Bangor University

DOCTOR OF PHILOSOPHY

Assessment of the feasibility of stock enhancement of mud crabs, Scylla paramamosain, in the Mekong Delta, Vietnam

Vu Ngoc, Ut

Award date:
2002

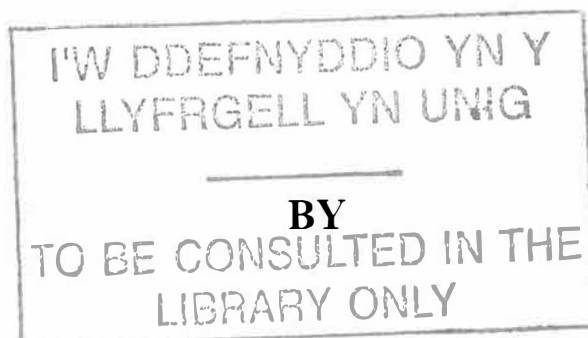*Awarding institution:*
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ASSESSMENT OF THE FEASIBILITY OF
STOCK ENHANCEMENT OF MUD CRABS,
SCYLLA PARAMAMOSAIN,
IN THE MEKONG DELTA, VIETNAM

BY

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**A thesis submitted in fulfilment of requirements for the degree
of Philosophiae Doctor in the University of Wales.**

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December, 2002

ACKNOWLEDGEMENT

Firstly, I would like to thank my supervisor Prof. Dr. David Jones, for giving me this opportunity to finish my doctorate and for his guidance.

From the bottom of my heart, I am sincerely grateful to my co-supervisor Dr. Lewis Le Vay, for his excellent suggestions, advice, criticism and guidance throughout the field work, data analysis and write-up of this thesis, especially his perceptive comments on various aspects of the work. Without his help this study would never have been completed. To him I express my sincere appreciations and respect.

I am very grateful to Dr. Vu Do Quynh and Drs. Truong Trong Nghia, former Directors of Institute for Marine Aquaculture, Agriculture College, Can Tho University, for their guidance and support from the very beginning and throughout the doctorate.

I gratefully acknowledge Dr. Nguyen Anh Tuan, Dean of College of Aquaculture and Fisheries, Can Tho University, for his encouragement and support during writing up of this thesis.

I am obliged to Le Van Liem, Tran Thi Hong Hanh and Huynh Thanh Toi, staff of College of Aquaculture and Fisheries, Can Tho University for their great assistance during the laboratory and field work. Many thanks are also due to all my colleagues in College of Aquaculture and Fisheries, Can Tho University for their support and friendship.

Special thanks are expressed to Mark Walton, staff of School of Ocean Sciences, University of Wales, Bangor for his help and friendship during my time in Bangor.

Last, but not least, I would like to express my gratitude to my beloved wife for her encouragement, understanding, patience and standing beside me throughout my work, especially for her suffering the cold to take care of me during the last 6 months of my doctorate work in the UK. My endless love is for her forever.

SUMMARY

In an attempt to investigate the feasibility of stock enhancement of the predominant mud crab species, *Scylla paramamosain* in the Mekong Delta, Vietnam, a number of investigations were undertaken including collection of background fishery information, abundance, recruitment, population dynamics, growth, effects of tagging on growth and survival of crabs, and fitness of hatchery-reared and wild crabs. Results from the investigation of seasonal abundance and recruitment of *S. paramamosain* in a selected estuarine mangrove area revealed that they are persistent throughout the year, despite the freshwater period during the monsoon season. However, the peak in abundance coincided with the dry season when salinity is higher (15-20ppt). Salinity tolerances/preferences in juveniles under laboratory conditions indicated that crabs are not able to survive in 0ppt for more than one week and the most preferable salinities are 15-20ppt. This range of salinity is consistent with the peak abundance of crabs in the wild but the mechanism for survival of wild crabs in freshwater is not known. Application of microwire tagging in *S. paramamosain* showed no significant effects on growth and survival of crabs. Juvenile crabs as small as 20 mm CW can be tagged successfully. Using this tagging method in a mark-recapture study has, for the first time, enabled determination of growth rate in *S. paramamosain* under natural conditions. The growth rate was similar to that obtained in pond conditions and indicates that crabs may attain an adult size 3-4 months after release. This is encouraging for future potential stock enhancement. However, abundance estimates for *S. paramamosain* from the mark-recapture study showed that monthly recruitment in the selected study area over a twelve month sampling period in 2000 was fairly high, with a sharp peak in March of 1,269,809 month^{-1} falling to 114,512 month^{-1} in November. This indicates that a restocking program in this area may be unworkable as a huge number of hatchery-reared juveniles would need to be produced to make a significant difference to the already substantial natural influx of recruits. Fitness of hatchery-reared crabs was found to be affected by several factors including nutrition and rearing techniques and still needs to be improved, although they were found to exhibit superior growth than their wild conspecifics in one experiment. Production of hatchery-reared juveniles through the nursery phase may be successful, with application of bricks as shelters and shrimp as diet. However,

further investigations of suitable stocking densities, use of shelters/substrates and diets to support economical effectiveness are required.

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CHAPTER I

GENERAL INTRODUCTION

INTRODUCTION

The mud crabs *Scylla* spp., are portunid crabs which are distributed throughout the Indo-Pacific region, typically found associated with mangroves, especially in estuaries and sheltered muddy coastal habitats. They are considered one of the most commercially important mangrove species for fishery and aquaculture, supporting artisanal, small-scale fisheries as well as supplies of juveniles for pond culture. However, the production of mud crabs has recently declined due to both over-fishing 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 (Le Vay, 2001).

Aquaculture of mud crabs may offer potential to compensate for these losses. Artificial production of mud crabs could help to mitigate pressure on fishery resources (Overton and Macintosh, 1997). However, due to the low level of success in hatchery production of mud crabs, fisheries are still playing a very important role in supporting aquaculture. Consequently, Sivasubramaniam (1992), Fortes (1999) and Cholik (1999) all report that seed supply for pond culture relies on wild caught juveniles. Proper management of fisheries is an important component of aquaculture development.

In an attempt to maintain the stability of the natural production of mud crabs in the Mekong Delta, the study of population dynamics can support evaluation of the potential for stock enhancement. Release of hatchery-reared mud crabs coupled with mangrove reforestation and protection could help restore the wild crab populations to the level of sustainable management (Overton and Macintosh, 1997). Keenan (1999) mentioned that one of the major problems in effective mud crab management and aquaculture is the existence of a number of genetically distinct species. However, this was recently resolved by Keenan *et al.* (1998) who have described four species of *Scylla* existing in the Indo-Pacific region, with two species present in the Mekong Delta (*S. paramamosain* and *S. olivacea*). Little is known about the biology, ecology, population dynamics, fishery and culture of these two recently separated species. However, information of biology, ecology, fishery and culture of the genus *Scylla* are

well documented in the literature, and should be reviewed as basic information for further studies.

BIOLOGICAL ASPECTS

Taxonomy

Until recently the taxonomy of the mud crabs, *Scylla* spp. has been considerably confused (Keenan *et al.*, 1998). According to Overton (1999), previous studies on the taxonomy of the genus had been based on traditional descriptive methods involving relatively few specimens and/or samples from a restricted area. This has created discrepancies between published descriptions, leading to much of the confusion on the taxonomic status of the genus. Many papers have described species of *Scylla* found in the Indo-Pacific region (Forskal, 1775; Fabricius, 1798; Dana, 1852). Forskal (1775) described the species as *Cancer serratus*, but did not mention the type locality. Fabricius (1798) described *Portunus tranquebaricus* based on a specimen obtained from Tranquebar, India. De Haan (1833) chose the genus name *Scylla* and Dana (1852) described *Scylla tranquebarica*, variety *oceanica*. Estampador (1949) used the colour markings on the walking legs, swimming legs and chelipeds, spines on the carpus of chelipeds, the position of the frontal teeth and their habitats to recognise two species and one variety of *Scylla* in the Philippines, namely *S. oceanica, S. tranquebarica* and variety *paramamosain*. Estampador's (1949) work was supported by Serene (1952), who also recognised four forms from Nha Trang, Vietnam based on spination and colour.

However, Stephenson and Campbell (1960) when studying the systematics of Australian portunid crabs did not agree with Estampador (1949) and Serene (1952) in their recognition of three species and one subspecies of *Scylla* and suggested that more work was needed. Further studies on the taxonomy of portunid crabs in India tended to agree with Estampador (1949), that two species were present, namely *S. serrata* and *S. tranquebarica* (Kathirvel, 1981; Radhakrishnan and Samuel, 1982; Joel and Raj, 1983). Kathirvel and Srinivasagam (1992) concluded that there are at least two distinct species in India, namely *S. serrata* and *S. tranquebarica*. These were

characterised by differences in size, spines on the outer border of the carpus of the cheliped and habitat preferences.

In a study of genetic variability of the genus *Scylla*, Fuseya and Watanabe (1996) used horizontal starch gel electrophoresis to study variation in 11 enzymes at 17 allozymic loci. Their results suggested that the genus included at least three species.

With the aim of understanding the biological basis for the presence of more than one species of *Scylla* in southeast Asia, Overton (1999) applied genetic, morphometric and ecological techniques to study crabs from several locations within the region, two located in the southwest of Thailand, one in the Mekong Delta of Vietnam and one in the east of Malaysia. Three more additional sites were selected as Chantaburi, northern Gulf of Thailand, Thai Binh province, northern Vietnam and Paikgasir, southern Bangladesh. Results of a canonical variate analysis showed there were three clusters, suggesting three phenotypic groups of *Scylla* from the seven locations sampled in southeast Asia. Genetic studies by Sugama and Hutapea (1999) in Indonesia indicated there were three species of *Scylla* in this area too, with little genetical difference between these species.

In an attempt to clarify three genetically different species existing in Thailand, Klinbunga *et al.* (2000) examined *Scylla serrata* (Forskaal), *S. oceanica* (Dana), and S. *tranquebarica* (Fabricius), collected from two locations in eastern Thailand (Chanthaburi and Trat) by randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). The authors concluded that there are three clearly different species rather than a single panmictic species exhibiting different morphs.

Recently a definitive study carried out by Keenan *et al.* (1998) used morphological and genetical techniques to revise the taxonomy of the genus. According to these authors, there are four distinct species, *S. serrata*, *S. paramamosain*, *S. tranquebarica* and *S. olivacea*. These four species can be distinguished by the slope of the frontal lobe, presence or absence of spines on the outer face of the carpus of the chelipeds and colouration (Keenan *et al.*, 1998).

The systematic taxonomy of *Scylla* genus, following Keenan *et al.* (1998) is:

Phylum:	Arthropoda
Class:	Crustacea
Order:	Decapoda
Family:	Portunidae
Genus:	*Scylla*
Species:	

Scylla serrata (Forskal, 1775)

Scylla paramamosain (Estampador, 1949)

S. olivacea (Herbst, 1796)

S. tranquebarica (Fabricius, 1798)

Because of the confused taxonomic history, it is not always clear in the literature which species has been studied. This review will therefore use the generic term *Scylla*, except where species identity is known.

Distribution

Distribution of *Scylla* species through the Indo- Pacific waters in separate or sympatrical territories is summarised in the following Table.

Table 1: Distribution of *Scylla* genus, according to Keenan *et al.* (1998)

Species	Region
Scylla serrata	- Indo-West-Pacific
	- Red Sea
	- South Africa
	- Western Australia
	- East coast of Australia
	- Pacific Ocean: Fiji, Solomon Islands and New Caledonia
	- Gulf of Carpentaria
	- Philippines
	- Okinawa, Japan

S. paramamosain	-	Mekong Delta, Vietnam
	-	Central Java, Indonesia
	-	South China Sea: Xiamen, China
	-	Hongkong
	-	Singapore
	-	Cambodia
S. olivacea	-	Indian Ocean: Western Australia; Phuket, Thailand; Karachi, Pakistan
	-	Pacific Ocean: Philippines; Thailand, Singapore, Vietnam, Malaysia, Southern Taiwan
	-	Arafura Sea: Timor, Indonesia; Gulf of Carpentaria
S. tranquebarica	-	Indian Ocean: Karachi, Pakistan; Penang, Malaysia
	-	Pacific Ocean: Panay Is., Philippines
	-	South China Sea: Malaysia; Singapore

Scylla olivacea is found mostly in the Philippines and Malaysia. Both *S. olivacea* and *S. tranquebarica* would appear to be centralised in the South China Sea, where *S. serrata* is almost completely absent (Keenan, 1999).

Recent study on the mitochondrial DNA of *Scylla serrata* carried out by Gopurenko *et al.* (1999) found that there are two clades of *Scylla serrata,* in which one is strictly confined to northern Australia and the other is widespread throughout the Indo- West-Pacific. This result was supported by a very recent study of Fratini and Vannini (2002), confirming the occurrence of *Scylla serrata* populations within a larger area of the Indian Ocean region.

In Vietnam, the *Scylla* species have also been confused in terms of nomenclature and species names. They had all been considered to be *Scylla serrata* for many years (Hoang Duc Dat, 1992; Nguyen Anh Tuan *et al.*, 1996; Nguyen Van Tien *et al.*, 1996). Following Keenan *et al.* (1998) it is clear that there are two species present in

Vietnam, *S. paramamosain* and *S. olivacea*. The species *S. serrata* is not reported for this region.

Life cycle

The first description of the early stages of mud crab larvae was made by Ong (1964). After hatching the first zoea undergoes five moult stages becoming zoea 5 within a period of 17-20 days. From zoea 5, the larvae metamorphose into megalopae and become juveniles within 8-11 days after only one other moult. The juveniles progress through 16-18 moults to become mature within at least 338-523 days (Ong, 1966). Observations of the post-larval life history of *Scylla serrata* reared in the laboratory showed that the minimum developmental period from the time of hatching to the metamorphosis into the first crab instar was 30 days. Generally, the life cycle of crabs is divided into 4 stages, namely the larvae, the juveniles (20-70 mm CW), the sub-adult (70-150 mm CW) and the adult (150 mm CW or above). Thomas *et al.* (1987) suggested that *S. serrata* may live for 3 years.

Larval development

Larval development of *Scylla spp* from zoeal stages to early crab stages was studied and described by Ong (1964, 1966). Recent work by O'Kelly *et al.* (1998) describes the development of *S. paramamosain* larvae. Under laboratory conditions, at temperatures between 18-24 °C, hatching of the eggs takes place over a 1-2 h period (Hill, 1974). Sivasubramaniam and Angell (1992) state that from eggs to first zoeal stage, the hatching process takes 16 to 17 days at a temperature of 23-25°C. The first 4 zoeal stages take 2 or 3 days for each intermoult, while the zoea 5 spends 3 or 4 days before moulting to become a megalopa. The megalopa requires 7 or 8 days to metamorphose to the first crab stage. According to Jamari (1992) it took 25 to 28 days for larvae of *Scylla* sp to develop from zoea 1 stage to crab 1. However, studies by Marichamy and Rajapackiam (1992) on the larval rearing of *Scylla* sp. in India revealed that the minimum period for metamorphosis of the larvae into the crab stage from hatching is 24-30 days. Lee (1992) studying the ecology and fisheries of *Scylla serrata* in Queensland stated that incubation of eggs took 12 days at 27 °C and 32ppt, metamorphosis of larvae to first crab stage occurring after 37 days. Anil and Suseelan

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(1999) successfully reared *Sc ylla* sp. from egg to crab instar under laboratory conditions. They reported a maximum period of egg incubation of 13 days, with time from hatching to megalopa of 17 days and from megalopa to crab 1 a further 6 days.

Effects of salinity, temperature, light regime, food and antibiotics on the development of larvae have been studied by DuPlessis (1971), Brick (1974), Hill (1974), Heasman and Feilder (1983), Djunaidah *et al.* (1998) and Baylon and Failaman (1999). Heasman and Feilder (1983) indicated that low temperature is a likely critical factor accounting for extremely poor survival rate of larvae. They also observed that the zoeal development time in *Scylla* sp. varies only marginally over rearing temperatures in the approximate range 22-28°C and salinity range 30-35ppt. Temperature, salinity and their interaction effect on the development of the crab larvae were also studied in detail by Hill (1974). Marichamy and Rajapackiam (1992) reported that temperature and salinity have a significant effect on survival and development of the larvae. According to these authors, the most suitable range of temperature is 27-30°C, with salinity around 35 ppt. In a recent study on the effect of salinity on the metamorphosis of *Scylla serrata*, Zhongli *et al.* (2001) found that the larval cycle is extended and survival rate decreased if salinity is lower than 10ppt. Manjulatha and Babu (1998) reported that *Scylla serrata* and *S. oceanica* could survive sudden fluctuations of salinity, but prolonged exposure to lower salinity below 10ppt in *Scylla oceanica* resulted in poor feeding and reduced growth. In an attempt to clarify suitable salinity for culture of *Scylla serrata* and *S. oceanica*, Babu (1998) found that *Scylla serrata* prefers comparatively low saline water <20ppt and *Scylla oceanica* is a good species for brackish water aquaculture where the salinity is >10ppt.

It is clear from various reports that a critical period of larval rearing of mud crab is during the zoeal stage, especially Zl and Z2. Thorson (1950) cited by Harms and Seeger (1989) stated that the main factors affecting larval survival are temperature, predation and food limitation. Brick (1974) found that survival of *Scylla sp.* zoeae was enhanced by high concentrations of food organisms. He indicated that an increase in the concentration of *Artemia* nauplii from 5 to 16 nauplii per ml increased the rate of metamorphosis successfully from zoea to megalopa. In addition, the rate of larval production could be considerably increased with improved feeding strategies and water quality management (Marichamy and Rajapackiam, 1992).

Experiments on the effects of diet density on feeding rates of *Scylla* spp. from hatching through metamorphosis showed that with larval development and strengthened swimming ability, the mud crab larvae probably shifted from passive feeding at early stages to actively pursuing prey at the later stages (Zeng and Li, 1999). The results also suggested that when early larvae of the mud crab were subjected to unfavourable feeding conditions, including both quality and quantity of diets, they tended to intensify their feeding at later stages to compensate for the nutrition deficits inflicted at the early stage. This was confirmed by Baylon and Maningo (2001) who studied the feeding of *Scylla olivacea* zoeae and megalopae on *Brachionus* and *Artemia* nauplii and reported that feeding behaviour of the larvae changes as they grow. In the early stages, they are passive feeders but as they grow, they are able to pursue prey actively.

Jamari (1992) found that a feeding regime for the larvae including *Brachionus* sp. and frozen *Artemia* nauplii during the zoea stages resulted in a better survival rate than live *Artemia* nauplii alone. Results of a recent study carried out by Takeuchi *et al.* (2000) on the necessity and suitable feeding schedule of *Artemia* nauplii for larval *Scylla tranquebarica* revealed that larvae fed *Artemia* nauplii at zoea 3 stage showed higher survival rate than those fed earlier or later. This study also indicated there is requirement of n-3 HUFA (highly unsaturated fatty acid) in live food to attain high survival rate of first crab stage. Kobayashi *et al.* (2000) reported that mud crab larvae require EPA (eicosapentaenoic acid, 20:5n-3) for survival, while DHA (decosahexaenoic acid, 22:6n-3) is required for the growth of the carapace.

Growth

In *Scylla* spp. male crabs grow faster than females (Manganpa *et al.*, 1987). The males grew at an average rate of 1.3 g. day^{-1}, while the females grew only 0.9 g. day^{-1}. Triño *et al.* (1999) also reported that the performance of male crabs in ponds is better than females. They found males attained a significantly higher final weight and specific growth rate than females. The crabs raised as mixed sex groups grew more slowly than males or females kept separately.

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Growth of mud crabs is affected by temperature. Luo and Wei (1986) reported that juveniles of *Scylla* sp. moult more frequently and grow rapidly with a rise of water temperature within the suitable temperature range. Lee (1992) observed that growth of mud crab ceases in winter, when temperatures drop below 20°C. Growth of *Scylla* was also measured in laboratory conditions by Ong (1966) who suggested that the average growth of *Scylla* under natural conditions is probably faster than even the fastest observed under laboratory rearing conditions. This conclusion was supported by Manjulatha and Babu (1998) as they stated that the growth of *Scylla* sp. attained in the ponds is significantly higher than in the laboratory, in spite of good water quality. Ong (1966) reported that the crabs undergo several moults during their growth but moult frequency and moult increment are not the same for every stage. In addition, the percentage moult increments, based on the mean carapace widths of the different instars, were greater for the early crab stages and decreased in the later stages. As a result fast growers are at a more advanced stage than slower growers. Also individuals of the same instar varied in size. Van Engel (1958) cited by Ong (1966) reported that female portunids may be the only crustaceans known to complete their growth at the time they become sexually mature, so that the difference in size of mature females would be due to the different percentage increases in size at each moult. Thomas *et al.* (1987) studied growth of *Scylla* sp in India and found that growth determined by a monthly mode curve indicates that *Scylla serrata* can grow up to 112, 151.5 and 187.5 mm carapace width in the first, second and third years, respectively.

Stephenson and Campbell (1960) cited by Ong (1966) recorded *Scylla* specimens from the natural habitat as large as 190 mm CW for males and 165 mm CW for females; and berried *Scylla* ranging from 92-151 mm in carapace width. However, differences in maximum sizes reported in the literature are likely to represent different species (Le Vay, 2001).

In the culture condition, growth of juvenile crab can be induced by treatment with diethylstilbestrol (Wang and Li, 1989). The authors revealed that with 14 or 18 μ g diethylstilbestrol per gram diet per 3 days resulted in notably accelerated growth.

Feeding

Feeding habits of mud crabs are reported to change with age and remain to be studied in detail. Prasad and Neelakantan (1988) found detritus was the major food group in gut analysis of juvenile mud crabs (<70 mm CW) while in sub-adults (81-110 mm CW) and adults (>110 mm CW) it was the remains of crustaceans and fish. Adult *Scylla* sp. in India are reported to be omnivorous scavengers, feeding predominantly on molluscs and crustacea, as well as fish, plants and detritus (Kathirvel and Srinivasagam,1992). Jayamanne (1992) indicated that mud crab juveniles of 20-70 mm CW feed mainly on crustaceans, sub-adults of 70-130 mm CW prefer bivalves and gastropods while the larger crabs consume small crabs and fish.

Analyses of the foregut contents of *S. serrata* in South Africa show that it is a predator of sessile or slow-moving benthic macro-invertebrates, chiefly molluscs (Hill, 1976). Plant material was not uncommon in *S. serrata* foreguts. It may be part of the natural diet of juveniles (Veerannan, 1974), but it is not known whether this plant material forms part of the normal diets of adults or whether it was merely swallowed along with other food (Hill, 1976). Hill (1976) also reported that in both South African and Australian crabs, 50% of the identifiable material in the foreguts are molluscs, 20-22% crustaceans, and the remaining 28-30% consisting of small amounts of plants and debris. In *S. serrata*, where the foreguts were less than 50% full, inorganic material made up nearly 100% of the content, thus showing that the crabs tend to swallow a great deal of apparently indigestible material. Genodepa (1999) investigated the growth rate of mud crabs in mangrove pen culture and suggested that the crabs did not benefit much from the presence of mangroves although plant or vegetable material as observed as part of their natural diet.

Like many crustaceans, *Scylla serrata* in South Africa and *Scylla* sp. in India were found to be nocturnal in their feeding activity (Hill, 1976; Joel and Raj, 1986). Becker and Wahl (1996) also found that *Scylla* sp. in Thailand are nocturnally active, the crabs spending most of the night either walking over the bottom or stationary, but not buried. The former behaviour is of obvious importance in locating slow-moving or sessile prey. The latter case is part of hunting behaviour as crabs may catch any animals wandering within catching distance. Similarly, Lavina and Buling (1977) also concluded that mud crabs feed more at night.

Temperature is a factor affecting feeding and activity of crabs. Hill (1980) found that maximum rates of feeding and activity in *Scylla serrata* occur around 25°C. When the temperature drops below 20°C, feeding and activity decreases sharply. Salinity is also reported to be a factor affecting feeding of crabs (Manjulatha and Babu, 1998). The authors indicated at salinity lower than 10ppt, feeding activity of *Scylla* sp. was severely curtailed.

Under culture conditions, daily diets for crab larvae and juveniles need to be supplemented with fatty acids and cholesterol to enhance survival and growth. Youzhu *et al.* (2001) reported that elevation of the content of eicosapentaenoic acid (EPA) and decoxahexaenoic acid (DHA) will favour the survival and growth of larval *Scylla serrata*. Sheen (2000) found that there is a requirement of cholesterol in juvenile *Scylla serrata* which improves survival and growth. The optimal dietary cholesterol requirement was found to be of 0.5%.

Maturation

The size of female crab at first maturity is 120 mm CW but seems to vary with region and probably species (see Table 2). The estimated pre-spawning and post-spawning fecundities are around 3 and 1.5 million (Jayamanna and Jinadasa, 1993). Devi (1985) found size at which *S. serrata* bear eggs is from 57 mm to 118 mm carapace length, while Prasad and Neelakantan (1989) reported that females of *S. serrata* attained sexual maturity after reaching 80 mm carapace width and above. A sharp transition at 80 mm CW indicates that morphological changes accompany sexual maturity.

Quinn and Kojis (1987) studied the reproductive biology of *Scylla* spp. in Labu estuary in Papua New Guinea and reported that *Scylla serrata* becomes sexually mature at a small size (100 mm carapace width) while *S. paramamosain* reaches maturity at a bigger size (120 mm CW). A recent study carried out by Overton and Macintosh (2002) on size at sexual maturity for females of two sympatric species, *S. paramamosain* and *S. olivacea* in Ban Don Bay, Thailand indicates that 50% of female *S. paramamosain* and *S. olivacea* are mature at 110.5 mm ICW (inner carapace width) and 91.2 mm ICW, respectively. The authors also recorded the

smallest sizes at mature for female *S. paramamosain* and *S. olivacea* are 101 mm ICW and 83 mm ICW, respectively.

Recent studies in northern Australia have shown the transition of immature *Scylla* sp. to physiological maturity probably occurs between 90-110 mm carapace width (Heasman *et al.*, 1985; Knuckey, 1996). In a similar study in South Africa, Robertson and Kruger (1994) revealed that 50% of male crabs produced sperm at 92 mm carapace width. However, Knuckey (1996) studied maturity in male *Scylla serrata* and identified 3 stages in male maturity. The first transition characterised by small-clawed "adolescent" crabs occurred from 90-110 mm carapace width (CW) as immature crabs attained physiological maturity. The second transition was from 140-160 mm CW crabs as adolescent males developed the large-claw "adult" morphology. The functionally mature males are characterised by presence of "mating scars" on their front ambulatory legs and sternum. Nagabhushanam and Farooqui (1984) found that secretory activity of the Y-organ cells of *Scylla* sp. shows a correlation with testis maturation, indicating that Y-organ factors are probably responsible for testis maturation.

Temperature affects the maturity of mud crabs. Fielder and Heasman (1978) suspected that higher water temperature in the tropics may increase the crab growth rates and therefore decreases time to maturity. Heasman (1980) concluded that *Scylla serrata* populations appear to reach sexual maturity at a smaller size in the tropics compared to subtropical populations. According to Poovachiranon (1992), in the tropics, *Scylla* sp. become sexually mature at a smaller size compared with crabs from subtropical regions. However, again this may reflect species differences (Le Vay, 2001).

Mud crabs are highly fecund producing up to 2 million eggs at a time (Hill, 1974), from 1 to 7 million eggs per spawning (Lee, 1992), 2-3 million per female (Hongxi *et al.*, 1998) or even from 2 to 21 million embryos per spawning (Onyango and Kudoja (1995). Tiensongrusmee and Pratoomchat (1999) stated that between 9.3- 10.6 cm CW a mud crab produces from 1.7 to 3.2 million eggs. Studies on decapod life histories and reproductive dynamics in relation to oceanography off southern Africa, Pollock and Melville-Smith (1993) reported that despite having different larval life

histories, both the shallow-water continental shelf spiny lobster and crab (*Scylla serrata*) have substantially smaller eggs and higher values of egg per recruit than the deep water spiny lobster. They raised the hypothesis that due to high rate of mortality of larvae and juvenile stages in the nearshore coastal environment an evolutionary response of these species was to have a higher egg per recruit value, so that a small sized egg is an adaptation to allow for larger brood sizes and greater egg production in shallow-water species.

Details on age and size at maturity are given in Table 2.

Table 2: Size at maturity of *Scylla* spp.

