of the 36 loci examined for three of the four species, 14 loci showed no apparent genetic variation in the amino acid structure of their enzymes (proteins).

These loci were *ENOL, FBALD, GAPDH, GDH, G3PDH, IDH, LDH, MDH-1, MDH-2, MDHp, PGK, PNP, SOD-1,* and *SOD-2* (Keenan 1996). At 16 loci, polymorphism was observed **within** one or more species (*AAT-H, AAT-M, ADA-H, ADA-M, AK, AMY, ARGK, bGAL, GenProt, GPI, MPI, PEP-GL, PEP-LG1, PEP-LG2, PGDH* and *PK*). Polymorphism within species was detected for *S. olivacea* at 13 loci, *S. serrata* at 5 loci and for *S. tranquebarica* at a single locus. The previously unidentified fourth species, *S. paramamosain* (Keenan et al. 1998) was observed to be polymorphic at only the *GPI* locus, for the 30 specimens examined.

Mitochondrial DNA

The COI gene sequence presented here is 594 bases long and its corresponding amino acid sequence is 198 codons. Similarly, the data obtained for the 16s sequence were 483 bases long. Table 3 summarises the within and between species variation, using Tamura (1992) genetic distances, for the CO1 sequence. Within species variation is clearly at least an order of magnitude less than the between species variation, which confirms the definition of the groups as species. The samples examined from the same identified species are from geographically spaced locations and further samples from additional locations would most likely provide more information on population structure and relationships within each species. Within species variability may also increase from the results of such studies.

Table 2. Allele mobilities of four species of mud crab at 19 loci. Relative mobilities are based on the mobility of the most common *S. serrata* allele as the reference point (100). Polymorphic loci are identified by the presence of more than one allele. ? = data missing, usually a result of poor staining intensity.

		S. paramamosain		*S. serrata*		*S. olivacea*		*S. tranquebarica*	
No.	Locus	common	additional	common	additional	common	additional	common	additional
1	*AAT-H*	100		100	77	100		?	
2	*AAT-M*	100		100		100	130, 60	100	
3	*ADH*	75		100		75		75	
4	*AK*	100		100		100	140	100	
5	*ALAT*	100		100		95		95	
6	*ARGK*	75		100		75	100	75	
7	*ENOL*	100		100		100		100	
8	*FBALD*	100		100		100		100	
9	*GAPDH*	100		100		100		100	
10	*GPI*	100	133,158?	100	158, 66	100	133, 58	42	100
11	*IDH*	100		100		100		100	
12	*LDH*	100		100		100		100	
13	*MDH-1*	100		100		100		100	
14	*MDHp*	100		100		100		100	
15	*MPI*	100		100	103	95	90	100	
16	*PEP-GL*	100		100		100	78	100	
17	*PEP-LG1*	100		100		150	200	100	
18	*PEP-LG2*	100		100		100	120, 75	100	
19	*PGM*	100		100		85		107	

Table 3. Within (in brackets) and between species variation in Tamura's (1992) genetic distance. Thalamita species, from the Family Portunidae, are included for comparison.

	S. serrata	*S. tranquebarica*	*S. olivacea*	*S. paramamosain*	*Thalamita* sp.
S. serrata	(0.0164)				
S. tranquebarica	0.1100	(0.0097)			
S. olivaceous	0.1814	0.1613	(0.0098)		
S. paramamosain	0.1198	0.0910	0.1704	(0.0045)	
Thalamita sp.	Average over four species = 0.2058				(0.0018)

Both within and between species variation in the COI gene was greater than for the 16s RNA gene. This is expected because the COI gene, as a protein-coding gene, has the potential to vary at silent sites in the third codon position. Between species variability was more than 10 times greater than within species variability for COI.

To define the generic and evolutionary relationships correctly, the data should be compared with outgroup taxa, to determine the most primitive and derived species. The most useful outgroups are other genera from the Portunidae, e.g., Thalamita and Portunus. Unweighted pair-group [clustering] method using arithmetic averages (UPGMA) (Sneath and Sokal 1973) analysis of Tamura's (1992) genetic distance has been used to illustrate within and between species relationships for the cytochrome oxidase subunit I (COI) genes (Figure 1) and the 16S

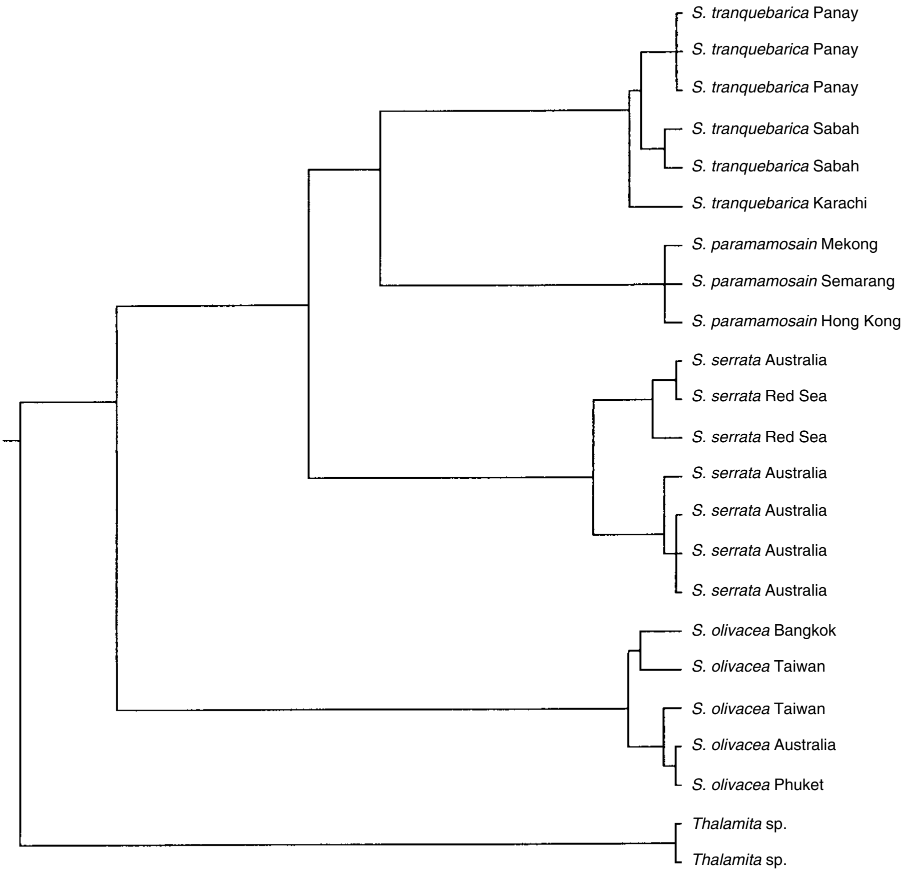

Figure 1. UPGMA dendrogram of Tamura's (1992) genetic distance for the COI mtDNA subunit, showing the *Species name* and location of each sample.

ribosomal RNA subunit (Figure 2). These figures clearly show that samples obtained from within a species, over a wide geographic range, show less than 2% sequence difference compared with between species sequence differences of greater than 9%. This provides additional conclusive evidence that there are at least four distinct species of mud crab.

Discussion

The absence of heterozygotes (i.e., hybrids) between the different species (Table 2), at loci where fixed differences were observed, provides strong evidence that there is no genetic exchange between these groups. As no heterozygotes were found between these species in sympatric samples, then there is strong evidence that speciation has developed to a stage where hybridisation can no longer occur and that they constitute good 'biological species'. Further, the large genetic distances observed between these species based on mtDNA sequence data (Table 3, Figures 1 and 2), compared to the small genetic distance observed between geographically isolated specimens within each species, confirms the distinct, species level differences.

However, the pattern of fixed differences in enzyme mobility differs from that usually observed between closely related species. It is unusual in that no one allele is species specific. It is only through the unique combination of alleles that any of the species can be identified. Almost all of these alleles are also shared with other species. At only one locus, *PGM*, there are unique alleles for three of the four species, when separated on the TRIC buffer system. This unusual distribution of alleles suggests that the ancestral species must have been, prior to the speciation events, polymorphic for the loci where the alleles are now distributed between the species (Keenan 1991). *GPI* still does not demonstrate fixed differences between species, although there are significant gene frequency differences. Loci which exhibit shared polymorphic alleles have been shown to be important in understanding the speciation process (Keenan 1991).

Genetic theory predicts that after isolation, polymorphic loci tend to fixation. From these results, it is

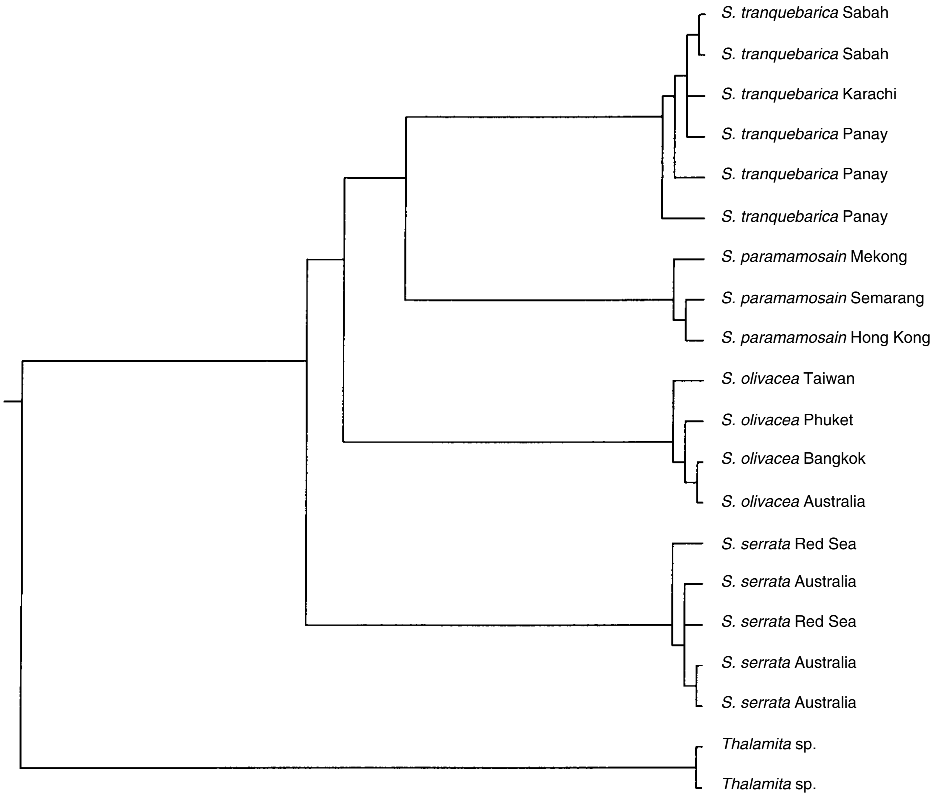

Figure 2. UPGMA dendrogram of Tamura's (1992) genetic distance for the 16s mtDNA subunit, showing the species name and location of each sample.

reasonable to conclude that speciation in *Scylla* has been a relatively recent event. Genetic divergence, both in terms of the fixation of alternate alleles at polymorphic loci and the evolution of new unique alleles, has not had sufficient time to produce fixation at all loci.

Using the techniques developed by this study, species discrimination can be accomplished by the electrophoresis of muscle tissue using EBT and TM (tris-maleate) buffers. By slicing the gel in thirds and staining for the enzymes *MPI* (which distinguishes *S. olivacea* from the other three species), *ADH* (which distinguishes *S. serrata* from the other three species), and *ALAT* (which discriminates *S. paramamosain* and *S. serrata* from *S. olivacea* and *S. tranquebarica*) all four species can be separated. *S. tranquebarica* has a different allele pattern for these loci; with the *S. serrata* allele for *MPI* and the *S. olivacea* allele for *ADH* and *ALAT*, as tabulated below (Table 4). *ARGK* could also be used on the TM buffer to distinguish *S. serrata* from the other three species, noting that the *ARGK*100* allele is also found in *S. olivacea* at a lower frequency than the *ARGK*75* allele.

The sample sites of mud crabs that have been positively identified by electrophoresis are detailed in Table 5, and some broad deductions regarding species distribution can be drawn. *S. serrata* is the most widely distributed species, ranging from the east African coast (South Africa, Mauritius and Yemen),

Table 4. Species-discriminating loci for the TM or EBT gel buffer systems.

	Allele mobility at diagnostic loci (first allele common, second if polymorphic)				TRIC Buffer
Species	*ADH* (EBT)	*MPI* (EBT)	*ALAT* (TM)	*ARGK* (TM)	PGM
S. paramamosain	75	100	100	75	100
S. serrata	100	100, 103	100	100	100
S. olivacea	75	95, 90	95	75, 100	85
S. tranquebarica	75	100	95	75	107

Table 5. Summary of the number of positively identified Scylla specimens by location, based on allozyme patterns.

S. paramamosain	*S. serrata*	*S. olivacea*	*S. tranquebarica*	Location
–	2	1	–	Australia – Gulf of Carpentaria
–	25	–	–	Australia – Moreton Bay
–	23	–	–	Australia – Northern Territory
–	–	3	–	Australia – Western Australia
–	7	–	–	Fiji
9	–	–	–	Hong Kong
–	1	5	–	Indonesia – Kupang
10	–	–	–	Indonesia – Semarang
–	7	–	–	Japan – Okinawa
–	–	–	8	Malaysia, Sabah
–	–	56	4	Malaysia, Sarawak
–	5	–	–	Mauritius
–	6	–	–	New Caledonia
–	–	1	3	Pakistan – Karachi
–	–	3	–	Philippines – Mindanao
–	–	4	–	Philippines – Negros
–	2	27	12	Philippines – Panay
–	–	8	–	Singapore
–	9	–	–	Solomon Islands
–	12	–	–	South Africa
–	1	7	–	Taiwan
–	–	4	–	Thailand – Bangkok
–	–	6	–	Thailand – Phuket
11	–	8	–	Vietnam
–	7	–	–	Yemen - Red Sea
30	107	133	27	Totals

through Australia (Northern Territory and Moreton Bay) and north Asia (Japan, Philippines and Taiwan) to the eastern Pacific Ocean (Fiji, Solomon Islands and New Caledonia). *S. serrata* and *S. olivacea* are sympatric from five areas; Gulf of Carpentaria, Western Australia (Taylor 1984), Panay, Taiwan and Kupang. Three species are only seen in one collection, from Panay Island, Philippines.

S. olivacea is the most numerous in the collection, with strong representation in the collections from the Philippines and Malaysia. It is sympatric with *S. tranquebarica* in three locations; Karachi, Sarawak and Panay, as well as Singapore (personal observation). Both *S. olivacea* and *S. tranquebarica* would appear to have a distribution that is centralised in the South China Sea, where the *S. serrata* is almost completely absent. However, as both *S. olivacea* and *S. tranquebarica* are observed in the Karachi collection, at least three species may be found around the Indian subcontinent and three species are also reported from Japan (Fuseya and Watanabe 1996). *S. tranquebarica* and *S. paramamosain* have not been reported from Australia, but because of their similar morphology to *S. serrata*, they may just be unrecognised.

Acknowledgments

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References

Brown, I.W. 1994. Mangrove Crabs. Chapter 19, In: Wright, A. and Hill, L. ed. Inshore Marine Resources of the South Pacific: Information for Fishery Development and Management, University of the South Pacific, Suva, Fiji.

BOBP 1992. The Mud Crab. In: Angell, C.A. ed. A report on the Seminar on Mud Crab Culture and Trade held at Surat Thani, Thailand, November 5–8, 1991. Bay of Bengal Programme, Madras, India.

Estampador, E.P. 1949. Studies on *Scylla* (Crustacea: Portunidae). I. Revision of the genus. Philipp. J. Sci. 78(1): 95–108, plates 1–3.

Fuseya, R. and S. Watanabe, 1996. Genetic variability in the mud crab genus *Scylla* (Decapoda: Portunidae). Fish. Sci., 78(1), 95–109.

Holmes, S. 1979. Henderson's Dictionary of Biological Terms. Ninth Edition. Longman, London.

Joel, D.R. and Raj, P.J.S. 1980. Taxonomic remarks on two species of the genus *Scylla* de Haan (Portunidae: Brachyura) from Pulicat Lake. Journal of the Inland Fisheries Society of India, 12(2), 39–50.

Keenan, C.P. 1991. Phylogeny of Australian species of flatheads (Teleostei, Platycephalidae) as determined by allozyme electrophoresis. Journal of Fish Biology, 39 (Supplement A), 237–249.

Keenan C.P. 1996. Genetic relationships of mud crabs, genus *Scylla*, throughout the Indo-west Pacific. In: Evans, L. ed. Proceedings, Mud Crab Workshop, Broome, 1995. Aquatic Science Research Unit, Curtin University of Technology, Perth, WA, 11–23.

Keenan, C.P. and Shaklee, J.B. 1985. Electrophoretic identification of raw and cooked fish fillets and other marine products. Food Technology in Australia, 37, 117–128.

Keenan, C.P., Mann, D.L., Lavery, S. and Davie, P. 1995. Genetic relationships, morphological identification and taxonomy of mangrove crabs, genus *Scylla*, from throughout the Indo-Pacific. ACIAR Project Report, QDPI, Brisbane.

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.

Kumar, S., Tamura, K. and Nei, M. 1993. MEGA: Molecular Evolutionary Genetics Analysis, Version 1.01. The Pennsylvania State University, University Park, PA 16802.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. and Erlich, H. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Sym. Quant. Biol., 51, 263–273.

