

Breeding Technique and Embryonic Development of Mud Crab, *Scylla serrata*

Nilufa Aktar



Fisheries and Marine Resource Technology Discipline

Khulna University

Khulna, Bangladesh

August, 2012

Breeding Technique and Embryonic Development of Mud Crab, *Scylla serrata*

A Thesis

By

Nilufa Aktar

Examination Roll No: MS-110609

Session: 2010-11



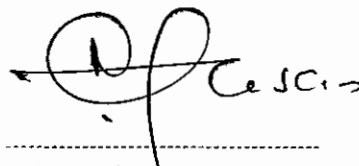
A submitted in partial fulfillment of the requirements

For the degree of Master's of Science in Fisheries and Marine Resource Technology

August, 2012

Breeding Technique and Embryonic Development of Mud Crab, *Scylla serrata*

Approved as to the style and content by



Professor Dr. Md. Nazmul Ahsan

Head

Fisheries and Marine Resource Technology Discipline

Khulna University

Khulna, Bangladesh

Declaration

Breeding Technique and Embryonic Development of Mud Crab, *Scylla serrata*

The results presented in the thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

Nilufa Aktar

(Signature of the candidate)

Nilua Aktar

Examination Roll No: MS-110609

Session: 2010-2011

(Signature of the Co-supervisor)

Dr. Mohammed Mostafa

Professor

Fisheries and Marine Resource Technology Discipline
Khulna University, Khulna, Bangladesh



[Signature] 12.08.2012

(Signature of the supervisor)

Dr. Md Golam Sarower

Professor

Fisheries and Marine Resource Technology Discipline
Khulna University, Khulna, Bangladesh

August, 2012

***DEDICATED
TO
MY BELOVED PARENTS.....***



Acknowledgement

First of all my admirations go to the supreme creator and ruler of the universe, the Allah, for his great mercy to create and to keep me alive.

I would like to express my indebtedness and gratitude to my supervisor Professor Dr. Md. Golam Sarower, Fisheries and Marine Resource Technology Discipline Khulna University, for his enthusiastic supervision and guidance in completing this thesis.

I express my sincere gratitude to Professor Dr. Md. Nazmul Ahsan, Head of Fisheries and Marine Resource Technology Discipline, Khulna University for providing me the opportunity for this study.

I would like to thanks to Dr. Mohammed Mostafa, Professor of Fisheries and Marine Resource Technology Discipline, Khulna University, for his co-supervision and providing special guidance to complete this thesis.

Special thanks to my friend, Sk. Shahinur Islam and junior, Khandaker Jahid for aiding me to complete the lab work.

Nilufa Aktar

Khulna University

August, 2012



Abstract

Scylla serrata, commonly referred to as mud crabs, is an important species, both as a food item for local consumption as well as a commercially exportable product. The natural resource of this species is gradually declining due to human intervention and environmental modification that made the species a threatened one. Bringing this species into culture system can be a strategy to prevent it from being dwindled in the nature. With this aim in view, this study was conducted to know the brood development, breeding techniques and embryonic development of the species followed to develop a successful hatchery technology. The experiment was conducted "Sundarban Golda Hatchery" in Khulna. Ten brood crabs were collected from Munshigong crab farm and the average weight of crab was 180g. During breeding at hatchery salinity, temperature and pH of water were 30 ppt, 26 to 28 °c and 7.5 to 7.6 respectively. Matured brood crabs were hatched within over night. From the study, the average fecundity of the specimens was counted approximately 10, 00,000 and the average hatching was 4000 per 8 individual, hatching rate was approximately 5%. The embryonic development was checked and only one stage named blastula was possible to observe because of high embryo mortality. During larval rearing, newly hatched zoea was also observed. As the species is being rapidly declined from the natural waters, attention must be taken for bringing it under culture system and for adhere package in the larvae production and hatchery management technologies are necessary. This results promoted and be deemed as initial in this regard necessitating alternatives for conservation of the species.

Table of Contents

Chapter	Contents	Page
	Acknowledgement	I
	Abstract	II
	Table of contents	III- V
	List of tables	VI
	List of figures	VII
	Lists of Abbreviations	VIII
Chapter-1	Introduction	1-11
	1.1 <i>Objective of the study</i>	11
Chapter-2	Literature Review	12-21
	2.1 Brood Sock	12
	2.1.1 <i>Sourcing and Maturation</i>	12
	2.2 Spawning (Egg Extrusion)	16
	2.3 Incubation and Hatching	17
	2.4 Larval Rearing	19
	2.4.1 <i>Selection and Stocking</i>	19
	2.5 Water Quality Parameters	19
	2.6 Feeding and Nutrition	21
Chapter-3	3. Materials and Methods	22-25
	3.1 Fry Management	22
	3.2 Brood Management in the Circular Pond	22
	3.2.1 <i>Preparation of Pond</i>	22
	3.2.2 <i>Brood Collection</i>	22
	3.3 Prophylactic treatments of Broods	23
	3.4 Acclimatization of Broods	23

	3.5 Brood Stocking	23
	3.6 Feeding of Crabs	23
	3.7 Breeding Techniques	23
	3.7.1 <i>Breeding Seasons</i>	23
	3.7.2 <i>Spawning and Incubation</i>	24
	3.8 Observation of Embryonic Development	24
	3.9 Hatching Rate Determination	24
	3.10 Fecundity Estimation	24
	3.11 Data Analysis	25
Chapter-4	4. Results	26-34
	4.1 Brood Management in Captive Condition	26
	4.1.1 <i>Sources of Fry</i>	26
	4.1.2 <i>Water quality parameters for fry management</i>	26
	4.1.3 <i>Feeding of fry</i>	26
	4.1.4 <i>Maturation of Crabs</i>	26
	4.2 Brood management in circular pond	26
	4.2.1 <i>Sources of Broods</i>	26
	4.2.2 <i>Water quality management</i>	27
	4.2.3 <i>Stocking Density</i>	27
	4.2.4 <i>Feeding of the broods in the point</i>	28
	4.2.5 <i>Maturation of brood crabs</i>	28
	4.3 Breeding techniques in the Hatchery	28
	4.3.1 <i>Prophylactic treatment of brood</i>	28
	4.3.2 <i>Water quality parameters in the hatchery tank</i>	29
	4.3.3 <i>Water Treatment</i>	29
	4.3.4 <i>Spawning and Incubation</i>	29
	4.4 Observation of embryonic development	30
	4.5 Assessment of hatching rate	30
	4.6 Larval Rearing Stage	31
	4.7 Fecundity estimation	32

	5. Discussion	35-39
Chapter-5	5.1 Broodstock Management of Mud Crab	35
	5.2 Maturation of Mud Crab	35
	5.3 Breeding Techniques of Mud Crab	36
	5.4 Observation of Embryonic Development	36
	5.5 Assessment of Hatching Rate	37
	5.6 Larval Rearing Stage	37
	5.7 Fecundity	38
Chapter-6	Conclusion	40
Chapter-7	References	41-54
Chapter-8	Appendix	55-56



List of Tables

Table No.	Title	Page
Table 1	Various water quality parameters during brood management in the circular pond	27
Table 2	Total Weight, Carapace length and width of crabs during stocking in pond	27
Table 3	Various water quality parameters in the hatchery tank	29
Table 4	Calculation Hatching Success	31
Table 5	Fecundity estimation of mud crab, <i>Scylla serrata</i> .	32

List of Figures

Fig. No.	Title	Page
1	Immature eggs of female crab	28
2	Mature eggs of female crabs	28
3	Dead egg	30
4	Blastula stage	30
5	Newly hatched egg	31
6	Newly hatched Zoea stage	31
7	Relationship between Carapace Width and fecundity	33
8	Relationship between Total Gonad Weight and fecundity	33
9	Relationship between Total Weight and fecundity	34



List of Abbreviations

Abbreviate word	Elaboration
Spp.	Specis
UV	Ultra Violet
CL	Carapace Length
CW	Carapace Width
ppt	Parts per thousands

1. Introduction

Crabs of the genus *Scylla*, commonly referred to as mud crabs, are commercially important crabs in Southeast Asia distribution. *Scylla serrata* is the most widespread of the four recognized *Scylla* species (Keenan *et al.* 1998) and it has traditionally been an important fishery in coastal communities throughout its range. The coastal belt of Bangladesh endowed with diverse resources bears a significant scope in ensuring the security of food and livelihood of the coastal poor people. But this region is highly pruned to the effect of climate change. The effect of climate change is deteriorating food security and livelihood of coastal resource-poor people. Fortunately, the perpetual sea-level rise and salinity intrusion is making the coastal waters of this area economically and environmentally feasible for coastal aquaculture. Among the edible shrimps, lobsters and crabs, the large-clawed mud crabs are considered as one of the important seafood items for aquaculture in South west coastal region of Bangladesh due to their esteemed delicacy, medicinal value and demand for local and export trades (Ali *et al.*, 2004).

Mud crab of genus *Scylla*, also known as green crab or mangrove crab constitute an important secondary crop in the traditional shrimp or fish culture systems in the Asian countries including Bangladesh. Mud crab aquaculture has been practiced for many years in this region, based primarily on capture and fattening of juvenile crabs from the wild. There is an unmet demand for mud crabs and this has led to over-exploitation in many areas. The mud crab, *Scylla serrata* is widely distributed in the Pacific and Indian oceans. It is available in brackish coastal waters and estuaries and has a great potential for aquaculture. Crab culture and fattening are however, still in the experimental stage in South Asia (Samarasinghe *et al.* 1992). The importance of live mud crab as an export commodity has opened up great opportunities for crab farming. Considering the increasing demand of mud crab in the local and international markets, it has been gaining popularity among the coastal communities in greater Khulna and Chittagong regions (Azam *et al.* 1998).