Area	Species	Size at maturity	Source
Papua New Guinea	Not known	100 –120 mm CW	Quinn and Kojis (1987)
India	Not known	For females: 91-100 mm CW (50 % of females mature) For males 81-90 mm but at 97 mm (50% of male mature)	Prasad and Neelakantan (1989)
Thailand (Andaman Sea)	Not known	At 110 mm CW or maturity index value 0.88	Poovachiranon (1992)
Thailand (Ranong)	Not known	Great majority at 100-115 mm CW	Macintosh *et al.* (1993)
Thailand	Not known	85 – 90 cm CW, about 10-12 months old from juveniles	Tiensongrusmee and Pratoomchat (1999)
Thailand (Ban Don Bay)	*S. paramamosain*	Females: 110.5 mm ICW (50% of females mature)	Overton and Macintosh (2002)
	S. olivacea	Females: 91.2 mm ICW (50% of females mature)	Overton and Macintosh (2002)
Sri Lanka	Not known	120 mm CW	Jayamanna and Jinadasa (1993)
South Africa	*S. serrata*	Females: 123 mm CW (50% of	Robertson and

(Natal)		females mature)	Kruger (1994)
		Males: 92 mm CW (50% of males mature)	
South Africa	*S. serrata*	83-144 mm CW with age of 1-1.5 years	Hill (1975)
South Africa (Eastern Cape Estuarine)	*S. serrata*	Females: 130-140 mm CW Males: 120-130 mm CW	Robertson (1996)
China	Not known	108 mm CW with age of above 1 year	Hongxi *et al.* (1998)
Australia	*S. serrata*	Females: 128 mm CW Males: 165 mm CW	Heasman *et al.* (1985)
Vietnam	*S. paramamosain*	Females: 80 mm CW	Vu Ngoc Ut *et al.* (1998)

Mating behaviour

As in other portunid crabs (Hartnoll, 1965) copulation in *Scylla* can only occur between hard-shelled males and soft-shelled females within a day or so of the pubertal moult of the female. During copulation, a male approaches a female in premoulting condition climbs over her, clasps her with his chelipeds and the anterior pair of walking legs, and carries her around. They may remain so paired for 3 to 4 days until the female moults. The male then turns the female over for copulation, which usually lasts 7 to 12 h or even 26 h (Lavina and Buling 1977). These authors also observed copulation lasting for 7 days with a break in between. Although the spermatozoa of *S. serrata* are non-motile (Bhavanishankar and Subramoniam, 1997), sperm can be retained by the female and fertilisation may not take place for many weeks or even months after spawning (Chen, 1976). It was reported by Le Reste *et al.* (1976) that mating may precede spawning by as little as 5 weeks. However, according to Du Plessis (unpublished data cited by Heasman *et al.*, 1985) it may occur as much as 7 months prior to spawning. Perrine (1978) and Heasman (1980) reported that mating in *Scylla* species may occur between free-ranging partners or within the confines of intertidal burrows. Robertson and Kruger (1994) stated that mating activities of *Scylla*

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serrata occur in all months of the year in South Africa. In addition, Heasman *et al.* (1985) concluded that mating of *Scylla* occurs to some extent throughout the year in Moreton Bay, Queensland. However, mating activity in a southern Queensland population of *S. serrata* was at a maximum level in mid-spring and late summer-early autumn. These authors also suspected that mating intensity is related to seasonality as their results indicate a spring-summer maximum, decreasing to a winter minimum. However the results were not statistically significant and should be treated with caution. When studying the abundance, breeding and growth of *Scylla serrata* in South Africa, Hill (1975) found that mating crabs were caught in most months from October to May, with peak activity in December at a carapace width of 103 to 148 mm for females and 141 to 166 mm for males.

ECOLOGICAL ASPECTS

Habitat

Scylla species are predominantly found in shallow marine environments, estuarine and mangrove areas. They are strongly associated with mangrove swamps and nearby intertidal and subtidal muddy habitats throughout the Pacific and Indian Oceans and form the basis of substantial fishery and aquaculture operations (Keenan, 1999).

The distribution patterns of decapod crustaceans have been studied by Miller and Maurer (1973) and Mair (1980). They have suggested that among many field and experimentally measured variables, salinity was a principal limiting factor for a number of species. Chandrasekaran and Natarajan (1994) studied the seasonal abundance and distribution of juvenile mud crab in southeast India. They found that in October, when the salinity was very low (1.5-2ppt) due to maximum rainfall, the population density of juveniles of *S. serrata* was at minimum. Hill (1979) reported that a minimum salinity necessary for the survival of juveniles of *S. serrata* in South Africa was 2ppt. Therefore high mortalities of *S. serrata* may occur in periods of freshwater flooding (Macnae, 1968; Hill, 1975). Le Reste *et al.* (1976) also found an inverse relationship between rainfall and annual catch of adult *Scylla serrata* in South Africa.

Dhavale (1988) found that *Scylla* spp. can withstand high salinity and low temperature better than low salinity coupled with high temperature. Davenport and Wong (1987) indicated that *Scylla* sp. proved to be powerful osmoregulators in dilute media. These authors also mentioned that they osmoconformed at high salinity and showed no ability to discriminate between salinities. However, Keenan *et al.* (1998) suggest that the different *Scylla* species may have different salinity preferences, determining their distribution as well as their larval growth and survival. *Scylla serrata* is found predominantly in the oceans where salinity is always greater than 34 ppt. The other three species are generally found in areas where salinity is less than 33 ppt (Lewis & Campbell, 1967 cited by Keenan *et al.*, 1998). Keenan *et al.* (1998) described different habitats for the different species. *Scylla serrata* occurs in association with mangrove forest inundated with full salinity oceanic water for the greater part of the year. *Scylla paramamosain* associates with shallow denuded coral, shallow subtidal flats and estuarine ponds and mangrove forest. The other two species, *Scylla tranquebarica* and *S. olivacea* are associated with mangrove forest and coastlines inundated with reduced salinity seawater for a certain period a year such as during the wet season. However, further work is needed to determine the different habitat requirements of the four species.

Hill (1975) and Hill *et al.* (1982) studied the abundance and distribution of *Scylla serrata* populations in Australia (tidal flats) and South Africa (estuaries) by means of a mark-release- recapture method. They found that abundance of *Scylla serrata* in the water differs from stage to stage. Juveniles up to 80 mm carapace width were found abundantly on the intertidal flats, while sub-adults and adults were more abundant in subtidal areas. They estimated densities of about 1 crab.124 m^{-2}, and a production of 3.4g.m^{-2} per annum. Joel and Raj (1986) studied the relative abundance of the portunid crabs in Pulicat Lake, India in relation to ecological parameters such as salinity, dissolved oxygen, nature of the bottom, depth, turbidity, temperature, direction of wind, tidal current and found that optimum light is believed to be the major factor in the distribution of the two species of *Scylla* found in this area.

Observations on the movement of mud crabs, by Sivasubramaniam and Angell (1992) found that in long channels which extend further away from the sea, the females

moved over 6.6 km while males covered only around 3.7 km. Similarly Hyland *et al.* (1984) reported that two categories of movement of *Scylla serrata* occur, a free-ranging type and an offshore migration by females. They also mentioned that crabs in narrow creeks with mangrove-covered banks displayed little movement. However, in areas with large intertidal flats bare of mangroves, crabs underwent more movement and the adults generally moved over a distance of 3.9 km per day. Lee (1992) also reported that *Scylla serrata* move locally up to 4km.

Seasonality in spawning

Reproductive activity in *Scylla serrata* occurs year-round at low latitudes and seasonally at higher latitudes (Quinn and Kojis ,1987). Heasman *et al.* (1985) stated that the length of spawning season increases with decreasing latitude, so that distinct seasonal peaks in the spawning activity are not evident in populations from the equatorial tropics. They also hypothesised that in tropical estuaries the periods of peak productivity coincide with highly available nutrient periods when abundance of food organisms is available to potential spawners. Still, reproductive peaks are apparent in tropical populations, associated with seasonal rainfall.

The spawning season of *S. serrata* peaks from the end of May to the end of September (Arriola, 1940) in the Philippines. Peaks in the number of reproductive crabs were reported in Papua New Guinea from April-June and September-October (Quinn and Kojis, 1987). They also found that reproduction of *Scylla serrata* occurs continuously throughout the year.

According to Robertson and Kruger (1994), spawning in *Scylla* in South Africa appears to occur throughout the year with peak activity from late spring to early autumn. Although breeding all through the year, *S. serrata* exhibited two peaks, one between Dec-Mar and another in Sep-Nov.

Seasonality in spawning/ maturity of mud crabs *Scylla* spp. is summarised in Table 3.

Table 3: Spawning/maturity season of *Scylla* sp.

Area	Species	Periods	Source
Tropical areas			
India	Not known	- Peak in April-June and September-February - In Southwest coast, throughout the year. Peak season in Sept-February In Pulicat: peak in Mar-Apr and Sept-Oct - In Kakinada: Nov-April, peak in December and February	- Marichamy *et al.* (1992) - Kathirvel & Srinivasagam (1992) - Devi (1985)
Hawaii	Not known	Spawning in early May to late October	Brick (1974)
Sri Lanka	Not known	- 2 seasons: April and August	Jayamanne and Jinadasa (1992)
Philippines	Not known	June – September	Arriola (1949)
Indonesia	*S. paramamosain*	- All -year-around in lagoon	Suwarso and Wasilun (1991)
Papua New Guinea	Not known	- Peak in April-June and September-Octobor	Quinn and Kojis (1987)
Thailand (Ademan sea)	Not known	- Reproduction occurs continuously year around. Peak in GSI from Oct – Dec	Poovichiranon (1992)
Thailand (Ranong)	Not known	- GSI peak in September, main egg carrying and spawning period from July – December	- Macintosh *et al.* (1993)
Vietnam	*S. paramamosain*	- Peak from December- February	Hoang Duc Dat (1992)
Vietnam	*S. paramamosain*	- Year-round, September- October peak in mature females	Le Vay *et al.* (2001)
Tanzania		Peak of large females with ripe ovaries in November, reappear in January to	Akil and Jiddawi

	March		(1999)
Subtropical areas			
South Africa	*S. serrata*	- Spawning throughout the year, peak from late spring to early autumn	Roberston & Kruger (1994)
Australia	*S. serrata*	Peak mating activity in mid spring-early autumn. Spawning activity in Spring and early Autumn.	Heasman *et al.* (1985)

Spawning and recruitment

While most of the life cycle of *Scylla* is spent in inshore waters, especially estuaries, the females migrate offshore with the fertilised eggs attached to the pleopods, where they hatch in a few weeks (Hill, 1974; 1994). Mud crabs are reported to migrate offshore for spawning due to the environmental requirements of the first stage zoeae (Hill, 1975). When studying spawning of *S. serrata* in South Africa Hill (1975) noticed that spawning migrations usually followed a lunar cycle and salinity changes. Perrine (1978), cited by Heasman *et al.* (1985), also found that spawning migrations frequently followed a lunar cycle and that rapidly decreasing salinity stimulated both migrations and spawning. A study on some major factors influencing the spawning migration of mud crab by Poovachiranon (1992) in a mangrove area in Phuket, Thailand indicated that females move out of the estuaries when the mangrove area is influenced by fresh water. The study indicated that changing temperature together with fluctuation of salinity in the estuaries caused migration in female crabs. More details on spawning migration of female crabs are presented in Table 4.

Table 4: Spawning migration of mud crabs.

Area	Species	Observation	Source
India (Karwar)	Not known	Berried females were found inshore and offshore waters	Prasad & Neelakanatan (1989)
Philippines	Not known	Females migrate to the sea to spawn	Arriola (1940)

Malaysia	Not known	Berried females found in the sea, not in brackish waters	Ong (1966)
Haiwaii	Not known	Females migrate from brackish to sea	Brick (1974)
Thailand (Andaman Sea)	Not known	Females migrate offshore 15- 84 miles away from the mainland, at a depth 97- 200 m during October-February	Poovichiranon (1992)
S. Africa	*S. serrata*	Females migrated out of estuaries into the sea after mating	Hill (1975)
Australia (Queensland)	*S. serrata*	Females migrate as much as 45 km offshore to spawn	Lee (1992)
Australia (Queensland)	*S. serrata*	Females migrate up to 65 km offshore	Hyland *et al.*(1984)
Australia	*S. serrata*	Mature females migrate to the sea to spawn at 10 - 60 m depth, 3- 95 km offshore, return to coast after spawning.	Hill (1994)

The persistence of populations depends on the successful recruitment of juveniles to adult habitats, which is usually, especially in brachyuran crabs, problematic because their planktonic larvae are transported away from adult habitats by the net seaward flow of estuarine surface water (DeVries *et al.*, 1994). These authors investigated the abundance of larvae of five estuarine crab species associated with tidal variables in the Newport River Estuary, USA and found that crab megalopae and juveniles were most abundant on night time flooding tides. Newly recruited juvenile mud crabs prefer sheltered and shallow-water habitats amongst seagrass, algae and mangrove roots (Hill, 1979; Chandrasekaran and Natarajan, 1994) which make population census sampling difficult.

Quinn and Kojis (1987) studied the reproductive biology of *Scylla* spp in Papua New Guinea and found there was a portion of the population reproductive at all times of the year with no distinct year class structure in the population. They further supposed that the non-seasonal nature of reproduction probably resulted in continuous recruitment.

Chandrasekaran and Natarajan (1994) studied seasonal abundance and distribution of mud crab juveniles in Pichavaram mangrove, southeast India and reported that the juveniles peak in abundance during post-monsoon (January to February) and total absence occurs during monsoon (October to November). In Queensland Lee (1992) found the occurrence of larvae from November to June.

Hill (1979) stated that seasonal peaks in juvenile recruitment may be related to water temperature in subtropical temperate climate. In the tropics Poovachiranon (1992) supposed population juvenile abundance may be related to salinity. However, despite seasonal peaks, results from size-frequency sampling of crabs and plankton surveys indicate that recruitment can be continuous throughout the year in some temperate and tropical populations (Roberston, 1987; Forbes and Hay, 1988; Roberston and Kruger, 1994).

FISHERIES

Due to the high economic value and export potential of mud crabs (Ray, 1992; Cholik and Hanafi, 1992) fisheries have expanded tremendously in recent years especially in Indonesia (Cholik and Hanafi, 1992), Bangladesh (Khan and Alam, 1992) and in many other countries. Mounsey (1989) investigated the fishery industry in the Northern Territory of Australia and found that although the value and prices of mud crabs resulted in increased catch and effort over the past 4 years, the corresponding catch per unit effort has dramatically decreased, along with a higher percentage of poorer quality crabs reaching interstate markets. He suggested that regulation and management of the fishery should be considered. Similarly, Jayamanne (1992) reported that in Sri Lanka mud crab production has declined in recent years due to civil strife and overfishing. To overcome the problem, prohibition of the capture of immature crabs, educating fishfolk and developing aquaculture for mud crab have been strongly recommended. In Bangladesh mud crabs are a relatively unexploited marine resource, therefore information on mud crab occurrence, seasonal abundance, trapping techniques, post-harvest technology should be studied to make useful recommendations to improve culture practices and to encourage mud crab fishermen (Ahmed, 1992). However, Khan and Alam (1992) suggested that more government

attention is needed to manage the fishery and promote culture of mud crab in Bangladesh. Tiensongrusmee and Pratoomchat (1999) studied the status of the mud crab fishery in Thailand and warned that resources were overfished. They also suggested the causes of decline of Thai mud crab resources were the drastic decrease of mangrove forest, lack of regulation on the indiscriminate fishing of natural stock and rate of fishing mortality is exceeding the rate of natural recruitment.

The fishing season for crabs is locally variable, with peaks reported in the rainy season (December-May) in Indonesia (Cholik and Hanafi, 1992), from April-July (rainy season) in Bangladesh (Khan and Alam, 1992), in December in Sri Lanka (Jayamanne, 1992) or wide variations between different regions in India (Ray, 1992), throughout the year in Sundarbans, West Bengal (Mahapatra *et al.* (1996), from March-August, peak in May in Kerala (Nasser and Noble (1995). In Queensland, Lee (1992) reported that the landing data of mud crab show a strong seasonal trend. The catch rate drops in winter and factors influencing the catch are distance between pots, size and sex, moult stage and water temperature. Mud crabs are captured by a variety of fishing gears, which are typically different among the regions as shown in Table 5.

Table 5: Fishing gears used for catching crabs in different areas.

Countries	Gears	Commonly used gears	Cited
Indonesia	Trap-like with bait	Trap	Cholik and Hanafi, 1992
	Trap and lift net		Suwarso and Wasilun (1991)
Bangladesh	Bamboo trap, fishing hook, nets, bait stick	Boom (bamboo trap)	Khan and Alam, 1992
Sri Lanka	Gillnets, baited trap	Baited trap	Jayamanne, 1992
Madagascar (Malagasy north-west coast)	Hook, racket and line Drop dilly	Hook, racket	Razafimandimby (1989)
Thailand	Traps, gillnets,	Crab trap	Tookwinas *et al.*,

	bagnets		1992
Queensland	Baited pots, dillies, gillnets, hooking	Baited pots	Lee (1992)
Philippines	Gillnets, liftnets, baby trawl, beach seine, pots, fish corral, pushnets, castnets, handpicking,	Crab liftnets	Ladra and Mondragon, 1992
India	Gillnets, castnet, hook-and-line, trawlnet, boat seine, crab trap, handpicking scoopnet, dragnet Ring net called 'Nanduvala'	Gillnets	Kathirvel and Srinivasagam (1992), Ray (1992). Nasser and Noble (1995)
Vietnam	Gillnets, pushnet, handpicking, hooked, dragnet	Handpicking	Vu Ngoc Ut *et al.*, (1998)

CULTURE

Mud crabs are considered one of the important seafood items for aquaculture in southeast Asian countries due to their large size, delicacy, nutritive value and great demand (Dorairaj and Roy, 1996; Triño and Rodriguez, 2002). They are cultured in many parts of south east Asia (Davis, 1996). Cholik (1999) indicated that there are three types of culture of mud crab in ponds based on the final product: grow-out from juvenile to consumption size, fattening and gravid female production. Felix *et al.* (1995) also described crab culture types practised in Vietnam such as "moulting crab culture", "soft-shell" and "mature female crab culture". Triño and Rodriguez (2002) have reported that pen culture of mud crab in tidal flats with mangrove trees is a newly profitable culture model.

Several authors agree that the major constraint for mud crab aquaculture development is the supply of seed crabs, which has relied on natural sources (Sivasubramaniam and Angell, 1992; Fortes, 1999; Cholik, 1999). In Thailand, Suresh (1991) concluded that although mud crab culture has many economical advantages, the potential expansion of this sector is severely limited due to the lack of viable seed production technology. Moreover, Babu and Manjulatha (1991) reported that in spite of a great demand for edible crabs in India and also in other countries, the culture techniques for adult crabs on a large scale are lacking. Chong (1995) suggested that successful crab laviculture performance will overcome the problem of inadequate supply of seed for monoculture and for fattening purposes. Additionally, Overton and Macintosh (1997) warned that there are significant risks to all forms of crab farming if the increasing commercial interest in *Scylla* results in too rapid an expansion of the sector. They also indicated that one of the main conflicts which would occur is competition for wild crab seed.

Mud crabs *Scylla* sp. are stocked in various ways, monoculture and polyculture (Dat, 1999) and monosex culture (Triño et al, 1999). Monoculture consists of stocking crabs at different densities depending upon initial size, from 0.5-5 crabs m^{-2}. Polyculture is usually a combination of mud crabs with shrimp or milkfish (Lijiauco *et al.*, 1980). Rodriguez *et al.* (2001) demonstrated that in culture of *Scylla olivacea*, monosex culture of males is more profitable than females or mixed sex as the males attained higher body weight at harvest. In an attempt to determine the harvesting regime for pond production of *Scylla olivacea*, Triño *et al.* (2001) suggested that a bimonthly partial harvesting produced more profits than a single terminal harvest.

Cannibalism is a serious problem in the grow-out of mud crab in ponds. The decrease in survival rates with increased stocking density mentioned above is believed to be due to greater cannibalism at the higher stocking density. Samonte and Agbayani (1992) determined the economic feasibility of pond culture of mud crab at various stocking densities and found that mud crab monoculture in brackish water ponds is economically feasible at stocking densities of 5000.ha^{-1} and 10000.ha^{-1}. Samarasinghe *et al.* (1992) found one of the factors causing crab mortality after stocking was poor handling during collection and transport. Better care may considerably improve the survival rate of mud crabs. Gunarto and Rusdi (1993) stated that behaviour such as

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mating and migration also contribute to high mortality of cultured crab. Another factor that causes apparent high mortality is the ability of crabs to escape from the pond through holes by digging or climbing out over the dykes or fences (Sulaeman *et al.* 1993). 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^{-1}) compared to the other treatments. The highest growth rate at 1.3 g. day^{-1} 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 has been reported by Wedjatmiko and Yukarsono (1991), Sulaeman and Hanafi (1992) and Wedjatmiko and Dharmadi (1994). They reported that mud crabs will eat any kind of trash fish. Regarding feeding frequency, Wedjatmiko and Dharmadi (1994) stated that feeding once per day is sufficient during crab grow-out and the ration should be 6-8% body weight per day.

Culture of mud crabs in Vietnam

In Vietnam, mud-crab farming occurs along the entire coast, especially in areas where there is abundance of wild populations to provide seedling stock (i.e. in mangrove areas) and has been developed for about 10 years (Hoang Duc Dat, 1999). According to the Ministry of Fisheries (MoF), 78% of the country's annual output is produced in the southern provinces while the northern and central regions account for 13% and 9%, respectively (IFEP, 1996a). Provinces of central Vietnam are less suited for mud crab farming due to the lack of seed resources and the lack of extensive pond areas which are plentiful in the two large delta regions of the Red River (North Vietnam) and Mekong River (South Vietnam). The total annual crab production for 1995 was estimated to be around 4,500-5,500 m.t. (IFEP, 1996) with a total pond area of about 3,000 ha.

In the north, mud crab culture has developed since the early 90's, mainly in the

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provinces of Quang Ninh, Hai Phong, Nam Ha and Thanh Hoa (from north to south). However, the top production seems to be coming from the Thanh Hoa province which reported production of 100 mt of mud crabs in 1990 with at least 160 farmers registered for mud-crab pond culture for export purposes (Anonymous, 1990). In 1991, the number of farmers participating in mud crab culture activity increased to 250 with 200 ha of water surface, representing 5% of the total brackish water pond area (Anonymous, 1991). Quang Ninh province produced 2 mt of mud crab in 1996 out from a total aquaculture production of 2,805 mt (Do Phuc Truy, 1997). However, this figure may be underestimated due to the difficulty in controlling trade of sea products flowing to the nearby Chinese market.

In the central coast, Quang Binh province (northern central Vietnam) was reported to have a production of 30 mt of mud crab in 1994 which increased to 70 mt in 1995 with a pond area during 1994 of 375 ha (Tran Truong Luu, 1996). According to the author, crabs were mainly cultured during the rainy season. In Khanh Hoa province (Ninh Loc Village) 127 farmers practice mud crab culture on 300 ha, producing an annual harvest about 80 mt (Anonymous, 1997). The stocking period is around July at a density of around 0.2-0.25. m^{-2} with a culture period of 3 months upward.

In a survey to 6 provinces of the Mekong Delta (eastern coast) Nguyen Anh Tuan *et al.* (1996) reported that the total crab culture area was around 3,086 ha and production about 1,644 mt in 1995. The culture area increased considerably during the period 1993-1995 in some provinces, Tra Vinh having six -fold and Tien Giang a 3-fold increase.

Keenan (1999) indicated that in the Mekong Delta, one of the culture models which produce very high numbers of crab is extensive culture in mangrove silviculture systems. With this method, no supplemental feed is added and the crabs forage across the forest floor for natural food. However, the production reported from Minh Hai province is noticeably low (< 100 mt) while this province has the most extensive mangrove area in southern Vietnam and had reported in 1990 a potential production of 5-6 thousand mt per year of (frozen) soft-shelled crabs for export. This indicates a source of 10-12 thousands mt of crabs, as 2 kg of crab is needed to produce 1 kg of soft-shelled crab (Anonymous 1990).

At present, mud crab production has considerably declined due to the over-fishing of natural resources, rapid destruction of mangrove forests and lack of artificial seed supplementation for culture. In Vietnam, mangrove forests have been rapidly destroyed in the last 20 years resulting in a decline in production of mud crabs. Cutting away the mangroves to make shrimp ponds is the main reason for rapid changes in area of the mangrove forests in Vietnam. Reduction of mangrove forests in some coastal provinces of Vietnam is presented in Table 6.

Table 6: Reduction of mangrove forest in the Mekong Delta, Vietnam over time.

Provinces	Years	Area (ha)	Cited
Mekong Delta	1990	208,143	Vo Quang Minh *et al.*
	1992	114,536	(1999)
	1995	83,385	
Minh Hai (now is Ca Mau and Bac Lieu)	1983	117,745	Hansson *et al.*, cited by
	1988	72,989	Hoang Huu Cai, *et al.*,
	1991	49,920	(1997)
Ca Mau	1954	149,982	Nguyen Xuan Hoa (2002).
	1998	72, 593	
Kien Giang	Before 1975	10,000	Sub FIPI (1995).
	1976	7,800	
	1986	4,250	
	1992	3,250	
	1993	3,110	
	1997	3,080	
Soc Trang	1984	3000	Tran Duc Ngoc (1998).
	1987	351	
Tra Vinh	Before 1975	40,000	Nguyen Xuan Hoa (2002).
	2000	6,678	

Aims of this study

The overall objective of the present study was to investigate the feasibility of stock enhancement of mud crabs in the Mekong Delta. A number of study components were undertaken to support this objective:

a). The fishery for mud crabs in a selected estuarine mangrove area was evaluated. At the same time, seasonal abundance and recruitment of *S. paramamosain* were investigated. In addition, salinity tolerance/preference of crab juveniles in relation to seasonal abundance of the species in the wild was also studied under laboratory conditions. The aim was to determine the most suitable period for release of the animals into the wild and preferable salinities for pond culture.

b). Application of tagging methods using microwire coded tags (Jefferts *et al.*, 1963), which have been successfully used in other crab species (van Montfrans *et al.*, 1991; Fitz & Wiegert, 1992), was developed and tested in *S. paramamosain* in preparation for a mark-recapture study and stock enhancement trial.

c). Population dynamics of *S. paramamosain* in the estuarine mangrove study area were investigated using the previously developed tagging method in a mark-recapture study. The aim of this component of the study was to obtain information on abundance, recruitment, mortality and growth of *S. paramamosain* in the mangrove environment, as the basis for evaluation of the feasibilities for a potential stock enhancement programme.

d). Fitness of hatchery-reared *S. paramamosain* was evaluated in comparison with wild conspecifics to ascertain the feasibility of using hatchery-reared crabs for stock enhancement.

e). In the meantime, nursery techniques were also developed to investigate shelters/substrates, diets and stocking densities to support production of crab juveniles for future uses.

CHAPTER II

SEASONAL ABUNDANCE AND RECRUITMENT OF *SCYLLA PARAMAMOSAIN* IN AN ESTUARINE MANGROVE SYSTEM

INTRODUCTION

The potential high economic value of mud crabs has increasingly drawn interest from large numbers of people involved in aquaculture for these species in many Asian countries (Chong 1993, 1995; Felix 1995; Dorairaj and Roy, 1996). Aquaculture of mud crabs is undertaken with a variety of culture techniques consisting of crab fattening, production of gravid females, soft-shell culture, growout from seed stock in pond or pen systems, and in mono-culture or poly-culture. Development of these activities has substantially increased demand for seed supply. However, production of hatchery-produced juveniles has not yet been successful and therefore aquaculture has relied mainly on natural sources of seed (Sivasubramaniam and Angell, 1992; Overton & Macintosh, 1997; Fortes, 1999; Cholik, 1999). At the same time mud crabs are an important target for artisanal fishing communities in the Asian region (Ladra and Mondragon, 1992, Delathiere, 1992) and overexploitation of the resource is a serious risk. Over the last two decades, over-fishing of mud crabs has been reported in many countries (Mounsey, 1989; Jayamanne 1992), especially in south east Asia (Angell, 1992; Keenan, 1999). Without regulation, fishing activities may target all size classes of the population including seed crabs for pond culture, sub-adult crabs for fattening as well as mature females (Jayamanne, 1992; Angell, 1992). In addition, rapid deforestation of mangroves has caused loss of the habitat with which mud crabs are associated causing further decline in crab populations (Tiensongrusmee and Pratoomchat, 1999).

Despite the economic importance of mud crabs in estuarine and mangrove fisheries, relatively little is known of their biology and ecology. In addition, although mud crabs are found to be associated with mangroves, creeks and estuaries throughout the Indo-Pacific region, studies on their population dynamics are still limited (Macintosh *et al.*, 1993). Data on population dynamics including abundance, recruitment, habitat preference and population structure may help establish strategies for proper management and exploitation of the resource.

Estuaries are characterized by high turbidity, water salinity and total suspended solid fluctuations. Among these, salinity is reported to be a crucial factor affecting many

*Chapter 2: **Seasonal abundance and recruitment….***

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species (Miller and Maurer, 1973; Mair, 1980). Furthermore, other physico-chemical and biotic factors such as depth, photoperiod, turbidity of water column and food availability are also found to be key factors influencing the abundance of mud crabs (Prasad *et al.*, 1990). Joel and Raj (1986) also suggested that light might be a major factor controlling the distribution of *Scylla* spp.

Estimation of mud crab abundance has been practiced in a number of areas using trapping and marking techniques (Hill, 1975; Williams and Hill, 1982; La Sara, 2001). Using traps, abundance and habitat preference of *Scylla serrata* was quantified. However, the authors encountered problems in evaluation of abundance, as traps caused sampling bias associated with size and moult stage of the animals. Furthermore, trap-saturation as well as temperature effects also result in underestimation of the population (Williams and Hill, 1982; Robertson, 1989; Lee, 1992).

In the present study, seasonal abundance and recruitment of the mud crab *Scylla paramamosain*, which is the predominant species in an estuarine mangal habitat in the Mekong Delta was investigated. Instead of using direct sampling methods, catch per unit effort (CPUE) obtained from a group of fishers, as an indirect measure of abundance, was applied. The aim of this component of the study was to develop a baseline for monitoring the effectiveness of resource management and potential impacts of further habitat change on crab populations.

MATERIALS AND METHODS

Study area

The 30th April State Farm was selected as a site of potentially high abundance and recruitment for mud crabs. It is located on an island between two estuaries, Tran De and Dinh An, originating from the Hau river, one of the largest branches of the Mekong River (Fig. 1). To the southeast, the island is limited by a sandy-mud and mud tidal flat of about 10,000 ha, which is covered by about 1m of water at high tide. A belt of 1,000 ha of mangrove forest, predominantly *Sonneratia* and *Avicennia*, surrounds the farm and protects ponds which are located behind it. Within the island, there are numerous natural or mechanically excavated canals, all of which connect to the Hau river. The tide amplitude during spring tides at this site is about 2.5 m.