Ong, K.S. 1964. Early development stages of *Scylla serrata* Forskål (Crustacea Portunidae), reared in the laboratory. Proceedings of the Indo-Pacific Fisheries Council 11(2), 135–146.

Richardson, B.J., Baverstock, P.R. and Adams, M. 1986. Allozyme Electrophoresis. Academic Press, Sydney.

Sarich, V.M. 1977. Electrophoresis in evolutionary studies: rates, sample sizes and the neutrality hypothesis. Nature, 265, 24–28.

Siciliano, M.J. and Shaw, C.R. 1976. Separation and visualisation of enzymes on gels. In: Smith, I. ed. Chromatographic and Electrophoretic Techniques. Vol. 2. Zone Electrophoresis. Fourth Edition Heineman, London, 185–209.

Simon, C., Franke, A. and Martin, A. 1991. The polymerase chain reaction: DNA extraction and amplification. In: Hewitt, G.M. et al. ed. Molecular Techniques in Taxonomy. NATO ASI Series. Volume H57. Springer-Verlag, Berlin, 329–355.

Sneath, P.H.A. and Sokal, R.R. 1973. Numerical Taxonomy. Freeman and Co., San Francisco.

Stephenson, W. and Campbell, B. 1960. The Australian Portunids (Crustacea: Portunidae). IV. Remaining genera. Aust. J. Mar. Freshwater Res., 11, 73–122, plates 1–5.

Tamura, K. 1992. The rate and pattern of nucleotide substitution in Drosophila mitochondrial DNA. Mol. Biol. Evol., 9, 814–825.

Taylor, M.L. 1984. New species of mud crab found in Western Australia. FINS, 17(2), 15–18.

GROWOUT IN PONDS

Monosex Culture of the Mud Crab (*Scylla serrata*) at Three Stocking Densities with *Gracilaria* as Crab Shelter

Avelino T. Triño[1], Oseni M. Millamena[1] and Clive P. Keenan[2]

Abstract

The effects of three levels of stocking density (0.5, 1.5 or 3.0/m^2) and monosex culture (male or female) on the growth, survival and production of *Scylla serrata* were investigated. Juvenile crabs were stocked in 150 m^2 enclosures in earthen ponds with *Gracilaria* as shelter and fed a mixed diet of 75% fresh brown mussel flesh and 25% fish bycatch. There was no interaction between stocking density levels and monosex culture ($P<0.05$) so the data were pooled for each sex or stocking density treatment. Results showed that highest survival was obtained from a stocking density of 0.5/m^2 ($P<0.05$). Crab growth at different stocking densities was not significantly different ($P>0.05$). Highest return on investment (ROI) and lowest production costs were attained from 0.5/m^2. Partial budgeting analysis showed that no net benefit accrued from stocking beyond 1.5/m^2. Male crabs attained significantly better ($P<0.05$) final weight and specific growth rate than female crabs. Length, width, survival and production between male and female crabs were not significantly different ($P>0.05$). Male and female monoculture gave high net revenue and ROI of more than 100 but male monoculture is more profitable. Overall the results suggest that the culture of male or female mud crabs at 0.5–1.5/m^2 with *Gracilaria* is economically viable.

THE mortality of mud crabs during the grow-out phase has been largely attributed to cannibalism. Cannibalism affects survival and appears to be partly dependent on stocking density (Baliao et al. 1981). Mixed sex culture also enhanced cannibalism among the stock (Cholik and Hanafi 1992).

In other Indo-Pacific countries, crab shelters are often used in ponds to provide refuge for moulting and post-moult soft crabs (Fielder et al. 1988) to minimise cannibalism. Chen (1990) reported that crab farmers in Taiwan had reduced crab cannibalism by providing *Gracilaria* as crab shelters. Monosex culture and the use of *Gracilaria* as crab shelters were studied in the Philippines to improve crab survival and yield in ponds across a range of stocking densities.

This paper presents growth, survival and production of pond-reared mud crabs, *Scylla serrata,* initially stocked as small seed crabs to simulate aquaculture of hatchery reared crabs.

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines
[2]Bribie Island Aquaculture Research Centre, PO Box 2066, Bribie Island, Queensland 4507 Australia

Materials and Methods

The study was conducted at the Western Visayas Demonstration Fish Farm (WVDFF), Molo, Iloilo City (see cover photograph). A 2 × 3 factorial experiment was carried out for 4 months in a completely randomised design with three replicates for each treatment. The performance of male or female mud crabs (7.0–11.0 g) was determined at three stocking densities (0.5, 1.5, and 3.0/m^2) in 150 m^2 enclosures placed in six ponds.

The enclosures used nylon net (12 mm mesh and 2 mm twine diameter) to prevent crab stock from escaping. The pond bottom was sun dried for 5–7 days or until the soil cracked. Agricultural lime was applied at 1 tonne/ha, urea (45-0-0) at 25 kg/ha and ammonium phosphate (16-20-0) at 50 kg/ha. Ponds were then filled with water to about 10 cm and planted with *Gracilaria* at 10 cm in between hills at 10 g seed/hill (Ponce, pers. comm.). When good growth of *Gracilaria* in all ponds was obtained, pond water volume was gradually increased to a level of 80 cm over three days.

Crab juveniles, from Camarines Norte and Samar, were stocked two days after the pond water reached

80 cm. The water depth was maintained at 80–100 cm. Thirty percent of the water volume was drained and replenished for three consecutive days during spring tide periods. Plankton and *Gracilaria* growth were maintained with urea and ammonium phosphate at the rate of 12 kg and 25 kg/ha, respectively, after water replenishment. Water temperature, salinity, dissolved oxygen concentration, pH, and water depth were monitored daily at 0730.

The crabs were fed a mixed diet of 25% fish bycatch and 75% fresh brown mussel (*Modiolus metcalfei*) flesh at 8% of the biomass daily, equally divided at 0700 and 1700 feeding times. Stock sampling was done twice a month. The daily ration was then adjusted based on an overall estimate of the survival for all treatments and the estimated biomass for each treatment replicate.

Soil samples were collected before and after the experimental period for the determination of soil type, organic matter content, pH, available phosphate, sulfate, and iron of the pond soil.

The growth, apparent FCR, survival, production and cost of production were calculated from the total harvest. The means were compared by analysis of variance and Duncan's multiple range test (SAS Institute Inc. 1988). The economic feasibility of the culture methods was evaluated by cost-return and partial budgeting analysis (Shang 1990).

Results

Physico-chemical analyses of the pond soil samples taken before stocking and after the experimental period showed that organic matter content increased, but available phosphate, iron, and sulfate decreased after the crab culture period. This declining trend in the availability of these mineral components in the pond soil may be attributed to assimilation by *Gracilaria* and other macroalgal associates and photosynthesizing algae or to trapping in the pond sediment (Shilo and Rimon 1982).

Water quality parameters recorded for the duration of the experiment were: temperature, 25–27 °C; salinity, 25–29 ppt; D.O., 3.5–8.0 ppm; and pH 8–9. The ranges of values did not vary much for all ponds and were within the optimum ranges reported by Hill (1980) and Cholik and Hanafi (1992).

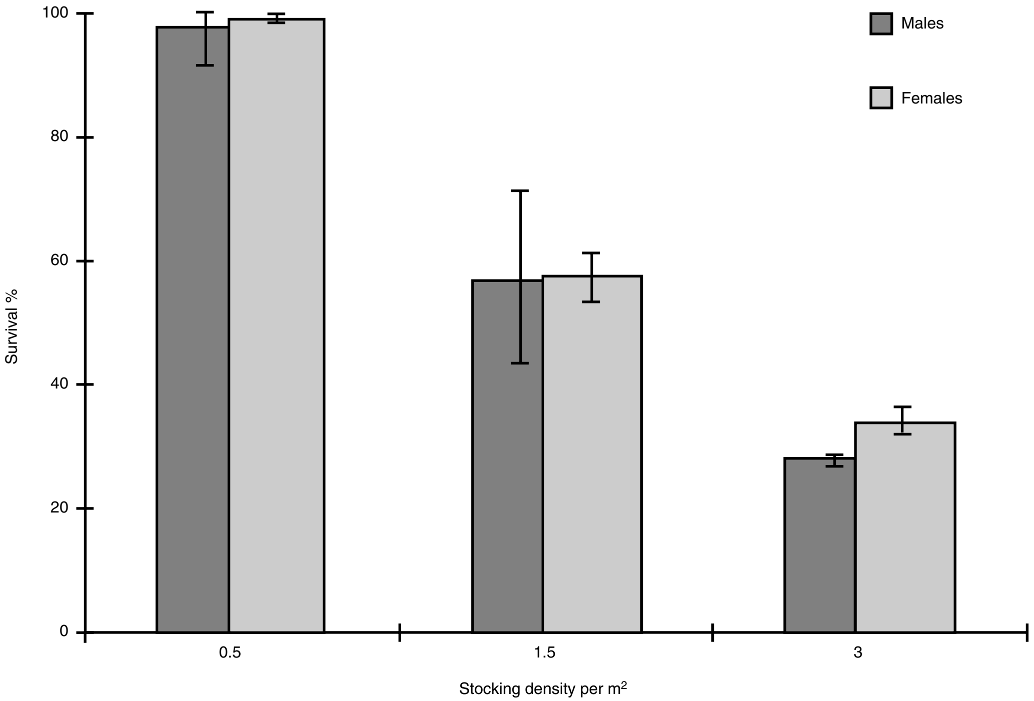

Figure 1. Survival of mud crabs after 120 days of monosex culture in ponds, at three stocking densities. The error bars indicate the survival range.

Table 1 shows the proximate composition of the mixed diet given to mud crabs. Crude protein content was 66.1% for fish bycatch consisting mainly of *Leiognathus* spp. and 61.3% for the brown mussel flesh, while crude fat was 6.9 and 9.5%, respectively.

Table 1. Proximate composition of the feeds given to mud crabs. Analysis according to AOAC (1984).

	Composition (% dry weight)	
	Fish bycatch*	Brown mussel flesh
Crude protein	66.14	61.34
Crude fat	6.91	9.54
Crude fibre	1.56	9.28
Nitrogen free extract	3.43	10.68
Ash	21.96	9.16

*Fish bycatch consisted mainly of *Leiognathus* sp.

Growth, survival and production of monosex pond-reared mud crab at three stocking densities are shown in Figures 1–3. Regardless of sex, survival rate of crabs significantly increased with lower stocking density (Figure 1, $P<0.05$). Production was highest at the highest stocking density although not significantly different ($P>0.05$) from that at the intermediate stocking density but significantly higher ($P<0.05$) than at $0.5/m^2$ (Figure 2). Growth in terms of weight, however, was not significantly different ($P>0.05$) between stocking densities (Figure 3). In the monosex culture, male crabs reached a significantly higher final weight than females ($P<0.05$). Mean survival and production, however, were not significantly different ($P>0.05$) across stocking densities.

The total investment was expressed in terms of capital cost and operating cost (variable and fixed costs). The capital cost consisted of cost of materials and labour for the construction of net enclosures, but pond development cost was not included in the analysis as it was assumed that the ponds were available and ready for use. Feed and crab juveniles comprised the major component of the variable costs (41–53% and 35–43%, respectively). Another major cost was materials for pond preparation (3–17%). Production costs are summarised in Figure 4 for the three stocking densities.

The sale price per kg of mud crab produced (~A$10 for females and A$9.50 for males, exchange rate P20 = A$1) was based on the farm gate price offered by exporters during harvest. Net revenue (A$16 546) was highest at $1.5/m^2$, primarily due to high yield, whereas production costs and ROI were lowest and highest, respectively, at $0.5/m^2$. Net

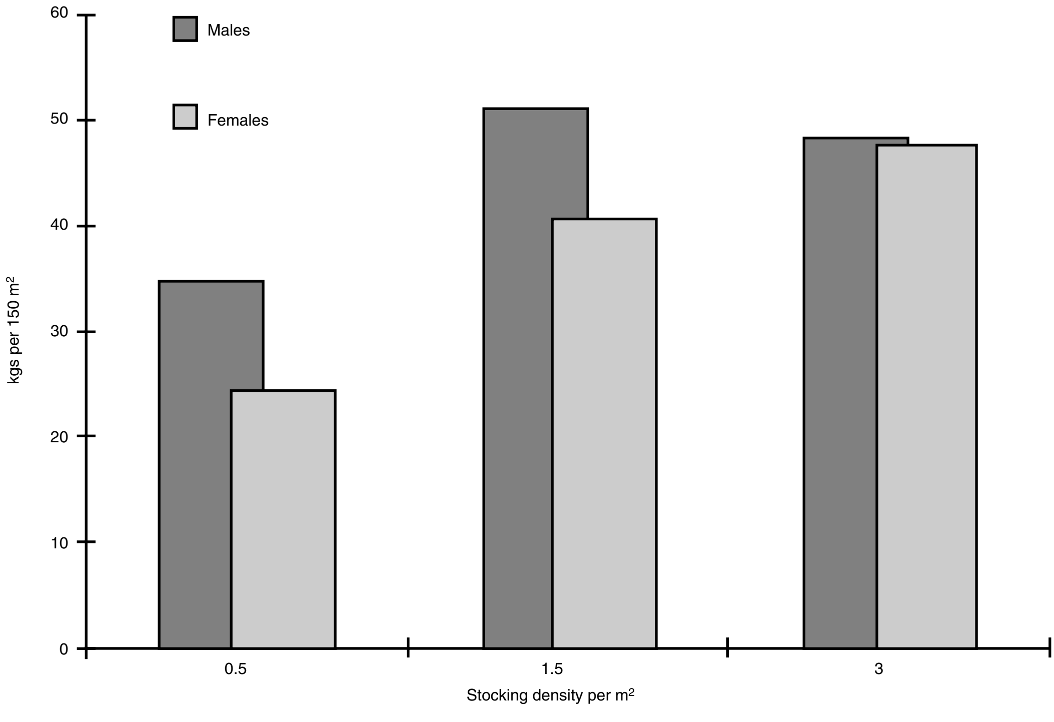

Figure 2. Pond production of mud crabs in monosex culture at three stocking densities.

revenue increased as stocking density level was increased from 0.5 to 1.5/m^2, but fell at 3.0/m^2 where production costs were doubled. Although both monosex cultures attained high net revenue and ROI of over 100%, cost-return analysis, showed that lesser production costs and a higher net revenue and ROI came from male rather than female monoculture. Partial budgeting analysis demonstrated that a larger profit (A$5240) can be earned by using male crabs for monoculture rather than female crabs.

Discussion

Poovachiranon (1992) and Jayamanne (1992) reported that male crabs gained more weight than females. This observation was confirmed in the present study with significant differences between the sexes. However, crab survival and production were not influenced by monosex culture; instead, crabs were more affected by stocking density levels. The three stocking density levels did not result in a significant difference in growth. Similar observations were reported by Refstie (1977) for rainbow trout and by Triño and Bolivar (1993) for seabass fry.

High mortality due to cannibalism is a common problem in mud crab culture and may be due to overcrowding (Baliao et al. 1981) and mixed sex culture (Cholik and Hanafi 1992). In the present study, the lower the stocking density the higher the survival, the highest survival of 98% was obtained from 0.5/m^2, compared with 57% and 30% at 1.5 and 3.0/m^2, respectively. *Gracilaria* may have been effective as crab shelters, minimising loss of stock due to cannibalism. Chen (1990) reported survival of 50–60% for crabs cultured in Taiwan with *Gracilaria* at a stocking density of 2–3/m^2. The importance of aquatic macrophytes as shelters for mudcrabs in the natural habitat was reported by Hill et al. (1982). Fielder et al. (1988) indicated also that the use of crab shelters increased survival by minimising agonistic encounters between crabs.

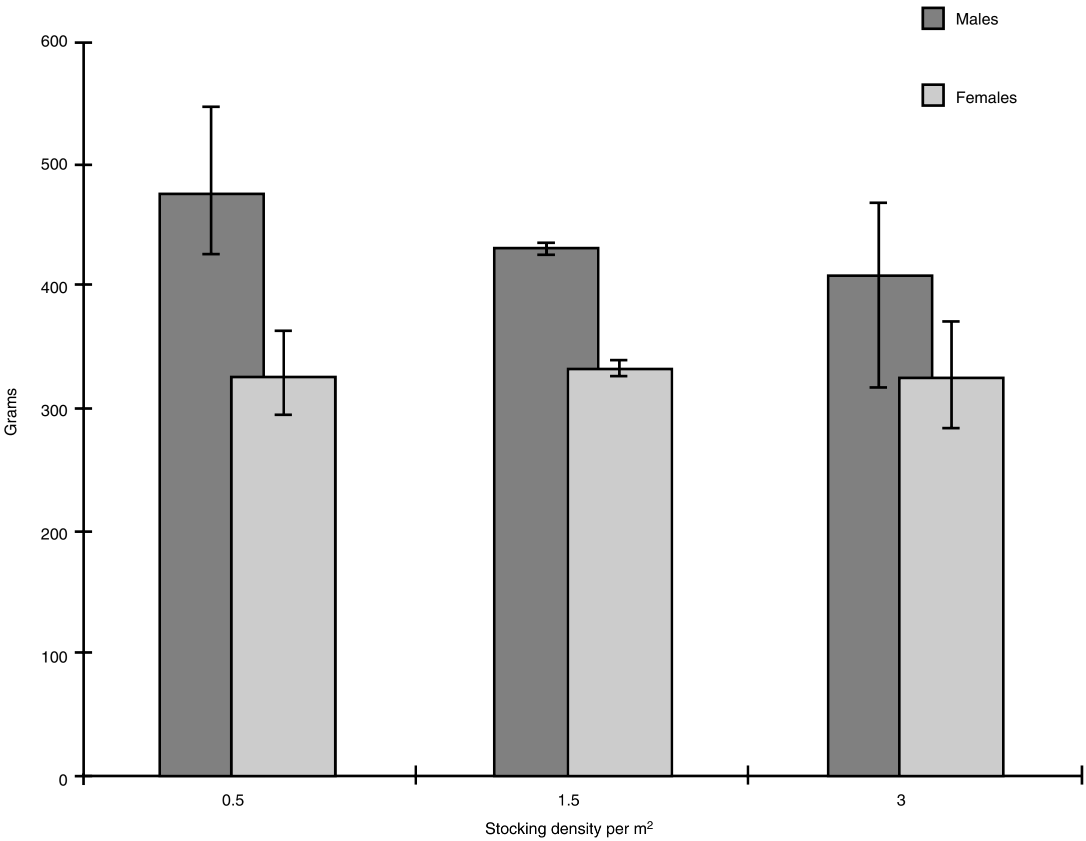

Figure 3. Growth of mud crabs after 120 days of monosex culture in ponds, at three stocking densities. The error bars indicate the growth range.