The annual production of crab is estimated to be more than 10,000 tons (Zafar and Siddique 2000). Crab fattening is widely practiced in Thailand, Taiwan, Malaysia, Singapore, India, Indonesia and Bangladesh. Gravid female with full orange-red egg masses are in great demand in seafood restaurants of the South-east Asian countries. Due to its high price, people



started to hold immature female crabs in some kind of enclosures and fed them until the gonads developed and filled the mantle cavity. In Bangladesh, coastal farmers have been practicing mud crab fattening mainly in encircled earthen ponds. In earthen ponds, some crabs manage to escape and cannot be caught in time due to burrowing inside the mud. High mortality and poor survival are the main constraints of crab fattening in existing systems. The introduction of mud crab fattening in cages is new in our country though it has been practiced in the Philippines, Indonesia, Malaysia and other South-east Asian countries. In the Philippines, only documented studies on fattening crabs were in bamboo (DA, Region VI 1988) and net cages (Kuntiyo, 1992). In Myanmar, the traditional mud crab fattening practice is done in ponds, bamboo enclosure and cage in river and canal system (Win 2000). Felix *et al.* (1995) reported mud crab fattening practice in cage in Vietnam. In Malaysia, floating cages (6m³ with a depth of 1m) are used to fatten mud crab (Sivasubramain and Angel 1992). It is very much needed to improve fattening techniques of mud crab to get higher production in Bangladesh.

The mud crab *Scylla serrata* is an important species of the class crustacea under the phylum arthropoda. Sixteen species of crabs have been so far reported from Bangladesh waters, of which the common ones are *Scylla serrata*, *Portunas pelagicus*, *P. sanguinolentus*, *Charybdis feriata*, *Charybdis rostrata*, *Matuta lunaris*, *M. planipes*, *Clappa lophos*, *C. pustulosa*, *Varuna litterata*, *Sartorina spinigera*, *Ocypoda cratophthalma*, and *Gelasimus annulipes*. The six important genera used as food crabs are *Scylla*, *Portunus*, *Charybdis*, *Matuta*, *Varuna* and *Sartorina*. Most of these species are economically important but the serrated mud crab, also known as mangrove crab, or *Scylla serrata* is now the most commercially important species and is widely distributed in the Indo-Pacific region, including the Bay of Bengal. Bangladesh earns about US \$ 6 million per year by exporting 1,500 m tons of live mud crab to Singapore, Hong Kong, China, Taiwan and Japan. They are common in mud flats of the littoral, parts of the supra littoral and the intertidal muddy zones of the Bay of Bengal. Bangladesh has a coastline of 710 km with 6, 18,780 ha of mangrove tidal flat and 80,000 ha of prime area which is suitable for brackish water aquaculture (Anon, 2003). In the Sundarban, there are 4 families and 16 species of crab. This species is also called mangrove crab or green crab. It is locally known as Shilla Kakra 'Haubba kakra', appears to be the most important crab species in Bangladesh for food and trade. It occurs in

the coastal waters of 15-30 ppt salinity and particularly dominates in the mangrove fauna. It is also harvested from shrimp (*Penaeus monodon*) ponds (gher) along the whole coastal zone of Bangladesh. They are essentially euryhaline, but die beyond 70 ppt. and rarely tolerate turbid waters (Khan and Alam, 1991). Shrimp and crab live in similar environmental conditions. They are quite abundant in places 40-50 km inland from the Bay, in the creeks and canals of the brackish water estuaries (Khan and Alam, 1991). Since the mid 70s, the crab marketing system has developed in Bangladesh with the export to foreign markets. Although mud crab export started in it 1977-'78 becomes a settled business in 1982 (SEAFDEC, 1998). In past mud crab was a virgin stock and marketed only locally in negligible quantities. Mostly Non-Muslims like Hindu, Buddhists etc. are traditional consumers. The majority of Muslim population does not accept mud crab as a food item. Nevertheless, attitudes are gradually changing in the Muslim community recently. Once considered as a minor fishery, the mud becomes an export commodity and ranks 3rd among frozen food export earnings. Of several crab species, the mud crab in Bangladesh holds the prime position in the market in term of price because of its excellent table quality and larger size than other countries.

After the disease outbreak in shrimp farming, crabs have become candidate species for aquaculture because of their export potential. Recently the demand and price of mud crab have increased significantly. Therefore, the entry of new fishers in crab harvesting from coastal waters has increased manifolds. As a result, there is huge overfishing exists on the species which could lead to extinction from our coastal waters if the present catching rate continues further. Export of mud crab started in 1977-78, and became a stable business in 1982 and ranked third among frozen foods exported from Bangladesh (SEAFDEC, 1998; Ali et al., 2004). During 2005-2006, Bangladesh earned US\$3,668,000, compared with 2006-2007, when the total export value increased by 65%. The export of live mud crabs from Bangladesh has increased many folds in the last decade (BEPB, 2004). Generally Hong Kong, Malaysia, Taiwan, Singapore, and European Union import mud crab from Bangladesh. The crab fattening technology feasible in the coastal belt of Bangladesh has been developed by the Bangladesh Fisheries Research Institute, Brackishwater station, Khulna (Saha and Ahammad, 1999).



Usually, mud crabs matures at a carapace width of 10 cm at the age of about six months and spawning takes place within 10 days after eyestalk ablation. Hatching takes place after about 17 days of spawning at temperatures between 23° and 25°C. A 250g female may produce up to 1.5 million first zoea (Z1) larvae which take 2-3 days for each intermoult to reach the megalopa stage. High salinity sea water (25-53 ppt) is best for larval rearing. Mud crab farmers still depend largely on natural wild seed. So, seed is a limiting factor for the expansion of crab culture in Bangladesh. Most farmers collect crab seeds from the tidal rivers and seacoast by using different nets. Crab fry may be available year round but the peak *Culture techniques*. The mud crab is widely distributed in Indo-Pacific including the Bay of Bengal. The culture techniques of this crab include (i) rearing of young crabs in large farms, and (ii) fattening of postmoult 'water crabs' as well as immature crabs to marketable sizes. Crab seeds are usually stocked in large ponds, and are reared until they reach the marketable size and have well-developed gonads. They are reared as a part of polyculture system along with shrimp, milkfish, and seaweeds in Asian countries including Bangladesh where traditional shrimp farms, the *ghers* are the "growout" ponds. In such farms young crabs of 10-100 g are held up to 6-8 months until they reach the marketable size (usually 300-500g).

Scylla serrata species is large, euryhaline crabs which as adults inhabit sheltered coastal habitats across the Indo-West Pacific. Larger populations are usually associated with mangrove forests, particularly in estuaries (Le Vay, 2001) where they tolerate a wide range of environmental conditions (Hill, 1979). They are primarily carnivorous, eating benthic molluscs and crustaceans (Hill, 1979), but are also opportunistic scavengers and will eat a wide variety of animal protein and even vegetable matter such as filamentous algae (Williams and Primavera, 2001) and cooked maize (Rodríguez *et al.* 2003). The crabs are mostly nocturnal or crepuscular, hiding during the day in burrows and foraging on mud flats from late afternoon to early morning, particularly during flood tides (Dat, 1999a; Barnes *et al.* 2002). Maturation and spawning is continuous throughout the year with seasonal peaks in certain areas (Le Vay, 2001). Adults mate inshore when the exoskeleton of the female is still soft after moulting. Females undergo up to three post-pubertal moults. Mated, ovigerous females generally migrate offshore where they extrude the eggs, attach them to the pleopods and incubate them till they hatch (Brick, 1974; Hill, 1978; Perrine, 1979). Females then

return inshore and can spawn up to three times without needing to mate again. Little is known about the oceanic phases of the larvae (Fielder, 1995) except that the larvae develop through five pelagic zoeal stages after which they recruit into sheltered, shallow water habitats as semi-benthic megalopae (Brick, 1974; Hill, 1975, 1979). Keenan *et al.* (1998) recently revised the genus and identified four distinct species: *S. serrata*, *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. *S. serrata* is more abundant in high salinity habitats than the other three species and females migrate further offshore, often as far as the continental shelf (Heasman and Fielder 1983; Hill, 1994).

The production of mud crabs has recently declined due to both overfishing and aquaculture activities. In some areas, expansion of the shrimp culture industry, mangrove forests have been substantially destroyed, resulting in loss of habitat for these species with consequent decline in fishery production. Currently, there is an increasing interest in crab culture as an alternative to shrimp farming. The nutritional status of breeders has been known to affect reproductive performance and determines the quantity and quality of eggs and start-feeding larvae and eventually their hatchery performance (Teshima and Kanazawa 1983; Harrison 1990; Browdy 1998).

The continuous collection of wild seeds for grow-out culture due to the expanding export market for mud crab and the search for alternative culture species for shrimps has threatened the wild stock population. Seed production of *S. serrata* is still dependent on spawners that are captured from the wild. As the mud crab seed resources from the wild are limited, future expansion of mud crab farming will have to depend upon hatchery produced seed, which in turn depends on the availability of berried or egg bearing females. Artificial production of mud crabs could help mitigate pressure on fishery resources. Inadequate spawner supply is a major constraint to further development of the industry. An essential component of hatchery production is the establishment of a broodstock that can produce a predictable supply of fertilized eggs (Wickins and Lee 2002). Domesticated broodstock can be a convenient alternative to wild spawners for a steady supply of the seeds and stability in the production of larvae.

The crab culture is presently dependent on wild caught seeds, which are not sufficient. The natural seed availability is also declining due to indiscriminate collection of juveniles for

farming. The collected seeds are not of uniform size and availability throughout the year is also doubtful. To solve this problem developing hatchery technology is need of the hour. The mud crabs, *Scylla serrata* are commercially important and are available throughout the year in the coast. So far there is no established hatchery for these mud crabs and even if there is one the survival rate is very less *i.e.*, only 10-15% because of mass mortality in the first and second zoeal stages. Therefore a study on their embryology can form the baseline to assess the quality and efficiency of the eggs to hatch into healthy first zoea. So, an attempt has been made here to study the embryonic development of the mud crab, *S. serrata*.