The farm controls fishing activities within the mangrove area and 2,000m seawards over the mud flats. In the mangrove, mud crab fishing is monitored by fishing licence. A several-year contract has been established between the farm and a crab agency located on the western shore of the Hau River, allowing crab fishing to take place in the controlled areas. However, the agency in turn sub-contracts to local fishermen who guarantee to sell all daily catches to the agency. Twenty to twenty-five fishermen are allocated with a certain fishing fee derived from the division of the main contract by a factor 20/25. This amount is partly deducted from their daily catches.

The fishermen cross to the island in groups by boat at low tide and return at high tide. They catch crabs mainly by hand using shovels or hooks in burrows. All of crabs encountered are caught, regardless of size, maturity or species. Captured crabs are tightly tied and directly transported to the agency.

At the agency, crabs are graded into commercial sizes and maturity status as categorized in Table 1. Crabs are recorded in weight for different groups for each individual fisher. The small crabs (less than 3-5 cm carapace width) are counted and sold to the farmers in Camau province, where they are stocked in ponds, while the bigger ones are either used for pond fattening or market consumption.

Fishing activities on the mud flat are also controlled by another fishing licence. Similarly, contracts are allocated to 7-10 boats which usually carry about 5-7 fishermen each. Their fishing activities only take place at night at low tide. The main catches in this area are small crabs of 2-6 cm CW. The catches are also sold to the same agency.

Besides the crabs from the 30th State Farm mangrove, the agency also purchases crabs captured from the adjacent areas in Tran De estuary. These crabs are caught by several methods. Fishing boats operate on the mud flats in front of the main mangrove area, using standing nets 700m long, with 40m sections supported by upright poles with mesh size of between 2-5 cm. Numerous people also work hand-pulled seine nets on the mud flats. Within the river system, fixed nets along the mangrove edge and across narrow tidal channels catch fish, shrimp and crabs.

The island is isolated and boats are the only means of access. The numbers of fishers and fishing days are highly dependent upon either the weather conditions or the cultural activities of the fishermen. Bad weather such as heavy rain, typhoon or storm is usually the main reason for stopping fishing activities in a certain period. Most of the fishers are ethnically Khmer, who originate from Cambodia. Their culture includes frequent festivals during the year. During these periods, no fishing activities take place.

During the period of the present study, a landing point was established at the State Farm (Fig. 2) to facilitate the recording of fisheries data, especially for the later mark-recapture study (Chapter 5). Crabs caught by the fishermen in the mangrove were taken to this point for measuring before being sold to the agency in Long Phu.

Sampling methods

Salinity

Salinity was measured with a hand-held refractometer. Samples were collected at high and low tide from both surface and bottom water, at a station 100 m from the mouth of a creek opening on the Tran De estuary (see Fig. 1). Samples were taken in the

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period of new-moon spring tides from December 1997 to December 1998. Sampling
for salinity was repeated in August 1999 to July 2000. During this period samples
were taken at low tide in the mangrove and on the mangal fringe on the southeast
shore of the study area.

Frequency data

Data were collected monthly at the agency (for 1997-1998) and every fishing day at
the landing point (for 1999-2000). Crab carapace width (CW) was measured by
means of a caliper, accurate to 0.1 mm, and is defined as the distance between the two
last antero-lateral spines of the two sides of the carapace (Forbes and Hay, 1988).
Crabs were measured and classified into different size classes of 1 cm interval. The
number of crabs measured depended on the total number obtained from the fishermen.
Crabs were also sexed and separated by species. Based on the colour of the claw and
the body as well as the shape of the frontal lobe crabs were distinguished into two
species, locally named Green and Red crab. The Red crabs have dark red claws and
low stunted frontal lobes while the Green crabs have bluish-white claws and higher
pointed frontal lobes. They were identified as *S. paramamosain* (Green) and *S.
olivacea* (Red) following Keenan *et al.* (1998). Maturity of females was also
observed. The characteristics used to identify maturity of females are the width of the
abdomen and the appearance of numerous setae around it. The abdomen is round and
broadened and the horizontal dark patterns on the abdomen are obvious. According to
Ong (1966) the abdomen of a mature female is broadened and dark green in color. In
addition, by the experiences of local farmers who usually sort the round broadened
abdomen females for gravid crab culture.

Historical data

Within the sub-contracting system used by the agency described above, data of
catches were recorded for each individual fisher in log books. Historical records of
crab landing from 1995 to 2000 were provided by the agency. However, for some
years the records were found to be incomplete and were not used. Analysis was
undertaken for the most complete annual data sets in 1997, 1998 and 2000. These
consisted of data for daily catches of crabs caught by individual fishers in the

mangrove. Data were transcribed into spreadsheet format, as daily landings (kg) for each named fisher (see sample Table 2). In addition, the catch records were divided into classes of crabs, each with a different market value as indicated in Table 1. These allow the calculation of the relative abundance of size classes within the crab population. The fishermen travel in one boat, so that they all spend the same amount of time in the mangrove on a given day to collect all crabs encountered. This fishing activity is assumed to be non-selective in terms of crab sizes. The fishing methods were considered to be standardized and therefore data for hand-collected crabs was expressed as catch-per-unit effort (CPUE, kg person^{-1} day^{-1}). Data of small crabs of 3-5 cm CW collected on the mud flat of the mangal fringe at night were analyzed separately. This provided an opportunity to look at both long term and seasonal trends in recruitment within the fishery, using catch per unit effort (CPUE) as a measure of relative crab abundance. Only data recorded during 1998 and 2000 were obtained and CPUE was calculated as numbers of juveniles collected person^{-1} day^{-1}. For both intertidal (mangrove) and subtidal (mudflat) fishing activities, total monthly landings are recorded in commercial size-classes as shown in Table 1. Gravid females (determined by the fullness and orange colour of the ovary that can be recognized when lifting the posterior edge of the carapace) were recorded separately. In general, the data were analyzed in several ways:

- Total landings by month
- Total catch per unit effort (CPUE) (kg person^{-1} day^{-1}) for each month.
- Size classes, expressed as percentage (by weight) of the total catch by month
- Fishing effort (person days/nights) per month
- To study seasonality in recruitment, CPUE for juveniles was expressed as numbers person^{-1} day^{-1}.

Data for calculation of CPUE were pooled by month and tested for normality with the Anderson-Darling Test (Minitab program package). When the original data were not normally distributed, they were square root or log$_{10}$ transformed. Homogeneity of variances was tested for the normally distributed, transformed data. A non-parametric test was applied for all data sets, which were heterogeneous in variance. In this case median CPUE values were ranked by a Kruskal-Wallis test. ANOVA was used to compare the difference in CPUE from year to year.

RESULTS

Salinity

During the sampling period from December 1997 to December 1998, salinity at the surface and bottom, at high and low tide, was highest in April (21 ±1.6 ppt) and dropped down to 0 ppt through the months of the monsoon season (Fig. 3). During this period, freshwater was recorded at all water depths within the mangrove at high tide and low tide.

In the second sampling (August 1999 to July 2000), 0 ppt was also recorded during the period of peak freshwater flow (August to October). Higher salinity occurred in February, however the overall mean salinity during this period was lower than in 1998 (13 ± 0.2 compared to 21 ± 1.6, respectively. (Fig. 4)). This was believed to be the consequence of previous heavy flooding that had occurred in the inland areas.

Frequency data

In 1998-1999

2,785 crabs were measured and examined from February 1998 to March 1999. Two species were identified following Keenan *et al.* (1998) as *Scylla paramamosain* and *S. olivacea*. *Scylla paramamosain* was found to be predominant (2,696 individuals among the total sample) representing 96.8% of the sample. The sex ratio was different for the two species. In *S. paramamosain*, females were slightly more preponderant than males with a ratio of 54.1% to 45.9%. However, for *S. olivacea* the ratio for male to female was 73% to 27%.

The size-frequency structure for the *S. paramamosain* samples, both male and female, is shown in Fig. 5. Throughout the year the dominant size classes range from 40-70 mm CW. Relatively few crabs smaller than 40 mm and larger than 100 mm CW were found. There was no clear modal progression in the size frequency for this species, which suggests recruitment of this species takes place year-round with continuous

mortality or emigration. *S. olivacea* was found to have a similar upper limit in size distribution (Fig. 6), however, juveniles of less than 5 cm CW were rarely observed. In both species, females appeared to be in the puberty moult from 9 to 10 cm CW. This indicates crabs caught intertidally are sub-adult or juveniles.

In 1999-2000

Data were recorded at the landing point in the State farm during the period of September 1999 to August 2000. As in 1998-1999, *S. paramamosain* was the predominant species. Among a total of 25,536 crabs sampled, *S. paramamosain* (24,655) represented 96.5%, while *S. olivacea* was only 3.5% of the total sample. Size-frequency histograms for both species are shown in Fig. 7 & Fig. 8. There was no clear modal progression in the size frequency data for either *S. paramamosain* or *S. olivacea*, with modes for both sexes and species remaining between 4 and 7 cm CW throughout the sampling period. The lower size limit for *S. olivacea* remained unchanged, as small crabs (less than 5 cm CW) were hardly found.

A slightly higher number of males was recorded for *S. paramamosain* than females (54% and 46%, respectively). Similarly to year 1998-1999 males were more abundant than females for *S. olivacea* (84% and 16%, respectively). A fairly clear pattern of occurrence was observed for *S. paramamosain* females. They were more abundant during the dry season when salinity is high and a reduced number was found in the period of the monsoon (Fig. 9), which may indicate the offshore emigration for spawning in a certain number of mature females. However, for *S. olivacea* the number of females decreased rapidly in the early monsoon and almost disappeared during this period (Fig.10).

Historical data

Total landings and Catch Per Unit Effort (CPUE)

During 1997, the combined overall landings for both species of crabs caught interdially in the mangrove of the study area were highest in the dry season from February to May, reaching more than 900 kg month^{-1} in May, and declined to 150-400

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kg month[-1] in the rainy season (Fig. 11). Moreover, this seasonal variation appears to be due to higher fishing effort in the dry season. However, there is no correlation between fishing effort and CPUE for landing data ($p > 0.05$). The amount of crabs caught per person per day (CPUE) was highest in February (Fig. 12). Although the range of mean monthly CPUE was fairly narrow between 1.6 and 3.1 kg person[-1] day^{-1} (Table 3), there is still some significant variation between months (H=261.22, p<0.001). However, median CPUE values returned to close to the overall median in September-December, which suggests that crab abundance in the mangrove areas is fairly constant throughout the year, despite the prolonged periods of low salinity (freshwater) conditions in the rainy season.

The highest intertidal mud crab landings in 1998 were also recorded in the dry season, February- March at 400 kg month[-1] and declined during the rainy season (Fig. 13). This variable trend reflected the CPUE obtained, which was highest in February and lowest in September (Fig. 14). However, the range of mean CPUE was wider (0.4 and 2.4 kg person[-1] day^{-1}) than in the previous year indicating variation in abundance during the year. Combined total landings and CPUE values show that the catch was declining and varying from year to year. However, during 1998 fishing activities in the research area changed significantly. Due to the high prices for seed crabs as the result of high demand for pond culture, the fishers switched to collecting juvenile crabs at night on the mud flat. This practice targets crabs that are less than 4-6 cm carapace width. Fig. 15 shows the substantial increase in fishing effort during the year, especially during the late rainy season with a corresponding increase in the numbers of juvenile crabs collected with a peak in October-November of about 15,000-18,000 crabs. If CPUE value is used to measure the relative abundance of small crabs as number of small crabs fisher[-1] night[-1], there is some evidence of greater abundance of juveniles during August-September (Fig. 16). However, no data were available for January-February, so the pattern of seasonal variation is not complete.

Total landings for 2000 were recorded with a peak in May of about 400 kg month[-1] (Fig. 17). However, CPUE was highest in December, November and April (1.3, 1.1 and 1.0 kg person[-1] day^{-1}, respectively) and lowest in January (0.4 kg person[-1] day^{-1}) (Fig. 18). There was a significant variation of CPUE between months (H = 391.3, p< 0.001) (Table 4). Although there is no relationship between fishing effort and total

landings and CPUE, increasing in fishing effort is likely to result in increased total landings and lowering CPUE, especially during the monsoon period when crabs are less abundant (Fig. 19 & 20). The trend of declining overall landings was most obvious from 1997 to 2000. However, as in 1998, many fishers switched to catching juveniles resulting in lower total landings of larger crabs. The peak in juveniles occurred during February-March, reaching 55,000 individuals month^{-1} (Fig. 21). The number of crabs caught per fisher per night was highest in March with about 80 crabs person^{-1} night^{-1} (Fig. 22) compared to 40 crabs person^{-1} night^{-1} in 1998 (Fig. 16). However, there was no significant difference in CPUE between the two years (p>0.05).

There are differences in CPUE from total landings and small crabs recorded in 1998 and 2000. In 1998 two peaks of abundance of juveniles were observed, one in March (although no data available for January and February) and the other one in August. The highest CPUE of intertidally caught crabs for this year was recorded in January-February. In 2000, juvenile abundance peaked in March and the highest CPUE for intertidal crabs was observed in November-December. Total landings and CPUE of intertidal crabs of 1997 was significantly higher than that of 1998 and 2000 (p<0.001), but there was no significant difference between 1998 and 2000 (Fig. 23 & 24). Correlation test shows there was a relationship between salinity and CPUE (p<0.05 and r = 0.68) indicating likely effect of salinity on abundance of crabs throughout the year.

Population structure

The structure of the crab population was determined by the proportion of different size classes as categorized in Table 1. The proportion of crab landings for intertidal hand fishery in 1997 is shown in Fig. 25. Adult crabs (>400g) have a relatively low abundance (1.6-11.3%) while the juvenile animals (<200g) remain predominant throughout the year, at 50-89% of landings. As this analysis is based on landed weights, juvenile animals must be much more predominant on a numerical basis. In contrast, data for crabs caught outside the study area, throughout the Tran De Estuary, including sub-tidal fishing with nets (Fig. 26) show a relatively higher proportion of adult crabs than in the intertidal population. There is also some evidence of a period

of increased juvenile abundance during the dry season, possibly related to seasonal recruitment. This is supported by the high proportion of mature females found in the subtidal fishery with a strong peak in September, when they represented 28% of landings by weight. An increase in adult crabs (>200g) was found throughout the estuary during the middle of the rainy season. The peak of mature females is not evident in the intertidal population, possibly because the crabs would be expected to be moving into deeper water.

Data for intertidal landings in 1998 also show a high proportion of juvenile crabs (51-82%) in the population. They predominated throughout the dry season with a peak in May but rapidly dropped at the end of the rainy season. Another size class (200-400g) was observed with 16-31%, however this group was more predominant during October and December up to 51-64%. Adult crabs (>400g) including mature females were relatively scarce (Fig. 27). As in the previous year, landings recorded in the subtidal fishery reveal an abundance of adult crabs (>200g) through the year representing between 21.6 and 91% of the catch. Adult females (>400g) reached a peak of 69.2% of landings in September, followed by a peak in mature females in November of 19.9% of total landings by weight (Fig. 28).

For the intertidally-collected crabs in 2000, small animals (<200g) were predominant, with a high proportion during the dry season from February to June (Fig. 29). Adult crabs (>200g) were less abundant, while adult and mature females were hardly recorded during this period. However, adult males occurred fairly frequently (26-37%) from September to December. In the monsoon season (August-September), the proportion of small crabs decreased slightly when salinity dropped to 0ppt, the population structure of crabs caught subtidally and outside the study area shows a slightly higher proportion of adult crabs (>200g) throughout the year (compared to intertidal crabs). Small crabs (<200g) were also more abundant in the dry season (January-April) but with a lower proportion compared to the intertidal population. This peak re-occurred in November-December (Fig. 30).

Table 1: Size classes used to record crabs purchased by the selected agency

Notation	Class
G	Gravid female
FI	Female I >400g
MI	Male I >400g
MII	Male II >200-400g
FII	Female II >200-400g
Trash	Small <200g

Table 2. Example of data entry for crab landings at agency in Long Phu. Notations in table are categories of crabs (in Vietnamese) used by the agency as G: gravid females; Cai: females >400g; I: males >400g; II: males <200-400g); @: mature females; Xo: small crabs <200g.

Date	Name	G	Cai	I	II	@	Xo	Total
04/10/97(dl)	Them	0	0.3	0	1.75	0.5	1.7	4.25
	Phuon	0	0.35	0.35	0	0	0.95	1.65
	Lac	0	0	0.35	0.85	0	2	3.2
	Choi	0	0	0	0.35	0.2	0.9	1.45
	Phol	0	0	0	0	0	0.8	0.8
	Chia	0	0	0	3.1	0.65	1.3	5.05
	Hang	0	0	0	0.35	0	0.7	1.05
	Det	0	0	0	0.55	0	1	1.55
	Them	0	0	0	0.7	0	1	1.7
	Phuol	0	0	0	0	0.15	1.1	1.25
	Duol	0	0.25	0	0.45	0.2	1.15	2.05
	Chuong	0	0	0.4	0.25	0	2.4	3.05
	Kha	0	0	0.8	0.45	0.15	1	2.4
	Tien	0	0	0	0.5	0.5	1.1	2.1
	TuNa	0	0	0	0.9	0	1.8	2.7
	Canh	0.25	0	0	0.9	0.2	1.2	2.55
	Dung	0	0	0	0	0	1.4	1.4
	Tua	0	0	0.35	0.45	0	0.9	1.7
	Mai	0	0	0	1.45	0	1.7	3.15
	Mel	0	0	0	0.2	0.15	0.3	0.65
	Sang	0	0	0.35	0.2	0.15	0.5	1.2
05/10/97(dl)	Phuon	0	0	0.5	0.3	0	1.4	2.2
	Mai	0	0	0.3	1	0	1.1	2.4
								etc………

Table 3: Catch per unit effort analysis for mud crabs (predominantly *S. paramamosain*) in the intertidal hand-fishery during 1997. Data were square root transformed, due to non- normal distribution. Mean and median values are expressed as CPUE (kg person^{-1} day^{-1}), N= number of unit fishing days each month. Average rankings and z-scores show output from Kruskal-Wallis test (H = 261.22, DF = 11, P < 0.001 adjusted for ties).

	N	Mean ± se	Median	Average ranking	Z
January	77	2.08±0.14	1.95	1333.3	0.01
February	186	3.13±0.10	2.80	1901.6	10.47
March	310	2.53±0.11	2.10	1464.1	3.22
April	349	2.36±0.07	2.30	1489.7	4.13
May	418	2.24±0.06	2.20	1416.1	2.45
June	170	1.56±0.06	1.40	1030.3	-5.28
July	247	1.57±0.10	1.20	926.6	-8.69
August	249	1.65±0.07	1.40	1052.8	-6.01
September	128	1.92±0.09	1.92	1269.4	-0.94
October	138	2.07±0.09	1.90	1357.6	0.41
November	157	2.09±0.10	1.90	1348.3	0.28
December	234	2.02±0.09	1.80	1266.9	-1.35
Overall	2662	2.13±0.03	1.90	1331.5	

Table 4: Catch per unit effort analysis for mud crabs (predominantly *S. paramamosain*) in the intertidal hand-fishery during 2000. Data were $\log_{10}$ transformed, due to non- normal distribution. Mean and median values are expressed as CPUE (kg person^{-1} day^{-1}), N= number of unit fishing days each month. Average rankings and z-scores show output from Kruskal-Wallis test (H = 391.3, DF = 11, P < 0.001 adjusted for ties).

	N	Mean ± se	Median	Average ranking	Z
January	236	0.37±0.02	0.25	803.6	-9.86
February	449	0.44±0.02	0.30	919.7	-10.50
March	114	0.52±0.05	0.35	1023.2	-3.32
April	297	0.98±0.06	0.75	1567.6	8.37
May	660	0.63±0.02	0.50	1239.5	-0.06
June	65	0.88±0.09	0.60	1427.6	2.13
July	280	0.78±0.04	0.55	1350.4	2.71
August	85	0.82±0.09	0.55	1378.3	1.80
September	67	0.87±0.09	0.65	1503.2	3.04
October	68	0.64±0.04	0.55	1362.9	1.42
November	45	1.13±0.12	0.90	1711.8	4.45
December	115	1.26±0.07	1.20	1891.8	9.98
Overall	2481	0.85±0.06	0.59	1241.0	
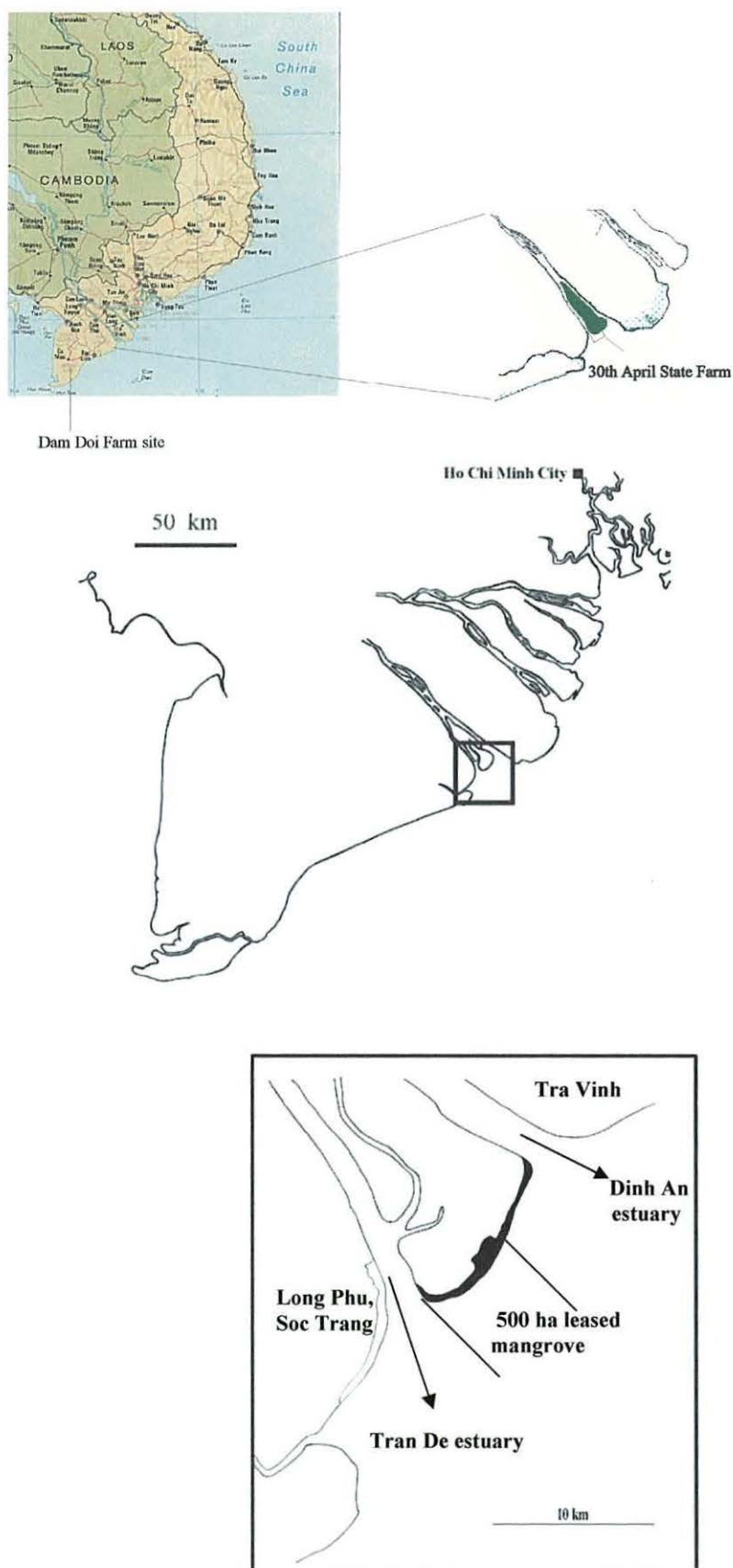
Figure 1: Location of the study area

Figure 2: Overciew of the study site. a) Mangrove study area; b) Fishers come across on one boat for fishing in the mangrove; c) Landing point = research station at the State Farm; d) Fishermen bringing crabs for measurement at the landing point.
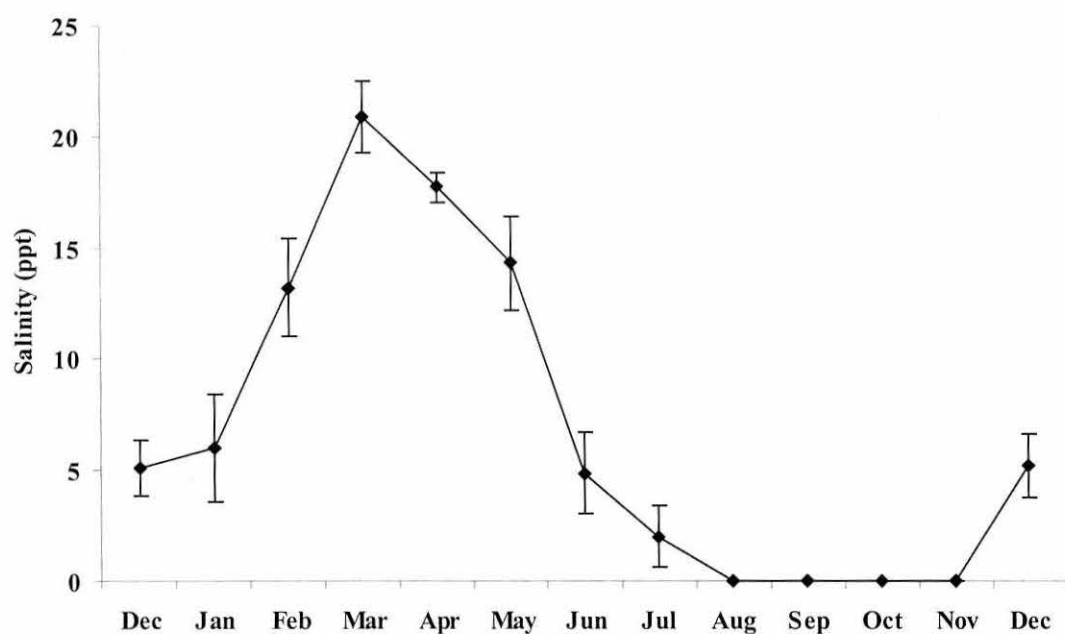
Figure 3: Seasonal variation in salinity (ppt) measured at a station 100 m from the mouth of a creek opening on the Tran De estuary during 1997-1998. Each value is mean (± sd) of measurements taken at high and low tide, from the surface and bottom (n=4-8 for each value).
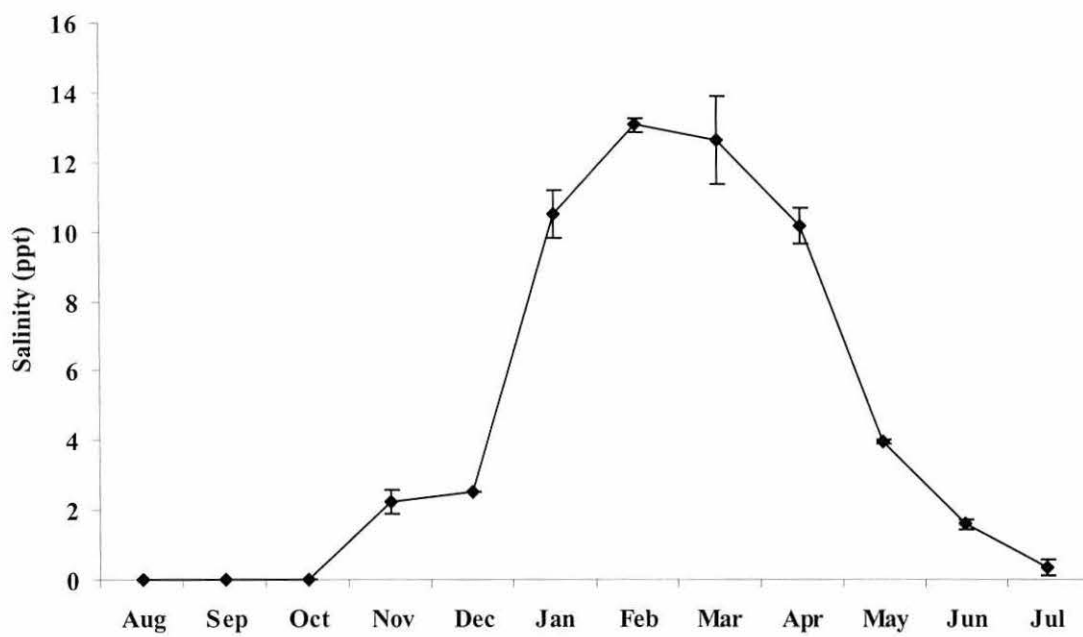
Figure 4: Seasonal variation in salinity (ppt) measured on the fringe at the western edge of the mangrove study site during 1999-2000. Each value is mean (± sd) of measurements taken at high and low tide, from the surface and bottom (n=4 for each value).