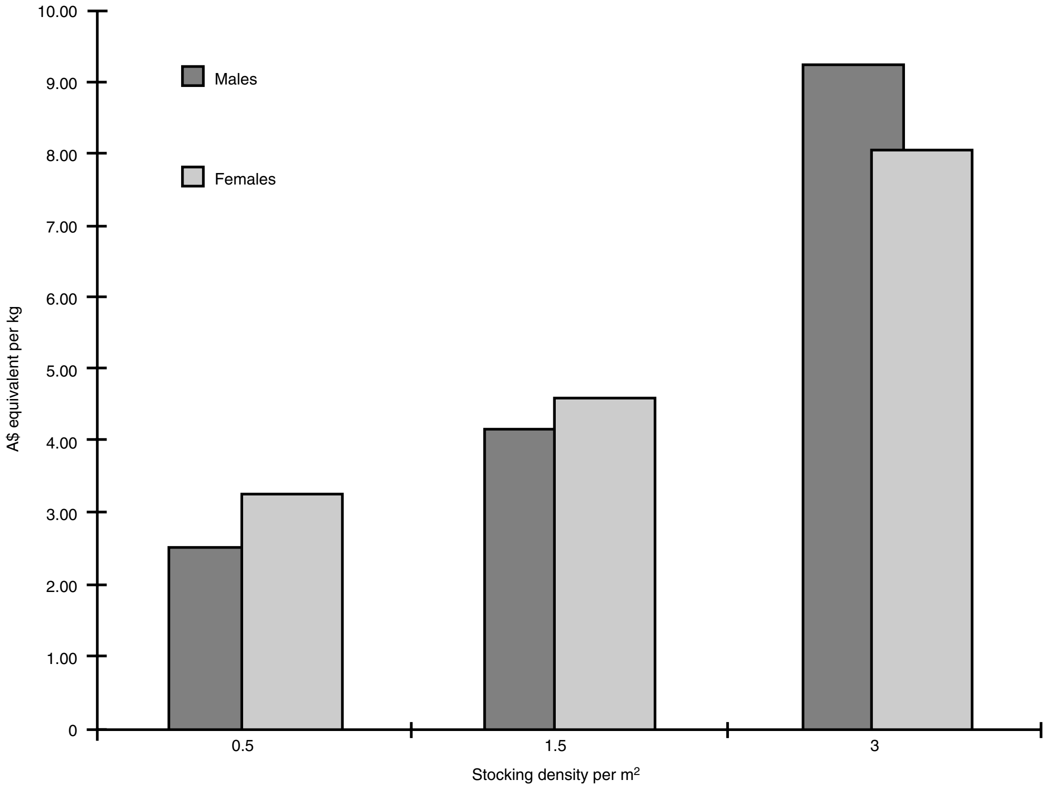

Figure 4. Cost of production of mud crabs in monosex culture at three stocking densities.

From the economic point of view, the study demonstrates that the use of *Scylla serrata* monoculture is a viable aquaculture venture in the Philippines, with stocking densities between 0.5 to $1.5/m^2$ being most profitable. Although the market price offered for female crabs is usually higher than for males, the price difference can be more than compensated by the significantly higher mean final weight attained by the male crabs. Thus more profit can be earned from male crab monoculture.

Acknowledgments

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References

Association of Official Analytical Chemists, 1984. Official Methods of Analysis, 14th edition. AOAC, Arlington, VA, 1141 p.

Baliao, D.D., Rodriguez, E.M. and Gerochi, D.D. 1981. Culture of the mud crab *Scylla serrata* (Forskål) at different stocking densities in brackishwater ponds. Quarterly Research Report. SEAFDEC Aquaculture Department, 5(1), 10–14.

Chen, L.C. 1990. Mud crab culture. In: Aquaculture in Taiwan. Fishing News Books, London, 142–149.

Cholik, F. and Hanafi, A. 1992. A review of the status of the mud crab (*Scylla* sp.) fishery and culture in Indonesia. In: Angel, C.A., ed., The Mud Crab. Report of the Seminar on Mud Crab Culture and Trade. Surat Thani, Thailand, 5–8 November 1991. 13–27.

Fielder, D.S., Mann, D.L. and Heasman, M.P. 1988. Development of intensive pond farming techniques for the mud crab *Scylla serrata* (Forskål) in Northern Australia. FIRTA Project Report 86/9, 37 p.

Hill, B.J. 1980. Effects of temperature on feeding and activity in the crab *Scylla serrata.* Marine Biology, 59, 189–192.

Hill, B.J., Williams, M.J. and Dutton, P. 1982. Distribution of juveniles, subadult and adult *Scylla serrata* (Crustacea: Portunidae) on tidal flats in Australia. Marine Biology, 69, 117–120.

Jayamanne, S.C. 1992. The mud crab fishery in Sri-Lanka. In: Angell C.A. ed., The Mud Crab. Report of the Seminar on Mud Crab Culture and Trade. Surat Thani, Thailand, 5–8 November 1991, 41–48.

Poovachiranon, S. 1992. Biological studies of the mud crab *Scylla serrata* (Forskål) of the mangrove ecosystem in the Andaman Sea. In: Angell C.A. ed., The Mud Crab. Report of the Seminar on Mud Crab Culture and Trade. Surat Thani, Thailand, 5–8 November 1991, 49–57.

Refstie, T. 1977. Effect of stocking density on growth and survival of rainbow trout, *Salmo trutta* L., to chronic crowding stress. Journal of Fish Biology, 24, 731–734.

SAS Institute Inc. 1988. SAS/STAT^{TM} User's Guide, Release 6.03. SAS Institute, Inc., Cary NC, 1028 p.

Shang, Y.C. 1990. Aquaculture economic analysis, an introduction. In:. Sander, P.A. ed. Advances in World Aquaculture, Volume 2. World Aquaculture Society, Louisiana State University, Baton Rouge, LA, 211 p.

Shilo, M. and Rimon, A. 1982. Factors which affect the intensification of fish breeding in Israel. Bamidgeh, 34, 101–114.

Triño, A.T. and Bolivar, M.E.C. 1993. Effect of stocking density and feed on the growth and survival of seabass fry *Lates calcarifer* (Bloch). International Journal of Tropical Agriculture, 11, 163–167.

Description of Mud Crab (*Scylla* spp.) Culture Methods in Vietnam

Hoang Duc Dat[1]

Abstract

In Vietnam, the culture of mud crabs has only been established and developed during the past 10 years, and is mainly located in coastal provinces. There are a number of culture procedures: growing seed crabs for flesh or eggs in ponds (200–500 g body weight); poly-culture with shrimps or fish in ponds, with a growing period of 3–5 months; fattening 'empty' crabs for market (300–800 g) with a very short growing period of 15 to 40 days; producing soft-shell crabs by feeding 30–80 g crabs without claws over 15–20 days, until moulting with a product weight of 50–120 g. Crabs produced by fishing or culture are used for both domestic consumption and export.

THE raising of mud crabs as a business throughout the world and in Vietnam has a very recent history of development in comparison with other sea species such as shrimp, fish and algae. The culture of S*cylla* species has been developed for just about 10 years and research reports dealing with the reproduction, growth, development and biological characteristics of this species are very few. There is still no full documentation about culture techniques for S*cylla* species, although there are some reports about the status of raising mud crabs in China, Taiwan, Philippines, Malaysia, Thailand, India and Sri Lanka.

Recently, mud crab culture in Vietnam has taken root and is being developed in some coastal provinces such as Quang Ninh, Hai Phong, Thanh Hoa, Thua Thien-Hue, Ba Ria-Vung Tau, HoChiMinh City, Ben Tre, Tra Vinh, Soc Trang, Minh Hai, Kien Giang. The production of cultured crabs has accounted for a remarkable portion of the total exploited yield of crabs.

The common species cultured in the Mekong Delta region is *Scylla paramamosain* (Keenan, these Proceedings). Various methods for crab aquaculture have been developed in the provinces, depending on local conditions. Therefore, a lot of technically useful experience has been established in mud crab culture.

[1] National Centre of Natural Sciences and Technology, Institute of Tropical Biology, 85 Tran Quoc Toan St., District 3, Ho Chi Minh City, Vietnam

In general, there are three kinds of commercial enterprises: raising immature crabs to flesh crabs, raising thin crabs to flesh crabs and raising soft-shell crabs. Certain aspects of crab rearing are common to all three commercial enterprises: seed crab supply and handling, feeding, pond water management, harvesting and pond construction. These are detailed below. Where variation exists between the three different operations, they have been highlighted.

Seed Crab Supply and Handling

Presently, seed crabs for culture are wild-caught. They are collected using small boats and fishing nets from the river bottoms or from marshes flooded by sea water. Crabs are available in the following sizes:

- Small: 60–120/kg;
- Medium: 25–40/kg;
- Large: 10–15/kg.

Collected seed crabs usually have their pincers firmly tied, before packing into suitable bags for transport to the farm. Newly caught seed crabs from neighbouring areas are preferred because they can be transported quickly to the culture site. Seed crabs of the same size are used for each pond. Small seed crabs (60–120 crabs/kg) are grown for 6–7 months for a successful harvest. This can be shortened to 4–5 months for medium (25–40 crabs/kg) and to 3–4 months for large seed crabs (10–15 crabs/kg).

The culture density for seed crabs is 3–5 crabs/m^2 for small, 2–4 crabs/m^2 for medium and 1–2 crabs/m^2 for large crabs. The seed crabs are evenly distributed around the pond. A sharp knife is needed for cutting ties. The crabs are placed on the edge of the pond so they can enter the water by themselves. This helps to check their health, as strong crabs usually run into the water quickly and swim out, while weak crabs tend to remain where placed or slowly enter the water. Weak crabs are collected and kept separate for better care; as soon they are healthy they can be returned to the pond. Each pond is filled with a sufficient number of crabs within one or two days. During their first days in the pond, the crabs spread out and look for a place to settle.

Feeding

Mono-cultured crabs live mainly on daily supplied food, as the quantity of natural food in the pond is insufficient. In poly-culture, during the initial months, crabs often use food already present in the ponds as their main food source. However, in the last months of the raising period when the crabs are well grown and have a greater demand for food, the farmer should supply additional nutrition. Crab food is usually raw and fresh and consists of crushed fish, small crabs, oysters, molluscs, shrimp or fish heads. The quantity of food supplied daily is 4–6% of the estimated total weight. The crabs usually search for food in the late afternoon and are therefore fed once a day between 1700 and 1900. Food is spread widely over the pond to prevent fighting for food.

Crabs should be fed every day, not every second day or longer, as the larger mud crabs can kill smaller ones. There should be a reserve of food like dried crushed fish and small shrimp in case fresh food is unavailable. However, these foods should be rehydrated before feeding. To measure the crabs' food consumption, put the food on a sieve/feed tray and place it into the water. Next morning check the sieve and increase the amount of food if the crabs have consumed the supplied food or reduce this amount if they have not. As soon as food is placed in a poly-culture pond, resident shrimp and fish also eat it. Thus, there should be careful calculation for a sufficient amount of food to be supplied to all the species present.

During culture, weigh and check some crabs every two weeks for condition, activity and development, and for any fungus disease outside or inside the shell. If crabs acquire any disease, efforts should be made to find the cause and a suitable treatment. Also, check the condition of the pond edges, outlet and fence regularly in order to find holes from which mud crabs may escape. Check fences carefully and avoid any big opening between railing bars as mud crabs tend to creep up at night and may escape through these openings.

By the end of the growing period, when the crabs are large enough for harvesting, more food is needed and thus the habitat is prone to contamination. At this time, it is critical to replace water and check the habitat more frequently. In some cases, the pond bottom will be filled with rotten excess food and it may be necessary to empty all the water, pick up crabs and clean the bottom by removing surface mud and rotten food. This food is often concentrated in the bottom of the canal that runs around the pond.

Pond Water Management

During their development stages young crabs usually live in brackish water of 15–25 ppt. However, crabs can withstand dramatic changes in salinity and can live and develop in water with salinities from 5–36 ppt.

The water needs to be clean with no pollution by industrial, agricultural and domestic sewage, especially when the mud crabs are kept at high density and fed with raw and fresh food. In areas with daily tidal movement, 30–50% of the pond water is replaced every day, and the water fully replaced once a week. To do this, empty a part or all the water prior to tidal rise, close the outlet, and when the tide rises towards the top of the tide, take in fresh water from the middle-layer or lower-layers. Surface water is avoided as it is often polluted and/or of lower salinity. The new water is fresh and clean and thus stimulates mud crabs to move, exercise, eat more and moult better and more often.

After harvesting, it is important to clean the pond. If the pH of the water is less than 6, empty the pond and spread lime powder (0.07–0.1 kg/m^2) evenly over the bottom, canal and the inner sides of the pond. Expose the bottom of the pond for two to three days and then fill with fresh water three to four times to empty all the contaminated water.

Harvesting

Poly-culture of crabs often results in uneven growth rate due to the various sizes of crabs stocked into the pond. Special nets, fishing rods or traps may be set to catch crabs. After poly-culture, all the crabs, shrimp and fish in the pond or lake should be collected by drain harvest. This process is done successively over three nights at high tide. Then drain all the water and catch the remaining crabs by hand. If the pond or lake is large, many people will be needed to catch the crabs, moving across the pond in a straight line. The collection time should be short so that the crabs are strong and can be transported for sale within the same day. Steel hooks can be used to catch crabs from holes. This method usually causes the crabs to drop their pincers, which considerably reduces their value. Therefore, it is best to empty all the water with a net set in one end of the outlet and catch the crabs by hand from the net.

Nets can be used to collect crabs grown in ponds or lakes. In this case, the ponds or lakes must have a flat bottom of solid or sandy soil so that crabs cannot burrow into the bottom when the nets are set and pulled up. However, this method does not collect all the crabs. The ponds and lakes are usually reformed or cleaned as soon as all the crabs are harvested, to make the next grow-out successful.

Harvested crabs are classified as special class (male crabs of 500 g or over), first class, second class, third class, fourth class and others. All the harvested crabs should be carefully weighed in order to work out the production and the best density for the improvement of grow-out procedures. If the crabs are not sold out quickly, they should be put in cool shade. Female crabs without a full ovary, 'empty' male crabs or small crabs should be transferred to smaller ponds for further fattening over a short time.

Crabs must be tied ready for sale; a rush or nylon tie is needed to retain the two pincers. This tie is wound around the legs and the paddles and a knot made between the carapace and the plastron. The crabs are then washed (put the crabs into water for some minutes so that they can eject mud and dirt) and placed in a special cage (back upwards). Each cage can contain 20–25 kg. Cover the top of the cage with rushes and protect with a wooden or bamboo net so that when another cage is placed on top, it does not injure the crabs in the lower cage. Spray some water onto the top of the cage to keep the crabs wet and put the cages in cool places and transport them for sale.

The loss of young mud crabs grown in ponds for 3–8 months can be relatively high (40–60% by number), particularly if stocking rates are high (see Triño et al., these Proceedings). However, the total weight of the crabs is increased by 3 to 5 times (seed crab 60–80 g are harvested at 250–350 g).

Pond Construction

General

Culture ponds are often large and need a great input from people and machinery to shape. The width of a bank's foot should be 3–4 m depending on the height of the bank. The top of the bank should be 1–2 m wide and at least 0.5 m higher than the highest tide. The foot of the banks are often made of bamboo nets for stability. The banks are firmly sealed by solid soil or clay to avoid leakage or slippage. Depending on local features, trees or blocks of woods are planted to prevent the destructive effects of waves, which can cause erosion and collapse.

Around the inside of the pond, a canal with a width of about 3–5 m and depth of about 0.5–0.7 m is dug. The excavated soil is used to build the bank. Brushes are often added to the canal to serve as shelters for crabs. There should be 1 or 2 outlets, depending on the area of the pond. One outlet is placed at the lowest point of the canal in order to drain away completely all water in the pond when necessary for harvesting, reforming or cleaning. The outlet's diameter depends on the pond area but is often 0.8–1.5 m. At the inlet, 2–3 valves are installed to control the flow. These openings have mesh to prevent the crabs from escaping when the pond is either emptied or filled. The choice of inlet and outlet material depends on financial capacity, but can be made of concrete, prefabricated concrete, prefabricated-concrete pipes, bricks or wood. Recently, composite pipes have been used as inlets and outlets at a reasonable cost; they are very convenient to install, and are highly durable (pressure resistant and not attacked by the teredo worm).