Aquaculture production currently relies on wild-caught seed for stocking ponds, as larval rearing at a commercial scale is still difficult. Laboratory experiments of the larval stages of each species should provide valuable information on each species' biological and ecological requirements. 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.

Practically very few hatcheries pay attention to the production of crab seed. Seed production of *Scylla serrata* in different hatcheries of Bangladesh is an uncommon phenomenon. But there is a great potential of this species to introduce in our aquaculture practices. Problems involved in seed production of *Scylla serrata* in different hatcheries are: Lack of availability of mature brood, smaller body size, slower growth rate etc.

Considering the problems, attempt would be made to improve the production of *Scylla serrata* in hatchery condition in order to maintain biodiversity & to protect this species from its extinction which will be helped for the supply of nutrients to the targeted consumers.



1.1 Objectives of the study

- To develop brood in captive condition.
- To observe the embryonic development of *Scylla serrata*.
- To improve the production potential of *Scylla serrata* in the hatchery condition.

2. Literature Review

A major bottleneck to the expansion of mud crab (*Scylla* spp.) aquaculture is a lack of hatchery produced seed. Although research on egg production and larval rearing techniques has been undertaken for the past 30 years (and intensively for the last decade), little information is available in the primary literature. Most of the technical information has been published in the "grey" literature and in research reports and is largely unavailable to the wider scientific community.

Research on the breeding of mud crabs started approximately 40 years ago and several early publications describe captive spawning and larval rearing (Ong, 1964; Ong, 1966; Du Plessis, 1971; Brick, 1974; Hill, 1974). In Japan, research on hatchery production of seed for restocking purposes has been ongoing since 1979 (Fukunaga and Uzumaki, 1982; Horiuti and Yamamoto, 1987; Hasegawa, 1989; Hamasaki, 2002). In the last decade there has been a substantial increase in research, fueled in part by the disease problems in the shrimp industry and partly by declining mud crab fisheries in some parts of South East Asia (Le Vay, 2001).

2.1 Broodstock

2.1.1 Sourcing and maturation

Broodstock are most commonly sourced from the wild. They are either caught specifically for this purpose (Mann *et al.* 1999a), are bought from markets (Dat, 1999a; Quintio *et al.*, 2001) or directly from fishers (Dat, 1999a; Marichamy and Rajapackiam, 2001). In areas where commercial culture is practiced, mature breeders are sourced from production ponds (Millamena and Quintio, 2000). The life cycle can be closed within 9 months (Quintio *et al.*, 2001).

Mud crabs readily mate in captivity. This occurs while the female is in a soft-shell condition following molting. However, adult females caught from the wild have usually mated (Robertson and Kruger, 1994) and carry spermatophores for extended periods, producing viable eggs up to 6 months after capture without the need for mating (Nghia *et al.*, 2001a). Before introducing wild broodstock into the hatchery, the crabs are commonly scrubbed to

remove mud, encrusting algae, infestations and detritus. Individual crabs are usually marked for identification by engraving (Millamena and Quinitio, 2000; Djunaidah *et al.*, 2003; Churchill, 2003) or by gluing an identification tag to the shell. They are then disinfected in a strong formalin bath (50-100 mg/L) for periods ranging from 1 hour (Millamena and Bangcaya, 2001) to overnight (Mann *et al.*, 1999b).

Females also mature their ovaries and extrude eggs in earthen ponds or netted pens. There is some indication in Vietnam that a higher percentage of crabs spawn in ponds, possibly due to reduced stress. The diet available to broodstock in ponds is more varied and probably closer to the one they would encounter in the wild. However, managing broodstock in ponds is more difficult than in tanks and the eggs from broodstock spawned in ponds are occasionally heavily infested with parasites (Quinitio and Parado-Estapa, 2003).

In smaller broodstock maturation systems the water is recirculated, while some larger systems operate on a flow-through basis (Marichamy and Rajapackiam, 2001; Millamena and Bangcaya, 2001; Quinitio *et al.*, 2001; Hamasaki, 2002) or on partial re-circulation (Williams *et al.*, 1998; Mann *et al.*, 1999a 1999b; Millamena and Quinitio, 2000; Baylon *et al.*, 2001a; Millamena and Bangcaya, 2001). Full strength seawater (30-35 g/L) is normally used for captive maturation and spawning of *S. serrata* in the hatchery (Mann *et al.*, 1999a). Lower salinities (20-25 g/L) can be used for *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. However, full strength seawater is still commonly used for maturing and spawning all four species.

Broodstock are generally kept between 25°C and 35°C. A lower critical temperature for egg development of 18°C has been identified (Heasman and Fielder, 1983; Hamasaki, 2002) but successful ovulation and spawning have been observed at water temperatures ranging from 20- 30°C (Mann *et al.*, 1999a; Millamena and Bangcaya, 2001; Davis *et al.*, 2003b). *S. serrata* broodstock extrude significantly larger eggs with higher hatch rates during cooler months (18- 22°C) Mann *et al.*, (1999a) suggesting that lower temperatures than normal should be applied.



Mud crabs are nocturnal and normally inhabit turbid estuaries (Hill 1978; Dat 1999a; Barnes *et al.*, 2002). For this reason broodstock crabs are maintained under low light conditions (Williams *et al.*, 1998; Mann *et al.*, 1999a; Quinitio *et al.*, 2001), although darkness is not required for spawning (Nghia *et al.*, 2001a). Spawning tanks are normally securely covered in order to prevent the crabs from escaping.

Broodstock are normally fed fresh or frozen foods including penaeid shrimp, squid, fish, bivalves such as clams and mussel, gastropod snails and annelid worms (Dat, 1999a; Mann *et al.*, 1999a; Millamena and Bangcaya, 2001; Quinitio *et al.*, 2001; Hamasaki, 2002). Enriched *Artemia* biomass has also been used as food, presented as agar bound pellets (Djunaidah *et al.*, 2003). The natural diet of mud crabs consists mostly of benthic mollusks and crustaceans (Hill, 1979) and it is generally agreed that these organisms must be present in the diet. The source and health status of feed organisms, particularly crustaceans, need to be carefully assessed as they can act as vectors for disease as WSSV (Chen *et al.*, 2000).

2.2 Spawning (egg extrusion)

Mud crabs adapt well to artificial conditions and spawn readily in captivity without intervention. Over 85% of wild caught crabs brought into the laboratory or hatchery normally spawn, mostly within 40 days of stocking (Williams *et al.*, 1998; Mann *et al.*, 1999a; Davis *et al.*, 2003b). The status of the ovary can be assessed by biopsy (Mann *et al.*, 1999a; Millamena and Bangcaya 2001) or by pushing down on the first abdominal segment which exposes the ovary under the carapace (Djunaidah *et al.*, 2003; Quinitio and Parado-Esteva, 2003). Mature ovaries are bright yellow to deep orange in colour and fill the body cavity (Quinitio and Parado-Esteva, 2003).

Captive mud crabs produce viable eggs year round. Some authors have reported seasonal changes and peaks in spawning activity (Mann *et al.*, 1999a; Nghia *et al.*, 2001a; Marichamy and Rajapackiam, 2001; Hamasaki, 2002; Davis *et al.*, 2003b) especially in areas that have extremes in seasonal water temperatures or salinity (Li *et al.*, 1999; Le Vay, 2001). However, these are usually not distinct and spawning patterns seem to vary between locations (Hai *et*

al., 2001; Nghia *et al.*, 2001a). Mud crabs are capable of spawning up to three times from a single mating (Dat, 1999a; Marichamy and Rajapackiam, 2001; Quintio *et al.*, 2001). Rematuration of the ovary in captive crabs occurs about one month after spawning (Hai *et al.*, 2001; Marichamy and Rajapackiam, 2001; Djunaidah *et al.*, 2003).

Broodstock matured in captivity are more fecund and produce eggs with a higher rate of fertilization than wild caught crabs. This is apparently due to enhanced environmental conditions and nutrition (Millamena and Bangcaya, 2001; Quintio *et al.*, 2001). Broodstock domestication is a high priority area of research.

2.3 Incubation and hatching

Brood crabs which have spawned are conspicuous. The large, bright yellow to orange egg mass ("sponge" or "berry") is carried prominently under the abdominal flap. Berried brood crabs are transferred to incubators as soon as possible after spawning. Flat bottomed glass aquaria of 60 L, plastic containers of 100 L or larger fiberglass tanks of approximately 300-500 L (Mann *et al.*, 1999a; Millamena and Quintio, 2000; Millamena and Bangcaya, 2001; Hamasaki, 2002) and 1000 L (Williams *et al.*, 1998) have been used as incubators. Water in the incubators is usually sterilized with UV light and exchanged either on a flow-through basis or recirculated through biofilters (Williams *et al.*, 1998; Mann *et al.*, 1999a; Hamasaki, 2002).