Frequency (%)

Carapace width classes (cm)
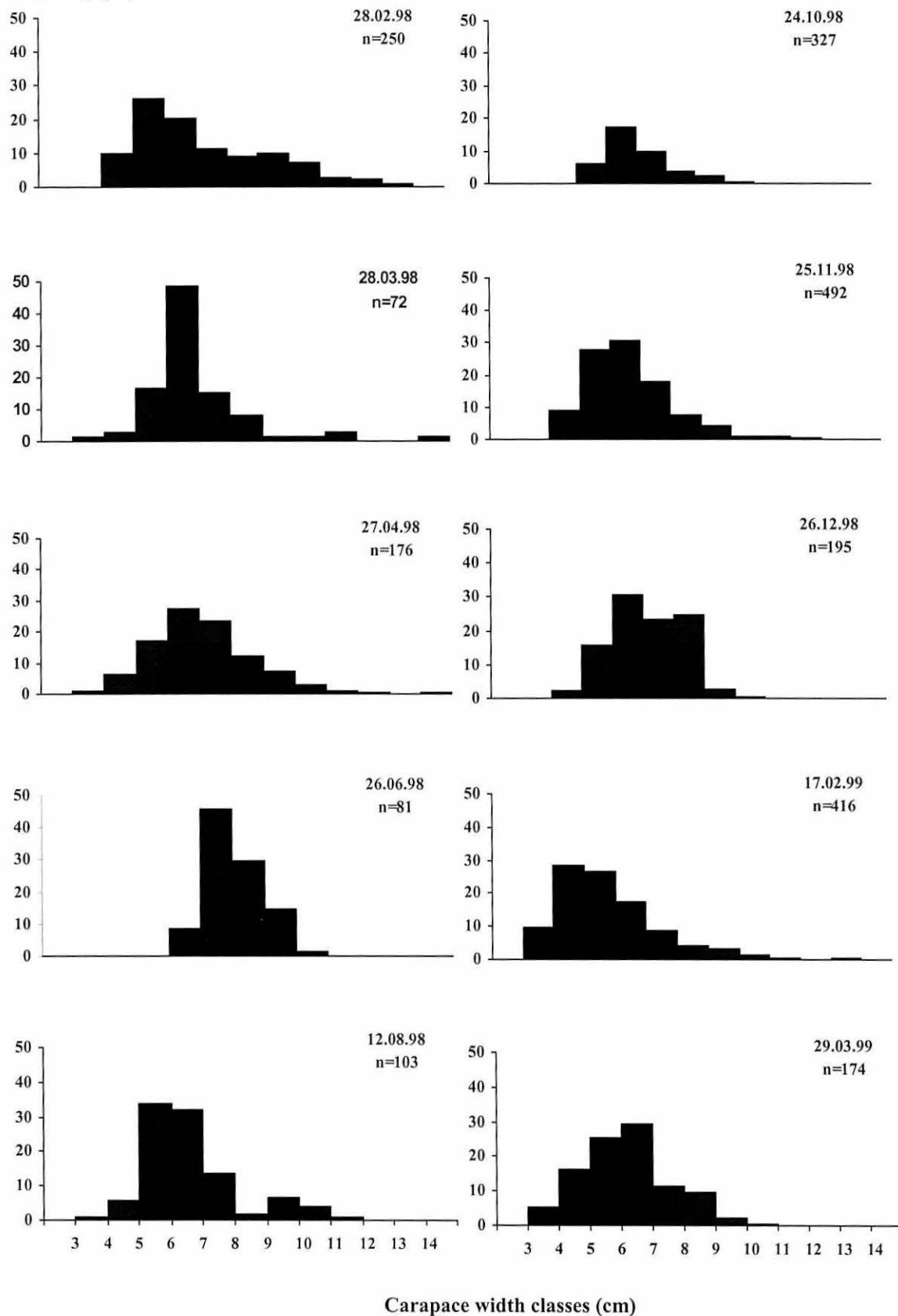
Figure 5: Size-frequency distribution for samples of *S. paramamosain* recorded from intertidal hand-fishery landings during 1998-1999. Pooled data for males and females.
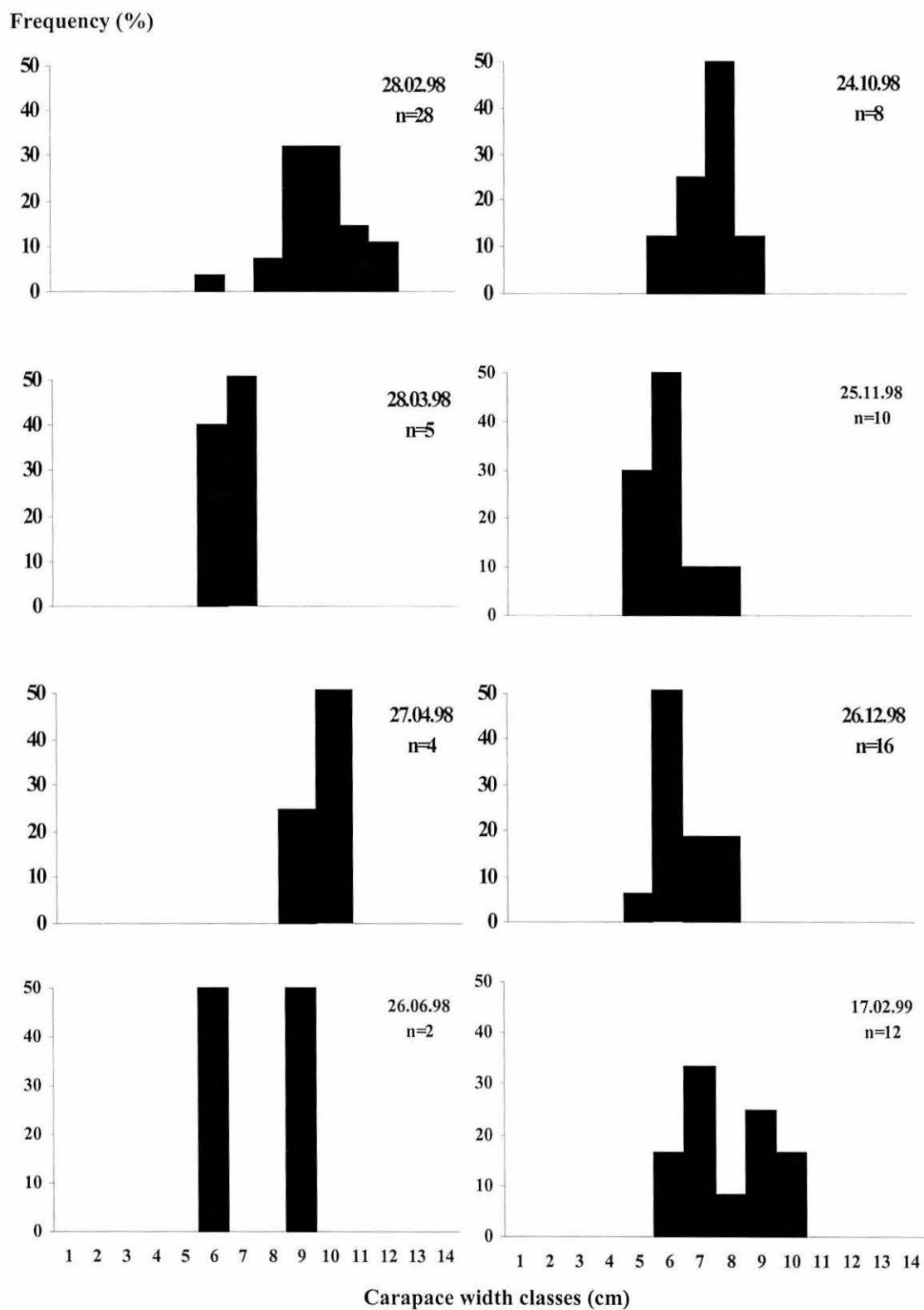
Frequency (%)

Figure 6: Size-frequency distribution for samples of *S. olivacea* recorded from intertidal hand-fishery landings during 1998-1999. Pooled data for males and females.

Frequency (%)

Carapace width classes (cm)
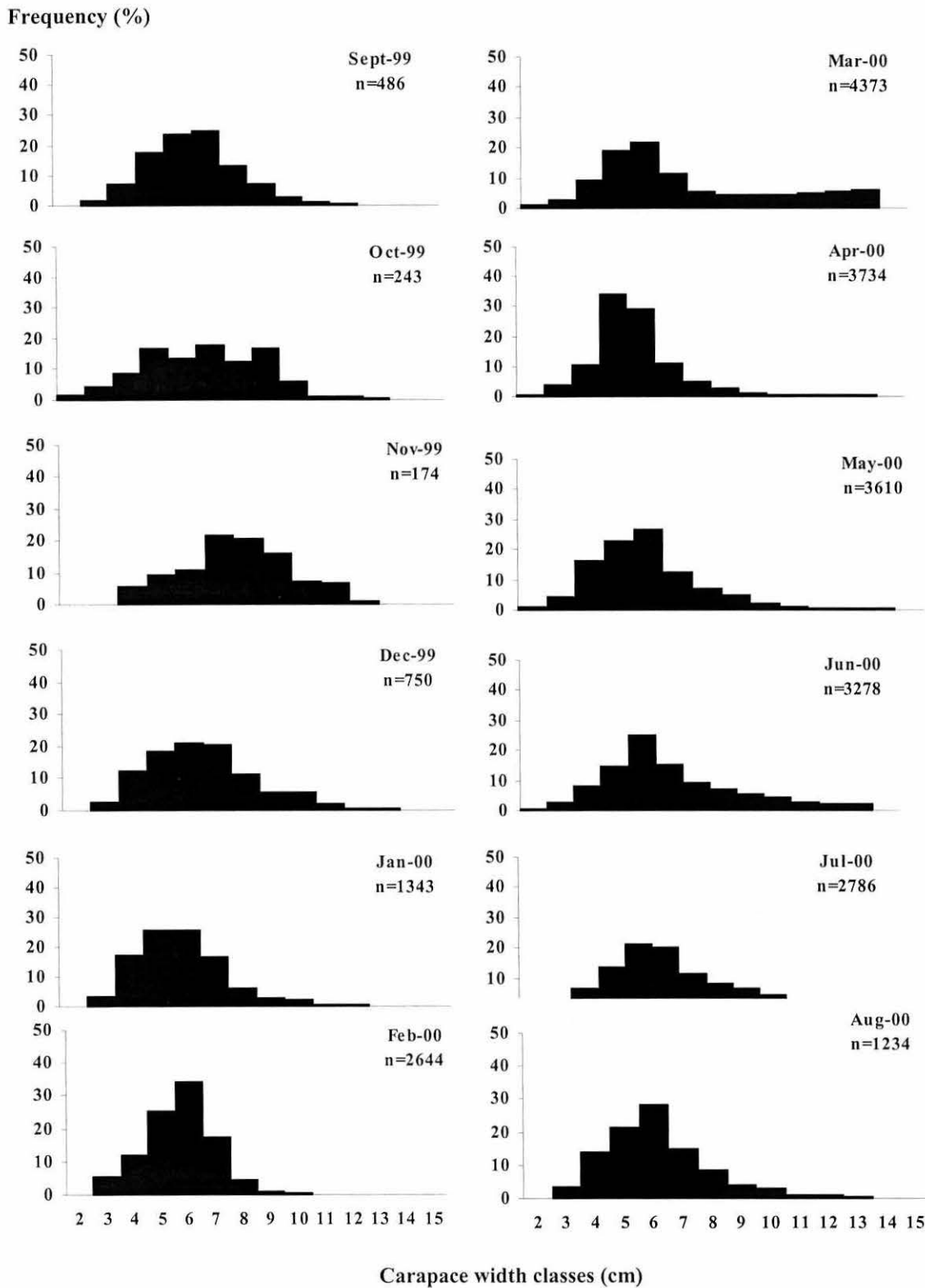
Figure 7: Size-frequency distribution for samples of *S. paramamosain* recorded from intertidal hand-fishery landings during 1999-2000. Pooled data for males and females.

50

Frequency (%)