In large extensive ponds, living conditions are often similar to the natural environment and crabs rarely escape. However, in some parts near the outlet where crabs are carried along by the current, when they cannot pass the outlet, they may try to climb the bank to the outside. Therefore, those parts of the bank near the outlet must be fenced. These fences extend 20–50 m from the edges of the outlet. Large ponds often have the same structure as the natural environment (with plants, mounds in the middle and space). In small ponds (1–3 ha), crab farmers can create mounds, or plant trees for shade, and often use bamboo or other kinds of fences around the bank to prevent the escape of crabs from the pond. These fences are 0.7 m or more in height, deeply driven into the inside edge of the pond bank.

In some places, instead of building ponds, farmers install bamboo fences enclosing large areas to raise crabs, shrimps and fish. Farmers can take advantage of the topography of the channel or bay to build a one sided or three sided fence to enclose an area of water for culturing. Bamboo is the most popular material used to build fences and stakes are driven deeply and diagonally into the bottom with supporting poles. The height of the fence must be 0.5–1 m higher than the highest tide. On top of the fence, a net can be placed to prevent the escape of crabs. It is very convenient raising crabs in fenced water areas because the living conditions are much the same as natural conditions. The farmers should install a harvest wing to catch the product.

Poly-culture

Poly-culture is usually extensive cultivation in combination with shrimp or fish raising. In some places, the growers also combine this with algal cultivation. The ponds range in size from one to tens of hectares, located in brackish-water coastal areas or saline-flooded areas. Large ponds are often run under natural conditions. The best sites have little wind or waves, with a low current and slope in order to avoid building high banks. The bottom of the pond has a deep layer of mud (up to 30 cm) or sandy-mud, or loamy soil mixed with sand. It is possible to have trees and mounds but they usually cover less than 30% of the area of surface water. In these semi-natural ponds, there is an abundant source of food.

Monoculture

Ponds for this type of culture are usually 500–5000 m^2 in area and the biggest ponds are limited to 2 ha. The pond shape depends on the topography. Generally, ponds are rectangular with a width equal to 40% of the length, with inlet and outlet on opposite ends. As feed is an important component of intensive monoculture, all stages should be carefully managed.

Fattening Empty Crabs

Empty crabs for fattening are of market size, but are unsuitable to eat because the male crabs have a thin, soft carapace with little flesh, and female crabs have little ovary tissue. They are purchased cheaply from fishermen and become available after harvesting. Thin crabs are fed for 25–35 days so that their shell hardens, muscle flesh develops, or in the case of females the ovaries develop, which increases their commercial value. Thin crabs are fattened in small ponds (200–500 m^2), enclosures (100–300 m^2) or cages. The density for fattening is 0.5–1.0 kg/m^2 for ponds and enclosures. However, this density is increased to 10–25 kg/m^2 for cages.

For areas where ponds or enclosures are not suitable, fattening cages are used. The cage is usually made of bamboo and a popular size is 2–3 m wide, 3–4 m long, and 1.0–1.2 m high. A wide opening in the top of the cage (0.6–1.2 m), covered with bamboo, is used for access and feeding. It must be tightly closed and locked. The cage is kept afloat by buoys, the top about 0.2–0.3 m out of the water, and is anchored by cables tied to stakes on the bank. It is ideal to set cages along canals, or at drain openings of big lakes with relatively strong water flows.

Crabs which are fattened for a short time in ponds, enclosures or cages at high density are carefully fed, cared for and managed. Thin crabs need large amounts of food, which is usually small fish, clams, solens, fiddler crabs etc. The quantity of food should be 5–8% of the biomass of crabs. During fattening, if the pond is heavily contaminated, empty all the water, collect the crabs, clean the bottom and remove excessive food. This can be carried out in cool weather taking in new water at high tide.

Soft-Shell Crabs

Soft-shell crabs are a specialised commercial crab product. In Southern coastal provinces, after the normal production season, there appear great numbers of crabs 25–60 g each, ideal for soft-shell crabs. Ponds for soft crabs are rectangular with an area from 100–200 m^2. The bottom of the pond is covered with a 20–25 cm layer of mud or sandy-mud.

Only strong crabs of 30–60 g are stocked. Prior to stocking both pincers and the three pairs of legs are removed from each crab. Cut the two pincers close to the body and hold the three legs together and turn them, the crab will shed these legs. The pleopods (oars) are kept so the crab can swim. The stocking density is 100–120 kg/100 m^2. Dead crabs are removed from the pond. After water replacement, if the crabs swim quickly, they are strong enough to commence feeding, often possible by the end of the second day.

The daily quantity of food used is 2–4% of the total biomass of the crabs. Crabs are fed twice a day at 0500 and 1700–1900, although this also depends on the tide, as they are fed as soon as water has been replaced. Avoid feeding crabs at high temperatures. In the first few days, the crabs tend to eat a great amount of food but from the ninth or the tenth day on, the crabs' food consumption capacity is reduced slightly. If a crab eats much and grows bigger in five days, its legs and pincers are developing. With time, the pincers grow larger, but are still covered in a delicate membrane which turns from light rose colour to darker rose.

By the eleventh or twelfth day when its pincers become big enough, the crab passes into a premoult stage, recognised by a breaking sound when a fingernail is slightly pressed on the lower edge of the carapace. When all the crabs pass into this stage, the pond is harvested by complete draining. Crabs which do not develop pincers or legs are sold. Those with developed pincers and legs but with incomplete maturity are returned to the pond, which is filled quickly to prevent any remaining crabs from desiccation. These remaining crabs are only fed once every day with half the food quantity.

Crabs with developing pincers and legs are selected for soft-shell production. They are placed in a special floating cage, which consists of a bamboo frame 1.5 × 1.0 × 0.25 m covered with curtain. Such a cage is stocked with 3–7 kg and placed into a cool pond with a good supply of fresh water. They are not fed, but are examined every two hours. Crabs that have just thrown off their shell are left in this cage from 20–40 minutes, then harvested and arranged on trays in a lateral position, resting against each other. The basket or tray is covered with a thin piece of cloth or a layer of young grass, kept in a cool shady position to avoid sun and wind, and carefully transported to either export crab purchase stations or local markets. Farmers of soft-shell crabs may profit 10–15% within a month (one breeding duration).

Preliminary Results of the Rearing of Mud Crab, *Scylla olivacea* in Brackishwater Earthen Ponds

Romeo Diño Fortes[1]

Abstract

An experiment was conducted to determine the effects of stocking densities (0.5 and 1.0 crab/m^2) and presence or absence of bamboo shelters on the production of the mud crab *Scylla olivacea* reared in brackishwater earthen ponds. The shelters were about 45 cm long, measuring 20–25 cm from the node and had a diameter of 12–15 cm. Preliminary results did not show significant differences among the four treatments. The mud crab production attained from the various treatments ranged from 141.9–87.0 kg/ha. The presence of bamboo shelters did not show significant differences (α>0.05). The low production may be attributed to: slower growth of this species of mud crab; the burrowing characteristics of the *S. olivacea* which made the harvesting very difficult; escape of the mud crab due to their natural habit of migration to the sea for spawning; and mortality of crabs entangled in the filamentous algae or from cannibalism and losses to poaching. While the results of this first trial did not clearly show any treatment effects on the production of mud crab in brackishwater earthen ponds, a number of significant problems were identified: (1) the design and other engineering aspects of the pond for mud crab aquaculture needs to be established; and (2) for each species of mud crab, their unique characteristics should be considered in developing suitable and appropriate culture techniques.

THE farming of mud crabs, *Scylla* spp. has received special interest in the past few years due to its importance as a source of high quality seafood. It is also very important to the economy of many Asian countries as it is an export commodity. In the Philippines, the mud crab, *Scylla* spp., has been identified as an export-winner in the country's agenda for national development. It is believed that the improvement of the culture techniques for the mud crab will boost its production as mud crab production in many countries in Southeast Asia has not yet really been developed.

Several attempts had been made in order to improve the culture techniques of the mud crab in several countries. In Taiwan, it originated in polyculture with milkfish, *Chanos chanos* (Chen 1990) and since then Taiwan has slowly developed its mud crab culture technology. In Ceylon, the growth and survival under pond conditions were observed and monitored (Raphael 1970) but very little progress was attained. In Thailand, tremendous efforts were exerted to produce mud crab in ponds which used relatively small ponds (1600 m^2); bamboo fences were installed to prevent their escape (Harvey 1990). In India the mud crab was cultured in Tuticorin Bay in different types of cages (Marichamy et al. 1986). In the Philippines, *Scylla* species have been reared in ponds, cages and even pens, particularly during fattening but there is still a lot of room for improvement. It can be said that in the Philippines, the technology for mud crab fattening has become more sophisticated but its culture in ponds has not yet progressed far. It is therefore very important that the existing pond culture technologies for the mud crab should be properly examined to develop culture techniques that will benefit every one.

The major objective of this study is to improve the production of the mud crab reared in brackishwater earthen ponds by providing a form of refuge during moulting to avoid the predation of their peers. Specifically, the effect of bamboo shelters and two stocking densities on mud crab production were tested.

[1]Institute of Aquaculture, College of Fisheries, University of the Philippines in the Visayas, Miagao, Iloilo, Philippines

Materials and Methods

Supply and stocking of crablets

The source of the stock was Barangay Baelan, Municipality of Pontevedra, Province of Capiz on the Island of Panay. A total of 3500 pieces of juvenile *Scylla*, the majority identified later as *S. olivacea* (Keenan et al. 1998) were transported in *kaing* (native container made of thinly sliced bamboos woven together to form a basket) each containing approximately 580 crablets. These were transported in a jeepney from the source in Pontevedra to the Brackishwater Aquaculture Center at Leganes, Iloilo, Philippines. This is a distance of more than 150 km or a total of more than 3 hours of travel time. When the crablets arrived at the Center they were allowed to rest before stocking.

The experiment used a 2 × 2 factorial design with 4 treatments replicated 3 times. The different treatments were:

1. 0.5 crablet/m^2; no shelter;
2. 0.5 crablet/ m^2; with shelter;
3. 1.0 crablet/ m^2; no shelter;
4. 1.0 crablet/ m^2; with shelter.

The different replicates of each treatment were randomly distributed in 12 units of 500 m^2 ponds. The density in treatments 1 and 2 were equivalent to 175 crablets per pond (3500/ha); while the density used in treatments 3 and 4 were equivalent to 400 crablets per pond (8000/ha).

The shelters tested in this experiment were made of bamboo cut to about 45 cm (20–23 cm each side of a node). These were based on the results of an experiment conducted in aquaria indoor (Cerezo unpublished). Each bamboo shelter had a diameter of 10–15 cm. The 3 replicate ponds with shelters having the lower stocking density (0.5 crab/m^2) received 88 bamboo shelters while the ponds with higher stocking density received 200 shelters each following a ratio of around 1 shelter : 2 crabs.

The experimental pond units were designed in such a way that the mud crabs were prevented from escaping. A fence made of nylon nets (mesh = 1 cm) was installed inside each pond about 80 cm from the dike. The edge of the net was buried 30 cm below the pond bottom and the upper portion of the net was clipped to the bamboo poles at 5 m intervals with bamboo slats. In addition to the net, a 45 cm plastic sheet was added overlapping 15 cm of the net.

The crablets were stocked in ponds starting the afternoon of 31 October and continuing to the morning of 02 November 1996. Stocking was very slow because the crablets' chelipeds had to be untied individually before they were stocked. Densities of stocking used were 0.5 and 1.0 crablets per square metre.

A random sample of 168 individuals was taken and their weight, carapace length and width measured. The results of these measurements were:

Average weight (g)	45.81
Average carapace length (mm)	41.70
Average width (mm)	62.42

Water depth was maintained at a minimum of 50 cm (the depth reached as high as 1.2 m depending upon the tide). Water was changed twice a month during spring tides. Physico-chemical parameters of the water such as temperature, salinity, depth and pH were monitored and recorded periodically.

The crablets were fed with trash fish given at a rate of 3% of their estimated biomass (about 5 to 10 kg per pond given daily, 7 days a week). Towards the middle of the experiment fresh trash fish became scarce and expensive thus we shifted to dried trash fish mixed with small crustaceans and molluscs (snails and squids).

Production and growth parameters

Production was measured in terms of recovery. This is the total weight of mud crabs recovered from each pond and extrapolated into per hectare production. Growth was determined in terms of carapace length or CL (from the point on the dorsal part of the carapace between the eyes to the base of the carapace).

Results and Discussion

Effects of stocking densities on production

On the basis of recovery which was very low (approximately 12% of the initial stocks), production was also low (Table 1). Analysis of variance (α>0.05) did not show significant difference among treatments in terms of production, weight gains, carapace length (CL) and increase in CL. It can be seen from Figure 1 that the mean production in Treatment 1 (141.9 kg/ha) is highest among the treatments (i.e., 106, 123.1, and 87 kg/ha for treatments 2, 3 and 4, respectively. The average gain in weight of the mud crab in treatments 1, 2, 3 and 4 are 144.55, 110.34, 122.9 and 105.05 g, respectively. The carapace lengths at harvest are shown in Figure 2. The average increase in CL for the mud crabs in treatments 1, 2, 3 and 4 are 25.11, 19.8, 24.22 and 18.95 mm, respectively.

While it can not be conclusively stated at this point that the lower stocking density without shelters is inferior to treatment with higher stocking density and with shelters, the results demonstrated the problems that can be encountered in mud crab aquaculture in ponds, particularly *S. olivacea.* The characteristic of this species to burrow and to move out from ponds or other enclosures during spawning was well demonstrated. Despite the enclosures along the perimeter of the ponds, the mud crabs still managed to escape by

Table 1. The initial carapace lengths and weights of the mud crab, *Scylla olivacea*, reared in brackishwater earthen ponds for 125 days.

Treatment	Initial		Final		Growth		Production
	CL (mm)	Wt (g)	CL (mm)	Wt (g)	CL (mm)	W-gain (g)	(kg/ha)
A–1	45.6	59.2	67.9	188.4	22.2	129.1	128.1
2	39.8	38.6	66.7	185.0	26.9	146.4	66.6
3	41.1	30.3	67.3	189.7	26.2	159.4	231.1
B–1	45.6	59.2	65.3	181.3	19.7	122.0	155.9
2	39.8	38.6					
3	41.1	30.3	64.9	157.9	23.9	127.6	56.8
C–1	45.6	59.2	65.5	169.0	19.9	109.7	106.6
2	39.8	38.6	71.1	160.0	31.3	121.2	165.3
3	41.1	30.3	62.6	167.9	21.5	137.5	97.4
D–1	45.6	59.2	60.2	145.8	14.6	86.6	99.8
2	39.8	38.6	61.8	144.9	22.0	106.1	69.4
3	41.1	30.3	61.3	152.8	20.2	122.5	91.7

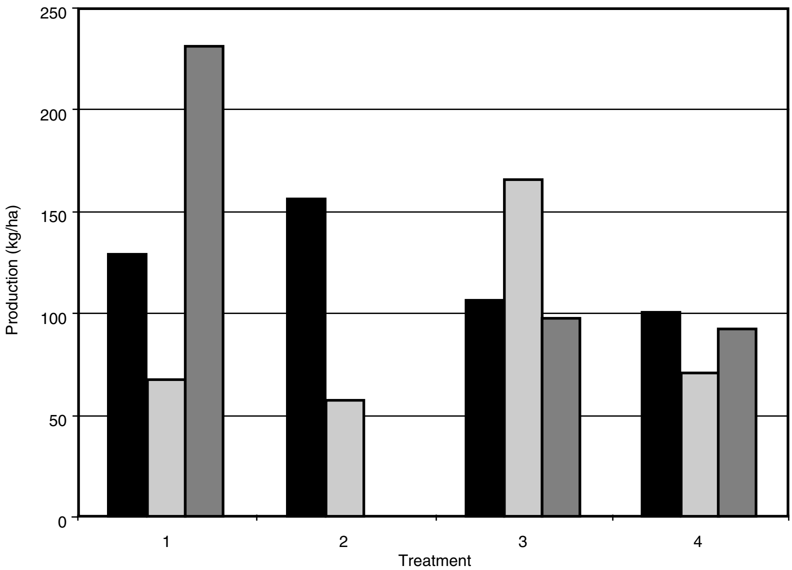

Figure 1. The production of the mud crab, *Scylla olivacea*, reared in brackishwater earthen ponds using bamboos as shelters at two stocking densities.

cutting the nylon nets. It has been documented that such characteristics are features that need attention in the culture of this species.

The escape of the mud crab from the ponds could have also caused the apparent insignificant effect of the shelters and the stocking densities used in the experiment. It should be noted that up to this time, efforts to recover the mud crab stocks are continuing and as of April 16, 1997, the recovery had increased to about 17%. The burrowing characteristic of the mud crab and their natural desire to get out of the ponds during spawning time and the escape during high tide, coupled with mortality caused by the entanglement of the mud crabs in the filamentous algae that bloomed in the ponds, contributed significantly to the low recovery.

Future plan

The immediate plan for this work is to carry out a second run using *Scylla serrata*. The same shelters shall be used but stocking density shall be changed (from 0.5/m^2 and 1.0/m^2 to 0.5, 1.0 and 1.5/m^2). The experimental units shall also be changed from 500 m^2 to 125 m^2 ponds. Feeding frequency shall be reduced from 2 times a day, 7 days a week, to 2 times a day every other day.