The eggs can be infected with a variety of parasitic worms, fungus (*Haliphthoros*, *Sirolopidium*, *Atkinsiella* and *Lagenidium* spp.) and ciliates (*Zoothamnium* spp.) (Hamasaki and Hatai, 1993a; Churchill, 2003). Parasitic infections generally occur if water quality is not adequately maintained and may result in poor embryogenesis or egg loss as a result of brood crabs tearing at the egg mass. The vulnerability of eggs to fungal infection decreases as their

development progresses (Hamasaki and Hatai, 1993a). Berried brood crabs can be bathed in formalin and/or malachite green as a prophylactic, or can be treated if infestations occur. There is evidence that formalin (25 mg/L) is toxic to eggs up to one day after spawning, although older eggs can tolerate high doses of both formalin (50-150 mg/L) and malachite green (50 mg/L) (Kaji *et al.*, 1991; Hamasaki and Hatai, 1993b; Churchill, 2003; Davis *et al.*,

2003). Heavy aeration is applied during the bathing process. Antifungal agents such as Treflan®1 (44% trifuralin) (0.05 - 0.1 mg/L) or formalin (25 mg/L) can be added to the incubation water resulting in a reduced fungal load of the eggs and preventing transmission of the fungus from the egg surface to the larvae at hatch (Kaji *et al.*, 1991; Qunitio *et al.*, 2001). At 27-28°C first cleavage occurs 5-8 hours after extrusion (Qunitio and Parado-Esteva, 2003). The developing embryo is visible under a dissecting microscope from day 4 after spawning (Djunaidah *et al.*, 2003). Embryonic development can occur successfully between 20 and 30°C but temperatures above 26°C are preferred (Li *et al.*, 1999; Mann *et al.*, 1999a; Qunitio *et al.*, 2001).

Hatching usually occurs in the morning (Hai *et al.*, 2001; Hamasaki, 2002). Brood crabs are often transferred to a separate tank 1-2 days prior to hatching (Hamasaki, 2002; Mann *et al.*, 1999a 1999b). Hatching tanks tend to be larger than 500 L, providing sufficient space for the hatched larvae to disperse and to delay deterioration of water quality. The water in the hatching tank is usually full strength seawater (30-35 g/L) that has been filtered and treated to reduce the microbial load. The larvae hatch as pre-zoeae, a non-swimming phase that typically moults into the first zoeal stage within 10 minutes post-hatch. The persistence of pre-zoeae after the normal initial moult time has elapsed is used as an indicator of poor batch quality (Dat, 1999b; Mann *et al.*, 1999a; Hai *et al.*, 2001).

2.4 Larval rearing

2.4.1 Selection and stocking

Once the eggs have hatched, the brood crab is removed from the hatching tank. Hatch success is generally high, ranging from 80-90%. Larvae are negatively buoyant and swim vigorously in order to maintain their position in the water column. In The Philippines, larvae are left in the hatching tank for approximately one hour after hatching, providing sufficient time for “poorer quality” larvae to sink to the bottom so that “better quality” larvae at the surface can be used for rearing (Qunitio and Parado-Esteva, 2003). The value of this practice needs to be weighed against the fact that Z1 larvae are highly susceptible to fungal infections both from a variety of sources including the egg envelope (Hamasaki and Hatai, 1993a) and in a typical hatching tank of 1 m³, bacterial numbers rise rapidly within an hour of hatching

(Mann *et al.* 1999b). This suggests that larvae be removed soon after hatching (10-15 minutes).

2.5 Water quality and parameters

The larvae require high quality water, free of potential pathogens, predators or parasites. Seawater is commonly filtered to 1 μm and then either chlorinated overnight and dechlorinated with sodium thiosulphate (Parado-Esteba and Qunitio, 1998; Williams *et al.*, 1998; Mann *et al.*, 1999b; Williams *et al.*, 1999; Qunitio *et al.*, 2001), ozonated and then recirculated through a biofilter, inoculated with nitrifying bacteria and settled for several days before use (Baylon and Failaman, 1999; Williams *et al.*, 2002) and/or re-filtered through activated carbon and sterilized with UV light (Dat, 1999b). Water in the rearing vessels is also allowed to stabilize for several days before introduction of the larvae (Parado-Esteba and Qunitio, 1998; Mann *et al.*, 1999b) and background algae are sometimes added for apparent antimicrobial qualities. Water pretreatment has been found to significantly improve survival through to megalopa (M) (Mann, 1999b; Williams *et al.*, 2002).

2.6 Feeding and nutrition

Larvae are fed as soon as possible after transfer into the rearing vessels. High protease activity in newly hatched zoeae indicates their ability to digest food immediately after hatch (Li *et al.*, 1999). Delaying feeding for up to 24 hours after hatching has no significant effect on survival (Lumasag and Qunitio, 1998). However the effects of such starvation on the later stages are not known. Starving larvae for longer than 48 hours induces high mortality despite resumption of normal feeding (Lumasag and Qunitio, 1998; Djunaidah *et al.*, 2003). The point of no return (PNR) for newly hatched larvae has been estimated at 30 hours and 96 hours for *S. paramamosain* and *S. serrata*, respectively (Li *et al.*, 1998 and 1999). In an earlier study Mann and Parlato (1995), found that *S. serrata* Z1 could survive for up to 142 hours without feeding but were not able to moult to Z2. Temperature significantly influences the PNR. Newly hatched *S. serrata* have a PNR50 of 57.6 hours at 28°C and 91 hours at 24°C (Lumasag and Qunitio 1998). Z1 and Z2 are usually fed on rotifers (*Brachionus* spp.) (Li *et al.*, 1999; Mann *et al.*, 1999b; Zeng and Li, 1999; Takeuchi *et al.*, 2000).

3. Materials and Methods

3.1 Brood management in the circular pond

3.1.1 Preparation of pond

At first, all water was removed from the place, and then the place was dried by sunlight. Then every point was prepared by barrier which was made by sand. Bamboo fancy was used inside the barrier. Height of barrier and bamboo fancy was 2 to 3 feet. Area of every point was 50 decimal. Then different types of fertilizer, such as urea, potash etc and calcium oxide were used for preparing every point. In the fry stock pond, crabs were cultured 6 to 7 months. Crabs were collected from the fry stock pond which weight was between 100 to 180 gm. Then, those crabs released in 10 broodstock management ponds and cultured 10 to 12 days for gonad development.

3.1.2 Feeding of broods

Tilapia, parsha and different types of trash fish were given as a live feed for fry of crabs. Feed were supplied twice daily (10% of the body weight). Feed were given in every morning and evening.

3.1.3 Spawning and Incubation

The female stored sperm in seminal receptacles within her body. Fertilized eggs were guarded under the females' abdomen. The incubation period was 12 days. During the incubation period, temperature and salinity were maintained properly.

3.2 Berried Brood collection

Berried crabs were collected from the broodstock management pond of Munshigong. Berried crabs were transformed (tied their chelete legs by roap) into plastic container. Then the broods were kept at "Sundarban Galda Hatchery", Khulna. 10 berried female mud crab, *S. serrata* were collected for the breeding.

3.2.1 Prophylactic treatment of broods

Berried mud crabs were disinfected by 0.4ml/L formalin for 30 minutes for disinfection. This formalin soak was provided for preventing from any kind of contamination. Normally, various fungi and other parasites kept with berried females and it causes higher mortality.

3.2.2 Acclimatization of Broods

After disinfection, the adult mud crabs were acclimatized by maintaining proper salinity concentration, before the initiation of trials on breeding.

3.2.3 Brood stocking

The collected broods were stocked in circular tank of “Sundarban Golda Hatchery” in Khulna with proper sheltering and proper stocking density. Temperature, salinity, and pH were maintained properly.

3.2.4 Feeding of crab

When the berried female mud crabs were kept at circular tank for breeding purpose, no feed was given at that time.

3.3 Breeding techniques

3.3.1 Breeding season

The mud crab, *Scylla serrata* breeds throughout the year. But, the peak season of *Scylla serrata* breeding is September to February. The experiment was conducted at the end of September.

3.3.2 Duration of hatching

Eggs were hatched within over night.

3.4 Observation of embryonic development

Released eggs of mud crabs were transferred at the “Fish Biology Laboratory at Khulna University” to observe the embryonic development by using microscope and photo of embryo were taken by microscopic camera. Eggs were transferred by 500 ml beaker.

3.5 Hatching rate determination

Total 10 berried female mud crabs were kept for hatching. Among them, 8 mud crabs were spawned. Total numbers of hatchling were kept at 500ml beaker. Then, one-fourth milliliter (0.25 ml) samples were counted and then multiplied.

3.6 Fecundity estimation

Fecundity was calculated by gravimetric method. For this, about 10 female broods were collected from the local market. Then the crabs were dissected in the fish biology laboratory and the viscera were removed. The total weight of the gonad was taken by electronic balance. A small portion of the gonad was cut from total gonad and weighed. Then it was allowed to soak in water contained in petridish and the number of the egg was counted with the help of a needle and magnifying glass. The number of egg of an individual was obtained by multiplying the total number of eggs taken from the sample portion with the total weight of the gonad and then by dividing this value by the weight of small portion of the gonad of that particular individual. Thus the fecundity was obtained by using the following formula:

$$F = \frac{\text{Total gonad weight} \times N}{\text{Weight of small portion of total gonad}}$$

Here, N= Number of eggs

3.7 Data analysis

The data obtained were analyzed by using SPSS software. During the time of fecundity estimation, correlation coefficient was calculated by using SPSS program.

4. Results

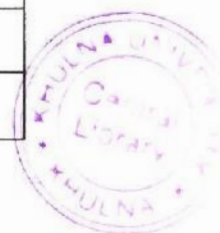
4.1 Brood management in circular pond

4.1.1 Water quality management

Various water quality parameters were maintained properly in the pond during gonad development.

Table 1: Water quality parameters during brood management in circular pond

Parameters	Measures
Water depth of the pond	1.5 m
Salinity of the pond	4 ppt
pH	7.5 to 8.2
Water Temperature	25-28°C



4.1.2 Stocking Density

For each pond, 150 crabs were stocked for gonad development. The Carapace Length (CL), Carapace Width (CW), and Total Weight of crab at the time of stocking in the pond were given as following table.