Carapace width classes (cm)
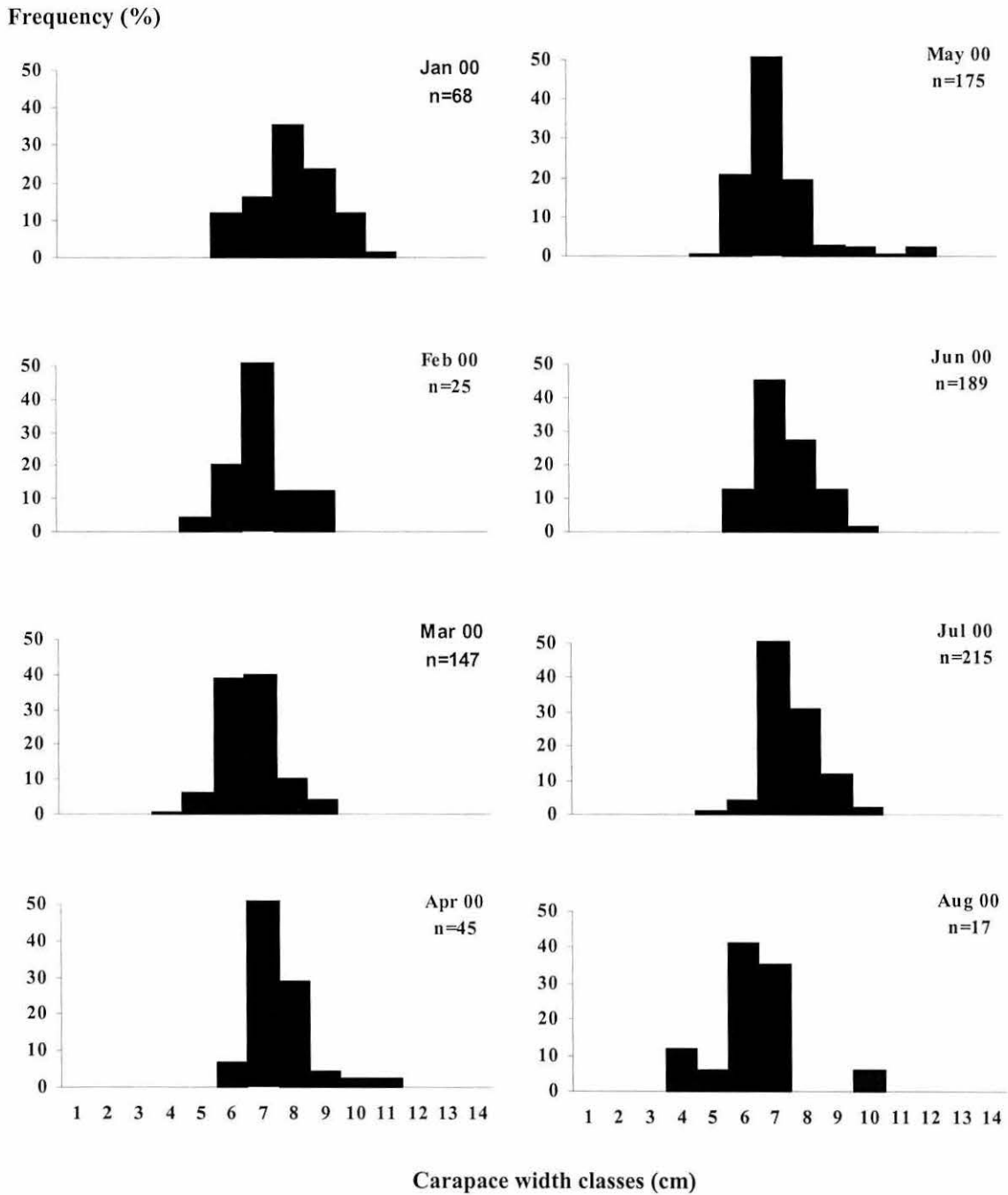
Figure 8: Size-frequency distribution for samples of *S. olivacea* recorded from intertidal hand-fishery landings during 1999-2000. Pooled data for males and females.
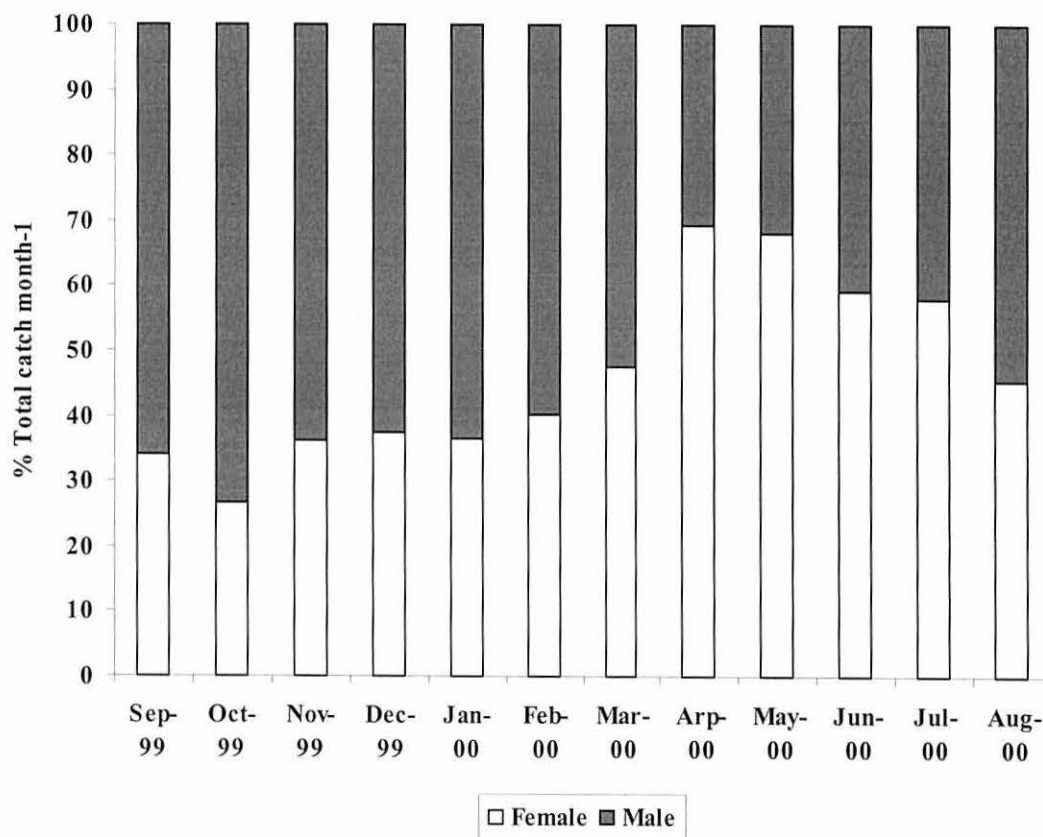
Figure 9: Ratio of females to males (in %) of *S. paramamosain* recorded in 1999-2000. More males found during monsoon period.
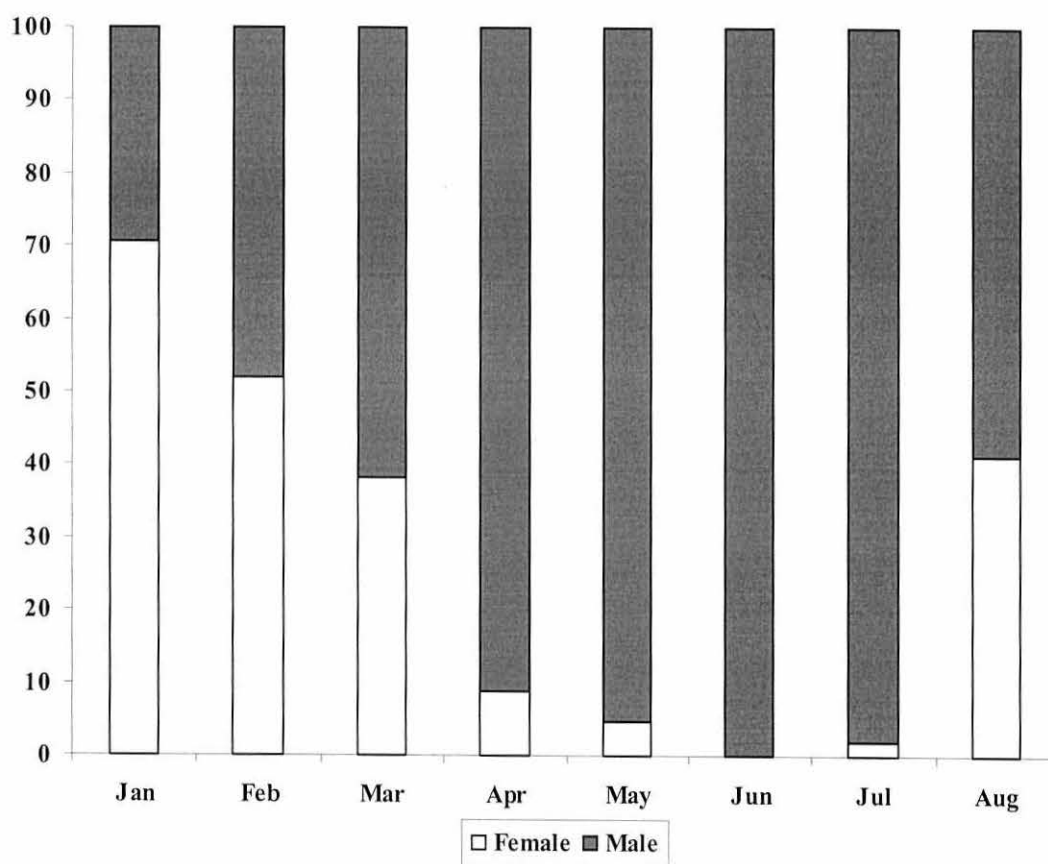
Figure 10: Ratio of females to males (in %) of *S. olivacea* recorded in 1999-2000. More males found in the end of the dry season onwards.
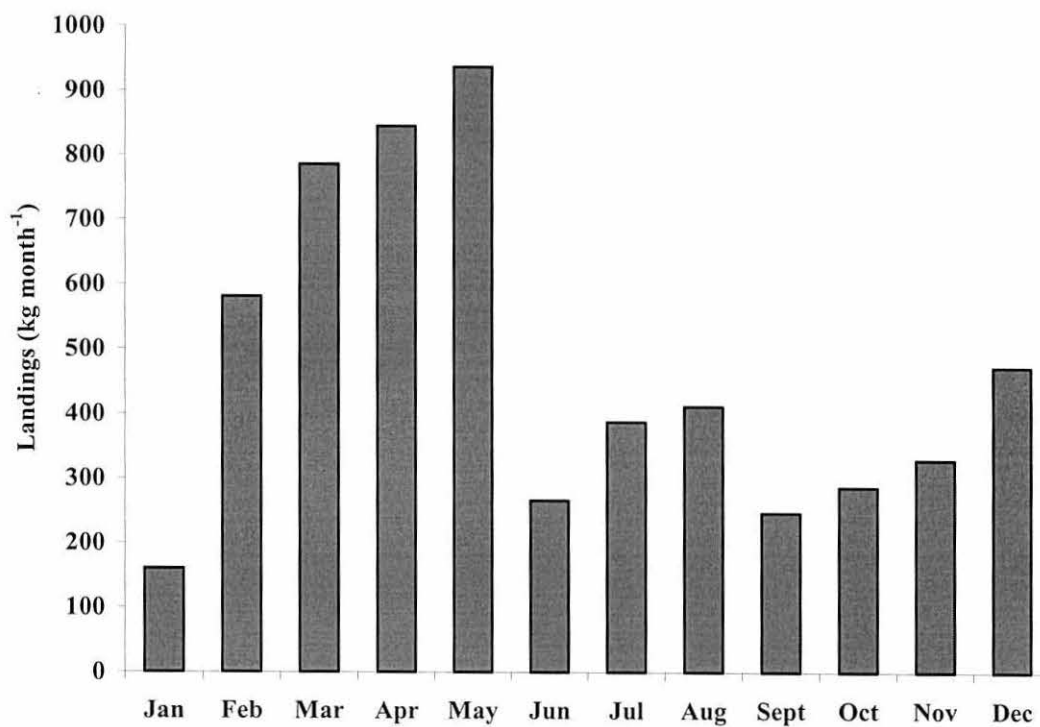
Figure 11: Total landings of mud crabs, predominantly *S. paramamosain*, in the intertidal hand-fishery during 1997.
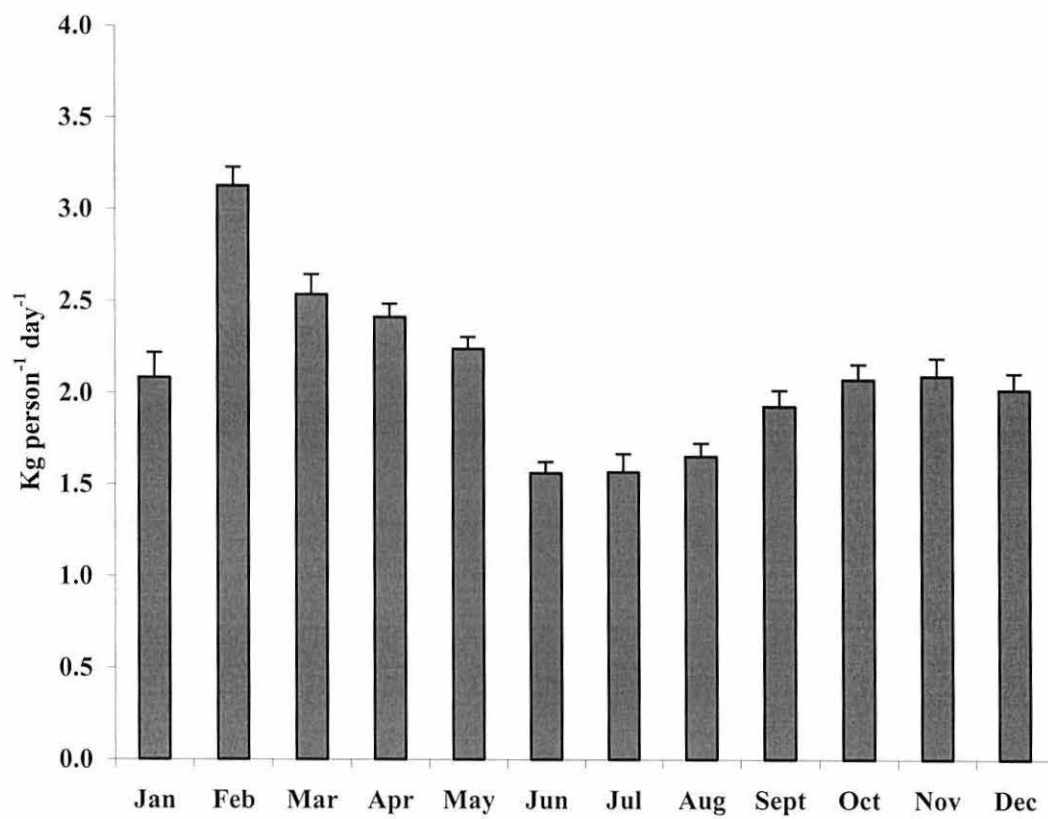
Figure 12: CPUE of mud crabs, predominantly *S. paramamosain*, collected at low tide in the intertidal mangrove study area during 1997.
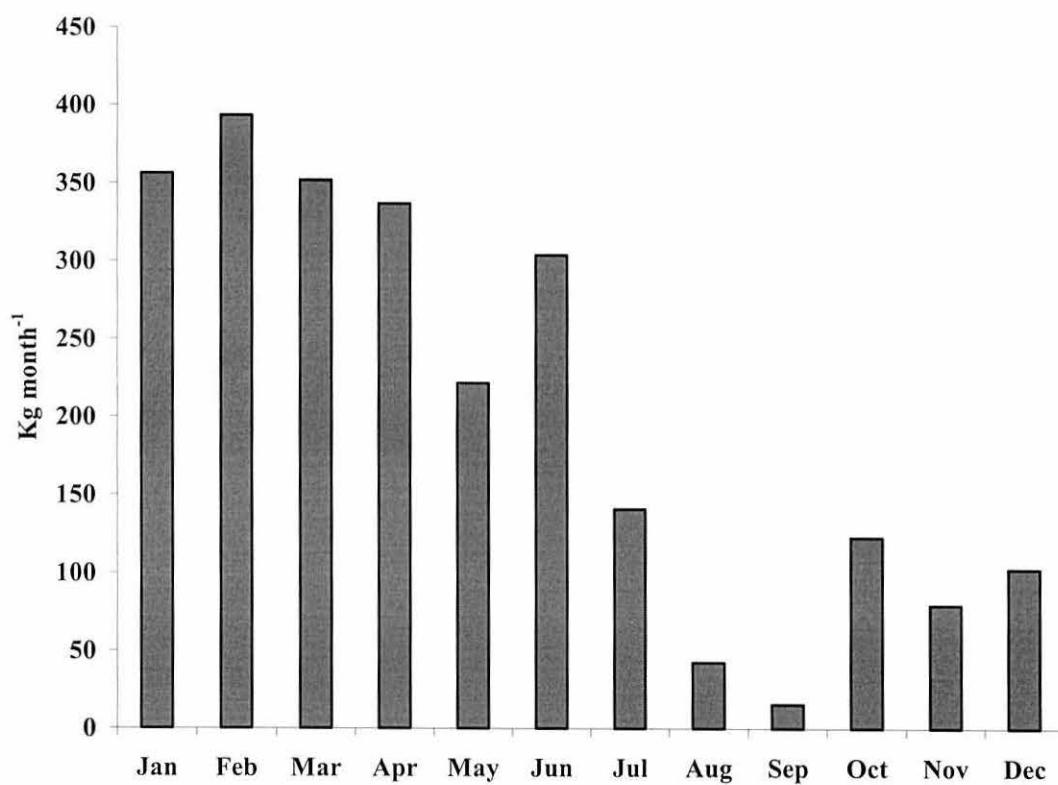
Figure 13: Total landings of mud crabs, predominantly *S. paramamosain*, in the intertidal hand-fishery during 1998.
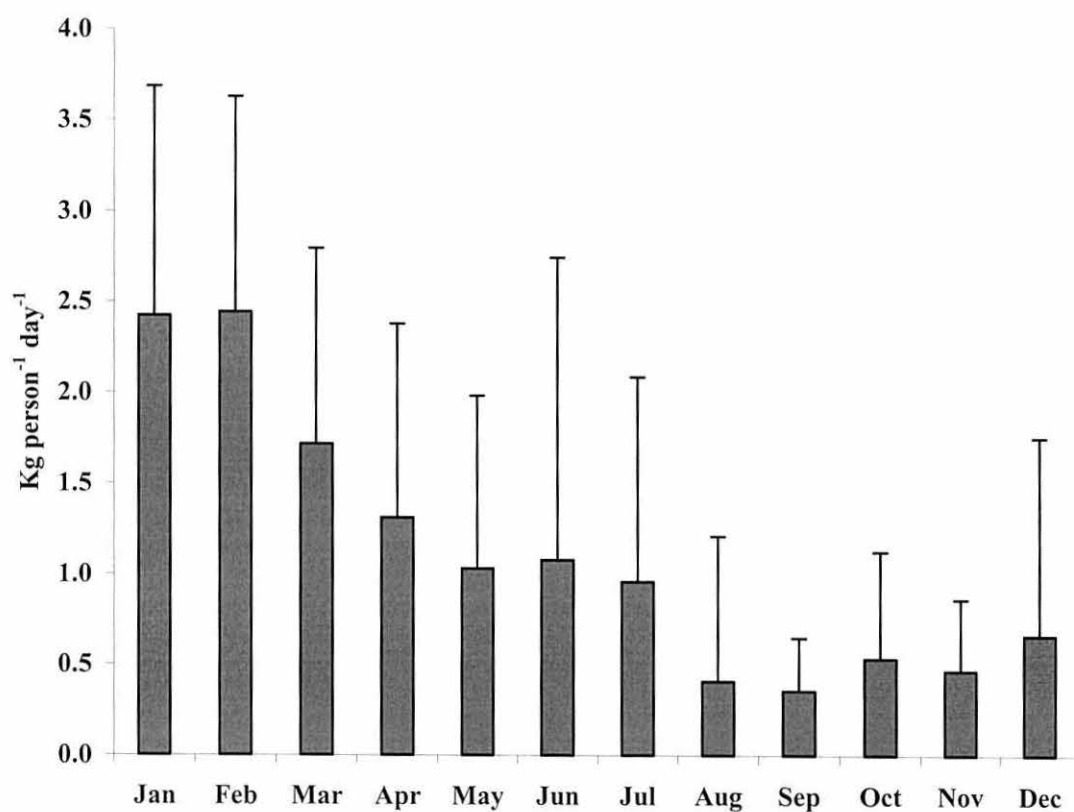
Figure 14: CPUE for mud crabs, predominantly *S. paramamosain*, collected at low tide intertidal mangrove study area during 1998.
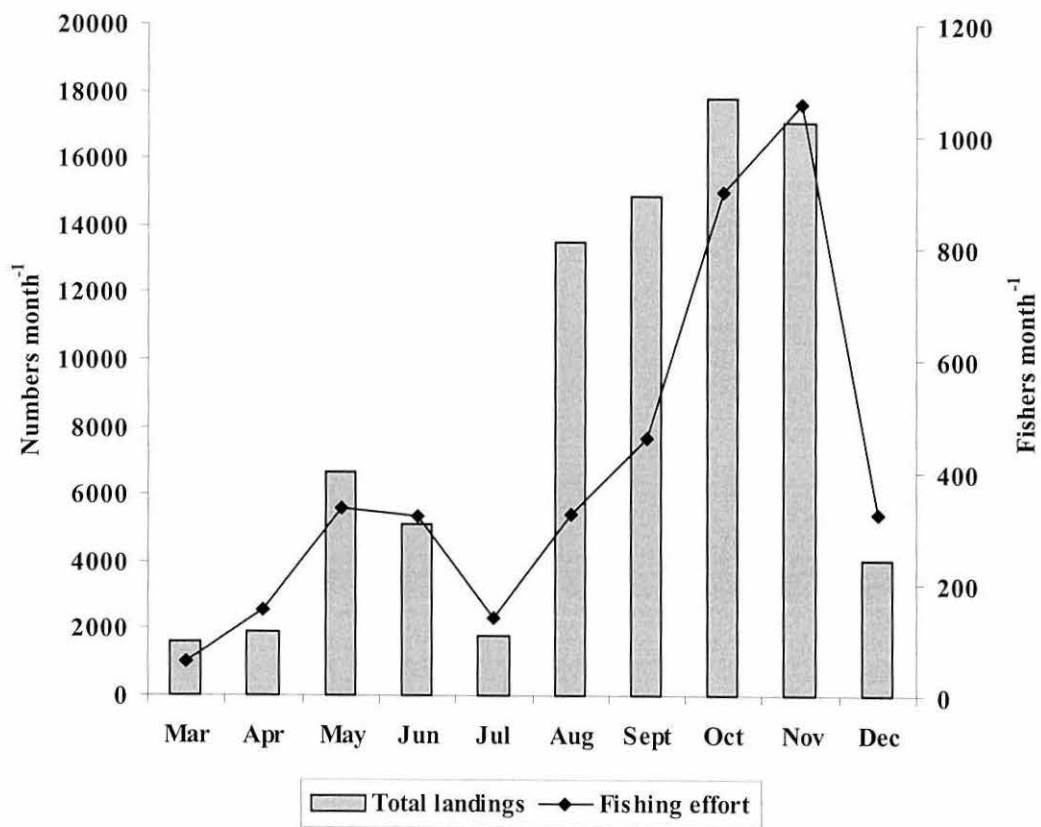
Figure 15: Increased fishing effort for night fishing corresponds to increase in number of small crabs during late rainy season 1998.
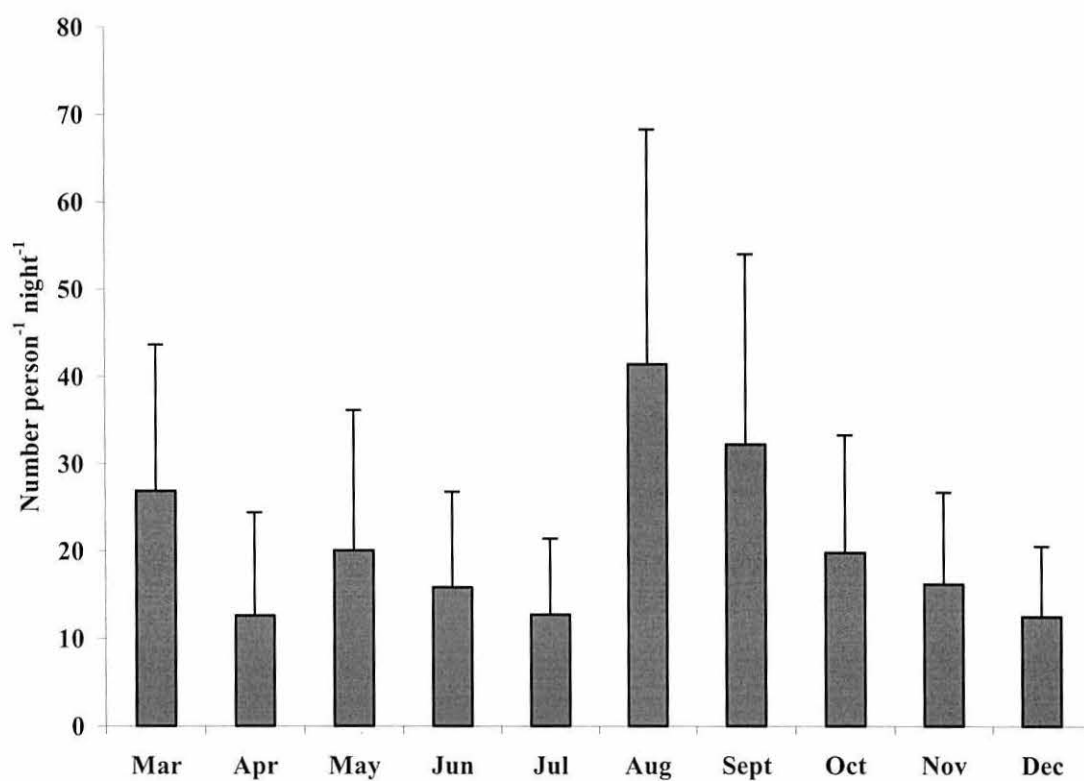
Figure 16: CPUE for crab juveniles, predominantly *S. paramamosain* collected at night on the intertidal mud flat of the mangrove study area during 1998.
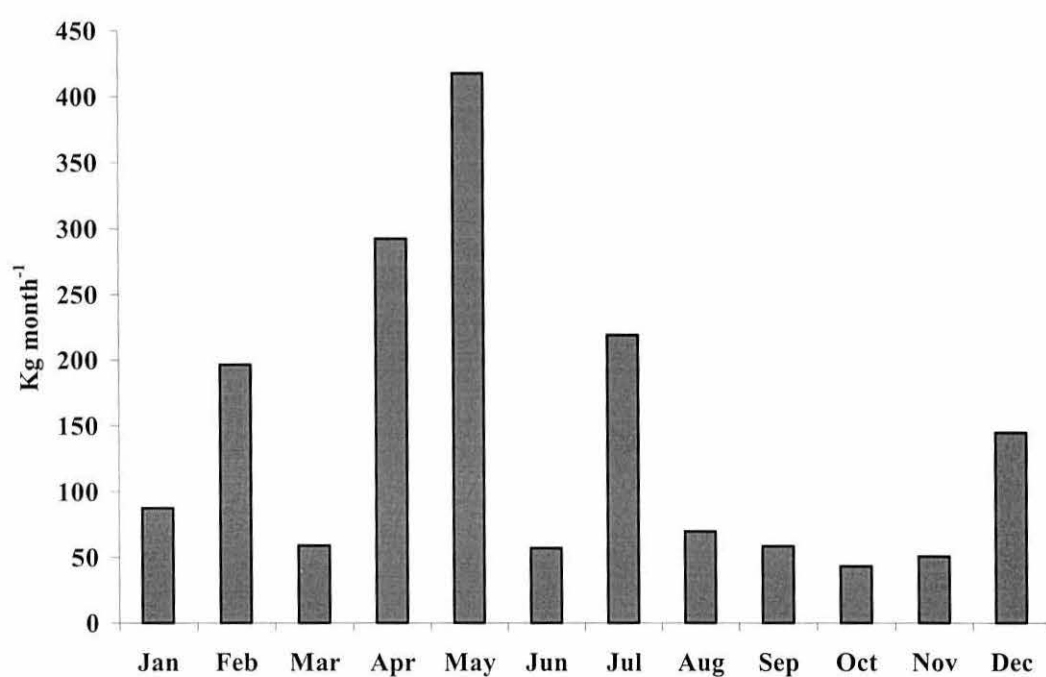
Figure 17: Total landings of mud crabs, predominantly *S. paramamosain*, in the intertidal hand-fishery during 2000.
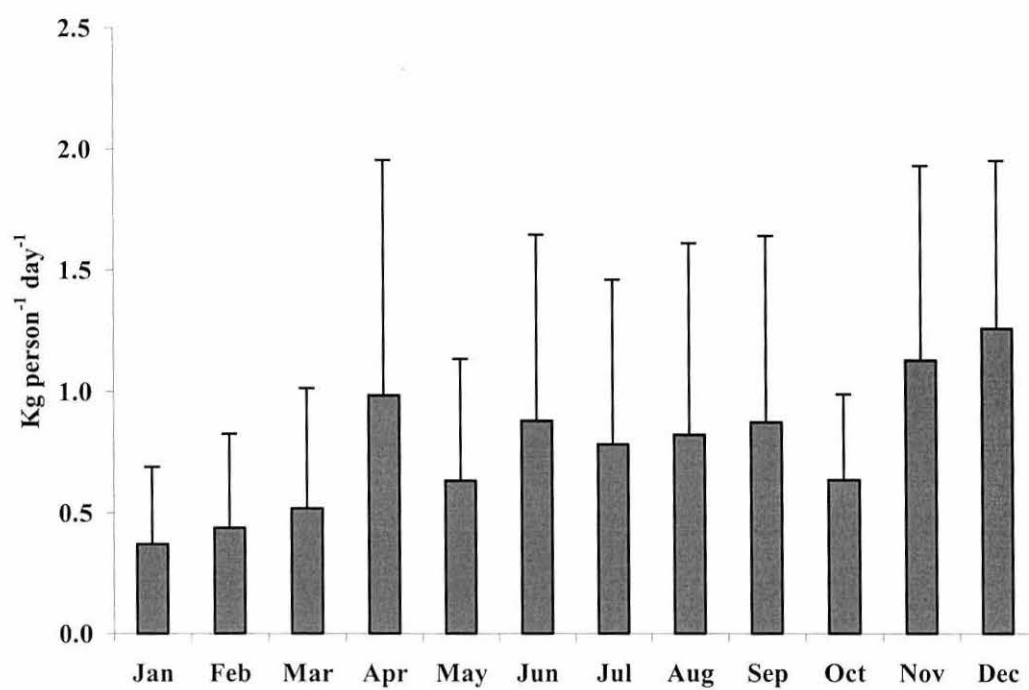
Figure 18: CPUE for mud crabs, predominantly *S. paramamosain*, collected at low tide intertidal mangrove study area during 2000.
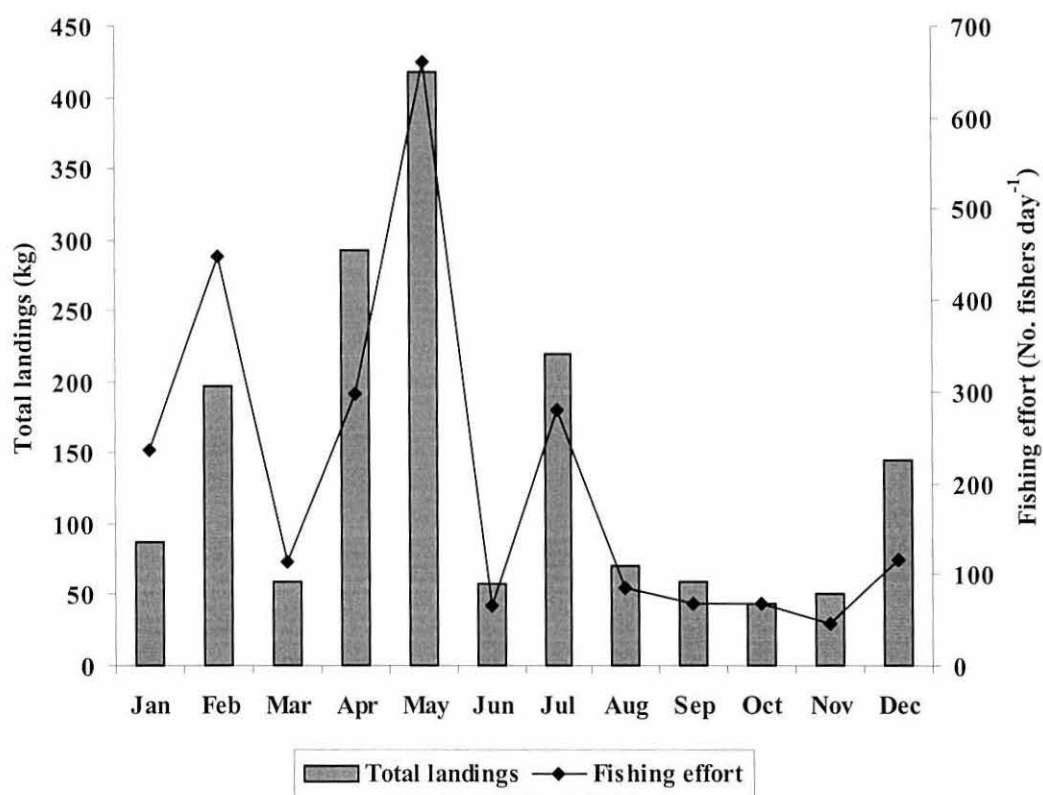
Figure 19: Relationship between fishing effort and total landings of intertidally caught crabs *S. paramamosain* during 2000 but no significant difference (p > 0.05).
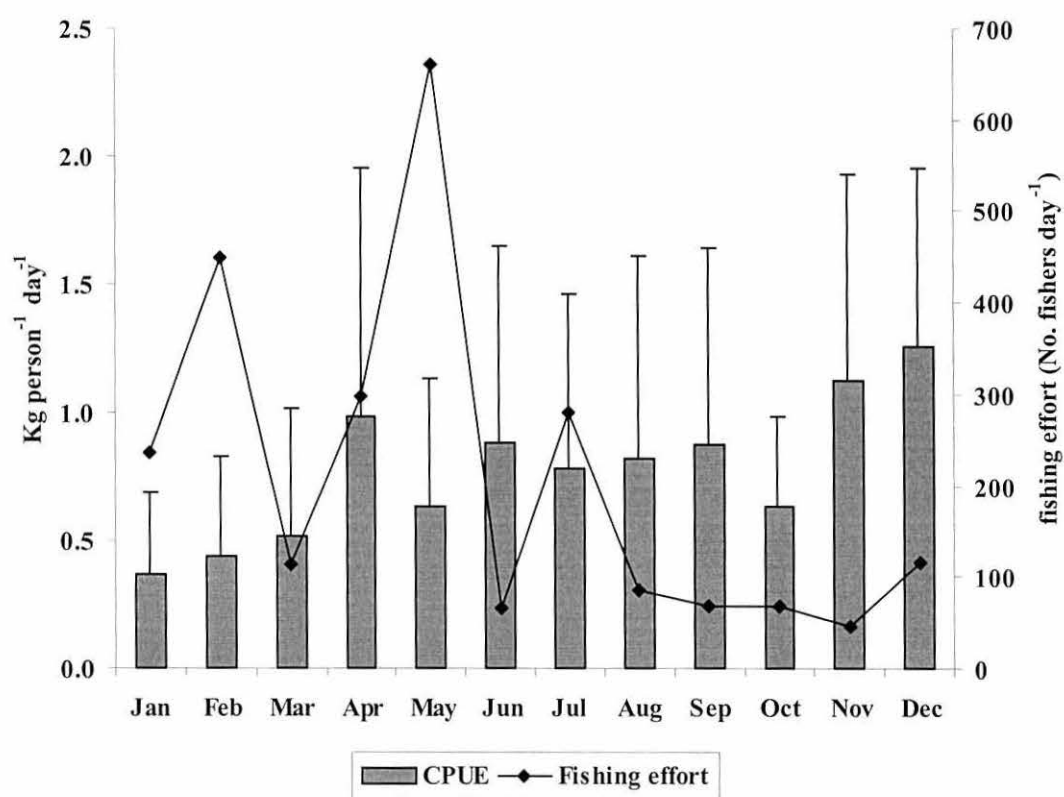
Figure 20: Relationship between fishing effort and CPUE of intertidally caught crabs *S. paramamosain* during 2000 but no significant difference (p > 0.05).
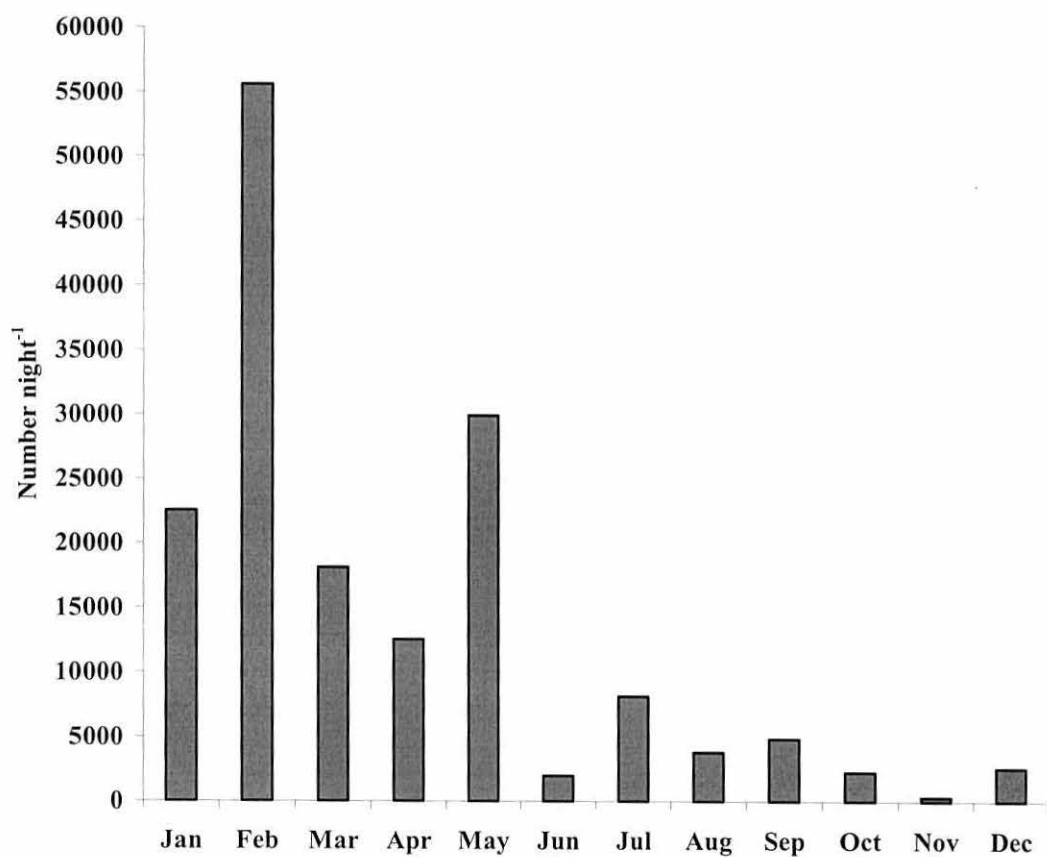
Figure 21: Number of juvenile mud crabs, predominantly *S. paramamosain*, collected at night on the intertidal mud flat of the mangrove study area during 2000.
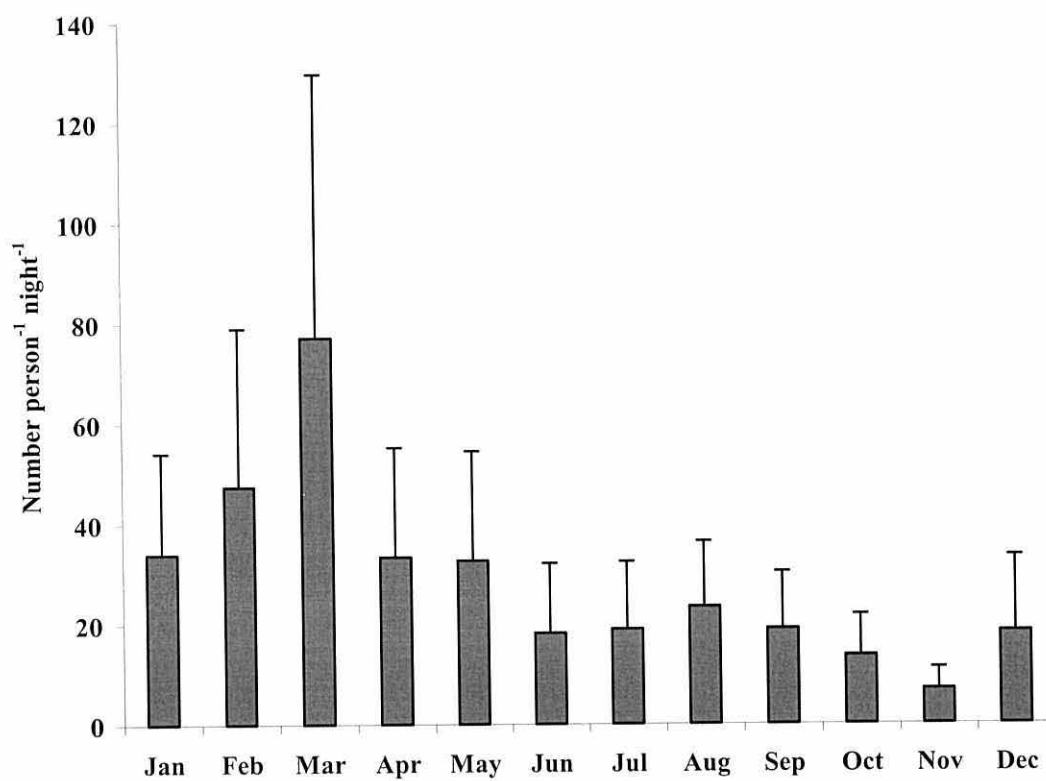
Figure 22: CPUE for crab juveniles, predominantly *S. paramamosain* collected at night on the intertidal mud flat of the mangrove study area during 2000.
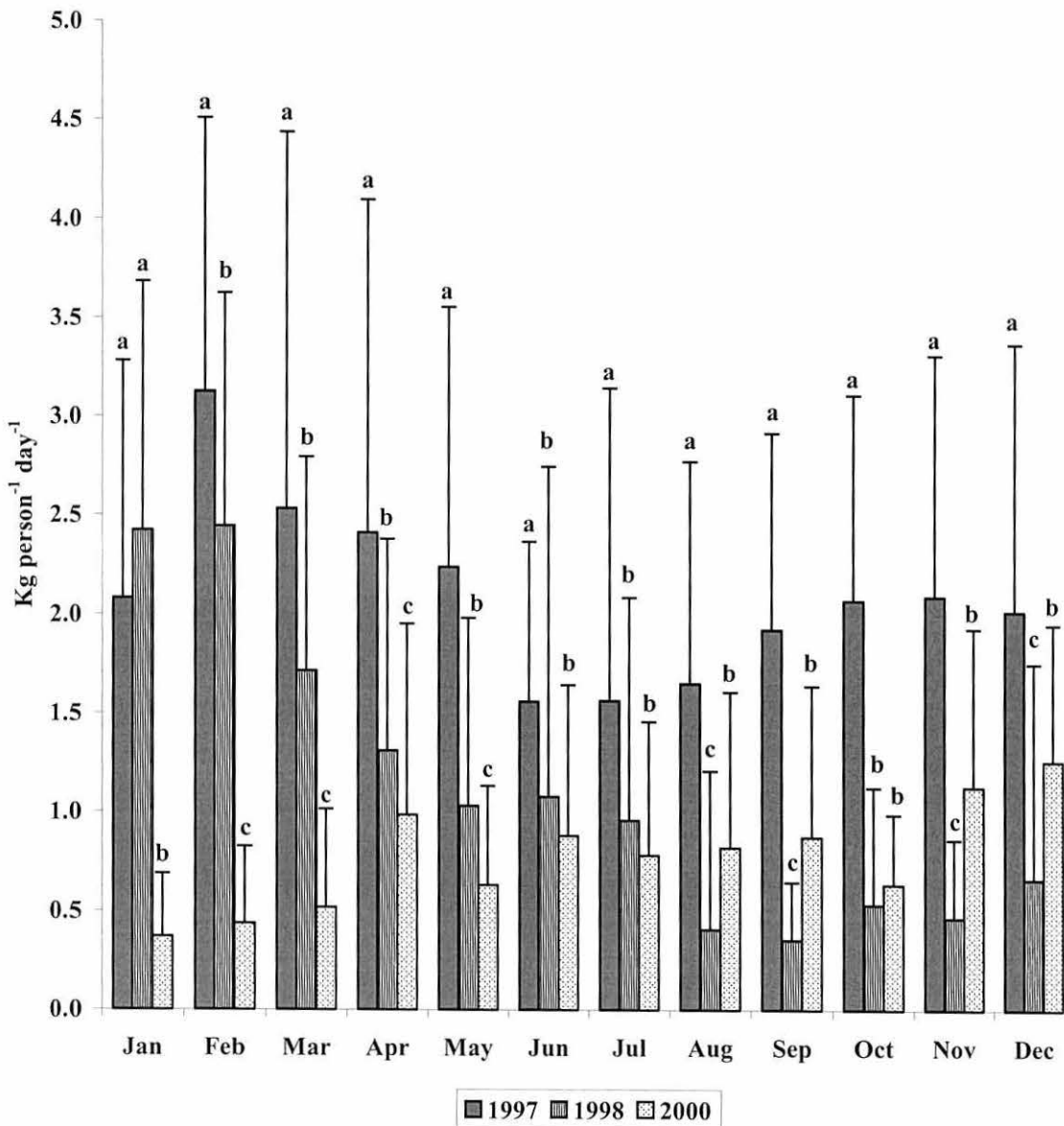
Figure 23: Comparison of CPUE (mean ± sd) for intertidal fishing of *S. paramamosain* between 1997, 1998 and 2000. Within each month, different letters indicate significant differences for CPUE between years (Kruskal-Wallis test, p<0.0001). However, for overall annual CPUE, only significant difference found between 97 and both 98 and 2000 (ANOVA, log transformed data, p<0.05), but no significant difference between 98 and 2000 (p>0.05).
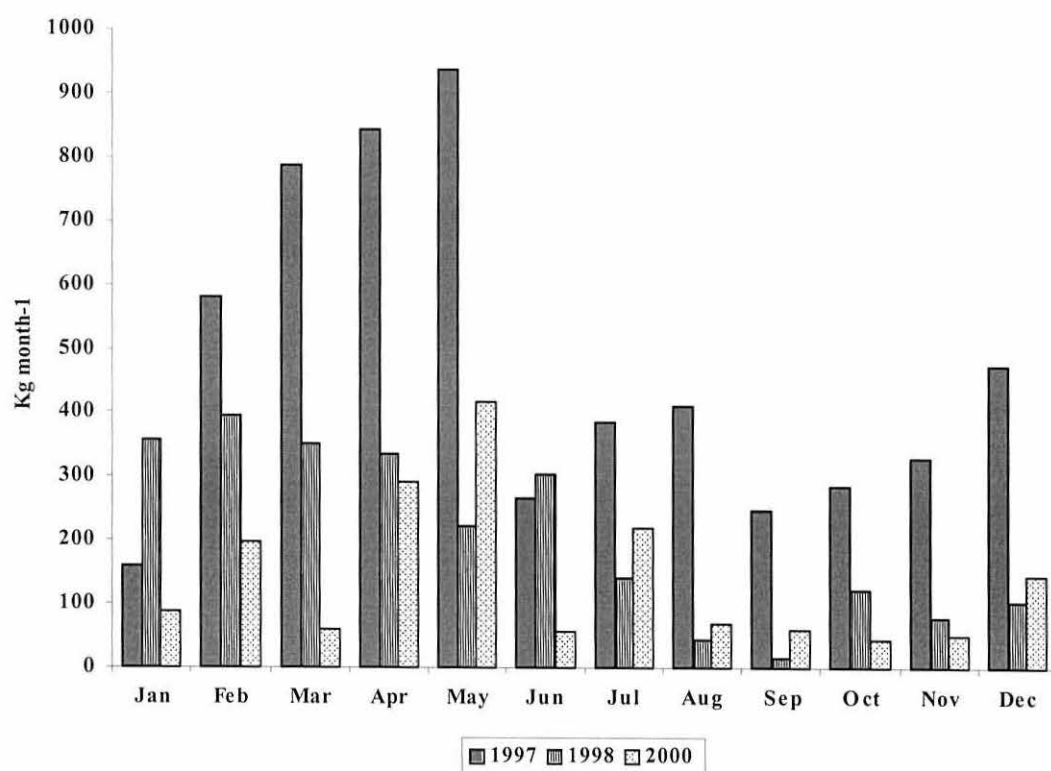
Figure 24: Comparison of total landings between 3 years, significant difference between CPUE of 1997 and both 1998 and 2000 (p< 0.05), but no significant difference between 1998 and 2000 (p> 0.05).
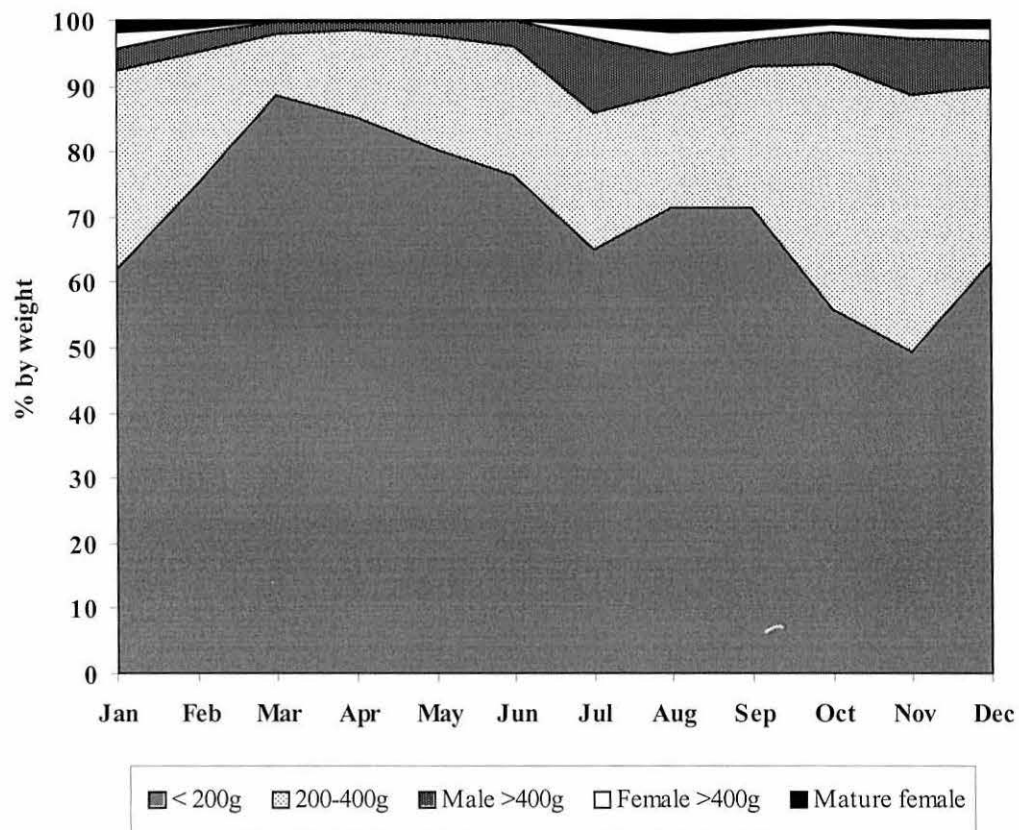
Figure 25: Commercial size classes (% of landings) for mud crabs, predominantly *S. paramamosain*, collected at low tide in the intertidal mangrove study area during 1997.
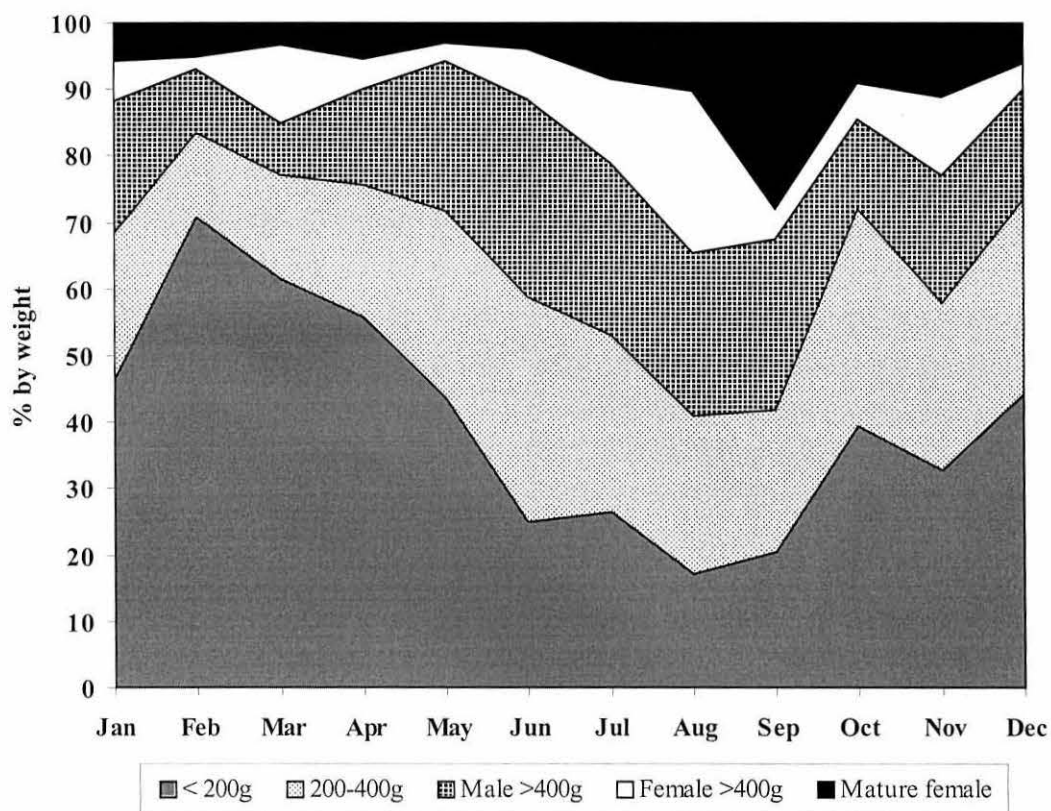
Figure 26: Commercial size classes (% of landings by weight) for predominantly *S. paramamosain* fished at high tide over the mud flats adjacent to the mangrove study area during 1997.
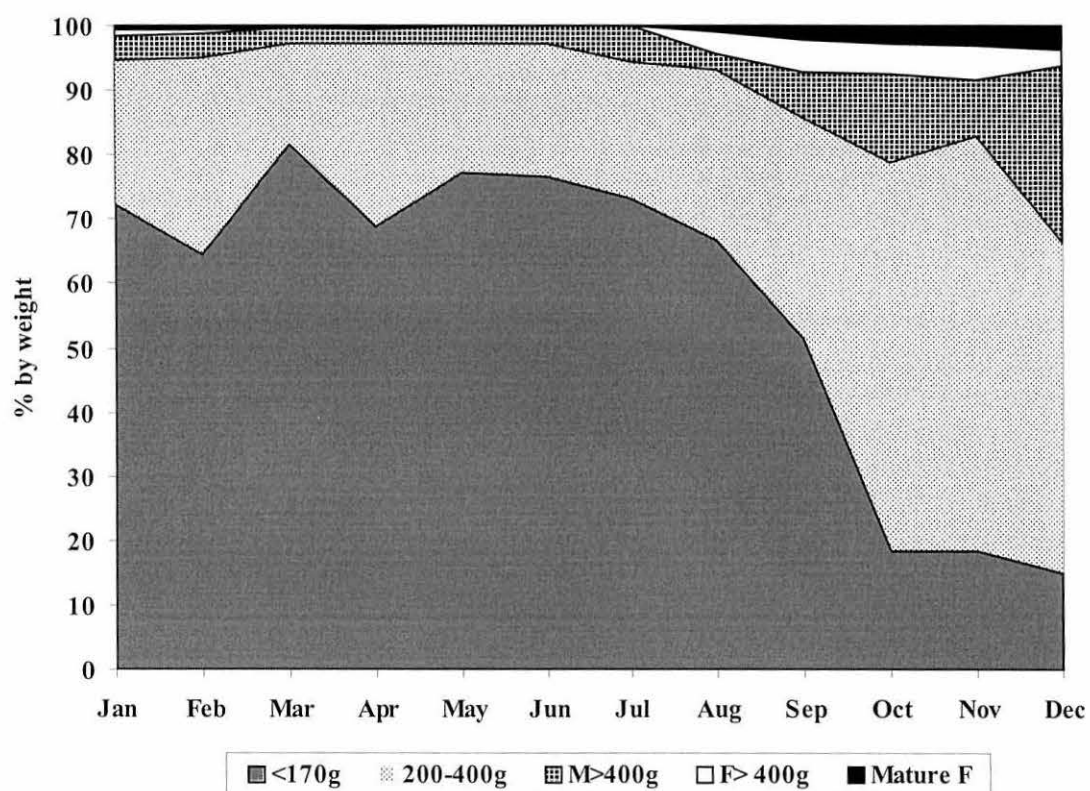
Figure 27: Commercial size-classes (% of landings by weight) for mud crabs, predominantly *S. paramamosain*, collected at low tide in the intertidal mangrove study area during 1998.
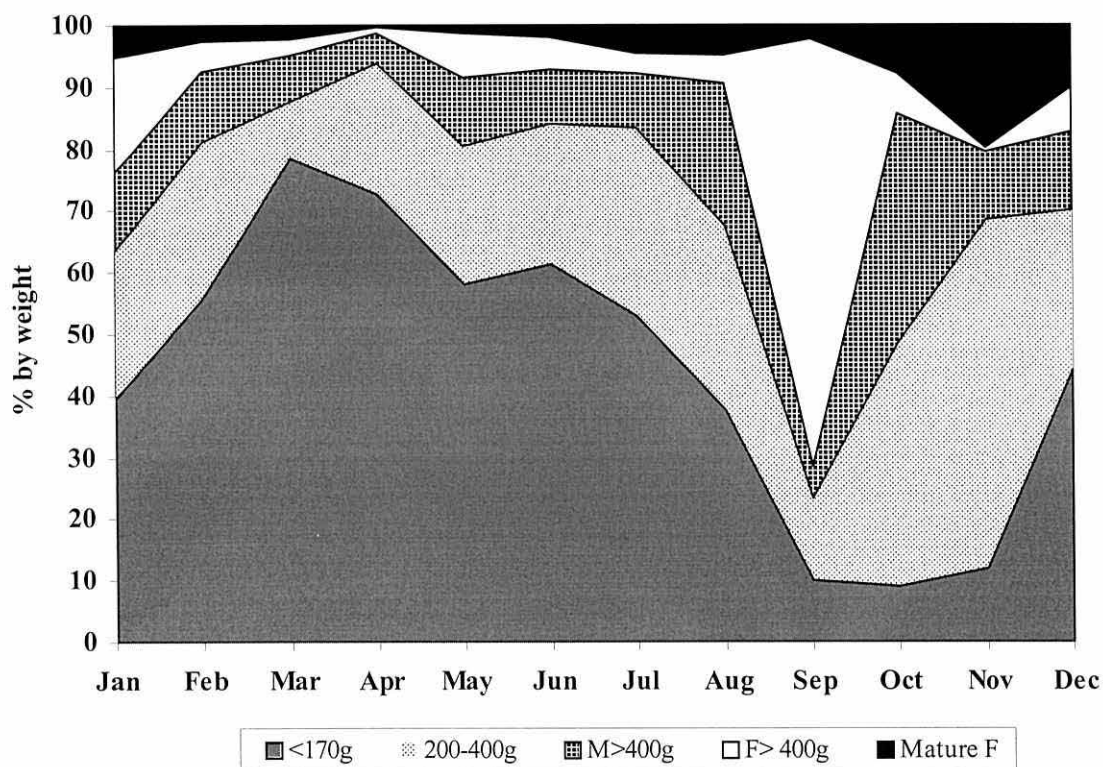
Figure 28: Commercial size-classes (% landings by weight) for mud crabs, predominantly *S. paramamosain*, fished at high tide over the mud flats adjacent to the mangrove study area during 1998.
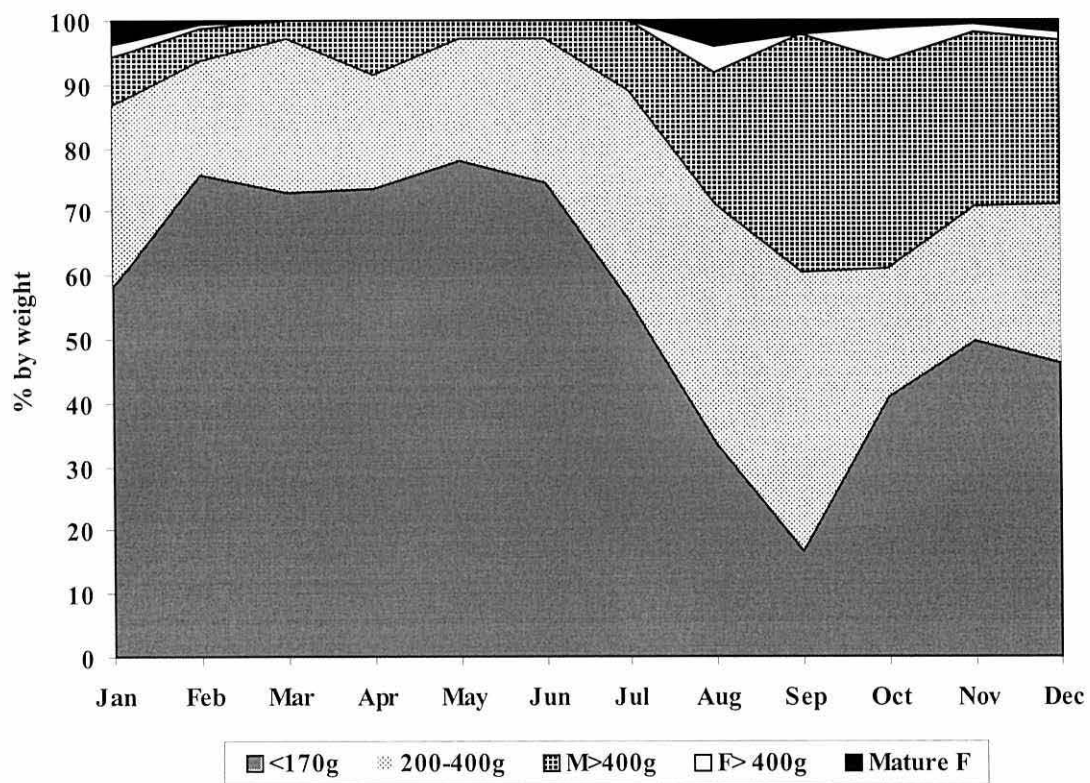
Figure 29: Commercial size-classes (% of landings by weight) for mud crabs, predominantly *S. paramamosain*, collected at low tide in the intertidal mangrove study area during 2000.
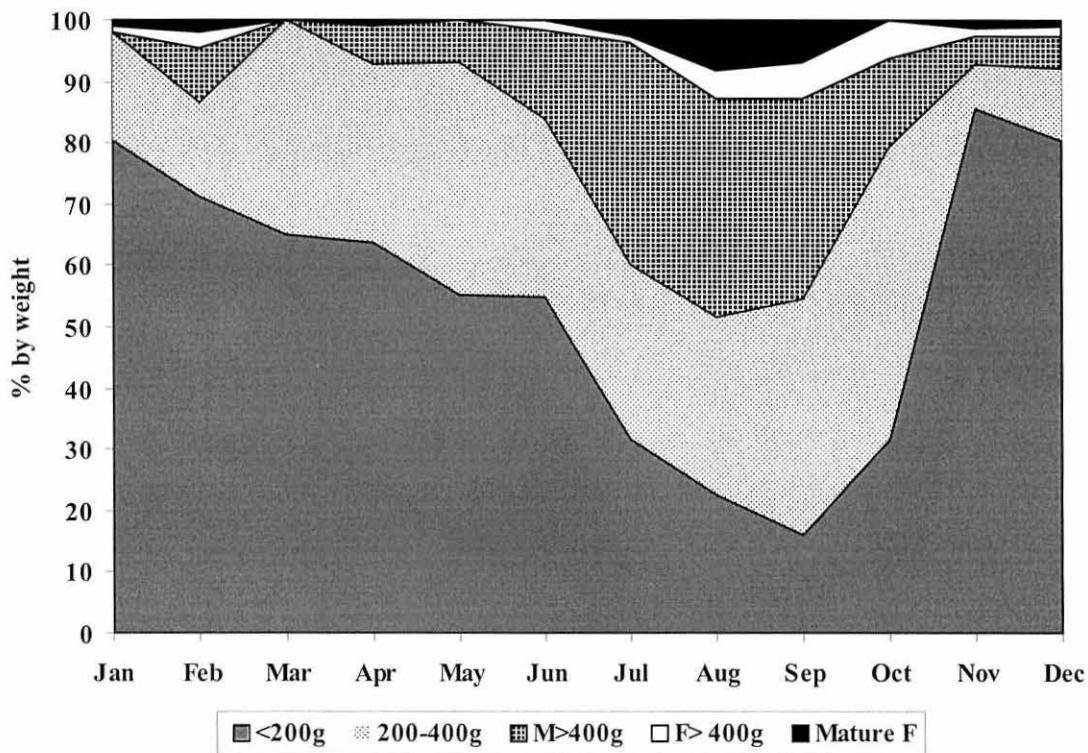
Figure 30: Commercial size-classes (% landings by weight) for mud crabs, predominantly *S. paramamosain*, fished at high tide over the mud flats adjacent to the mangrove study area during 2000.