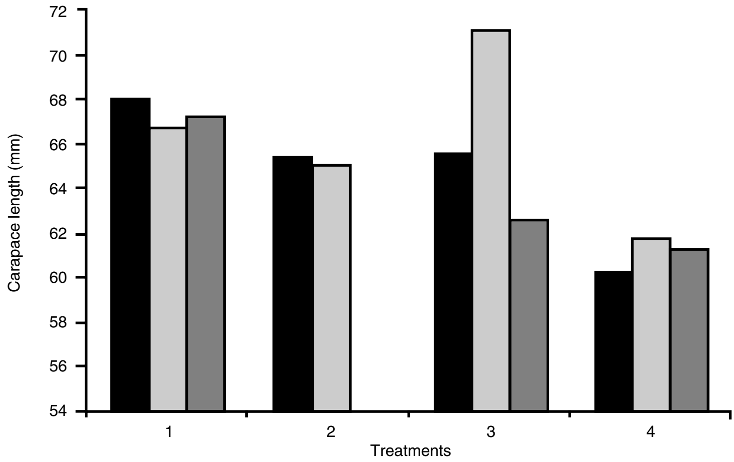

Figure 2. The carapace length (CL) of the mud crab, *Scylla olivacea*, reared in brackishwater earthen ponds at 2 stocking densities and using bamboos as shelters.

References

Chen, L-C. 1990. Aquaculture in Taiwan. Fishing (News) Books, Ltd. (Blackwell Scientific Publications Ltd.) Mead, Oxford, England.

Harvey, M. 1990. Mud crab culture in Thailand. Infofish International. June 1990.

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.

Marichamy, R., Manickaraja, M. and Rajapackiam, S. 1986. Culture of the mud crab *Scylla serrata* (Forskål) in Tuticorin Bay. Proc. Symp. Coastal Aquaculture, 4, 1176–1182.

Raphael, V.I. 1970. A preliminary report on the brackish-water pond culture of *Scylla serrata* (Forskål) in Ceylon. Proc. Fourteenth Session, Indo-Pacific Fisheries Council, FAO-UNDP, Bangkok, Thailand.

Preliminary Economic Analysis of Mud Crab (*Scylla serrata*) Aquaculture in the Northern Territory of Australia

Brian Cann[1] and Colin Shelley[2]

Abstract

Economic analysis was conducted on larval rearing and pond grow-out of the mud crab *Scylla serrata*: to examine the economic potential of mud crab aquaculture based on current knowledge; to identify important cost components to guide future research and development; and to generate discussion on appropriate farming systems for Australia. A break-even budget was used for larval rearing and a development cashflow budget for pond grow-out. The base set of parameter values for larval rearing produced a break-even cost of approximately 24 cents per crab instar1. This cost is considered too high. The major cost was labour. Future research will need to reduce cost by increasing the number of crabs produced per unit of labour. In the grow-out model, the base set of parameter values produced a return to capital of 51%. The most important parameters affecting the profitability of grow-out were sale price, finishing weight, survival rate, feed cost, feed conversion ratio and capital cost. The two analyses indicate that mud crab aquaculture has promising potential in the Northern Territory of Australia.

DESPITE its attractiveness as a seafood item, the aquaculture of mud crab has yet to develop into a significant industry in Australia. Considerable research can be required to overcome problems that confront commercial production of new aquaculture species. To this end, an ACIAR project has been funded which aims at overcoming these problems in both Australia and the Philippines. Typically, the aquaculture of new species requires research on broodstock maintenance, induction of spawning, larval rearing, nursery production, grow-out, marketing and economics.

The later two are often implemented late in the research and development (R&D) cycle, and sometimes not at all. This is somewhat surprising since the outcome of such R&D is usually to develop or expand a profitable industry. In this study, economic analysis has been commissioned early in the overall research effort to provide insights that improve the quality or direction of the other lines of research in developing a viable aquaculture industry. This can be achieved by identifying the important cost components or input/output parameter relationships affecting the profitability of hypothetical commercial enterprises. Alternative potential production systems and variation within these systems can be modelled.

In this study, two analyses are conducted, one on larval rearing and the other on nursery/pond grow-out.

It should be noted that the current study relates to Australia. In other countries, the relative cost and availability of inputs may be different. Such differences will affect the relative importance of input costs and input/output parameters, and impact on the most appropriate production system(s) for a particular country or region.

[1]Berrimah Agricultural Research Centre, NT Department of Primary Industry and Fisheries, PO Box 990, Darwin, NT, 0801, Australia
[2]Darwin Aquaculture Centre, NT Department of Primary Industry and Fisheries, PO Box 990, Darwin, NT 0801, Australia

Methods

The analysis of larval culture was conducted using a break-even budget, while for pond nursery/grow-out culture, a development cashflow budget was used. These were constructed using the spreadsheet program Excel®. In both cases, a hypothetical scenario, called the base scenario, was assumed and a sensitivity analysis was conducted. This involved calculation of the effect of variation in individual assumptions on the break-even cost or internal rate of return. In the case of larval culture, a break-even approach was used because it was envisaged that the scale of operation was not large enough to support a stand-alone enterprise. In the case of grow-out culture, a stand-alone enterprise was assumed and the rate of return on capital invested (internal rate of return) was calculated.

The larval culture budget has been based on the system currently used at the Darwin Aquaculture Centre (G. Williams, pers. comm.). The larvae are reared in 7-tonne tanks, which are outdoors, under shade structures. They are fed on algae and rotifers from zoea 1 (Z1) to day 2 of Z3, with algae levels being maintained through the rest of the culture period to promote good water quality. From day 2 of Z3 to crab instar1 (C1), newly hatched artemia are fed and this is supplemented with dried *Acetes* shrimp powder from the beginning of the megalopa stage to C1.

In the base scenario, survival rates are 40% from Z1 to megalopa and 50% from megalopa to C1. These rates have been reached in local trials and it is anticipated that they will be regularly achieved or exceeded over the next few years. Z1 larvae were assumed to be stocked at 5/L, the level used in current trials, although this is considered conservative. Labour input is estimated to be 30 days per batch, for two people costing $320 per day, inclusive of on-costs. The level of capital related costs in the larval culture budget are based on an estimated capital value of $50 000. An allocation of 20% of this value is estimated to cover capital related costs (i.e., return on capital invested, depreciation, and repairs and maintenance to capital items). Artemia nauplii are fed at a rate of 3.5 million per 7-tonne tank per day for the first 8 days and 50% higher for the remainder. The artemia cysts used in the research trials cost A$140 per 400 g can. Broodstock costs are based on feeding 10 females throughout the season with feed valued at $8/kg, at a rate of 5% of body weight/day. It is assumed that the purchase of 20 females at $15 each will be required over the season.

The pond based nursery and grow-out budget is modelled on a hypothetical 10-hectare enterprise located in the Gulf of Carpentaria, Northern Territory, Australia. This enterprise would produce 148 500 crabs per year weighing 56.4 tonnes, over two crops. This would require stocking of 540 000 C1 crabs per year. The enterprise would have 40 ponds, each covering 0.25 ha. The type of pond construction envisaged has minimal earthworks. The bunds between the ponds would be built by digging from a trench along the edge of each pond. This is similar to the design of some existing ponds currently used in Vietnam, although smaller. The lower estimate for the capital cost of such an enterprise is around $300 000 based on Northern Territory costs. This relatively low cost was used in the base scenario as it was anticipated that successful mud crab pond culture of mud crabs would rely on capital costs being kept to a minimum.

In the absence of data on which to base estimates for the nursery phase of pond culture, one hectare of the pond area is assumed to be used for nursery culture to raise juvenile crabs from C1 to C7. Mortalities are assumed to be 50% in the nursery phase. The cost of C1 crabs is assumed to be 10 cents each. Note that this is less than the break-even cost in the base scenario for larval culture, but is considered an achievable target. Stocking densities, mortalities and growth rates during the grow-out phase were as reported by Triño et al. (these Proceedings). These data were averaged between monosex male and female crabs grown at Iloilo City in the Philippines. Feed assumptions are based on the expectation that a suitable pelleted feed will be developed. The feed conversion ratio and feed price are based on performance and prices in cultured prawn production.

Pumping costs are based on pumping 10 megalitres per day, the equivalent of 100 millimetres over the entire pond area every day. The cost per megalitre is based on diesel-powered pumps and low pumping heads of less than 3 metres, which are likely to apply in the Gulf of Carpentaria. Capital repairs and replacement are estimated at 8% of capital value per year, which is $24 000 in the base scenario. The salvage value at the end of the ten-year budgeting period is assumed to be the same as the initial capital value (i.e., $300 000). Labour costs are assumed to total $90 000 per year, which includes two full-time employees and $25 000 worth of casual labour. Sundry costs were assumed to be $10 000. Income in the first year was assumed to be for only one crop, but two crops for other years.

The price for crabs is assumed to be $12/kg at the farm gate for live crabs bound and packed in crates. This price is based on prices on offer for wild caught crabs in the Gulf Region of the Northern Territory in 1996 (Calogeras, pers. comm.).

Results

Larval culture

The total cost of larval culture in the base scenario was $11 025 per batch or 24 cents per C1 crab. The major cost is labour at $9600 per batch or 17.14 cents per crab. Labour represented 73% of the total cost. Other major costs were those related to capital ($2000 per batch, 4.77 cents per crab), artemia ($656 per batch, 1.17 cents per crab) and thiosulphate ($504 per batch, 1.01 cents per crab).

The sensitivity analysis was conducted on the parameters of stocking density, survival rate and capital related costs. Stocking densities for Z1 larvae were varied from 5/L, the base scenario, up to 30/L, the upper level considered possible at present. Survival rate was varied from 5% to 50% in the sensitivity analysis. In the sensitivity analysis, capital related costs were varied from 50% of the base level to three times the base level. A summary of the results of the sensitivity analysis is presented in Table 1.

Pond grow-out

In the base scenario, the grow-out enterprise produced a peak annual gross income of $667 160, total operating costs of $399 398 and an attractive internal rate of return of 51%. Feed is the major cost item at $203 148, followed by labour at $90 000 and juveniles at $54 000. Operating costs are equivalent to $7.08/kg, although it should be noted that this does not include a return to capital.

The sensitivity analysis was conducted on the parameters, price, nursery survival rate, grow-out survival rate, turn off weight, feed conversion rate, feed price, labour cost and capital cost. In the sensitivity analysis, the internal rate of return, based on constant prices, is reported. This is a measure of the rate of return to capital invested. A summary of the results of the sensitivity analysis is presented in Table 2.

Table 1. Summary of the sensitivity analysis on larval rearing.

Stocking density (no./L)	5	10	15	20	25	30
Cost per C1 crab (cents)	23.97	12.64	8.86	6.97	5.84	5.08
Survival rate (% Z1–C1)	5	10	20	30	40	50
Cost per C1 crab (cents)	95.88	47.94	23.97	15.98	11.98	9.59
Capital related costs (% of base level)	50%	100%	150%	200%	250%	300%
Cost per C1 crab (cents)	22.18	23.97	25.76	27.54	29.33	31.11

Table 2. Summary of the sensitivity analysis on mud crab grow-out.

Price (A$/kg)	9	10	11	12	13	14
Internal rate of return (%)	18.5	29.7	40.5	51.1	61.4	71.6
Sale weight (g)	230	280	330	380	430	480
Internal rate of return (%)	16.4	28.8	40.3	51.1	61.3	71.2
Cost of juveniles (cents)	5	10	15	20	25	30
Internal rate of return (%)	57.2	51.1	45.1	39.2	33.5	27.8
Nursery survival rate (%)	20	30	40	50	60	70
Internal rate of return (%)	33.5	43.1	48.1	51.1	53.1	54.5
Grow-out survival rate (%)	25	35	45	55	65	75
Internal rate of return (%)	0.4	19.5	36.0	51.1	65.2	78.6
Feed conversion ratio (x:1)	2.75	2.5	2.25	2.00	1.75	1.5
Internal rate of return (%)	34.5	39.9	45.4	51.1	56.8	62.6
Feed price ($/kg)	2.10	1.80	1.50	1.20	0.90	0.60
Internal rate of return (%)	43.6	51.1	58.7	66.6	74.6	82.8
Labour costs ($000/year)	170	150	130	110	90	70
Internal rate of return (%)	33.7	38.0	42.3	46.6	51.1	55.6
Capital costs ($000)	800	700	600	500	400	300
Internal rate of return (%)	19.8	23.0	27.2	32.6	40.0	51.1

Discussion

Larval culture

The break even budget for larval rearing shows that further improvement in the performance and productivity of the current system are necessary to achieve an acceptable level of cost for C1 crabs. A cost of 10 cents per crab is used in the grow-out out budget. This represents what is believed to be an achievable target, although a lower figure should be the aim. Labour is the major cost of the larval rearing system. Alternative production systems should be directed at reducing labour costs or increasing the productivity of labour (i.e., crabs turned off per unit of labour) to decrease the cost per crab. Increasing the value of parameters such as stocking densities, survival rates and scale of operation are important to decrease the cost of labour per crab, megalopae or zoea produced using the current system. The estimate of capital value used in the base scenario is considered to be at the lower end of the scale of possible values for a stand-alone operation. A lower capital value per crab produced might be achieved with a larger scale operation or a mud crab hatchery that is part of an integrated aquaculture enterprise.

High labour costs associated with intensive hatcheries in Australia have led to the development of extensive green water rearing techniques for barramundi larvae (Rimmer and Rutledge 1996). This method might be investigated for part or all of the mud crab larval cycle once research in the more controlled intensive systems provides a clearer picture on the requirements for consistently good results in mud crab larval rearing.

Grow-out

The internal rate of return, a measure of profitability, for the base scenario is quite attractive. However, there are a number of variables about which there is little information, so considerable uncertainty exists. The sensitivity analysis demonstrated that profitability is quite sensitive to a number of variables. It should be noted that the sensitivity analysis considers changes in only one variable at a time. If, for example, there was a one step less favourable shift to the values of the variables; sale price, sale weight and grow-out survival rate, in Table 2, then the internal rate of return would drop from 51.1% to 18.1%.

The growth and grow-out mortality rates used in this study were taken from Triño et al. (these Proceedings) where *Gracilaria* was trialed as cover on the pond floor. In Australia, artificial shelters or planted mangroves are more likely to be used to reduce cannibalism. The effects of such differences are unknown.

The sale price for mud crabs is based on that paid for wild crabs which are bigger than the assumed turn off weight. It is not known if there is a large market for small crabs in Australia. The grow-out data of Triño et al. (these Proceedings) was based on 120 days. As only two crops a year were assumed in this study, it may be possible to grow-out crabs to a larger size using a longer grow-out period and/or a longer nursery phase. Alternatively, a fattening period in individual cages might form part of the production system.

In this study, the highest cost by a considerable margin was feed, followed by labour and juveniles. The cost of the latter is dependent on stocking rate, the size at stocking, mortalities and price per juvenile crab. Agbanyani et al. (1990) budgeted labour as higher than juvenile mud crab cost for stocking densities of 5000 and 10 000, but lower for stocking densities of 15 000 and 20 000 per hectare. Triño et al. (these Proceedings) budgeted labour as being well below the cost of juvenile crabs for all stocking densities, ranging from 5000 to 30 000 per hectare. The latter two studies were both conducted in the Philippines. In this study, labour was the second highest cost. Different assumptions on the cost of juveniles and juvenile mortalities could have produced a different result. The difference in the relative cost of inputs between this and the other two studies is indicative of such differences between countries and over time.

These can be expected to lead to different production systems, using different inputs and/or mixes of inputs, being developed in different countries or regions. Over time, the increasing relative cost of some inputs, such as labour in developing economies, will also affect the evolution of production systems.

References

Agbayani, R.F., Baliao, D.D., Samonte, G.P., Tumaliuan, R.E. and Caturao, R.D. 1990. Economic feasibility analysis of the monoculture of mud crab *Scylla serrata* (Forskål). Aquaculture, 91, 223–231.

Rimmer, M.A. and Rutledge, B. 1991. Extensive Rearing of Barramundi Larvae. Queensland Department of Primary Industries No Q191012. 6 p.

GROWOUT IN MANGROVES

Pen Culture of Mud Crabs, Genus *Scylla* in the Mangrove Ecosystems of Sarawak, East Malaysia

William Chang Wei Say[1] and Abdullah Mhd. Ikhwanuddin[2]

Abstract

The features of the pen culture of mud crabs, genus *Scylla*, in the mangroves of Sarawak, East Malaysia, are described. It is an ecologically friendly system in that it does not have any adverse effect on the mangroves. A crab pen requires only a small area (about 162 m^2) of mangroves. Observations on the 16 crab pens in the Sematan mangroves showed that the techno-economic performance was very promising. The crab pen culture project supervised by the Sarawak Department of Agriculture has been observed to have markedly increased the income of many artisanal fishermen. Issues related to the pen culture system are discussed. Most of these issues relate to the need for research and development to support further development of this culture system.

THE fattening and grow-out of mud crabs (*Scylla* spp.) is a new aquaculture undertaking in Sarawak. Crab culture was started in the late 1980s. The practice was to rear crabs in small, shallow earth ponds in areas that were subjected to tidal influence. The average size of the ponds was about 65–70 m^2 with the depth of about 0.91 m. The sides were lined with planks or asbestos cement sheets. Although this culture system is still used, it is not widespread. In view of the economic potential of crab culture undertakings, and the shortage of suitable lands for crab culture in many coastal villages, the Inland Fisheries Division of the Sarawak Department of Agriculture, in 1992, introduced the pen culture system in logged areas of the mangrove swamps in Sematan as a pilot project to assist the artisanal fishermen to raise their income. In this pen culture system, the crabs are allowed to grow in their natural habitat in enclosures in the mangroves. The mangrove vegetation is kept intact. As such, it is an ecologically friendly system. Since its introduction, this innovation has now spread to a number of districts in Sarawak.