Table 2: Total Weight, Carapace length and width of crabs during stocking in pond

Serial no.	Carapace length(mm)	Width (mm)	Total Weight (g)
1	106	71	180
2	96	76	180
3	81	60	100
4	83	60	140
5	88	68	139
6	86	66	138
7	91	68	142
8	88	63	122
9	91	66	120
10	88	66	125

4.1.3 Maturation of brood crabs

For the giving of feeds (Tilapia, parsha and different types of trash fish) at the rate of 10% of body weight, gonad development of crabs was occurred within 10 to 12 days and eggs were spawned within 30 days. When the female mud crab was ready to spawn, eggs were bright orange when extruded and turned almost black by the time they were ready to hatch.



Fig 1: Immature eggs of female crab



Fig 2: Mature eggs of female crabs

4.2 Breeding techniques in the Hatchery

4.2.1 Water quality parameters in the hatchery tank

Water quality parameter during breeding is very important. Various water quality parameters were maintained followed at table 3.

Table 3: Various water quality parameters in the hatchery tank

Parameters	Measures
Stocking density of berried female in hatchery tank	10 crabs were stocked
Salinity	30 ppt
pH	7.5 to 7.6
Temperature	26 to 28°C

4.2.2 Water Treatment

At first, water was filtered and then bleaching powder mixed with water at the proportion of 1 kg bleaching at 50 ton water. Then brine water was mixed with water and finally, salinity kept at 30 ppt. Then, water was aerated for 12 hours and again kept for additional 6 hours for settlement. Again, water was filtered.

4.2.3 Duration of hatching

Eggs were hatched within over night because of the maturation of eggs was so high.

4.3 Observation of embryonic development

Released eggs were transferred at the “Fish Biology Laboratory at Khulna University” by 500 ml beaker to observe the embryonic development. Because of no aeration was applied during transportation that’s why maximum embryos were died, some embryos were alive and reared at zoea stage. Embryonic development was observed by using microscope and photos were taken by microscopic camera. Due to the mortality of embryos during the experiment, only one stage named blastula was possible to observe.



Fig 3: Dead egg

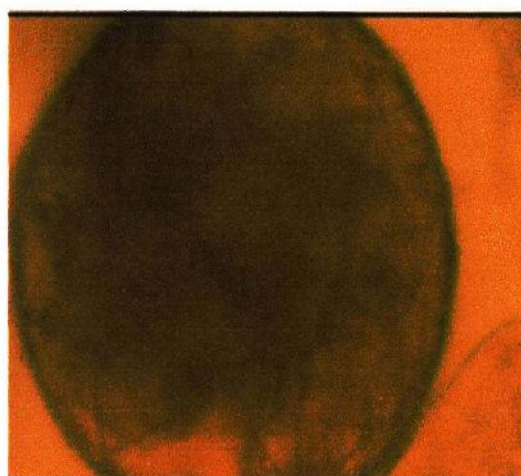


Fig 4: Blastula stage of mud crab

4.4 Assessment of hatching rate

Total 10 berried female mud crabs were kept at hatching tank. From them, 8 female crabs were spawned. Then the hatchlings were taken at 500ml beaker and $\frac{1}{4}^{\text{th}}$ ml (.25ml) sample was taken for three times from the beaker. Then the hatchlings were counted and found 3, 2, 1 number of hatchling respectively from the separate sample. Then the numbers of counted

samples were multiplied. The average hatching rate was 4000/ 8 individual, approximately 5% of hatching rate.

The result of the calculation of hatching success was given as following table no 4.

Sample no.	Hatchlings /0.25ml solution	Total no. of hatchling in each sample
1	3	6000
2	2	4000
3	1	2000

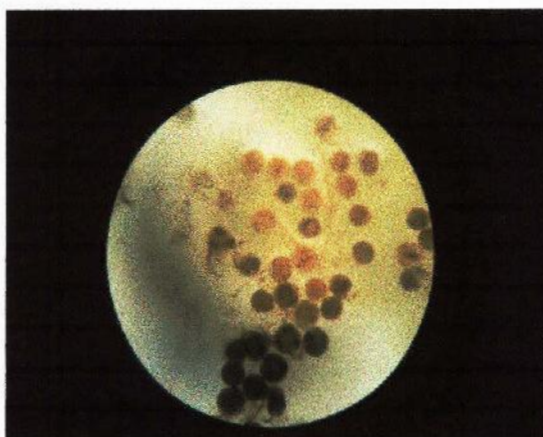


Fig 5: Newly hatched egg

4.5 Larval Rearing Stag

Newly hatched zoea was observed by microscope at Fish Biology Laboratory under Fisheries and Marine Resource Technology Discipline, Khulna University. Unfortunately, all the zoea was died before reaching at the megalopa stage. So, it was impossible to observe rest of larval stage of mud crab, *S. serrata*.

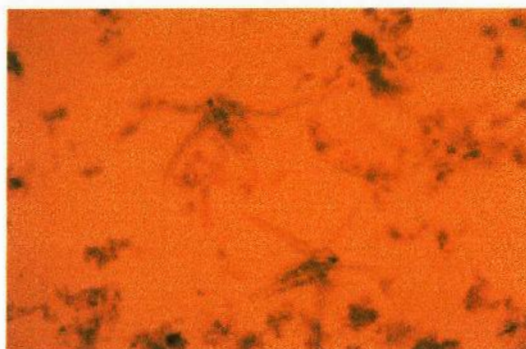


Fig 6: Newly hatched Zoea stage

4.6 Fecundity estimation

For fecundity study, about 10 specimens of various sizes of crabs were collected. The carapace widths of the experimental mud crab were ranging from 75 to 87. The total gonad weights were ranging from 34g to 45 g. And total weight of the specimens ranged from 175g to 225g. The lowest fecundity was found at 9, 00,000 and the highest was at 11, 00,000 and the average fecundity were observed to be 9, 75,000. Fecundity estimation of mud crab, *Scylla serrata* was showed at following table 5.

Table 5: Fecundity estimation of mud crab, *Scylla serrata*.

Serial No.	Carapace Width (mm)	Total Gonad Weight (g)	Total Body Weight (g)	Fecundity
01	76	34.0	177.5	9,00,000
02	84	42.2	218.5	10,00,000
03	77	35.0	180.0	9,30,000
04	87	45.0	225.0	11,00,000
05	80	38.4	200.0	9,50,000
06	85	43.0	220.0	10,10,000
07	75	34.0	175.0	9,00,000
08	80	38.0	190.0	9,30,000
09	86	43.0	217.0	10,80,000
10	81	38.2	210.0	9,50,000

Regression analysis was done by using SPSS software to show the relationship between carapace width and fecundity, total gonad weight and fecundity and between total body weight and fecundity. A strongly positive linear relationship was found for each case.

A positive linear relationship was found between fecundity and carapace Width (CW) and total gonad weight (Fig 7 and 8).

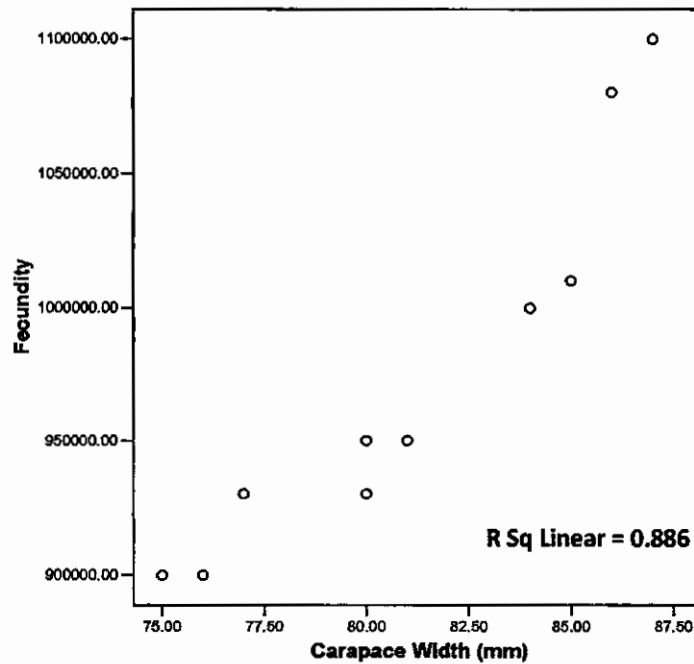


Fig 7: Relationship between Carapace Width and fecundity

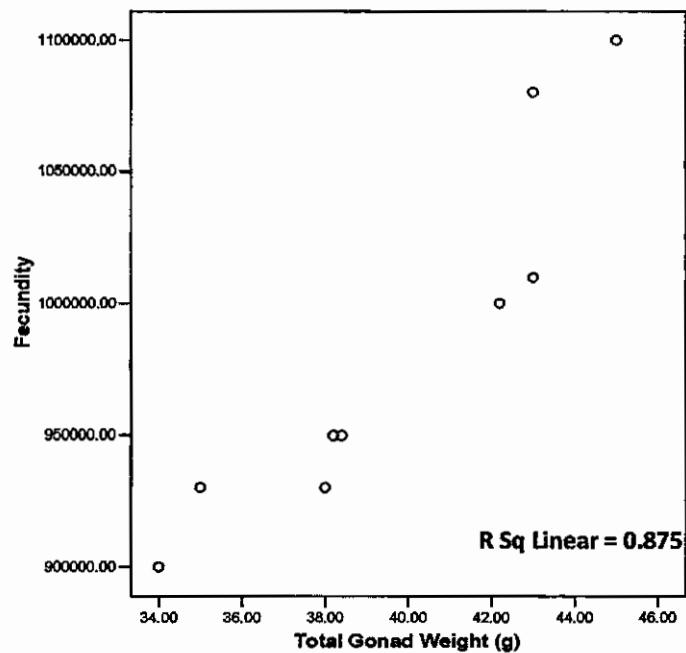


Fig 8: Relationship between Total Gonad Weight and fecundity

A positive linear relationship was found between fecundity and total weight (Fig 9).