DISCUSSION

Salinity is one of the most important factors limiting the distribution for a number of crustacean species (Miller and Maurer, 1973; Mair, 1980). In mud crabs, *Scylla* spp., distribution and abundance among the species (Keenan *et al.*, 1998) and within different stages of species (Hill, 1974 & 1975; Baylon *et al.*, 2001) may also be controlled by salinity. Keenan *et al.* (1998) stated that the distributions of the four *Scylla* species may be related to their salinity preference. They pointed out that the most widely distributed species, *Scylla serrata* is dominant in oceans where surface salinity is greater than 34ppt. The main distributions of the other species (*Scylla paramamosain*, *S. tranquebarica* and *S. olivacea*) are in the seas with salinity generally below 34 ppt and in mangal and estuarine habitats where seasonally low salinity occurs. In the present study, salinity was observed to vary widely throughout the year. Freshwater conditions dominated during the period of monsoon season, with salinity as low as 0ppt throughout the water column. This finding is consistent with previous studies in the Dinh An estuary by Wolanski *et al.* (1996), who reported that during the monsoon season freshwater reached a depth of 5m from the surface at the river mouth. As a result, persistent freshwater conditions are expected in the mangal through all tidal cycles at that time of year. Although there is a relationship between abundance of crabs and salinity, *S. paramamosain* did not disappear during the dominant freshwater period. This is consistent with the study of Keenan *et al.* (1998) who reported that *S. paramamosain* is found predominantly in estuary habitats with highly seasonal variation in salinity.

Macintosh *et al.* (2002) have confirmed two common mud crab species of the genus *Scylla* found in the mangrove ecosystem of the Mekong Delta, known as green crab (*S. paramamosain*) and red crab (*S. olivacea*). Their confirmation has strongly supported the results of the present study, with *S. paramamosain* as the predominant species. *S. olivacea* is reported to be associated with mangrove forest and lower salinity areas with direct freshwater run-off. It is perhaps surprising that it was not more abundant in the study area. This species might be found more abundantly in further inland mangrove forest systems. In addition, Macintosh *et al.* (2002) also

mentioned that the species composition varies from region to region in the delta and this may explain the predominance of *S. paramamosain* in the study area.

The lack of any clear modal progression in the size-frequency data for both species *S. paramamosain* and *S. olivacea* indicates continuous recruitment of young crabs to the population in the mangrove. The lower limits in the size-frequency data suggests that recruitment into the mangrove is taking place at a size greater than 20 mm CW. At the end of the size range, very few crabs of more than 100 mm CW were found in the area. This could be explained by continuous natural and fishing mortality and migration. Tongdee (2001) also studied recruitment of juvenile crabs *Scylla* spp in Ranong, Thailand with modes remaining between 75-90 mm CW and pointed out that left skew in size distribution, coupled with upper limit (greater than 110 cm) might indicate migration and mortality being insufficiently replaced from the recruitment of crab juveniles. In the present study, over-fishing, however, may be a possible factor that removed large crabs from this relatively accessible population resulting in greater limit of bigger animals in the study area.

The ratio of females to males of *S. paramamosain*, which was high in the dry season but remained lower during the monsoon period, may confirm the offshore migration for spawning by females. This is also in agreement with Tongdee (2001) who found the similar situation and refers to the migration of females out of the mangrove area during the spawning period. The extreme difference in male to female ratio found for *S. olivacea*, especially during the early monsoon may suggest a high rate of offshore migration of females during the spawning period, earlier than that seen in *S. paramamosain*. However, Branco (1990) studied the relative abundance of 15 crab species in a mangrove, pointed out that males were usually more prevalent than females. Similarly, Lari (1995) and La Sara (2001) also found a significant difference in sex ratio of *Scylla* spp. with more males than females. Thus the observed difference in male and female ratio may also be a biologically characteristic typical of this mangrove crab species.

Although there is variation within and between years, the CPUE data recorded from 1997 to 2000 generally indicates the persistence of populations of *S. paramamosain*, despite the large seasonal salinity variation. Continuous recruitment of juveniles

throughout the year in spite of a prolonged period with extremely low salinity may be the main factor in maintaining the population. DeVries *et al*. (1994) pointed out that successful recruitment of juveniles to adult habitats results in the persistence of populations. Robertson (1987), Forbes and Hay (1988) and Robertson and Kruger (1994) also suggested that despite seasonal peaks, crab recruitment could be continuous throughout the year in some tropical populations.

The peak of relative abundance of *S. paramamosain* occurs in the dry season when salinity was high, which is consistent with observations of previous workers (Hill, 1975; Chandrasekaran & Natarajan, 1994; Tongdee, 2001). Furthermore, Poovachiranon (1992) supposed that juvenile abundance may be related to salinity in tropical areas. However, the finding that the population was maintained throughout the period of freshwater during the monsoon season is not typical for mud crab populations studied elsewhere, possibly reflecting species differences. Chandrasekaran and Natarajan (1994) studied seasonal abundance and distribution of mud crab juveniles in Pichavaram mangrove, southeast India. They found that in October, when salinity was very low (1.5-2ppt) due to maximum rainfall, the population of juveniles was totally absent. Hill (1979) reported that a minimum salinity necessary for the survival of juveniles of *S. serrata* in South Africa was 2ppt. Therefore high mortalities of *S. serrata* may occur in periods of freshwater flooding (Macnae, 1968; Hill, 1975). However, Jones (1984) noted that many of the mangrove crabs can tolerate a wider range of salinities beyond the restrictive salinity regimes to the mangals themselves. In addition, Macintosh (1988) highlighted the capacity of tolerance of extremely low salinity affected by heavy rainfall is a feature of mangrove crabs. Davenport and Wong (1987) indicated that *Scylla* sp. proved to be powerful osmoregulators at low salinity and inability to discriminate between salinities. They also found the crabs could survive for several hours in freshwater under laboratory conditions. Furthermore, Forbes and Hay (1988) investigated the population of *S. serrata* in South Africa during the flooding period and confirmed the negligible effect of freshwater on recruitment and survival of the crab population. Thus the persistence of *S. paramamosain* recruitment during the rainy season is not inconsistent with some previous studies, but may indicate habitat specificity and environmental tolerance of this species.

The fact that relative abundance of crab juveniles increased to another peak during the post-monsoon in 1998 but not in 2000 may indicate variation of recruitment from year to year. This is consistent with Hill (1975), who found that for *Scylla serrata* in southern Africa recruitment of crabs was variable and irregular during four years of study. He also mentioned the reason for variation may be due to the effect of ocean current on movement of the larvae. However, in the present study water salinity and turbidity may be the factors.

Recruitment of small juveniles was in August-September comprising crabs about 3-4 cm CW. These juveniles are likely to be about 2-3 months post-settlement (Ong, 1966) indicating initial recruitment taking place in April- May when salinity was still high. In contrast, lower salinity was recorded during the same period in 2000 as a consequence of long-lasted floods that had occurred in inland areas. Freshwater may have been flushed further seaward, resulting in an unusually low salinity environment that may have deterred the immigration of early stage crabs in that year. In contrast, under more normal conditions, a second peak of juvenile recruitment was observed in 1998. Furthermore, strong outflow currents resulting from high freshwater flow may cause high turbidity in the subtidal areas. Joel and Raj (1986) suggested that optimum light is believed to be the major factor in distribution of *Scylla* spp., and high turbidity may interfere with light penetration into the water column during the freshwater period and could affect the crab abundance. In the normal estuarine conditions, Tongdee (2001) found two recruitment peaks for small crabs (*Scylla* spp.) in Thailand, with one of them in the post-monsoon season. Although a peak in recruitment of small crabs was recorded in the rainy season 1998, no subsequent peak was reflected by the CPUE data.

The patterns of size-composition recorded over the three years shows the difference in population structure between crabs collected in the mangal and the estuary. A higher abundance of small crabs obtained in the intertidal fishing during the dry season (from January to June) coupled with continuous loss of large crabs from the intertidal population suggests that the mangrove is acting as a nursery. More large crabs were caught in the subtidal fishery during the period of the monsoon season (August-October) indicating migration of large crabs out of the mangals into subtidal habitats. This is in agreement with similar results for *Scylla serrata* (Hill *et al.*, 1982) in

Australia. In addition, a similar size-distribution was reported for *Scylla serrata* (Hill *et al.*, 1982; Hyland *et al.*, 1984) indicating that they are subtidal species, as sub-adult and adult, migrating into the intertidal zone at high tide to feed. The seasonal peak in mature females is consistent with data published for *Scylla serrata* indicating peak offshore spawning activity during the rainy season (Arriola, 1940; Brick, 1974; Hill, 1975). Pripanapong (1993) cited by Tongdee (2001) showed two seasonal peaks of mature females in March and August-September. Data recorded in the present study show a seasonal peak of mature females observed in August-September. This is in agreement with Hill (1994) who noted that the season of mature female *Scylla serrata* in Australia was in September-October. In the Andaman Sea, peaks of mature females of *Scylla* spp. were found from October-December, and spawning migration periods were during October-February (Poovachiranon (1992).

In summary, the standardized, non-selective, hand-fishing methods used in the intertidal study area coupled with the availability of daily recording data set for each month's landings appears to be a powerful tool in studying seasonal relative abundance and long-term trends in the crab population. As the records are based on catches of individual fishers, they could also contribute to evaluation of the economic importance of crab fishing to local communities. The CPUE data of intertidally caught crabs and night fishing for juveniles indicate that continuous recruitment of crabs occurs through the year, despite a prolonged period of extremely low salinity. However, as smaller animals may be less tolerant of low salinity conditions, some further studies on salinity are required.

CHAPTER III

GROWTH AND SALINITY TOLERANCE IN JUVENILE *SCYLLA PARAMAMOSAIN* UNDER LABORATORY CONDITIONS

INTRODUCTION

The study of growth in crustaceans under natural conditions is problematic as the animals possess an exoskeleton which is periodically shed. Determination of growth for the animals has often been based on the observation of captive specimens or from tagging data (Hartnoll, 1982, 2001). Growth is reported to be controlled by a variety of intrinsic and extrinsic factors (Hartnoll, 1982). The intrinsic factors are mostly related to endocrine mechanisms, in which moulting hormones play the major roles. The extrinsic factors include temperature, salinity, food availability and light. Temperature and salinity, however, are considered the most important factors affecting growth in marine crustaceans, including crabs.

Salinity in combination with temperature has a major effect on growth, abundance and recruitment in crustaceans (Tagatz, 1971; Hill, 1974; Young and Hazlett, 1978; Hardy *et al.*, 1994; Gonçalves *et al.*, 1995) especially in temperate and sub-tropical regions. Although temperature is reported to be a critical factor affecting growth and survival of crabs, salinity is very important in species that inhabit estuaries (Guerin and Stickle, 1997).