This paper describes the design, cultural practice, and techno-economic performance of the culture system, and the impacts on the fishing communities and the mangroves. Those important issues and problems associated with the culture system are also addressed in this paper.

[1]HQ, Department of Agriculture, Sarawak, 93250 Kuching, Sarawak, Malaysia
[2]Sematan Fisheries Station, Department of Agriculture, Sarawak, 94100 Sematan, Sarawak, Malaysia

Pen Design and Structure

The crab pens are constructed in the logged areas of the mangrove swamp. The vegetation of the area is left intact to provide the natural environment for the crabs to grow and reproduce. The pen is constructed using the trunks of a type of palm *(Oncosperma tigillaria)* which is abundant in the coastal area and locally called 'Nibong'. This type of palm can last for many years in wet conditions. The trunks of the palm are split into strips of about 6 cm thick, 9–12 cm wide and 3.7 m long which are used for the fencing and plankwalk. For fencing, each strip is driven about 1.2 m into the soil with almost no gaps between strips. The dimension of the pen is 18 m by 9 m (162 m^2) and the fence is 2.4 m high to keep off predators and to prevent crabs from escaping. The fence is supported by posts at 3 m intervals and three levels of horizontal rungs of the same palm materials. The posts are 3.7 m long with 1.2 m in the soil. The rungs are 6 cm thick, 9 cm wide and 3.7 m long. These rungs are nailed horizontally to the fencing strips and the posts; one at ground level, one in the middle, and one about 0.3 m from the top of the fencing.

A perimeter plankwalk either made of timber planks or palm strips is constructed for ease of moving around the pen. Also, a small store is constructed in between pens.

Inside the pen, perimeter drains of 0.6 to 0.9 m wide and 0.8 m deep are dug. Usually, a small drain 0.3 m wide and 0.3 m deep is constructed across the pen. The soil dug out from the perimeter drains is accumulated at the foot of the fence to build a small bund. The perimeter drain is linked to the inlet/outlet drain outside the pen. An 18 cm elbow PVC pipe is installed at the entrance to the inlet/outlet drain with the elbow end on the inner side. The drains inside the pen are always filled with water. During high tide, the elbow end of the pipe is pressed down to allow fresh salt water to enter. The elbow end is pulled up during ebb tide. In pens located on higher ground, there is a need to install water pipes and water pump to irrigate the pens during the neap tide period when the tide cannot reach the area.

The pens are under the shade of the mangroves and crabs will make holes in between the mangrove plants and stay in there during low tide. Those bare areas where the mangrove plants have been removed are replanted using mangrove cuttings. This is to ensure a good canopy over the pens.

Cultural Practice

Stocking and stocking rate

A survey of 16 crab pens owned by different participants in Sematan in the first production period in 1992 and 1993 showed that the stocking rates were high, ranging between 973 to 5351 pieces per pen and averaging about 3249 pieces per pen (Table 2). It has been observed that the stocking rates in many pens in Sematan and other areas are now very much reduced to between 1000 and 1500 pieces per pen. The reasons for the reduction in stocking rate are the increase in the number of pens and the realisation that the crabs can grow faster and mortality reduced. Stocking of a crab pen normally takes about two months to complete.

Feeding

The crabs are fed with trash fish chopped up in pieces of about 9 cm by 12 cm and placed in the drains. Feeding is done once a day during high tides. During high tide, the crabs, being attracted by the inrushing fresh salt water, come out from their holes to feed. The survey of the 16 pens in Sematan in 1992 and 1993 showed that the total quantity of trash fish used per pen for the whole production period varied by pen. The average quantity of trash fish used per pen was about 604 kg (Table 3).

Water management

Water management in the crab pen is important to ensure good quality water for survival and growth of the crabs. Fresh salt water is allowed to enter the pen through the elbow pipe during high tide. At higher tide levels, the salt water will flow into the pen through the small gaps along the fence. Stale water in the drains is drained out at least once a week through the 15 cm elbow pipe.

Harvesting

From the survey of the 16 crab pens initiated between 1992 and 1993, crabs reached a marketable size between 4 to 7 months, the average being 5.2 months (Table 4). Partial harvesting is practiced and is done during the high tide by means of scoop nets and/or traps locally called 'Bento'. Crabs are starved for two days before harvesting. Most fishermen do the harvesting twice a month. Normally crabs of 300 g and above are harvested. However, if there is insufficient supply to meet the market demand, smaller sizes may be harvested. The number of crabs in the pen is maintained by restocking with small crabs of about 100 g size. From the 16 crab pens surveyed, the average production per pen was about 530 kg with an average size of 300 g (Table 4).

Techno-economic performance

The establishment costs (excluding the family labour) for one pen of 9 m by 18 m is estimated to be about RM3180 (approx. $A1600). The details of the establishment costs are given in Table 1.

A survey of 16 crab pens in Sematan mangroves was carried out in 1992 and 1993 to collect technical and economic data on the performance of the crab pen culture. These 16 crab pens were owned by 16 fishermen who were keeping records and were willing to cooperate with the Department of Agriculture in this exercise. Observations were made on the performance of the first production cycles, which were initiated between October 1992 and April 1993. Harvesting was started when nearly all the crabs reached marketable sizes (about 300 g). The quantity of feed (trash fish) used for each pen up to the time of last harvesting, quantity of crabs stocked and harvested were recorded. The data for the 16 pens are shown in Tables 2, 3 and 4 and the average techno-economic performance is summarised in Table 5.

The total operating costs essentially consist of the cost of stocking materials and trash fish. Other miscellaneous costs are considered not significant for inclusion in the operating costs. Labour is contributed by the family, and as such, it is not computed

Table 1. Cost of establishment per crab pen of 9.1 m × 18.3 m at Sematan, Sarawak.

No.	Item	Quantity	Price/unit (RM)	Cost (RM)
1	Nibong trunks (17.8 cm dia × 3.7 m)	18 pcs	2.00/pcs	36.00
2	Nibong strips (10.2 cm × 3.7 m)	1000 pcs	0.80/pcs	800.00
3	Timber rung (5.1 cm × 7.6 cm × 3.7 m)	54 pcs	6.00/pcs	324.00
4	Walking plank (2.5 cm × 20.3 cm × 3.7 m)	150 pcs	10.00 pcs	1500.00
5	Iron nail	40 kg	3.00/kg	120.00
6	Miscellaneous			400.00
	Total cost			3180.00

Table 2. Stocking of mud crab pens (9.1 m × 18.3 m /pen) from 16 crab pen participants, Sematan, Sarawak (1992–1993).

Crab pen no.	Date of stocking	Total no. of crabs stocked	Total weight of crabs stocked (kg)	Total cost of crabs stocked (RM)
1	April 1993	1826	213	783.38
2	January 1993	5035	455	1207.00
3	January 1993	5351	495	1089.00
4	October 1992	3789	321	707.85
5	October 1992	3616	312	689.38
6	November 1992	2836	251	553.74
7	January 1993	3119	296	260.90
8	October 1992	3367	293	646.14
9	April 1993	1227	127	458.20
10	January 1993	3149	301	663.30
11	April 1993	1227	127	458.20
12	January 1993	3031	269	693.74
13	February 1993	4585	391	778.00
14	January 1993	3005	272	693.95
15	October 1992	3583	320	704.77
16	January 1993	3497	274	874.00
	Total	51 989	4761	11 285.00
	Average per crab pen	3249.31	297.56	705.34

Table 3. Feeding of mud rabs in mangrove pens (9.1 m × 18.3 m/pen) with trash fish from 16 crab pen participants, Sematan, Sarawak (1992–1993).

Crab pen no.	Total no. of crabs stocked	Cultured period (months)	Total weight of trash fish (kg)	Total cost of trash fish (RM)
1	1826	4	308	265.70
2	5035	5	1660	498.00
3	5351	6	1939	581.70
4	3789	7	228	136.50
5	3616	7	233	140.00
6	2836	6	379	227.50
7	3119	5	1850	726.85
8	3367	7	175	105.00
9	1227	3	87	109.20
10	3149	4	414	248.50
11	973	3	163	36.90
12	3031	5	391	234.85
13	4585	5	425	275.00
14	3005	4	553	332.00
15	3583	7	245	147.00
16	3497	5	621	372.50
Total	51 989	83	9671	4637.20
Average per crab pen	3249.31	5.20	604.44	289.83

Table 4. Harvesting of mud crabs from mangrove pens (9.1 m × 18.3 m/pen) from 16 crab pen participants, Sematan, Sarawak (1992–1993).

Crab pen no.	Date of harvesting	Cultured period (months)	Total no. of crabs harvested	Total weight of crabs (kg)	Total sales (RM)
1	August 1993	4	949	202	1110.11
2	June 1993	5	3462	833	5204.00
3	July 1993	6	2829	718	4528.00
4	May 1993	7	1739	832	4992.00
5	May 1993	7	1349	603	3619.50
6	May 1993	6	1307	607	3642.00
7	June 1993	5	3000	681	3801.55
8	May 1993	7	1573	728	4371.00
9	July 1993	3	368	87	575.50
10	May 1993	4	1765	655	3935.40
11	July 1993	3	225	51	306.35
12	June 1993	5	1831	386	2296.45
13	July 1993	5	1910	538	3234.00
14	May 1993	4	1954	446	2715.00
15	May 1993	7	1635	742	4462.80
16	June 1993	5	1585	386	2363.50
Total		83	27 481	8495	51 157.16
Average per crab pen		5.19	1717.56	530.94	3197.32

in the cost. From Table 5, the average operating costs per pen per production cycle is calculated to be about RM995 (approx. $A503).

The average production per pen was about 531 kg. At the average price of RM6.02/kg (approx. A$3.04/kg), the average gross income per pen per production cycle was RM3197.32 (approx. $A1615). The average net income per pen per production cycle (which is the gross income minus the operating costs) is calculated to be RM2204.15 (approx. $A1110). At the average production period of 5.2 months, the average net income per pen per month is calculated to be RM424.31 (approx. $A214).

The average mortality rate of the 16 pens surveyed was high, about 47.1%. This is probably attributed to the very high stocking rates among the pens. The average feed conversion ratio was fairly low, about 2.6.

Socio-Economic Impact of the Crab Pen Project

From the observations of the 16 crab pens each separately owned by a fisherman, the monthly net income was about RM424 (approx. $A214). A number of the fishermen have now established 4 to 8 pens; that means a monthly income of RM1696–3392 (approx. $A857–1713). This crab pen project clearly has markedly increased the income of the participating fishermen. The crab pen culture project, which uses a very small area of the mangroves, could help to reduce the incidence of poverty in the fishing community.

Ecological impact of the crab pen project

The mud crabs kept in the crab pens in the mangroves appear to breed very freely. Presently, there are 110 crab pens in the Sematan mangroves. The records (Table 6) in the last three years (1994 to 1996) show that there were quite a large number of berried females being harvested and supplied to the Sematan Fisheries Research Station nearby. These were only a portion of the berried female crabs developed in the pens. The remaining berried crabs were left in the pens and were of no market value. Looking at the morphological characteristics of the crabs found in the Sematan ecosystems and in many of the crab project areas in Sarawak, the crabs do not have the features of *S. serrata*. They have been identified as *S. tranquebarica* and *S. olivacea* by Keenan (1995) and Keenan et al. (1998). *S. olivacea* species tends to be more predominant in the Sematan mangroves.

Table 5. Performance of 16 crab pens in Sematan mangroves, Sarawak (from 1992/93 survey).

1. Average cost of stocking	
(a) Average cost/pen	RM705.34
(b) Average biomass/pen	597.56 kg
(c) Average price/kg = (a) ÷ (b)	**RM2.37/kg**
2. Average cost of feeding	
(a) Average cost/pen	RM289.83
(b) Average quantity of feed/pen	604.44 kg
(c) Average price/kg Trash Fish = (a) ÷ (b)	**RM0.48/kg**
3. Average production per pen	
(a) Per production period/pen	530.94 kg
(b) Average production period	5.19 months
(c) Average production/month = (a) ÷ (b)	**102.10 kg**
4. Average price of crab sold	
(a) Average gross income/production period/pen	RM3197.32
(b) Average biomass of harvested	530.94
(c) Average price of crab sold = (a) ÷ (b)	**RM6.02/kg**
5. Average gross income per pen	
(a) Average gross income/production period/pen	RM3197.32
(b) Average production period	5.19 months
(c) Average gross income/pen/month = (a) ÷ (b)	**RM616.05**
6. Average net income per pen (excluding cost of labour)	
(a) Average gross income/production period/pen	RM3197.32
(b) Average cost of stocking	RM705.34
(c) Average cost of feed/production period/pen	RM289.83
(d) Average production period	5.19 months
(e) Average nett income/pen/month = {[(a) − (b)] × 100%}÷ {(a)}	**RM424.31**
7. Mortality rate (including unharvested crabs)	
(a) Average no. of crabs stocked/pen	3249.31
(b) Average no. of crabs harvested/pen	1717.56
(c) Mortality rate = {[(a) − (b)] × 100%}÷ {(a)}	**47.14%**
8. Feed conversion ratio (FCR)	
(a) Average quantity of feed/pen	604.44 kg
(b) Average biomass of harvested/pen	530.94 kg
(c) Average biomass of stocking	297.56 kg
(d) FCR = [(a)] ÷ {(b) − (c)]	**2.59**

Laboratory tests at Sematan Fisheries Research Station have shown that the eggs carried by the berried female can hatch very well under a salinity regimen between 20–35 ppt. The salinity at the crab pen site has been found to be between 20 and 32 ppt., with the lower part of the range occurring in the wet season. It is believed that there is a strong possibility that the berried female crabs can hatch their eggs in the pen under this salinity condition during the high tide and release the larvae into the river, thereby contributing to the recruitment of crabs in the Sematan mangrove ecosystems. This belief is further reinforced by the observations of fishermen of an increase in the number of crabs in some of the tributaries of Sematan River.

Table 6. Number of berried female crabs brought to the Sematan Fisheries Station, Sarawak for hatching from farmers' crab pens (1994–1996).

Month	Year		
	1994	1995	1996
January	57	26	3
February	13	5	0
March	58	6	6
April	15	11	33
May	19	40	43
June	3	10	36
July	11	8	56
August	19	5	55
September	24	16	59
October	18	24	98
November	7	9	24
December	8	0	30
Total	252	160	473

Moreover, in the one-year period between December, 1994 and November, 1995, two years after the initiation of the crab pen culture project, biological studies were made on the mud crabs in the Sematan ecosystems. It was found that there was a high proportion of young crabs (less than 100 g); 36.3% male and 36.7% female from the sample caught (Ikhwanuddin 1996). In the period between 1992 and 1994, there were 60 crab pens established in the Sematan ecosystems. The total number of crabs harvested for stocking in the two-year period is estimated to be about 780 000, taking the average stocking rate per pen per cycle as 3250 (Table 2). In spite of this large number of young crabs being caught for pen culture, there was still a high proportion of young crabs as found in the Sematan ecosystem studies. This high proportion of young crabs in the population tends to suggest considerable recruitment of young crabs into the mangrove ecosystem. It is believed that this recruitment is contributed by the pen culture system.

It was observed that the fishermen adopting the pen culture system were practising replanting of mangrove plants in bare areas in and around their pens to improve the canopy over the pens. This practice helps to conserve the vegetation in the mangrove areas.

Issues Related to Crab Pen Culture Project Development

There are a number of important issues related to the crab pen culture in the mangroves in Sarawak.

Shortage of crab seed

Shortage of crab seed is the most critical issue in all culture systems. Shortage of crab seed has affected the expansion of crab culture projects in all the districts of Sarawak. The supply of crab seed has to depend on catching in the wild. Although, in a number of districts, there are considerable stocks in the rivers, the supply of crab seed is limited by the number of fishermen catching crabs.

Research on artificial breeding was initiated in 1994 in the Sematan Fisheries Research Station. So far, the station has managed to produce about 1000 juvenile crabs using over 160 berried crabs per year. The slow progress is attributed to lack of knowledge and experience among the fisheries research officers in carrying out research work on crab breeding. Moreover, it was only recently realised that the Sematan Research workers were not dealing with *Scylla serrata* but with different species where few studies on breeding have been done.

A search in the literature has found that all the research work carried out on breeding has been on *S. serrata*. Laboratory observations on the larvae in Sematan station have shown that the morphological characteristics of the larvae appear to be different. A lot of work is still required to see a breakthrough in the breeding of the two species found in Sematan.

Stocking rate

The present stocking rate as practised by the fishermen is too high, resulting in high mortality presumably due to cannibalism. Attempts are now being made to advise the fishermen to reduce the stocking rate. Reducing the stocking rate would help to reduce the seed requirement per pen. However, there is still a need to carry out research work to determine the optimum stocking rate.

Feeds and feeding

Presently, trash fish are used as feed. The main problems with the use of trash fish are availability especially during the rainy season. The other problem is the additional costs incurred on the supply of electricity and the freezer to store the trash fish. Prawn pellet feed was tried by the Sematan Fisheries Research Station and the results appeared to be promising (higher growth rate and faster gonadal development). One problem with the use of pellet feed is that the pellets are too small. Bigger pellet size (5 mm to 7 mm) would be better to reduce wastage. However, more research is needed to study the nutrition of the crabs and to formulate suitable artificial feeds for the various stages of growth of the crabs.