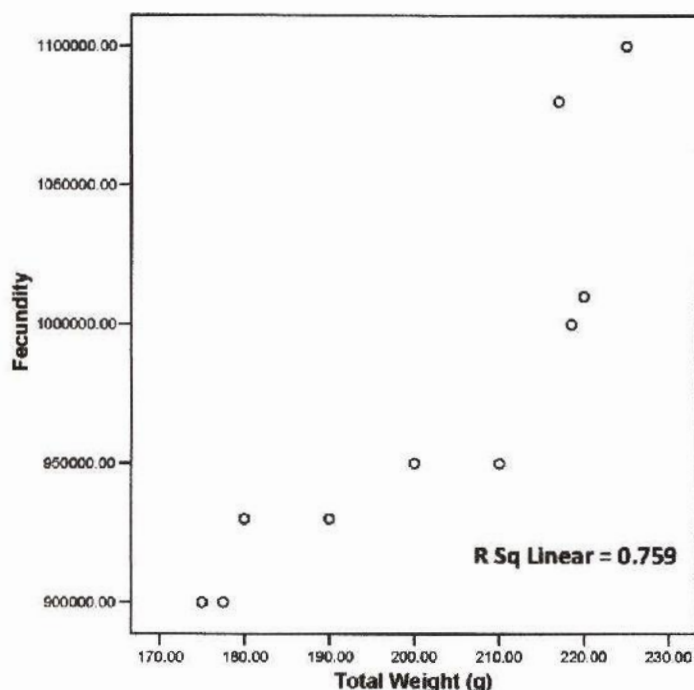


Fig 9: Relationship between Total Weight and fecundity

The correlation co-efficient for the relation between fecundity and carapace width (Fig 7) was 0.886 and for the correlation between fecundity and total gonad weight (Fig 8) was 0.875. This indicated that both the two relationships were so strong. Again the correlation co-efficient for the relation between fecundity and total body weight (Fig 9) was 0.759 that means fecundity was strongly correlated with the body weight.



5. Discussion

This experiment demonstrated the breeding techniques and the embryonic development of mud crab, *Scylla serrata*. In view of food value of *S. serrata*, it is very important to have data on the brood stock development, reproductive biology of the species particularly in relation to fecundity and hatching, for better scientific management conservation of the species.

5.1 Broodstock management of mud crab

In the current study, various water quality parameters were maintained during broodstock management. The salinity of water was 4 to 8 ppt, temperature of water was 25 to 28°C and pH was 7.5 to 8.2. Stocking density of fry was 150m per 50 decimal pond area. Whereas, Hill (1994) showed for the broodstock development salinity is needed 15 ppt and temperature at 31.2 °C.

Various trash fish such as tilapia, chingri and snails were given for attaining very fast gonad development. The feeding frequency was once in daily and usually it was given in the morning. It is generally accepted that broodstock feed quality and quantity are important determinants of egg quality and viability (De Culuwé *et al.* 1995a). This has led to numerous investigations on the relationships between broodstock diet and the resulting lipid and fatty acid composition of eggs and larvae (De Caluwé *et al.* 1995a, Furutia *et al.* 2000, Millamena and Quinitio 2000).

5.2 Maturation of mud crab

In the current study, it is observed that mud crab eggs were bright orange when extruded and turned almost black by the time they were ready to hatch. Whereas, the color of the crab eggs ranged from pale yellow to orange-red. Previously egg color was considered to be an indicator of egg quality in *Scylla serrata* (Ong 1966, DuPlessis 1971).

In this study, it is found that the carapace width ranged from 7.5 to 9 cm during maturation. According to Jayamanna and Jinadasa (1993), wild *S. serrata* crab attained first maturity at 12 cm CW and produced about 3,00,000 larvae. However, Robertson and Kruger (1994) obtained a wild mature female with only 10.4 cm CW. Another studies indicated *Scylla serrata* become reproductively mature starting at around 90 mm carapace width, often within the first year of life (Robertson and Kruger 1994, Knuckey 1996).

5.3 Breeding techniques of mud crab

In the current study, it is found the temperature at 26 to 28°C and the incubation period was 12 days. Whereas, Marichamy and Rajapackiam (1991) reported 7 to 14 days at 25 to 30°C which supported my result. The incubation period of *S. serrata* eggs at 18 to 20°C was two to three times longer than at 26 to 28°C (Heasman and Fielder 1983).

5.4 Observation of embryonic development

In the current study, maximum mortality of embryos was found due to the low dose of formalin and lack of aeration during transformation of embryos. For this reason, only one stage named blastula was possible to observe. Whereas, Ates *et al.* (2011) found 10 distinct stages of embryo of mud crab, *S. serrata*. In another study, Soundarapandian and Tamizhazhagan (2006) observed five stages of embryonic development of *S. serrata*.

5.5 Assessment of hatching rate

In the current study, the average hatching was found 4000/ 8 individual, approximately 5% of hatching rate. On contrary, Soundarapandian and Tamizhazhagan (2006) found 75% of hatching rate of *S. serrata*. Hai *et al.* (2001) observed hatching in the incubator tank occurred in the morning for 94% of the crabs.

5.6 Larval Rearing Stage

In the current study, newly hatched zoea was observed. Unfortunately, all the zoea was died before reaching at the megalopa stage. So, it was impossible to observe rest of the stage of life cycle of mud crab. Whereas, Marichamy and Rajapackiam (1991) reported 3 to 4 days duration for each of the 5 zoea stages and 8 to 11 days for megalopa at 27 to 30°C. Mortality was highest during metamorphosis from zoea 5 to megalopa and from megalopa to crab 1 as many dead larvae were siphoned out from the tank bottom.

Another study Marichamy and Rajapackiam (1984) have described the morphological features and development process of various stages of larvae. There were five zoea stages, each of duration 3-4 days, and a megalopa stage of 8-11 days in the complete larval development of *S. serrata*

Due to the lacking of berried crabs, another trial of this experiment wouldn't possible. This is a drawback of this research work. Further research is needed to get better findings of embryonic development and hatching rate, observation of larval rearing of mud crabs.

5.7 Fecundity

Fecundity among egg-laying animals is defined as the number of eggs being readily for the next spawning by a female (Royce, 1972). Fecundity appears to bear some broad relationship to the care or nurture accorded to the eggs (Lagler *et al.* 1967). Knowledge about fecundity of a species is essential for evaluating the commercial potentialities of its stock, life history, practical culture and actual management of the fishery (Lagler 1956; Doha and Hye, 1970). According to Vazzoler (1982); Lobao *et al.* (1985) and Valenti *et al.* (1989), the knowledge of fecundity of a species is important to evaluate its potential for commercial farming as well as for estimation of reproductive potential and the stock size of natural population. Santos (1978) defined fecundity as the number of eggs laid by one female per hatching. However, Bond and Buckup (1982) defined fecundity as the number of eggs laid per hatching that was found to adhere to the female pleopods. Shrivastava (1999) referred fecundity as the number of eggs borne in the brood pouch during a single spawning act. The number of eggs contained in the ovary of a fish is termed the fecundity (Nikolsky, 1963).

Fecundity in most crab genera generally increases with size (prasad and Neelakantan 1989; Jones 1993; Haddon 1994; Lardies and Wehrtmann 1996; Mantelatto and Fransozo 1997). Although this trend was observed for mud crabs in this study, high variability within size classes resulted in a poor CW and fecundity. Mud crabs are known to be able to fertilize more than one batch of eggs (usually 2 to 3) from a single mating (Ong 1966; Heasman *et al.* 1985; Millamena and Quinitio 2pQO; Djunaidah *et al.* 2001).

In the present study, the carapace widths of the experimental mud crab were ranging from 75 to 87mm. The total gonad weights were ranging from 34g to 45 g. And total weight of the specimens ranged from 175g to 225g. The lowest fecundity was found at 9, 00,000 and the highest was at 11, 00,000 and the average fecundity were observed to be 9, 75,000. Whereas, Srinivasagam *et al.*, (2000) found fecundity 10,00,000 to 23,00,000 with the carapace width 85 to 137 mm, Jyamanna and Jinadasa (1993) found 15,00,000 to 30,00,000 fecundity and Millamena and Bang-caya (2001) found the fecundity of 12,00,000 to 16,00,000 for the species of *S. serrata*.

The correlation co-efficient for the relation between fecundity and carapace width (Fig 7) was 0.886 and for the correlation between fecundity and total gonad weight (Fig 8) was 0.875. This indicated that both the two relationships were so strong. Again the correlation co efficient for the relation between fecundity and total body weight (Fig 9) was 0.759 that means fecundity was strongly correlated with the body weight.

6. Conclusion

After the disease outbreak in shrimp farming, crabs have become candidate species for culture practices because of their export potential. Recently the demand and price of mud crab have increased drastically. Consequently, the access of new fishers in crab harvesting from coastal waters has increased manifolds. As a result, there is huge overfishing exists on the species which could lead to extinction from our natural source if the present catching rate continues further. The continuous collection of wild seeds for grow-out culture due to the expanding export market for mud crab and the search for alternative culture species for shrimps has threatened the wild stock population. Practically very few hatcheries pay attention to the production of crab seed. Seed production of *Scylla serrata* in different hatcheries of Bangladesh is an uncommon phenomenon. But there is a great potential of this species to introduce in our aquaculture practices. The results of the present study could be further tested for continued better production of seeds of this species. Attempt would be made to improve the production of *Scylla serrata* in hatchery condition in order to maintain biodiversity & to protect mud crab from its extinction.