Effects of salinity have been widely studied in crabs in an attempt to determine their tolerances and preferences (McGaw and Naylor, 1992; Anger, 1996; Anger *et al.*, 1998; Spivak, 1999; Anger and Charmantier, 2000) and changes in osmotic regulation (Dehnel, 1962; Smith, 1967; Foskett, 1977; Zanders and Rojas, 1996; Charmantier *et al.*, 1998). Salinity tolerance or preference in crustaceans may be different between species (Guerin and Stickle, 1997; Spivak, 1999) or even within species which are geographically/regionally separated (McGaw and Naylor, 1992; Kumlu and Jones, 1995). All the studies agree that inherent differences or ecological adaptation may result in divergence in congeneric species. In addition, marine invertebrates inhabiting differing salinities are known to exhibit intraspecific variation in physiological response patterns to salinity (Guerin and Stickle, 1997). In general, most estuarine crabs are considered to be euryhaline (Foskett, 1977; Zanders and Rojas, 1996; Anger and Charmantier, 2000 and see Kinne, 1971 for more review). Ontogenetic studies in

crabs have indicated that the establishment of hyper-/hypo-osmoregulatory capability in crabs increases during their development (Charmantier *et al.*, 1998; Anger and Charmantier, 2000). This has enabled the animals to be more tolerant to highly variable salinities at the later life stages.

The recent revision of the genus *Scylla* and confirmation of the four species (Keenan *et al.*, 1998) has encouraged study of the biology and ecology of each species. Little is known about salinity tolerance and osmoregulation capability of *Scylla*, as yet. Although previous studies in this genus have indicated that they are hyper-/hypo-osmoregulators (Davenport and Wong 1987; Chen and Chia, 1997), few studies have examined the capability of the known species to tolerate low salinities. In other crabs, studies of the effects of reduced salinities on growth and survival showed a decreasing trend for both, especially at extreme low salinities (i.e. 5ppt) in *Callinectes* (Guerin and Stickle, 1997), grapsids (Anger, 1996; Spivak, 1999; Anger and Charmantier, 2000) and *Carcinus maenas* (Anger *et al.*, 1998). Since salinity tolerance is associated with osmoregulation, osmoregulatory capability of crabs was typically measured when animals were subjected to test salinities. The results revealed that osmoregulation increases the metabolic rates of crabs in dilute media (Mantel and Farmer, 1983; Spivak, 1999). Ammonia, urea and total nitrogen excretion have also been measured following the exposure of crabs to different salinities. Chen and Chia (1996) found an increasing trend of ammonia-N excretion with decreased salinities. In another study, Chen and Chia (1997) followed *Scylla* sp. subjected to different salinities and reported that the crabs are hyperosmotic to the medium below 33ppt, but osmoconform above this level.

The mud crabs, *Scylla paramamosain* has been reported to be predominant in the Mekong Delta (Keenan *et al*, 1998; Le Vay, 2001; and results from Chapter 2). They are found to be intertidally associated with mangrove in the estuary where they are likely to encounter variable salinity levels. They are abundant throughout the year, including the monsoon period, which raises the question of whether they are still able to survive and grow well at low salinities in captivity.

In the present study, effects of different salinities, especially low salinities, on performance of *Scylla paramamosain* juveniles under laboratory conditions were investigated. The aim was to study survival at the lower salinities (0-5ppt), and relate this to the observed persistence of this species in the estuary during the monsoon (see Chapter 2). In addition, the study aimed to determine suitable salinities for nursery and grow-out of juveniles in tanks and ponds. At the same time, the growth rate of hatchery-reared *S. paramamosain* was also studied.

MATERIALS AND METHODS

Source of wild crabs

Wild crabs were purchased from an agency in the eastern coastal area of Bac Lieu province where all crabs collected on the intertidal mud flats along the coast by the fishermen are landed. The fishers use push nets, scoop nets or collect crabs by hand during low tide. Crabs of all sizes are captured, but a high number of tiny crabs (3-6 mm CW) is often found in the landings. From the agency, crabs are taken for pond culture, especially integrated culture with shrimp in mangrove ponds by the farmers in Camau (a neighbouring coastal province to Bac Lieu).

Source of hatchery- reared crabs

Hatchery-reared crabs were produced at the hatchery of the Institute for Marine Aquaculture of Cantho University. Crab larvae were hatched from healthy gravid females obtained from the market which were originally caught from the coastal areas of the Mekong Delta. The larvae were reared in 30 l grey PVC cones connected to a biofilter system. They were fed with enriched rotifers from day 1 to day 4. Rotifers were cultured on Culture Selco and then enriched in micro-algae with 8000 cells/rotifer or in DIS (Dry Immune Selco, an experimental enrichment product of INVE technologies NV, Belgium) for 8 hrs before feeding. From day five onwards enriched *Artemia* nauplii were supplemented. The light regime was 12 hours light and 12 hours dark. Salinity was maintained at 30 ± 2 ppt and temperature was kept stable at 29 ± 0.5 °C. After the metamorphosis to megalopa, they were transferred to 60 l rectangular tanks. Frozen *Artemia* biomass and peeled shrimp were maintained as feed until they moulted to crab stages and were used in the experiments.

Rearing facilities

The rearing facilities are generally described below and will be indicated specifically in different experiments.

Small plastic jars 7 cm in diameter and 7.5 cm in height (200 ml in total volume) were used to hold crabs individually. The jars were perforated to allow exchange of water. All jars were labeled on the top. In some experiments, round plastic baskets of 14 cm in diameter and 8.5 cm high were used to contain the bigger crabs (Fig. 1). Rearing tanks consisted of 60 l black rectangular tanks or 500 l round blue tanks. Water level was maintained at 30 l in 60 l tanks and 150 l in 500 l tanks. Jars and baskets were remained floating in the rearing tanks during the experiments.

In Can Tho, experiments were carried out in a re-circulation system with biofilters consisting of two 100 l buckets containing limestone chips (3 x 5 cm in size) as substrate for bacteria, connected to a 60 l plastic tanks system (Fig. 2). The system was inoculated with NH_4Cl (5ppm) to encourage growth of nitrogenous transformating bacteria, for 2 weeks before starting. Water was recirculated in the rearing tanks at a rate of 800-1000 ml minute^{-1}.

At Vinh Chau, a batch exchange system was used for experiments (Fig. 3), in which, water was completely renewed every 2 days. Sea water (25 –30 ppt) used for renewal was stored in 1 m^3 sedimentation tanks and diluted with freshwater to obtain the experimental salinities. Salinities were checked with a hand refractometer to the nearest 1ppt.

General methods

In all experiments, crabs were measured (carapace width) and weighed and kept individually in the jars or baskets with labels. Where crabs were exposed to different salinities, they were acclimatized for a period of 24-72 hours depending on treatments. During the experiments crabs were fed with chopped peeled shrimp to excess once a day in the afternoon. The left-over feed was completely removed on the following day. Crabs were checked for moulting and mortality twice a day, in the morning and in the afternoon when the feed was added. Newly moulted crabs were measured (CW) and weighed in the day after or when the shells had hardened.

Temperature and pH was recorded daily. In the re-circulation system NO_2^- and NH_4^+ were measured every 3-4 days.

Statistical analysis

Statistical analysis of growth data was done by one-way ANOVA. Non-parametric tests were used for development data when the Anderson-Darling Normal test showed they were not normally distributed. The Kruskal-Wallis *H*-test was employed in multiple comparisons of mean values, after appropriate checks for equality of variance.

Growth

Two experiments were undertaken to determine some growth parameters of hatchery-reared juvenile crabs including sizes (carapace width and body weight), moult duration, moult increment (% increment in CW and weight at each stage). For convenience, the first crab stage after metamorphosis from megalopa is named as stage 1 crab or crab 1. The second instar is crab 2, and so on.

Experiment 1: Growth in a recirculation system

Stage 1 crabs were transferred from the larval tanks to be reared individually in labelled perforated plastic jars in the 4 m^3 recirculation system tank (water depth 0.5 m) at the Can Tho hatchery. Salinity was maintained at 30ppt during the experiment period. As the experiment was set up indoors, temperature was recorded at 26-28°C in the morning and 28-31°C in the afternoon. Crabs were fed with frozen *Artemia* biomass and chopped shrimp to excess once daily. Moulting was recorded every day. Crabs were measured (carapace width) and weighed at each stage with a caliper and electronic balance with accuracy to 0.01g. Date of moulting was also noted for each stage. The system contained about 300 individuals at crab 1 stage. Sizes, moult duration, moult increment for each stage was recorded as the mean of a group that were at the same stage.

Experiment 2: Growth in a batch exchange system

Crabs were cultured in a batch exchange system in the Vinh Chau field station, in which water was renewed every two days. Small crabs were transferred from the hatchery in Can Tho to Vinh Chau at Crab 2 and held separately in jars as in Experiment 1. Salinity was maintained at 25ppt and temperature was recorded at 25-26°C in the morning and 28-30°C in the afternoon. Crabs were fed live *Artemia* biomass and chopped peeled shrimp once a day in the afternoon. Thirty- two crab 2 were followed continuously through their moults until stage 9. Thus, sizes, moult duration and increment were based on the same individuals. Recording of sizes and moult duration was undertaken as in Experiment 1. However, due to the unavailability of an accurate electronic balance, weights for crabs of stages 2, 3 and 4 were not recorded.

Salinity

Experiment 3: Wild crabs in a recirculation system

The aim of this experiment was to compare the growth and survival of wild juvenile crabs at different salinities. The experiment was conducted in a bio-recirculation system in Cantho. A total of 286 wild crabs with a size range of 5.0 mm - 16.5 mm CW were randomly distributed in 4 treatments at 30 ppt, 20 ppt, 10 ppt and 5 ppt. Three 60 l rectangular tanks, each with a total of about 70 crabs were allocated for each treatment.

Experiment 4: Hatchery-reared crabs in a recirculation system

In this experiment hatchery-reared crabs were exposed to a range of different salinities reflecting those observed in Chapter 2 (from 5th April 1999 to 18th May 1999). Four treatments of 0 ppt, 10 ppt, 20 ppt and 30 ppt were set up. The aim of this experiment was to determine the salinity tolerance of small hatchery-reared crabs.

The mean initial carapace width and wet weight of small crabs were 6.6 ± 0.5 mm and 0.06 ± 0.01 g, respectively. Fifty crabs were randomly allocated to each treatment.

Experiment 5: Wild crabs in a batch exchange system

The experiment was carried out at the Vinh Chau station. The purpose was to repeat the salinity tolerance of wild juvenile crabs (Experiment 3) but in the batch water exchange system. Crabs of a mean size of 11.7 ± 0.4 mm CW were randomly allocated to five treatments (0ppt, 5ppt, 10ppt, 20ppt and 25ppt) with approximately equal numbers. They were held individually in jars floating in 500 l round plastic tanks. Water depth was maintained at 30 cm during the experiment and renewed every 2 days. Details of the experimental set up are indicated in Table 1.

Experiment 6: hatchery-reared (H-R) crabs in a batch exchange system

The aim of this experiment was to repeat Experiment 4, but in the batch water exchange system at the Vinh Chau station. Six treatments were established including 0, 5, 10, 15, 20 and 25ppt. Each treatment (one 500 l round tank) contained 30-35 crabs of a size range of 8-13 mm CW (mean 12.7 ± 1.93 mm CW). Details of the experimental design are shown in Table 2.

Experiment 7: Salinity tolerance with size

The experiment was to test the hypothesis that, at different stages crabs might have different responses to different salinities. A large size range of wild crabs was selected from 11.5 mm to 54 mm CW (Fig. 4) and allocated in 0ppt after being acclimated for 72 hours from 25ppt. The experiment was carried out in Can Tho in one 4 m^3 concrete tank (water depth 0.5 m). Crabs were maintained individually in baskets. They were fed once a day with chopped peeled shrimp.

Experiment 8: Salinity tolerance with size

In this experiment, Experiment 7 was repeated using a wider size range of wild crabs, from as small as 5.5 mm up to 52.7 mm CW (Fig. 5) with 0ppt. The smaller crabs (5 - 25 mm CW) were held individually in jars while the bigger ones were kept in baskets. The whole size range was randomly distributed into 0ppt (109 crabs) and 20ppt (90 crabs) as the control. This time crabs were acclimatized to 0ppt more slowly (more than 6 days from 25ppt). They were reared in two $2m^3$ composite tanks with a 20 cm water level.
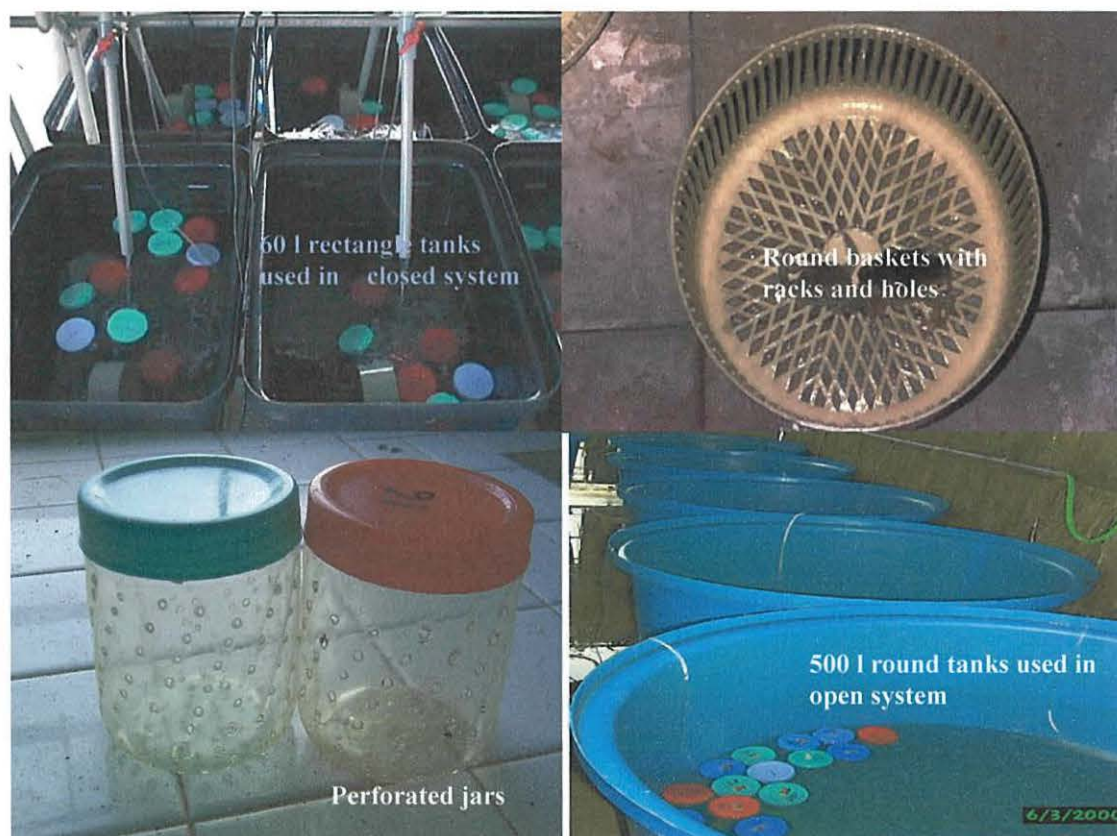
Figure 1: Rearing facilities used for salinity experiments

Figure 2: The recirculation system used for salinity experiments carried out in Can Tho laboratory.

Figure 3: 500 l round and 4m^3 rectangle tanks used as a batch system for salinity experiments carried out in Vinh Chau station.

RESULTS

Growth

Experiment 1: Growth in a recirculation system

After a two month period, eight moult stages were obtained. The carapace width increased from 3.1 ± 0.1 mm for Crab 1 to 17.9 ± 0.2 mm for Crab 8, whereas wet weight increased from 6.9 ± 0.9 mg to 782.1 ± 94.1 mg, respectively. Sizes for each stage are illustrated in Fig. 6. There was no significant difference in carapace width increment between stages ($H=12.14$, $p=0.059$). However, weight increment was significantly different between stages ($H=89.68$, $p<0.001$). In contrast to the absolute increment of CW and weight, the percentage CW and weight increment decreased from stage to stage. A significant higher percentage CW increment was observed for stages 1, 2 and 3 than that of the rest ($p< 0.05$). No significant difference was found between stages 5, 6, 7 and 8 ($p>0.05$). A similar trend was also observed for the percentage weight increment. Increase in CW and weight increment and decrease in percentage increment are shown in Fig. 7. Intermoult duration increased with stage (Fig. 8). The results indicate that moult intervals for crabs with sizes of less than 10 mm CW are less than one week, whereas crabs of larger sizes require a period of one to two weeks between moults.

Experiment 2: Growth in a batch exchange system

After 56 days, most crabs had moulted 7 times from stage 2 to stage 9. Sizes of carapace width and weight for each stage are shown in Fig. 9. Compared to those kept in the closed system in Experiment 1, the crabs in this experiment were larger at the later stages. This indicates that at the early instars there are no differences in growth between crabs in the two systems. However, the differences became larger in later stages (Fig. 10). Carapace width of crabs in Experiment 1 attained only 80.5%, 72% and 64.3% of that of crabs at the same stages (6, 7 and 8, respectively) in the batch

exchange system. The percentage increase in CW and weight increment of crabs in this system was higher than that of crabs in the closed system.

Increment in carapace width and weight increased with stages, in contrast to the decrease in the percentage increment (Fig. 11). There were highly significant differences in percentage increment in CW and weight between stages (H=66.8, p<0.001 and H=26.29, p<0.001, respectively).

Intermoult duration of crabs also increased with stage (Fig. 12). Intermoult was however shorter than that of crabs reared in the recirculation system (Fig.13). Within a period of one week one may expect to obtain Crab 6 (17 mm CW) in the batch exchange system but only Crab 5 (11 mm CW) in the recirculation system. The results also indicate that crabs with size of 30 mm CW can be obtained in two months reared in the batch water exchange system.

Salinity

Experiment 3: Wild crabs in a recirculation system

The experiment lasted 62 days. The environmental parameters recorded during the experiment including temperature, pH, were in suitable range except NO_2^- which reached levels of 0.06-0.23 ppm (Table 3).

Crabs in 30ppt grew significantly more slowly than those in other treatments (p<0.05). No significant difference was found for growth between 5ppt, 10ppt and 20ppt (Fig. 14). The percentage increment in carapace width and weight decreased from moult to moult for all treatments. There was no significant difference (p>0.05) in percentage increment between treatments and from moult to moult for both CW and weight (Fig. 15). The moult interval tended to increase from moult to moult as crabs became bigger in size. There was no difference in moult interval between treatments for the first, second and fourth moult, however, crabs in 30ppt had a longer moult interval for the third moult than those in other treatments. Number of crabs attaining

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each developmental stage also decreased from moult to moult in all treatments (Fig. 16). In contrast to growth rate, highest survival was obtained in the 30ppt treatment. There was a trend of decreased survival with lower salinity (Fig. 17).

Experiment 4: H-R crabs in a recirculation system

Hatchery-reared crabs were tested in different salinities for a period of 43 days. Crabs in 0ppt did not survive more than 3 days. In the other treatments, survival was also low at 35%, 35% and 39% for 10ppt, 20ppt and 30ppt, respectively (Fig. 18). There was no overall significant difference ($p > 0.05$) in growth rate (CW and weight) between treatments (Fig. 19). However, a significant difference ($p < 0.001$) was found for the percentage increment in CW at the second moult, with the highest in crabs in 10ppt. There was no significant difference in percentage weight increment between 10, 20 and 30ppt (Fig. 20). Moult interval was not different between treatments ($p > 0.05$), although crabs in 30ppt had a longer moult interval than the others for the third moult (Fig. 21).

Experiment 5: Wild crabs in a batch exchange system

The experiment ran for 2 months. As water was completely replaced in each tank every 2 days, measurements for pH, NH_4^+ and $NO2^-$ were not taken. Temperature was reasonably stable at 25-26 °C in the morning and 30-31 °C in the afternoon during the course of the experiment.

However, due to a technical problem, sudden mass mortality of crabs occurred in the 10ppt treatment two weeks prior to termination. Growth rate of crabs in this treatment therefore has been calculated based on the sizes recorded just before the accident. Crabs in 5ppt and 10ppt had a significant lower growth rate ($p < 0.0001$) than those in other treatments, but there was no significant difference ($p > 0.05$) between other treatments (Fig. 22). The highest survival was obtained in 20ppt and the lowest in 5ppt (Fig. 23).

At 0ppt crabs were only able to survive for up to one week. During the acclimatization period, 40% of crabs exposed to 0ppt moulted. Among the moulted crabs 33% were still moulting when salinity had dropped to 0ppt. Percentage increment in carapace width for the first moult recorded in this treatment was similar to that in other treatments (Fig. 24). Although there was no overall significant difference in CW and weight increment between the other treatments for all moults, in the second moult, the percent CW increment for crabs in 5ppt was significantly lower ($p<0.05$). The percent weight increment in crabs for 25ppt was significantly higher at the first moult ($p<0.05$).

A lower number of moults and moulting was observed in the 5ppt and 10ppt treatments, while a higher number of moults and moulting was obtained in 20ppt. Longer moult intervals were observed in crabs in 5ppt and 10ppt although there was no significant difference at some moults (Fig. 25).

Experiment 6: H-R crabs in a batch exchange system

The experiment was maintained for a period of 75 days. There was a significant difference in growth rate (CW) between treatments ($p<0.05$). The highest growth rate was observed in 15ppt and the lowest was recorded for 5ppt. Growth rate (wet weight) was not different ($p>0.05$) between 15ppt, 20ppt and 25ppt but these were significantly higher than 5ppt ($p<0.05$) (Fig. 26). In 5ppt crabs performed only 3 moults with a decreasingly lower percentage moulting at each moult. More moults and higher number of moulting crabs were observed in higher salinities, especially in 15ppt. The moult interval was also different between salinities. At 5ppt, after the first moult crabs exhibited significantly longer moult intervals for the second and the third moults. In 15ppt crabs moulted faster than those in other treatments, as the moult intervals were shorter (Fig. 27).

The percent increment in CW and weight also decreased with moults for all treatments. Crabs in 5ppt and 10ppt had significant lower percent CW increment than those in other treatments but not at all moults. Similarly, significant higher CW

increment was found in 15ppt and 20ppt. Percent weight increment showed similar patterns to that of CW (Fig. 28). Differences of growth increment between moults in each treatment are illustrated in Fig. 29. This figure indicates that the percent increment in CW and weight at the first moult is always significantly higher than second moult, which is not the case for subsequent moults.

No crabs survived in 0ppt for more than 5 days. Only 38% had died after 2-3 days at 0ppt before mass mortality occurred at day 5. In 5ppt, crabs died gradually during the experiment. There was no mass mortality observed for this treatment. Survival of crabs increased with increasing salinity. The lowest survival was obtained in 5ppt (16.2%) and steadily increased to 71.1% at 25ppt (Fig. 30).

Experiment 7 & 8: Salinity tolerance with size

There was no difference in tolerance to 0ppt in crabs of different sizes. In experiment 7 most (80%) crabs survived in freshwater for 5 days. However, these died at day 6, for all size classes (Fig. 31).

In experiment 8, crabs survived in freshwater for only 3 days. All crabs died by day 4. In comparison, 97.8% of crabs in the control (20ppt) survived (Fig. 32).

Table 1: Number of wild crabs and their mean sizes in different treatments in Experiment 5

Treatments (ppt)	Number of crabs	Mean CW (mm)	Mean Weight (g)
0	50	11.46 ± 4.21	0.36 ± 0.39
5	48	12.14 ± 4.28	0.39 ± 0.41
10	49	11.32 ± 4.01	0.34 ± 0.36
20	39	11.47 ± 4.55	0.37 ± 0.42
25	41	12.05 ± 4.97	0.43 ± 0.66

Table 2: Number of hatchery-reared (H-R) crabs and their mean sizes in different treatments in Experiment 6

Treatments (ppt)	Number of crabs	Mean CW (mm)	Mean Weight (g)
0	37	12.8 ± 2.06	0.4 ± 0.17
5	37	12.6 ± 1.87	0.3 ± 0.15
10	39	12.8 ± 2.13	0.3 ± 0.19
15	36	12.6 ± 1.9	0.3 ± 0.18
20	38	12.7 ± 1.89	0.3 ± 0.19
25	38	12.8 ± 1.94	0.3 ± 0.18

Table 3: Temperature, pH and NO_2^- recorded during Experiment 3 in different treatments.