Need for research work on the ecological impact of the culture system

It is acknowledged that very little study has been made on the biology and ecology of the species of mud crabs in Sarawak. Moreover, research on the ecological impact of the culture system needs to be carried out. The knowledge of the biology and ecology of the species would enhance the research in this area of ecological impact. In view of the shortage of trained research personnel in the Inland Fisheries Division of the Sarawak Department of Agriculture, there is a need to carry out research work in collaboration with those institutions that have the necessary expertise. This collaborative research work would enhance the research capability of the local research workers in Sarawak.

Conclusion

The crab pen culture system as adopted in the mangroves of Sarawak offers promise for the fishing communities to increase their household income. It is an ecologically friendly system in that it does not adversely affect the mangroves. The crab pen requires a small area of mangroves but the return has been shown to be comparatively high. As such, it is a suitable culture system for the artisanal fishermen to adopt to raise their income above the poverty line.

References

Keenan, C.P. 1995. Genetic relationship of mud crabs, genus *Scylla*, throughout the Indo-west Pacific, paper presented at the Mud Crab Workshop, 27 October, 1995, Broome, Western Australia, Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology.

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.

Ikhwanuddin, A. Mhd. 1996. Biological studies of the mud crab, genus *Scylla* in the Sematan Mangrove Ecosystem. Unpublished paper presented at the Research Officer Conference, 4 Nov. 1996, Department of Agriculture, Sarawak.

Pen Culture Experiments of the Mud Crab *Scylla serrata* in Mangrove Areas

Jerome G. Genodepa[1]

Abstract

The effect of four treatments, using the combination of stocking densities of 2.5 and 5.0/m^2 with feeding rates of 0% and 3% body weight, were evaluated after 5 months of culture in 200 m^2 net enclosures, in an attempt to develop technology for grow-out culture of the mud crab *Scylla serrata* in mangrove areas. Survival was significantly lower in treatments with no feeding compared to treatments fed at 3% body weight daily regardless of the stocking density. In treatments with feeding, the increase in stocking density significantly affected survival; decreasing as stocking density increased. The average body weight (ABW) at harvest was inversely proportional to survival, indicating a high influence of cannibalism on growth. The lack of animal food in treatments with no feed caused the mud crabs to resort to cannibalism rather than feed on available plant sources. There is no clear indication that the presence of mangroves has some positive effect on growth or survival of mud crabs. Recommendations on research priorities to pursue the general objective of developing techniques for grow-out culture are indicated.

MUD CRABS (*Scylla* spp.) are abundant in brackish-water areas and have been a traditional by-product of milkfish and prawn culture in the Philippines. Although mud crab culture has been practised for quite some time, technology has been very limited and has remained traditional. So far, success stories on mud crab culture are limited to fattening or straight culture from small sizes at low densities (Angell 1992). Recently, the potential of high-density mud crab culture has increased due to the need for alternatives to the collapsing prawn industry. Among the local species, *Scylla serrata* locally known as 'king crab' is getting the attention of fishfarmers because it grows fast and attains much bigger sizes at harvest.

The common method of mud crab culture is in ponds. These ponds have usually been developed in mangrove areas, which are the natural habitat of mud crabs, but considering that these ponds have been totally cleared of mangroves, the system does not conserve or manage the natural crab environment.

This research project was conducted in an attempt to derive a scheme to increase mud crab production and at the same time preserve the remaining mangrove areas; it seeks to improve the technology for grow-out culture of mud crabs (*Scylla* spp.) by utilising mangrove areas for pen culture and to assess the use of natural productivity to grow crabs in mangrove systems.

This paper reports on the first study conducted under this general objective, and evaluates the effect of two treatments, stocking density and feeding rate on the growth and survival after 5 months of culture. Two stocking densities, 2.5 and 5 crabs/m^2, and two feeding rates, 0 (no feeding) and 3% body weight, were used in a randomised block design with three replicates per treatment.

Materials and Methods

Site selection and construction of set-up

A mangrove forest within the UPV Land Grant in Batan, province of Aklan, Philippines, was chosen as the study site. Portions of the mangrove area easily reached by high tides and having a more or less even distribution of trees were chosen for the construction of the experimental set-up, consisting of 12 enclosures (200 m^2) grouped into three blocks. The enclosures were made of plastic netting (1 cm mesh size) supported by bamboo framework.

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

The bottom portion of the enclosure was buried in the ground to approximately 60 cm and its upper portion lined with plastic sheets to prevent climbing crabs from escaping. Peripheral canals, 60 cm deep, were built within each enclosure to retain water during low tides and the excavated soil was used as mounds.

Procurement of stocks and stocking

The seed crabs used for stocking the enclosures, collected from the wild, were purchased through a crab dealer from the province of Samar, Eastern Visayas and consisted totally of *S. serrata* (Keenan et al. 1998). They were transported, with pincers removed, inside pandan bags (30 × 60 × 75 cm) at 400 pieces per bag. The crablets were constantly moistened with seawater as part of the transport procedure. The total transport time from the source to the study site was approximately 18 hours. On arrival at the study site, the crablets were individually counted and mass weighed before they were stocked in the various compartments. Mortalities occurring within 24 hours from stocking were replaced.

Stock management and monitoring of physico-chemical parameters

Trash fish were given daily at 3% of the biomass, for treatments with feeding. The daily ration was divided into two feedings and given at 0600 and 1800. Stocks were sampled at 15-day intervals and the amount of feed was adjusted based on sampling results. Salinity and temperature were monitored at regular intervals.

Harvest and data analysis

The experiment was terminated after 147 days of culture. An inventory of the stocks was conducted and the crabs were weighed individually and their carapace length and width were measured.

The experimental data were evaluated using analysis of variance (ANOVA) to determine differences among the treatments. Duncan's multiple range test was used to evaluate specific differences among treatments at $P = 0.01$ and $P = 0.05$ significance levels.

Results and Discussion

The periodic average body weights (ABW, every 30 days) under the four treatments are presented in Figure 1. Analysis of variance of ABW showed no significant difference among treatments from day 0 to day 120 but there was a significant difference ($P<0.05$) at harvest (day 147). The best growth as indicated by the highest ABW at harvest was in Treatment III (5.0/m^2; no feed) and this was significantly different from the rest of the treatments. Other treatments were not significantly different from each other.

Survival after 147 days is shown in Figure 2. Analysis of variance showed that there was a significant difference in survival among treatments ($P<0.05$). Duncan's multiple range test showed that treatments with feeding (Treatments II and IV) had significantly higher survival compared to treatments with no feeding (Treatments I and III). In treatments without feeding, the increase in stocking density, from 2.5–5 pieces/m^2, produced no significant difference in survival, suggesting they had reached a base level. In treatments with feeding, the increase in stocking density significantly affected survival; decreasing as stocking density was increased. These results are similar to the data obtained by Baliao et al. (1981) and Triño et al. (these Proceedings) for grow-out culture in ponds wherein survival was significantly lower at stocking densities of 1.5 and 3/m^2 compared to 0.5 and 1/m^2.

Figure 3 shows that growth increased as survival decreased, indicating that the better growth in treatments without feeding may be due to cannibalism. The lack of animal food in treatments without feeding may have caused the crabs to resort to cannibalism rather than feed on plant food; consequently, survival was very low but growth was better. Cannibalism results in better growth because the predator is able to derive more nutrients by preying on the same species compared to eating other types of food. These results are similar to those observed in predatory fishes like sea bass (*Lates calcarifer*) and groupers (*Epinephelus* spp.).

Aside from cannibalism, an increase in temperature can be a factor in mortality although this is not reflected in the data gathered during the study. Temperature records taken between 0600 and 0700 ranged from 27 to 30 °C but the maximum temperature for the day was not monitored. However, considering that the culture period (November to April) was towards the hot season with an increasing trend in salinity (Figure 4), it is safe to assume that the maximum daily temperature increased towards the end of the culture. Also, during neap tides the water level in the set-up was low and therefore there were times when temperatures during daytime were high.

It was observed during harvest that the number of crab holes was few but each contained about 5 to 6 crabs. As observed in grow-out ponds, the 'king crab' *Scylla serrata* does not dig holes like *S. tranquebarica* and *S. olivaceous* but in this experiment the high temperature could have forced them to seek refuge in holes. This situation perhaps further increased the incidence of cannibalism.

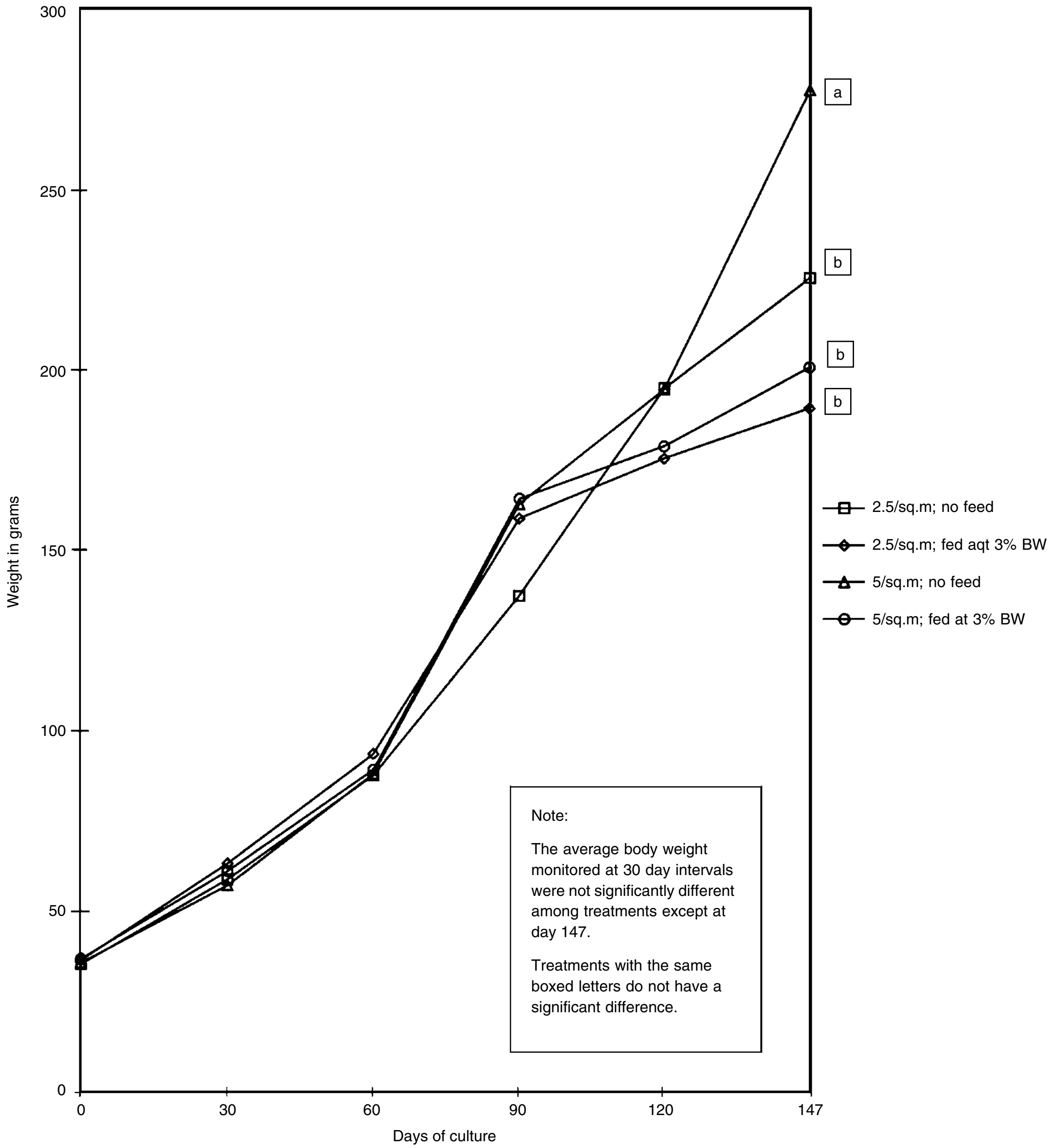

Figure 1. Periodic ABW of mud crab cultured in pens in a mangrove area.

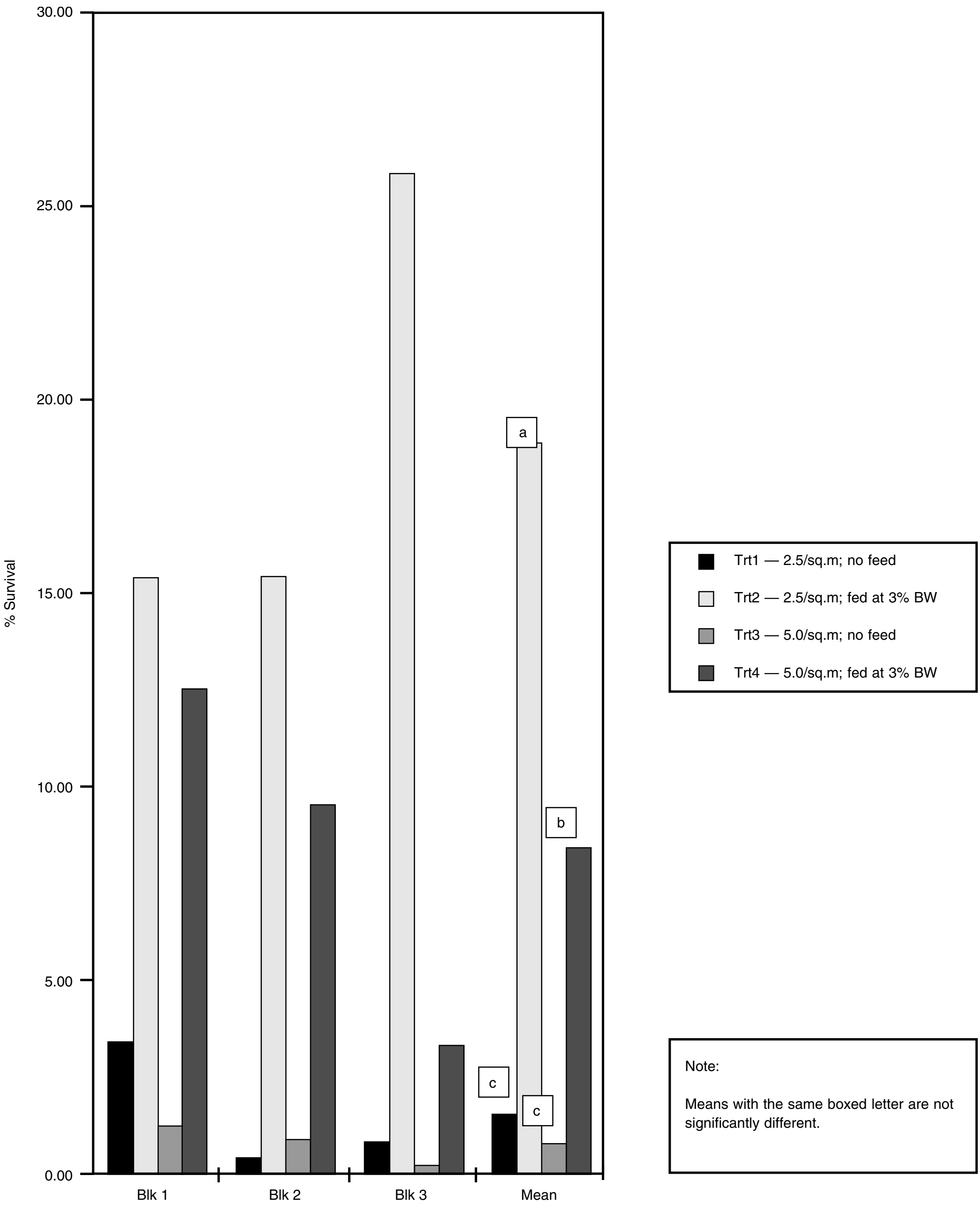

Figure 2. Survival after 147 days of culture.

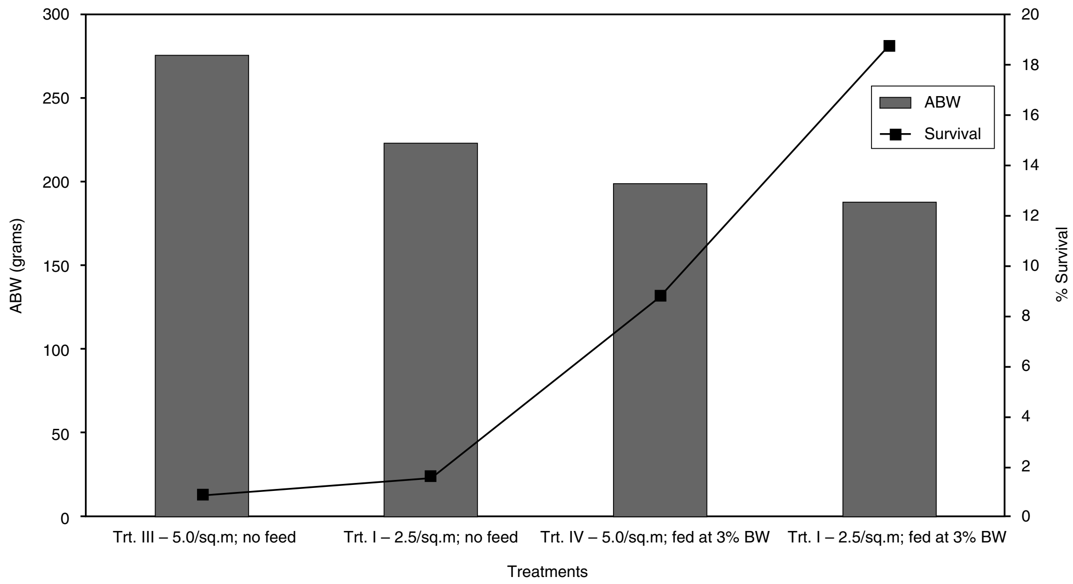

Figure 3. Growth and survival of mud crab in pens after 147 days.