7. References

- Ali, M.Y., Kamal, D., Hossain, S.M.M., Azam, M.A., Sabbir, W., Mushida, A., Ahmed, B. and Azam, K. 2004. Biological studies of the mud crab, *Scylla serrata* (Forskål) of the Sundarbans mangrove ecosystem in Khulna region of Bangladesh. Pakistan J. Biol. Sci., 19967 (11): 1981-87.
- Azam, K., D. Kamal and M. Mostafa. 1998. Status and potential of mud crab (*Scylla serrata*) in Bangladesh. In: Rahman, M.A. M.S Shah, M.G. Murtaza, and M.A. Matin (eds.). Proc. Nat. Sem. Integr. Manage. Ganges Floodplains and Sundarbans Ecosystem, July 16-18, 1994. Organized jointly by Khulna University, Bangladesh Agricultural Research Council and Department of Agricultural Extension. Khulna University, Bangladesh, pp. 150-160.
- Baylon, J.C. Failaman, A. and Tanate, J. 2001a. Comparison of different feeding schemes and feeding density on survival and development of the mud crab *Scylla serrata* zoea. Page 15 in Book of Abstracts: 2001 Workshop on Mud Crab Rearing, Ecology and Fisheries. Institute for Marine Aquaculture, Can Tho University, Vietnam, 8th-10th January 2001.
- Bond, G. and Buckup, L., 1982. O ciclo reprodutor de *Macrobrachium borellii* (Nobili, 1896) e *M. potiuna* (Müller, 1880) (Crustacea, Decapoda, Palaemonidae) e suas relações com a temperatura. Rev. Brasil. Biol., 42(3): 473-483 pp. (Publish in Portuguese).
- Brick, R.W. 1974. Effects of water quality, antibiotics, phytoplankton and food on survival and development of *Scylla serrata* (Crustacea: Portunidae). Aquaculture. 3: 231-244.
- Catacutan, M.R. 2002. Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. Aquaculture, and offspring quality in mud crab (*Scylla paramamosain*) broodstock fed different diets. Aquaculture International. 11(1-2).

- Catacutan, M.R., Eusebio, P.S. and, Teshima, S. 2003. Apparent digestibility of selected feedstuffs by mud crab, *Scylla serrata*. *Aquaculture* 216: (1-4) 253-261.
- Chang, C.F. and Wu, C.H. 1985. Studies on the production of mud crab, *Scylla serrata* (Forskål) – observations on the larvae development. *Bulletin of Taiwan Fisheries Research Institute*. 39: 33-45.
- Chen, L.L., Lo, C.F., Chiu, Y.L., Chang, C.F., and Kou, G.H. 2000. Natural and experimental infection of white spot syndrome virus (WSSV) in benthic larvae of mud crab *Scylla serrata*. *Diseases of Aquatic Organisms*. 40: (2) 157-161.
- Cowan, L. 1984. Crab farming in Japan, Taiwan and the Philippines. Information Series Q184009, Queensland Department of Primary Industries, Brisbane, Queensland. 85 mCrustaceana 70(2): 214-226.
- Davis, J.A., Van Blerk, L.L., Kirby, R. and Hecht, T. 2003b. Genetic variation of the mud crab *Scylla serrata* (Forskål, 1775) (Crustacea: Portunidae) in South African estuaries. *African Zoology*. In press. 208(1-2): 113-123.
- De Caluwé, J., Lavens, P. and Sorgeloos, P., 1995a. The influence of *Macrobrachium rosenbergii* broodstock diet on egg and larval characteristics. Larvi '95 – Shellfish Larviculture Symposium. European aquaculture society, special Publication No. 24, Gent, Belgium. 1995.
- Department of Agriculture (DA), Region VI. 1988. Fattening mud crabs *Scylla serrata* in bamboo cages. Fish Extension Rep., Dept. of Agriculture, BFAR, Philippines. Development, dry weight and chemical composition of larvae of the mud crab *Scylla paramamosain*. Pages 159-166 in Keenan, C.P. and Blackshaw, A. W. (Eds.), Mud crab aquaculture and biology: Proceedings of an international scientific forum April 21-24. 1997. Australian Centre for International Agricultural Research, Canberra.



- Djunaidah, I.S., Wille, M., Kontara, E.K. and Sorgeloos, P. 2003. Reproductive performance and offspring quality in mud crab (*Scylla paramamosain*) broodstock fed different diets. *Aquaculture International*. 11(1-2).
- Doha S, Hye M A. 1970. Fecundity of the Padma River *Hilsa ilisha* (Hamilton). *Pakistan J Sci* 22, 176-183.
- Du Plessis, A. 1971. A preliminary investigation into the morphological characteristics, feeding, growth, reproduction and larval rearing of *Scylla serrata* Forskål, held in captivity. Unpublished report of the Fisheries Development Corporation of South Africa. 24 pp.
- FAO, 2002. Farming freshwater prawns. A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). Fisheries Technical Paper 428. FAO, Rome, Italy.
- Felix, S., Gazendran, M. and Subramaniam, S. 1995. Aquaculture of mud crab. *Seafood Export. J.*, 26(6):5-6
- Fukunaga K. and Uzumaki K. 1982. Production of hatchery-raised juvenile crab of *Scylla tranquebarica*. *Saibaigiken*, 11(1), 45-53.
- Furutia, H., Tanaka, H., Yamamoto, T., Shiraishi, M. and Takeuchi, T., 2000. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 187, pp. 387-398.
- Hai, T. N., Hassan, A. Law, A.T. and Shazili, v.N.A. 2001. Some aspects on maturation and spawning performance of mud crabs (*Scylla* sp.) in captive conditions. Page 8 in Book of Abstracts: 2001 Workshop on Mud Crab Rearing, Ecology and Fisheries. Institute for Marine Aquaculture, Can Tho University, Vietnam, 8th-10th January 2001.

- Hamasaki, K. and Hatai, K. 1993a. Experimental infection in the eggs and larvae of the swimming crab *Portunus trituberculatus* and the mud crab *Scylla serrata* with seven fungal strains belonging to *Lagenidiales*. Bulletin of the Japanese Society of Scientific Fisheries. 59(6): 1059-1066.
- Hamasaki, K., Suprayudi, M.A. and Takeuchi, T. 2002a. Effects of dietary (n-3) HUFA on larval morphogenesis and metamorphosis to megalops in seed production of the mud crab, *Scylla serrata* (Brachyura: Portunidae). Suisanzoshoku. 50(3): 333-340.
- Hamasaki, K., Suprayudi, M.A. and Takeuchi, T. 2002b. Mass mortality during metamorphosis to megalops in the seed production of mudcrab *Scylla serrata* (Crustacea, Decapoda, Portunidae). Fisheries Science. 68(6): 1226-1231.
- Harrison, K.E., 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. Journal of Shellfish Research 9(1):1-28.
- Hasegawa Y. 1989. Mud crab. 1987 Annual Report of the Kochi Prefectural fisheries experiment station. Asian Fisheries Science. 14(2): 201-210.
- Heasman, M.P. and Fielder, D.R. 1983. Laboratory spawning and mass larval rearing of the mangrove crab *Scylla serrata*, from first zoea to first crab stage. Aquaculture. 34: 303-326.
- Hill, B.J. 1979. Biology of the crab *Scylla serrata* (Forskål) in the St Lucia system. Transactions of the Royal Society of South Africa. 44(1): 55-62.
- Hill, B.J. 1979. Biology of the crab *Scylla serrata* (Forskål) in the St Lucia system. Transactions of the Royal Society of South Africa. 44(1): 55-62.

- Horiuti, T. and Yamamoto Y., 1987. Production of hatchery-raised juvenile crab of *Scylla tranquebarica*. Annual Report of Shizuoka Prefectural Thermal Effluent Utilization Research Center. 1986: 63-69.
- Jayamanna, S.C. and J. Jinadasa 1993. Size maturity and spawning periodicity of the mud crab *Scylla serrata* (Forsk.) in Negombo estuary. Journal of Natural Science Council- Sri Lanka 21:141-152.
- Jones, D.A, Yule, A.B. and Holland, D.L. 1997. Larval nutrition. Pages 353-389 in Abramo, L., Conklin, D.E. and Akiyama, D.M. (Eds.), Crustacean nutrition – advances in world.
- Keenan, C.P. 1995. Genetic relationships of mud crabs, genus *Scylla*, throughout the Indo-West Pacific. Pages 11-23 in Evans, L. (Ed.), Proceedings of a mud crab workshop held on 27th October 1995 at the Aquatic Science Research Unit, Muresk Institute of Agriculture, Curtin University of Technology, Broome, Western Australia.
- Keenan, C.P. and Blackshaw, A. W. (Eds.), Mudcrab Aquaculture and Biology. Proceedings of an International Scientific Forum held in Darwin, Australia, 21-24 April 1997. ACIAR proceedings No. 78. Watson Ferguson and Company, Brisbane, Australia.
- Khan, M.G. and Alam, M.F. 1991. The mud crab (*Scylla serrata*) fishery and its bio-economics in Bangladesh. BOBP/REP 51: 29-40.
- Kuntiyo, A. 1992. Fattening of mud crab, *Scylla serrata* Forskal , in net cages installed in the drain canal of intensive prawn ponds fed with trash fish and prawn pellet. MS Thesis. University of the Philippines in the Visayas. pp. 60.
- Lagler, K.F. 1956. Enumeration of fish eggs. In freshwater fishery biology. 2nd Edn. W.M. Brown company publishers. Dubuque: 106-110.
- Lagler, K.F., Bardach, T.E. and Miller, R.R. 1967. Ichthyology. John Willey & Sons. Inc. New York, London, Sydney: 271-274.

- Lardies, M.A., and I.S. Wehrtmann, 1996. Aspects of the reproductive biology of *Petrolisthes laevis* (Guerin 1835) (Decapoda, Anomura, Porcellanidae). Part I: Reproductive output and chemical composition of eggs during embryonic development. *Archive of Fishery and Marine Research*. 43(2): 121-135.
- Le Vay, L. 2001. Ecology and management of mud crab *Scylla* spp. *Asian Fisheries Science*.
- Li, S., Zeng, C., Tang, H., Li, F., Wang, G., Cheng, Y. and Lin, Q. 1999. Investigations into the reproductive and larval culture biology of the mud crab, *Scylla paramamosain*: a research overview. Pages 121-124 in Keenan, C.P. and Blackshaw, A. W. (Eds.), *Mud Crab Aquaculture and Biology. Proceedings of an International Scientific Forum held in Darwin, Australia, 21-24 April 1997*. ACIAR proceedings No. 78. Watson Ferguson and Company, Brisbane, Australia.
- Liong, P.C. 1993. The culture and fattening of crabs. *INFOFISH International* 3:46-49.
- Lobão, V. L., Valenti, W. C. and Mello, J. T. C., 1985. Fecundidade em *Macrobrachium carcinus* (L.) do Rio Ribeira de Iguape. *Bol. Inst. Pesca*, 12(3): 1-8pp. (Publish in Portuguese).
- Lumasag, G. and Quintio, E.T. 1998. Ingestion rate of mud crab (*Scylla serrata*) larvae after delayed feeding. Page 29 in *Extended Abstracts: International forum on the culture of portunid crabs, Boracay, The Philippines, 1-4 December 1998*.
- Mantelatto, F.L.M., and A. Fransozo. 1997. Fecundity of the crab *Callinectes ornatus* Ordway, 1863 (Decapoda, Brachyura, Portunidae) from the Ubatuba region Sao Paulo, Brazil.
- Marichamy, R. and Rajapackiam, S. 2001. The aquaculture of *Scylla* species in India. *Asian Fisheries Science*. 14(2): 231-238.
- Millamena, O.M. and Bangcaya, J.P. 2001. Reproductive Performance and Larval quality of mud crab *Scylla serrata* (Forskål). Pages 41-48 in Evans, L. (Ed.), *Proceedings of the Mud crab workshop conducted by The Aquatic Science Research Unit Muresk*

Institute of Agriculture Curtin University of Technology. 27 October 1995, Broome, Western Australia.

- Millamena, O.M. and Quintio, E.T. 2000. The effects of diets on the reproductive performance of eyestalk ablated and intact mudcrab *Scylla serrata*. Aquaculture. 181(1-2): 81-90.
- Nghia, T.T., Wille, M. and Sorgeloos, P. 2001a. Effects of light, eyestalk ablation and seasonal cycle on the reproductive performance of captive mud crab (*Scylla paramamosain*) Broodstock in the Mekong Delta, Vietnam. Pages 4-5 in Book of Abstracts: 2001 Workshop on Mud Crab Rearing, Ecology and Fisheries. Institute for Marine Aquaculture, Can Tho University, Vietnam, 8th-10th January 2001.
- Nikolsky, G.V. 1963. The Ecology of Fishes. Academic Press London, London, 352 pp.
- Ong, K.S. 1964. The early development stages of *Scylla serrata* reared in the laboratory. Proceedings of Indo Pacific Fisheries Council, (11): 135-146.
- Ong, K.S. 1966. Observations on the post-larval life history of *Scylla serrata* reared in the laboratory. Malaysian Agricultural Journal. 45: 429-445.
- Prasad, P.N and Neelakantan, B., 1989. Maturity and breeding of the mud crab, *Scylla serrata* (Forsk.) (Decapoda: Brachyura: Portunidae). Proceedings of the Indian Academy of Science (Animal Sciences), 98(5), pp. 341-349.
- Quintio, E.T. and Parado-Esteva, F.D. 2001. Simulated transport of *Scylla serrata* zoeae at various loading densities. Asian Fisheries Science. 14(2): 225-230.
- Quintio, E.T. and Parado-Esteva, F.D. 2003. Biology and seed production of mud crab. SEAFDEC/AQD extension manual. In press.
- Robertson, W. D. and Kruger, A. 1994. Size at Maturity, Mating and Spawning in the Portunid Crab *Scylla serrata* (Forsk.) in Natal. South Africa. Estuarine, Coastal and Shelf Science. 39: 185-200.

- Royce W F. 1972. Introduction to the Fishery Science. Academic Press, New York. 251p
- Saha, M.R. and Ahammad, S.U. 1999. *Fattening Technique of Mud Crab*. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. 8 pp.
- Samarasinghe, R.P., Fernando, D.Y. and de Silva, O.S.S.C. 1992. Pond culture of mud crab in Sri Lanka. In : The mud crab. C.A. Angel (ed.). pp 161-164. Proceedings of the seminar on mud crab in Surat Thani, Thailand, 5-8 November 1991. Bay of Bengal Programme, Madras.
- Santos, E. P., 1978. *Dinâmica de populações aplicada à piscicultura*. Hucitec/Edusp, São Paulo, 130pp. (Publish in Portuguese).
- Sastry, A.N. 1983. Pelagic larval development. Pages 215-282 in Bliss, D.E. (Ed. in chief). The biology of crustacea volume 7: behaviour and ecology. Vernberg, F.J. and Vernberg, W.B. (Eds.), Academic Press, New York, USA.
- SEAFDEC. 1998. News letter Vol. 21, No. 1, January to March. http://www.spc.org.nc/Coastfish/News/Fish_News/85/NIAR_4.htm.
- Sheen, S.S. 2000 Dietary cholesterol requirement of juvenile mud crab *Scylla serrata*. Aquaculture. 189 (3-4): 277-285.
- Shen, Y. and Lai, Q. 1994. Present status of mangrove crab (*Scylla serrata* (Forskål)) culture in China. Naga, the ICLARM Quarterly, January 1994: 28-29.
- Shrivastava, C.B.L., 1999. A text book of fishery Science and Indian fisheries. Kitab Mahal, India.
- Sivasubramain, K and Angel, C.A. 1992. A review of the culture, marketing and resources of the mud crab. In : The mud crab (ed. C.A. Angel). Bay of Bengal Program, Madras. India. pp. 5-12.



3
Srinivasagam, S., Kathirvel, M. and Kulasekarapandian, S. 2000. Captive broodstock development, induced breeding and larval stages of mud crabs (*Scylla* spp.). Bulletin No. 12. March 2000. Central Institute of Brackishwater Aquaculture (Indian Council of Agricultural Research), Chennai, India.

Takeuchi, T., Kobayashi, T., Shimizu, T. and Sekiya, S. 2000. The necessity and suitable feeding schedule of *Artemia* nauplii for larval mud crab. Bulletin of the Japanese Society of Scientific Fisheries. 66(6): 984-992.

Tan, E.S.P. 1999. Malaysian Crab research. Pages 25-26 in Keenan, C.P. and Blackshaw, A. W. (Eds.), Mud Crab Aquaculture and Biology. Proceedings of an International Scientific Forum held in Darwin, Australia, 21-24 April 1997. ACIAR proceedings No. 78. Watson Ferguson and Company, Brisbane, Australia.

Teshima, S. and A. Kanazawa. 1983. Variation in lipid composition during the ovarian maturation of the prawn. Bulletin of the Japanese Society for Science and Fisheries, 49(6):957-962.

Triño, A.T. Millamena, O.M. and Keenan, C.P. 2001. Pond culture of the mud crab *Scylla serrata* (Forskål) fed a formulated diet with or without vitamin or mineral supplements. Asian Fisheries Science. 14(2) 191-200.

Valenti, W. C., Mello, J. T. C. and Lobão, V. L., 1989. Fecundidade em *Macrobrachium acanthurus* (Wiegmann, 1836). Rev. Bras. Zool., 6: 9-15pp. (Publish in Portuguese).

Vazzoler, A. E. A., 1982. Manual de métodos para estudos biológicos de populações de peixes: reprodução e crescimento. CNPq, Programa Nacional de Zoologia, Brasília, 108p. (Publish in Portuguese).

Warner, G.F. 1977. The Biology of Crabs. Paul Elek (Scientific Books) Ltd., London. 800 pp.

Wicklins, J. F. and D. O'C. Lee. 2002. Crustacean farming. ranching and culture, second

Zafar, M. and Siddiqui, M.Z.H. 2000. Occurrence and abundance of four Brachyuran crabs in the Chakaraia Sundarban of Bangladesh. The Chittagong Univ. Jour. Sci., 24(2): 105-110.

Photo Gallery



Fig: Mud Chari at Laboratory



Fig: Aeration for Berried Female Crab



Fig: Salinity Checking



Fig: Temperature measurement



Fig: Hatchling of *Scylla serrata*



Fig: Counting of Hatchlings

Photo Gallery

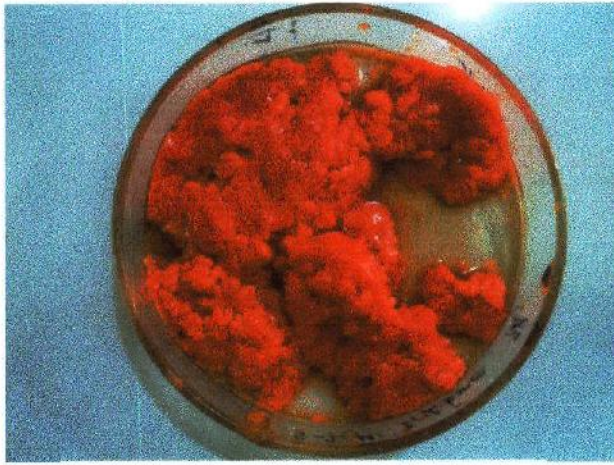


Fig: Gonad of mud crab



Fig: Counting of eggs of *Scylla serrata*