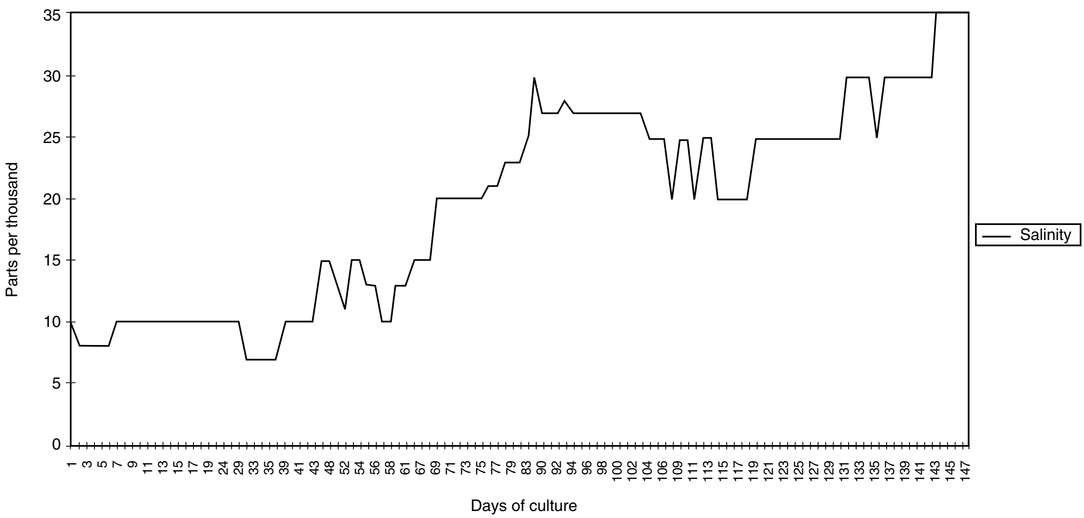

Figure 4. Salinity during pen culture of mud crab in mangrove.

Conclusion/Recommendation

Results of the study showed no clear indication that the presence of mangroves enhanced the growth or survival of *Scylla* spp. The high mortality due to cannibalism has overshadowed the expected benefit of the mangroves in the culture system. The increase in temperature due to frequent low water levels in the experimental set-up forced the crabs to seek refuge in holes and this condition further increased the chances of cannibalism. Cannibalism was highly pronounced during moulting and this was aggravated by lack of food, lack of shelter and limited space.

It is therefore recommended that the set-up in mangrove areas be extended to include deeper waters. It is also recommended that more studies be conducted to deal with factors affecting survival in grow-out culture at higher stocking densities such as:

1. feed ration and feeding frequency;
2. types of shelters and their ratio to the number of crabs or culture space.

References

Angell, C.A. 1992. ed. Report of the seminar on the mud crab culture and trade. Surat Thani, Thailand, November 5–8, 1991. Bay of Bengal Programme, Madras, India. 246 p.

Baliao, D.D., Rodriguez, E.M. and Gerochi, D.D. 1981. Culture of mud crab *Scylla serrata* (Forskål) at different stocking densities in brackishwater ponds. Quarterly Research Report, SEAFDEC Aquaculture Department 5(1), 10–14, Iloilo, Philippines.

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

Mud Crab Culture in the Minh Hai Province, South Vietnam

Danielle Johnston[1] and Clive P. Keenan[2]

Abstract

Disease outbreaks in the Minh Hai region during 1993–1994 led to a dramatic decline in shrimp yields. Low and highly variable post larval densities have continued in recent years. Farmers have found that mud crab farming has a higher profit margin than shrimp. Mud crabs grow extremely quickly with crabs ready to harvest at marketable size after only 3–5 months. Litter fall from mangroves adjacent to crab ponds may be responsible for the fast growth as it may promote benthic food chains in the pond. Crabs are stocked, at a low stocking rate, directly into ponds open to the mangrove forests. Aside from initial investment costs to purchase crab seed, costs are low as the crabs rely on natural food within the forest. At present, mud crab culture within mangrove forests is uncommon in the Minh Hai Province. Although not observed within the Minh Hai Province, there are two other recognised crab culture systems operating in southern Vietnam: moulting crab and fattening crab culture.

DURING the past 10–15 years, there has been a rapid expansion of shrimp culture in the Minh Hai Province of southern Vietnam (Figure 1). Unfortunately, this expansion has been at the expense of mangrove forests which have been cleared at a rate of approximately 5000 ha/year (Hong and San 1993), to less than half their original area (<50 000 ha) between 1982–1991 (Minh Hai Fisheries Dept.). If this rate of loss continues, mangrove forests will be unable to meet the projected demands for firewood and construction materials in the region.

To compound these land use issues, disease outbreaks in 1993–1994 led to a dramatic decline in shrimp yields, with farm incomes falling to 10% of the previous year. Since this time, low and highly variable post-larval densities in local canals and rivers (ACIAR PN 9412) have continued the poor yield (<350 kg/ha/year). Attempts to stock ponds with hatchery reared *Penaeus monodon* have so far failed due to stresses associated with lengthy transport periods and water quality shock, which in turn have heightened their disease susceptibility. Poor water quality in the region, in particular low dissolved oxygen, high suspended solids, acidity of the pond bottom and extreme salinity fluctuations will continue to hamper successful shrimp culture in the future.

Unreliability in shrimp yields has subsequently forced farmers into alternative strategies to support their families. Mud crab culture has been a highly successful and increasingly popular alternative over the past 4 years. It offers a number of benefits over shrimp culture. Crab culture provides a more reliable income, as crab survival is high because of superior adaptation to the mangrove environment. Farmers also have higher profit margins with crabs earning better prices per kg than shrimp. In most cases, the return is 3–4 times the initial investment made on crab seed and it is possible to earn up to US$1000 per harvest. Mud crab growth rates are extremely good with crabs ready to harvest at marketable size (300–400 g) after only 3–5 months. This is achieved with little or no capital or food input, and allows a second harvest per year, which further raises income potential. It is likely that litter fall from mangroves adjacent to crab ponds is partly responsible for these high growth rates as it provides detritus, a major dietary component of mud crabs (Prasad and Neelakantan 1988) and promotes benthic food chains in the pond. Finally, farmers have identified crabs as a lower disease risk than local shrimp, the latter being associated with white spot and brown gill.

[1]Australian Institute of Marine Science (AIMS), PMB 3, Townsville Qld 4810, Australia
[2]Bribie Island Aquaculture Research Centre (BIARC), PO Box 2066, Bribie Island Qld 4507, Australia

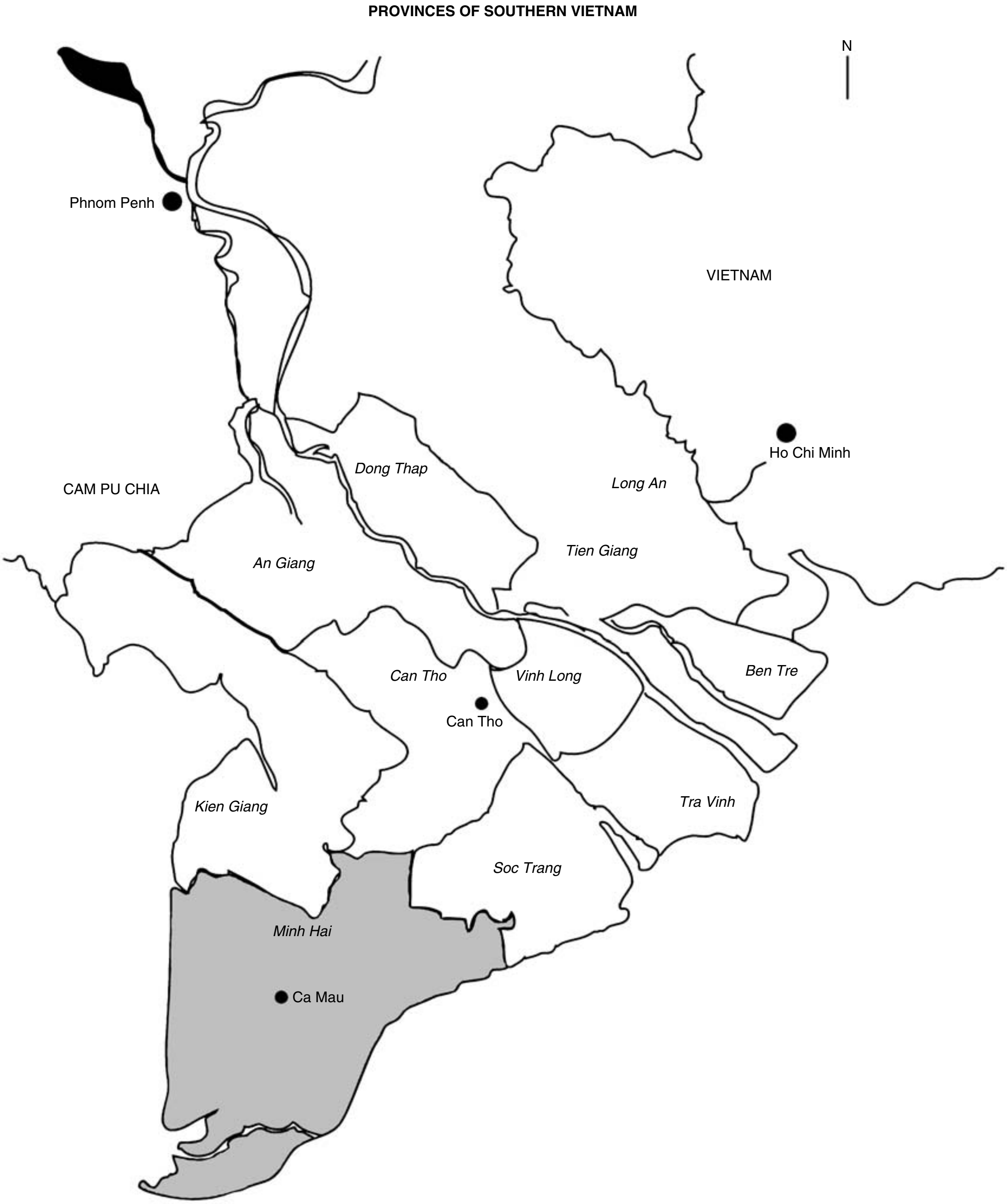

Figure 1. Location map of the Minh Hai Province in southern Vietnam.

Culture Techniques

Farmers in the Minh Hai Province purchase crabs from fishermen, who capture them from local canals and from coastal waters using bottom seine nets. The cheapest time to buy is from November to March (25–50 g each) at US$2.00/kg, with the most expensive time during June/July (80–100 g each) at US$3.00–4.00/kg. These price differences presumably reflect the natural fluctuation in crab seed abundance in the region. Crabs are stocked, at the low stocking rate of 0.05 crabs/m^2, directly into ponds, which are usually separate from dedicated shrimp ponds but are open to the mangrove forests. They are then left to grow for 3 to 5 months, during which time they rely on natural food supplies in the forest. The ponds are bounded by cleared channels 30–70 cm deep which carry water into the mangrove forest and have intermittent tidal exchange via the farm sluice gate.

Protective structures may be placed around the pond wall to prevent crabs escaping. One such system is a perimeter of wooden poles 1 m apart, each with a flag of plastic at the top which flaps in the wind to scare crabs back into the pond. Low plastic fences may also be found along the top of the banks. Another alternative is to grow or fatten the crabs in large (2 × 1 × 1 m) wooden enclosures or pens which are partially submerged within the pond. These allow the crabs to be monitored closely and provide considerable protection from predators particularly during the early stages. In this case, feed is added to the cage.

For the last 10–15 days, the crabs may be removed from ponds and placed into a large wicker basket which floats in the pond. During this time, significant additional food is added and the farmers monitor ovarian development through slits in the back of the carapace to determine the optimal time to harvest. When the ovaries are mature (red-orange colour), the crabs are sold to 'middle men' for US$8.00–12.00/kg, each crab weighing 300–400 g. One farmer reported stocking 57 kg of crab seed which he harvested after 3–4 months at 200 kg, which further demonstrates the high growth potential of mud crabs in the region.

Aside from initial investment costs to purchase crab seed, the most significant problem reported to date in the Minh Hai Province is children stealing the crabs from ponds during the nights before harvest. The situation has become so serious that farmers have organised groups of adults to guard the ponds at this time. It is likely that most farmers will adopt protective enclosures during the later stages of grow-out if the problem continues.

It is also important to note that, although crab culture in the Minh Hai Province is relatively small scale with low stocking densities, its increasing popularity will threaten future seed supplies and possibly the wild fishery. Expansion of crab aquaculture should therefore be based on the production of crab seed in hatcheries.

In addition, sustainable stocking densities that maximise production and minimise mortality will need to be identified and promoted. Incorporation of mud crabs into existing mangrove forest silvicultural practices is a suitable option, not only to prevent further forest destruction that has reached critical levels due to shrimp culture expansion, but also to maximise forest productivity.

For these reasons, a crab culture experiment has been incorporated into the ACIAR project PN 9412 to determine the maximum sustainable stocking densities, without the addition of food, at three forest ages within the Minh Hai Province.

Incorporation into mangrove forests

At present, mud crab culture within mangrove forests is uncommon in the Minh Hai Province. However, it is being promoted as a successful practice in other Asian countries such as Malaysia (Sarawak), Thailand (Ranong) and the Philippines (refer to papers in these Proceedings).

In the natural situation, a mutually beneficial relationship exists between mangroves and crabs. Crabs promote mangrove growth by increasing nutrient levels and facilitating nutrient recycling via defaecation and mortality, as well as oxygenating the anaerobic mud and reducing salt accumulation at root tips by burrowing. Mangroves increase crab survivorship by providing protection from predators and physical parameters by reducing sunlight and heat exposure. The forest floor is also a rich source of food, providing detritus and surface algae on leaf, propagule and branch litter, as well as supporting a large invertebrate population including gastropods and crustaceans and also fish, all of which are important dietary components of mud crabs (Prasad and Neelakantan 1988).

Nevertheless, if crab culture is to be successful within mangrove forests, stocking densities must be conservative and sustainable, particularly if crabs are dependent on natural food supplies. By raising predator (crab) densities above sustainable limits, farmers will disrupt the natural food chain and quickly deplete all food sources. The large-scale and longer-term effects of increased nutrient levels within mangrove forests are also unknown and need to be addressed in the future.

Other crab culture systems

Although not observed within the Minh Hai Province, there are two other recognised crab culture systems operating in southern Vietnam: moulting crab and fattening crab culture. Moulting crab culture involves capturing small (<100 g) low value crabs during their migration upstream from estuaries (January–August) using conical fixed nets (Hung 1992). Moulting is induced by removing the pinchers and walking legs and crabs are stocked into ponds (or rice fields) at 100 kg per 300–500 m^2 pond. Crabs are fed with trash fish and crustaceans at 3–5% body weight/day and the majority moult between 14–20 days after leg removal (Hung 1992). The ponds are drained to collect pre-moulting crabs, which are transferred to net *hapas* (1.0 × 2.0 × 0.8 m) where moulting crabs are easily recognisable (Hung 1992). Soft-shell crabs are removed and kept on a humid substrate for transferral to traders for freezing and export. The value of moulting soft-shell crabs is five to ten times higher than hard-shell small crabs, with a net benefit from a 300–500 m^2 pond reaching US$50–70/month (Hung 1992).

Fattening crab culture involves the fattening of large (>100 g) thin-bodied crabs in ponds fenced with nipa palm fronds or bamboo to prevent escapes (Hung 1992). Crabs may also be fattened in bamboo cages (1.0 × 2.0 × 1.0 m) floating in ponds or rivers. In contrast to the system in Minh Hai Province, these crabs are stocked at high densities of 100 kg/300 m^2 pond, or 10 kg/2 m^3 cage, and fed for 15–20 days with trash fish and crustaceans at 5–10% body weight (Hung 1992). Fattened crabs are worth three times the value of thin crabs with a net profit of US$100–150/month from a 300 m^2 pond (Hung 1992).

Fattening crab culture is practiced from August to November, whereas from October to December farmers prefer to culture mature female crabs as these fetch high prices for export (Hung 1992). Mature females are stocked at 50–100 kg per 300 m^2 pond and fed with fiddler crabs at 10% body weight/day for 15–20 days while their ovaries enlarge to 70% of body cavity. Net profits can be as high as US$200/month from these ponds (Hung 1992). Over-exploitation of mature females will, however, aggravate the already low seed supplies and regulations will need to be enforced to prevent the situation worsening, particularly as crab culture popularity increases in southern Vietnam.

Acknowledgments

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References

Hong, P.N. and San, H.T. 1993. Mangroves of Vietnam, IUCN Bangkok, Thailand.

Hung, L.H. 1992. Integration of crustacean aquaculture with coastal rice farming in Vietnam. NAGA 15, (2), 27–29.

Prasad, P.N. and Neelakantan, B. 1988. Food and feeding of the mud crab *Scylla serrata* Forskål (Decapoda: Portunidae) from Karwar waters. Indian Journal of Fisheries 35, (3), 164–170.