Parameters	5ppt	10ppt	20ppt	30ppt
t°C at 7am	27.9 ± 0.7	28 ± 0.7	28 ± 0.6	27.9 ± 0.6
t°C at 2pm	29 ± 0.8	29 ± 0.8	29.1 ± 0.8	29 ± 0.8
pH	8 ± 0.2	7.9 ± 0.2	7.9 ± 0.2	7.7 ± 0.2
NO_2^- (ppm)	0.15 ± 0.25	0.06 ± 0.04	0.2 ± 0.13	0.23 ± 0.21
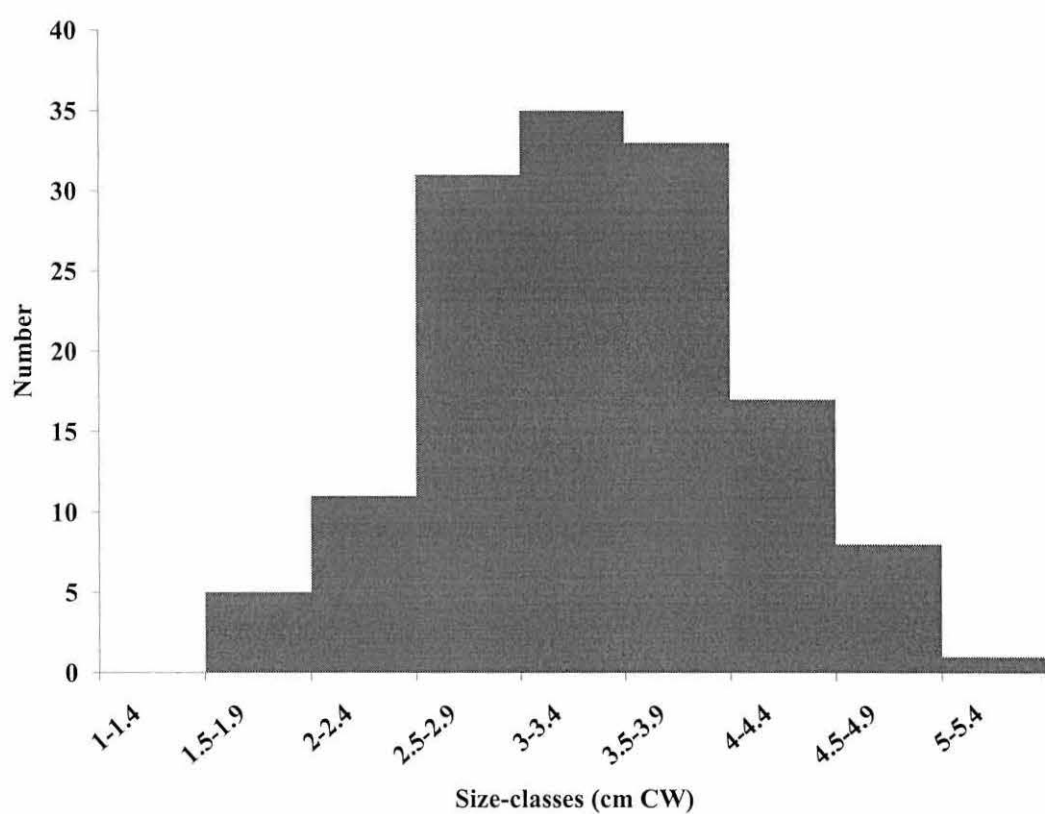
Figure 4: Experiment 7: Size-classes (cm CW) of crabs selected for testing at 0ppt in 4m³ concrete tank in Can Tho.
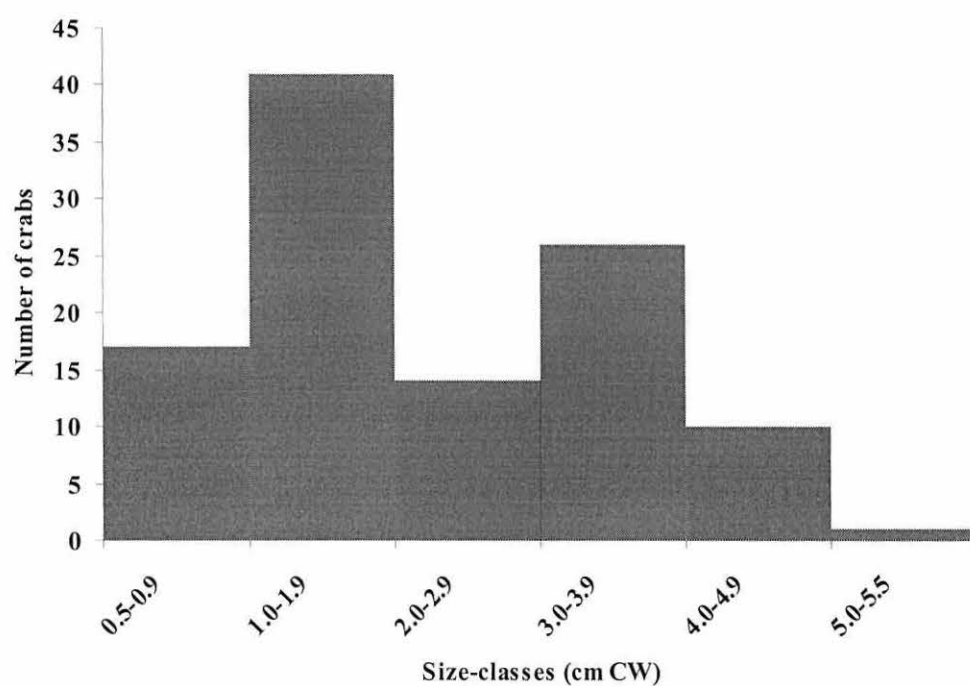
Figure 5: Experiment 8: Size-classes (cm CW) of crabs selected for testing at 0ppt in 2m^3 composite tank in Vinh Chau.
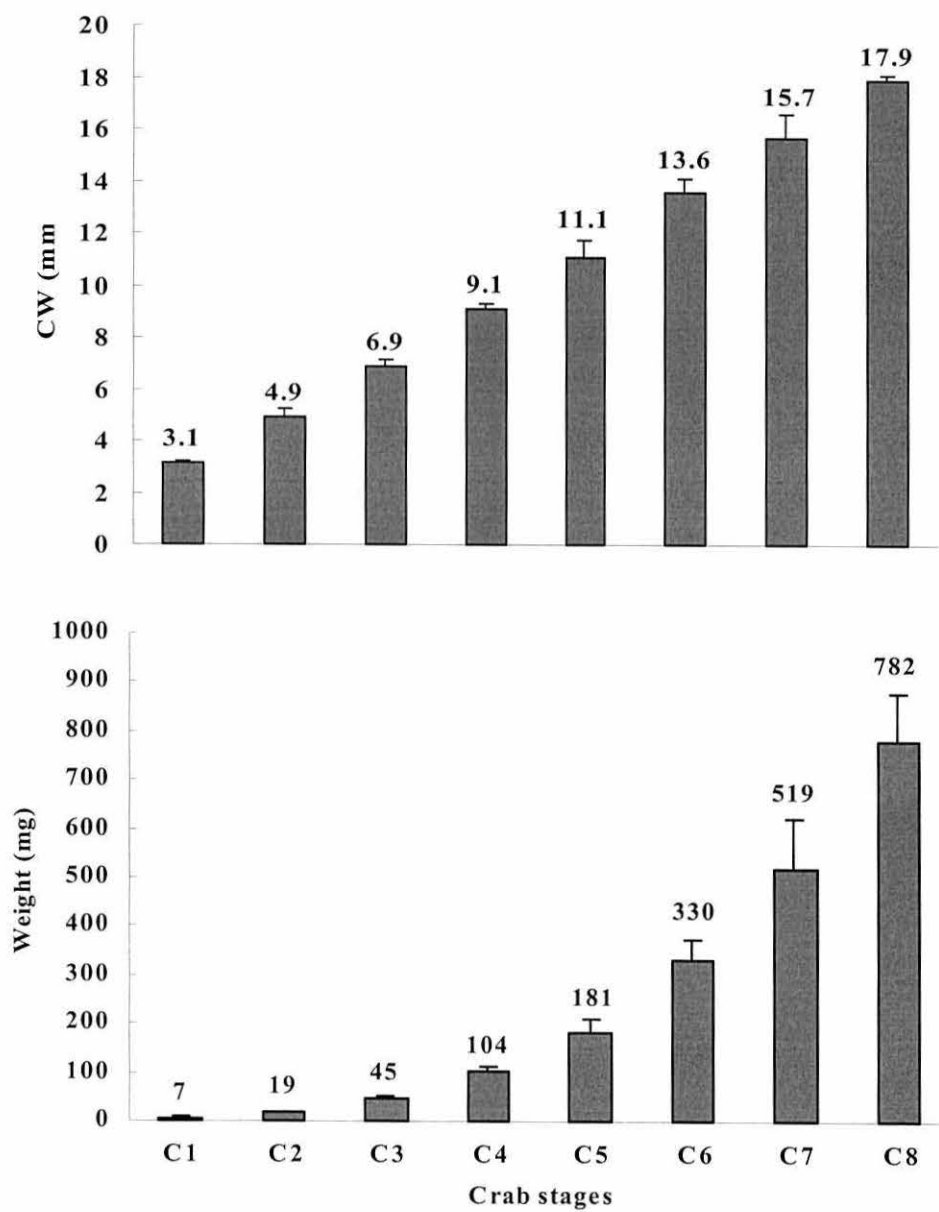
Figure 6: Experiment 1: Carapace width (mm) and weight (mg) of crab 1 to crab 8 reared in the recirculation system.
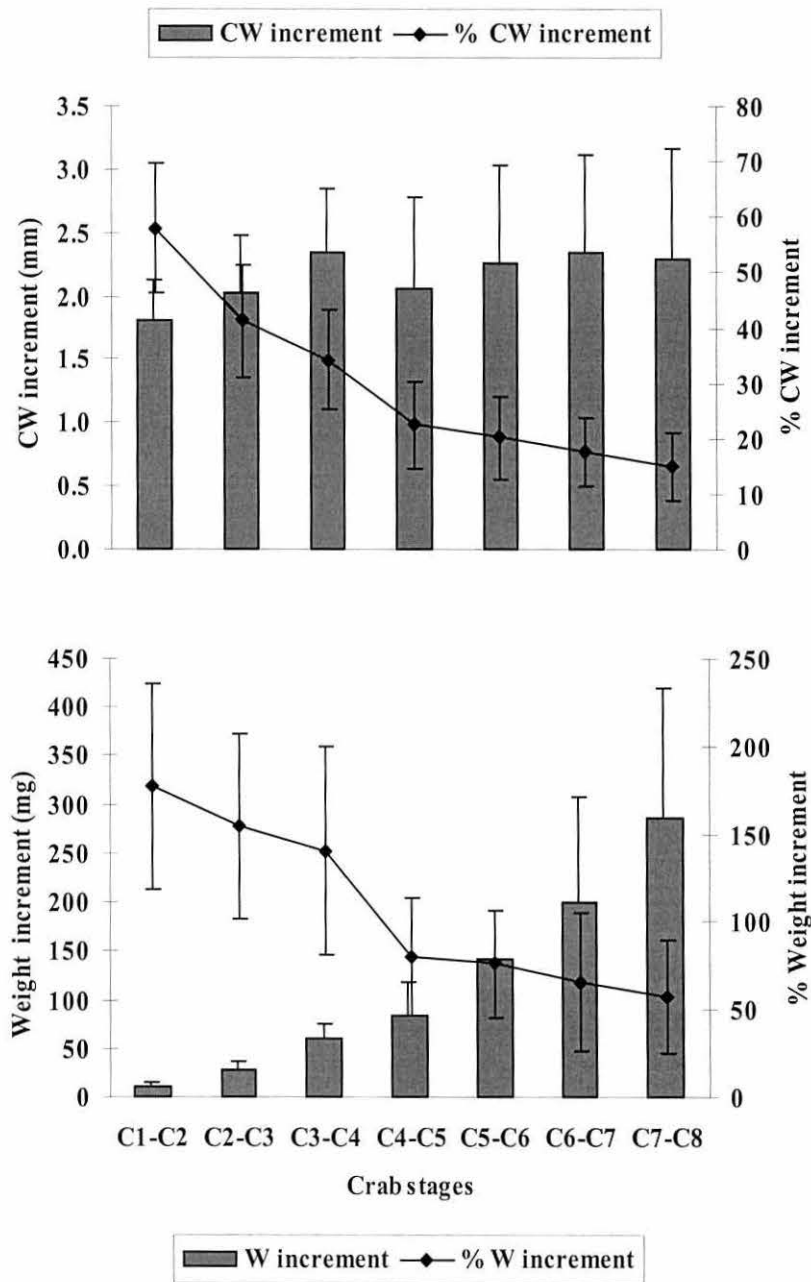
Figure 7: Experiment 1: Moult increment CW and weight from stage to stage of hatchery-reared crabs reared in the recirculation system.
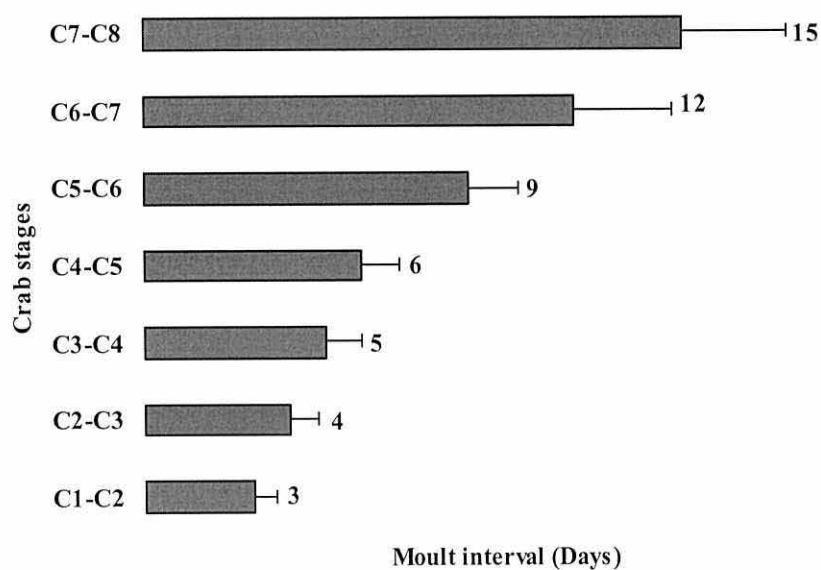
Figure 8: Experiment 1: Moult intervals of hatchery-reared crabs *S. paramamosain* reared in a recirculation system from crab 1 to crab 8.
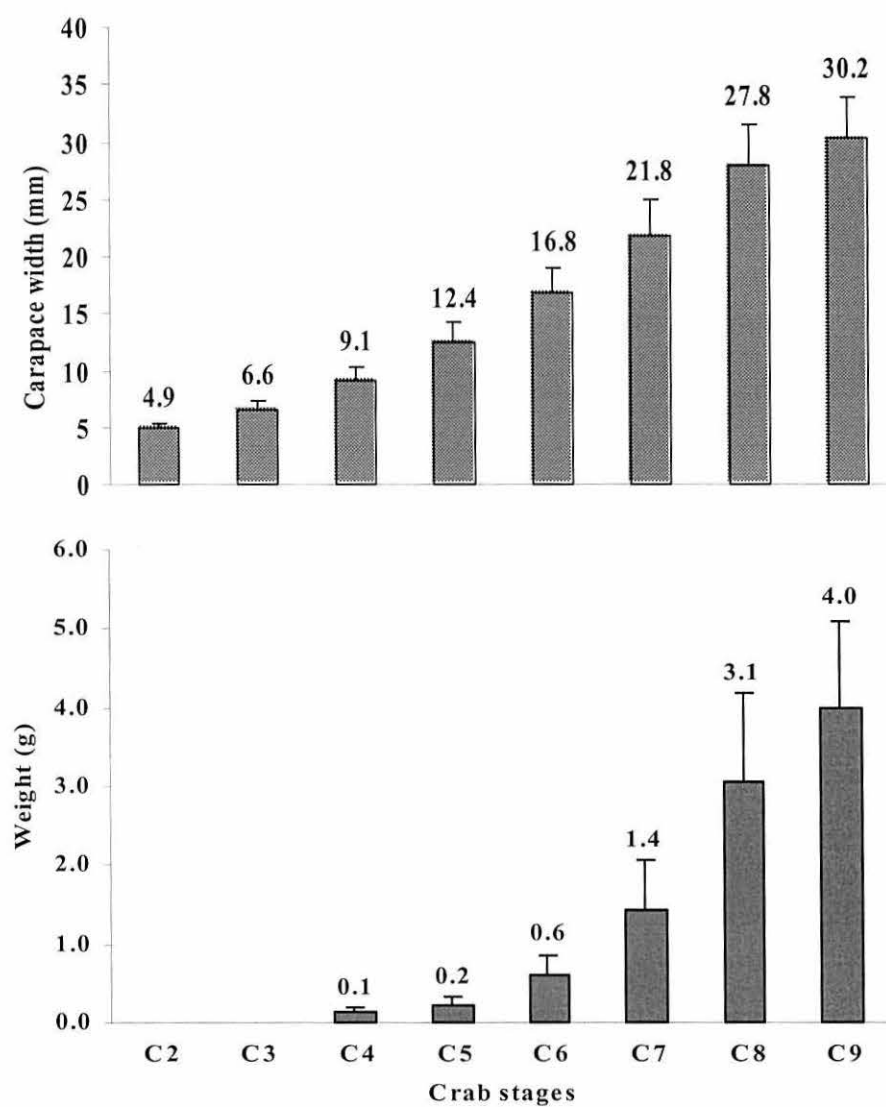
Figure 9: Experiment 2: Carapace width (mm) and weight (g) of hatchery-reared *S. paramamosain* reared in a batch system from crab 2 to crab 9.
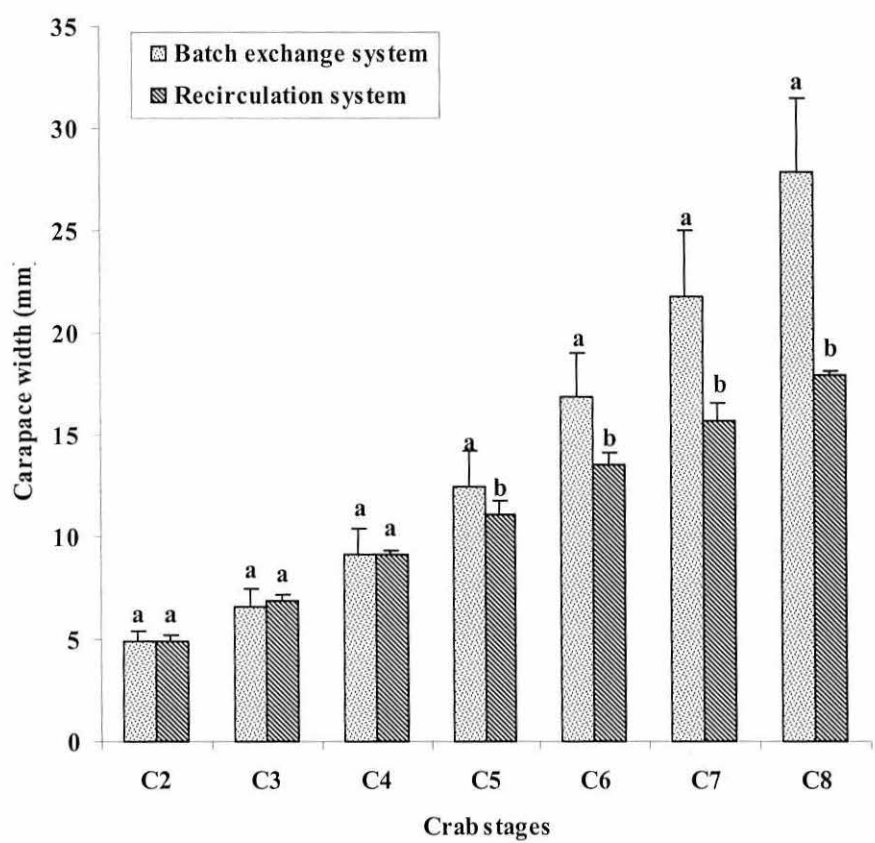
Figure 10: Comparison of CW between hatchery-reared *S. paramamosain* reared in recirculation system and batch exchange system.
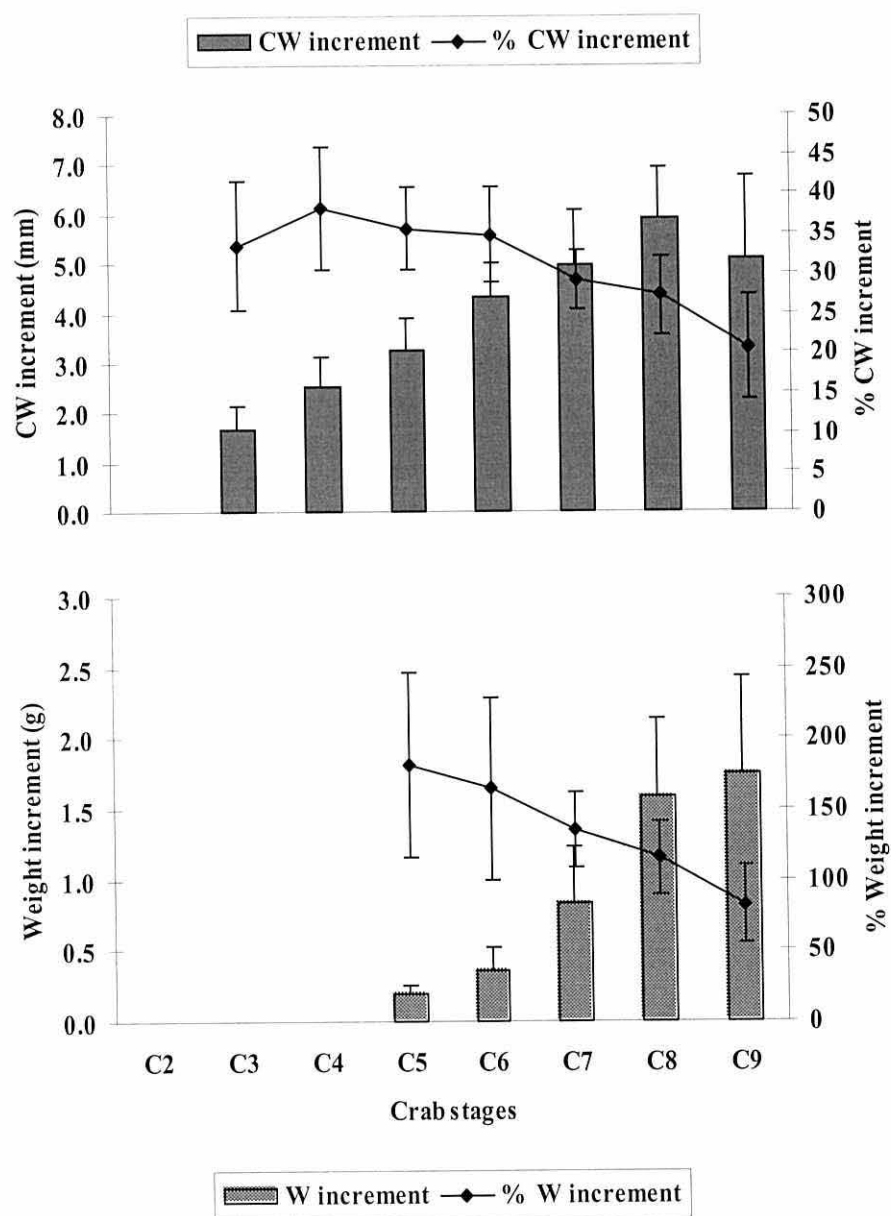
Figure 11: Experiment 2: Increment of CW and weight at different moult stages of hatchery-reared *S. paramamosain* reared in batch exchange system.
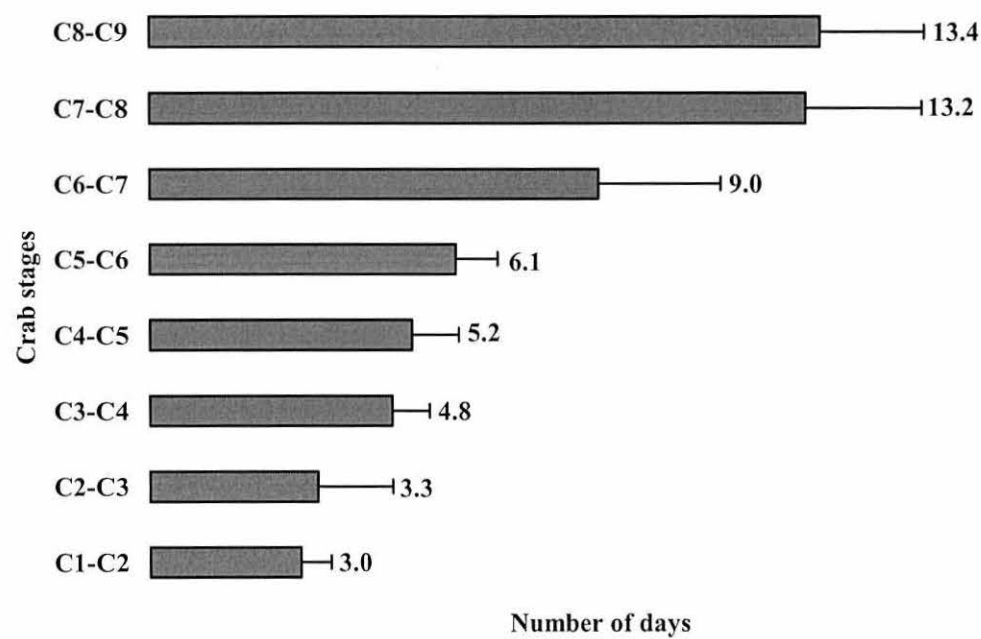
Figure 12: Experiment 2: Moult intervals of hatchery-reared *S. paramamosain* reared in a batch exchange system.
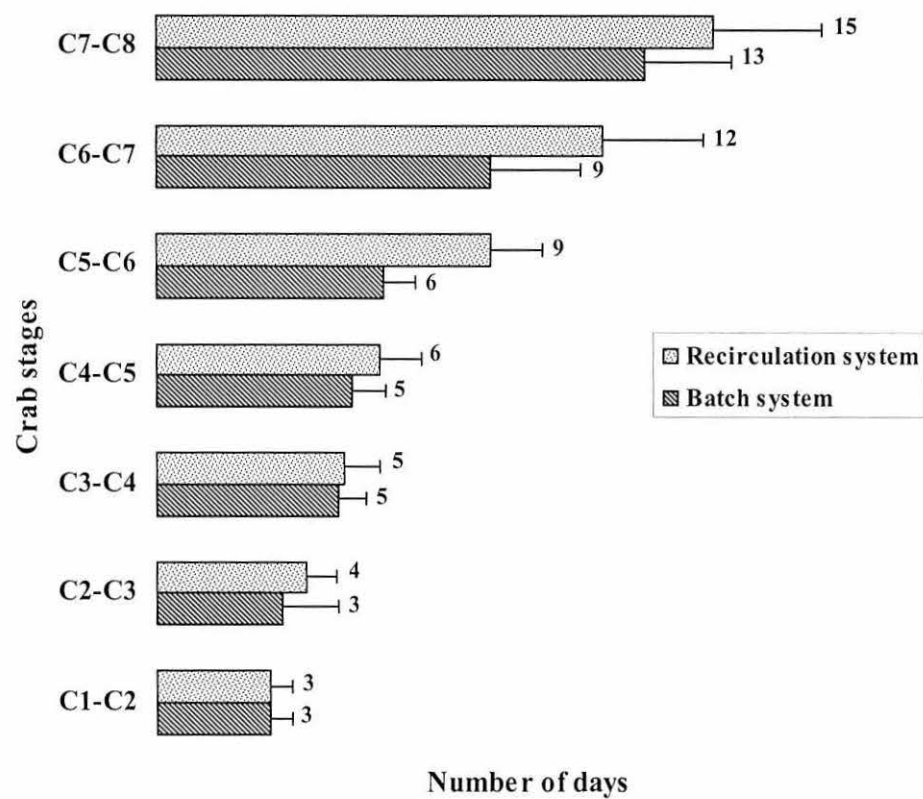
Figure 13: Experiment 1 & 2: Comparison of moult intervals of *S. paramamosain* between the recirculation and batch system.
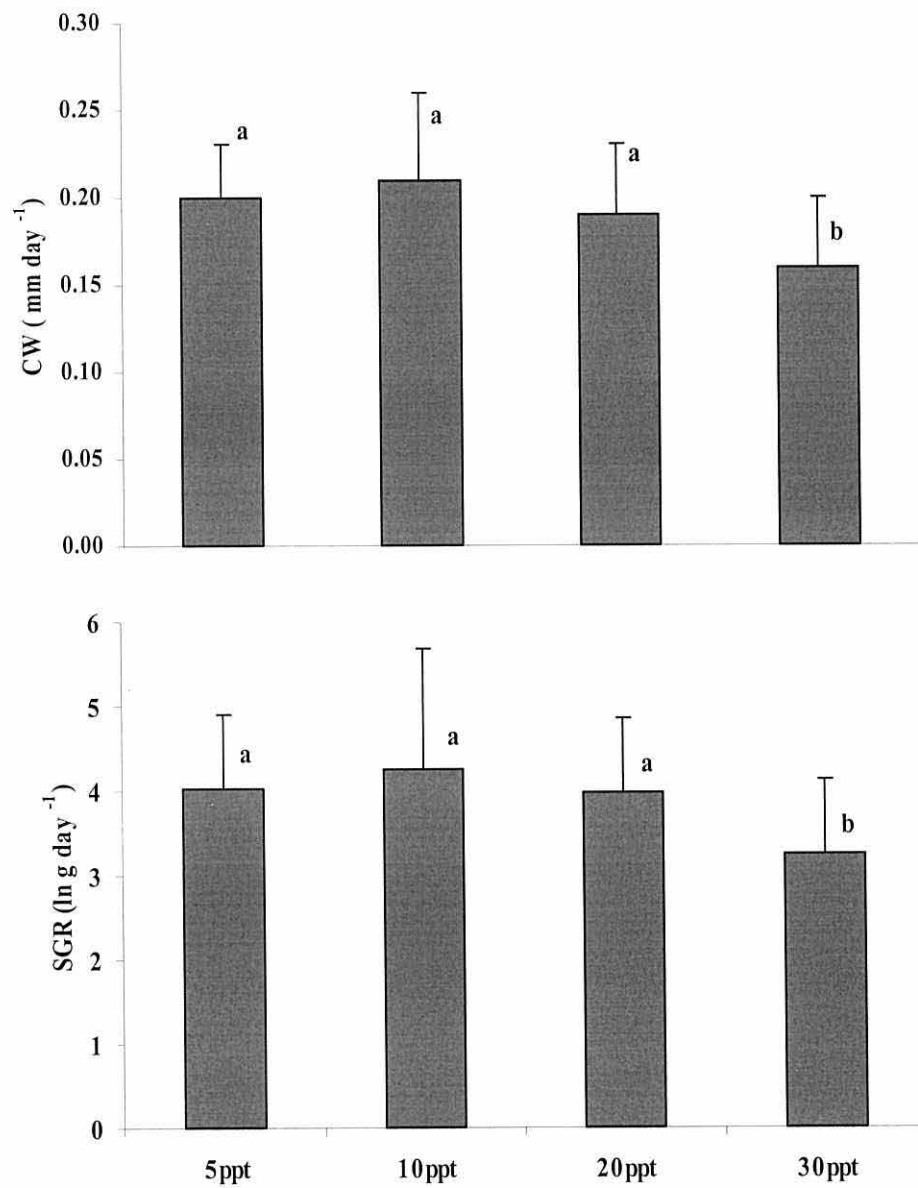
Figure 14: Experiment 3: Growth rate of wild crabs *S. paramamosain* tested in different salinities in a recirculation system. Treatments marked with different letter are significantly different from each other (p<0.05).
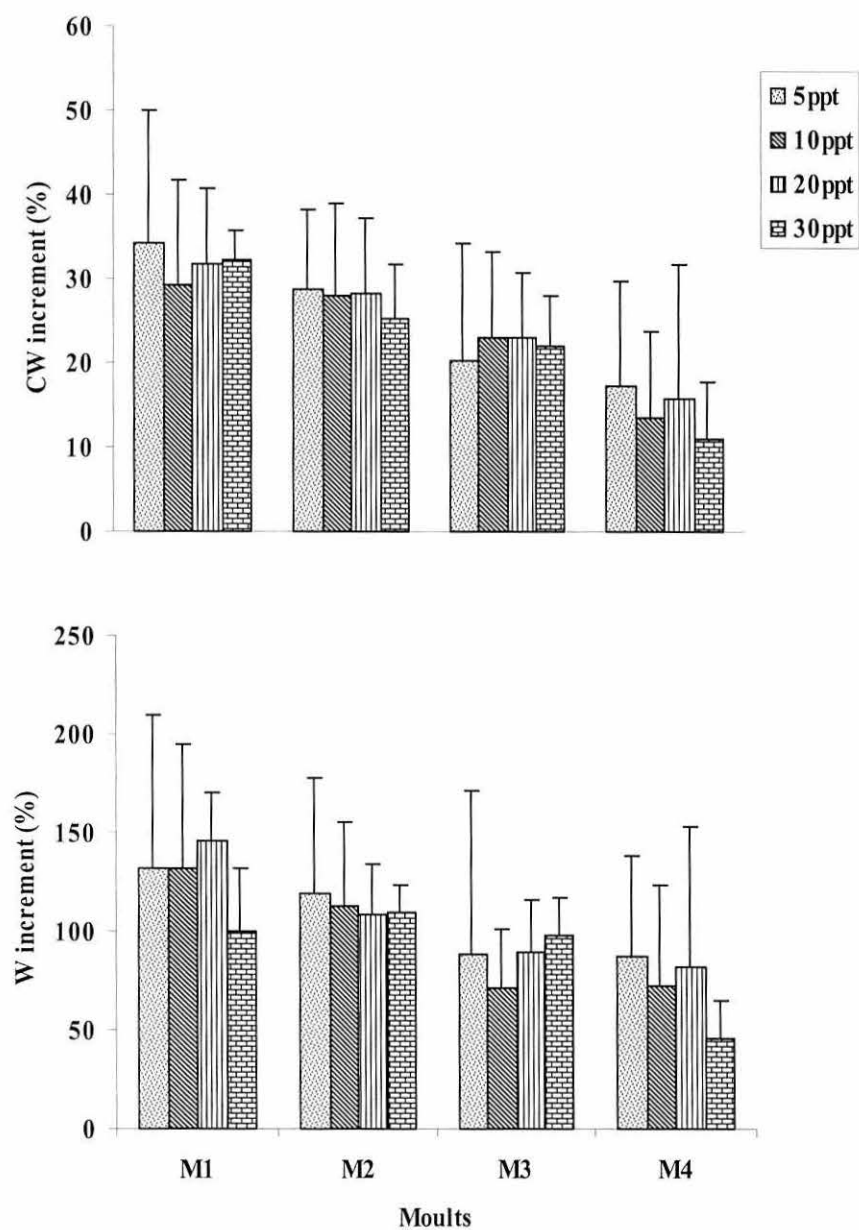
Figure 15: Experiment 3: Percentage increment of CW and weight of wild crab *S. paramamosain* tested in different salinities in a recirculation system. There was no significant difference ($p > 0.05$) between treatments at each moult for both weight and CW.
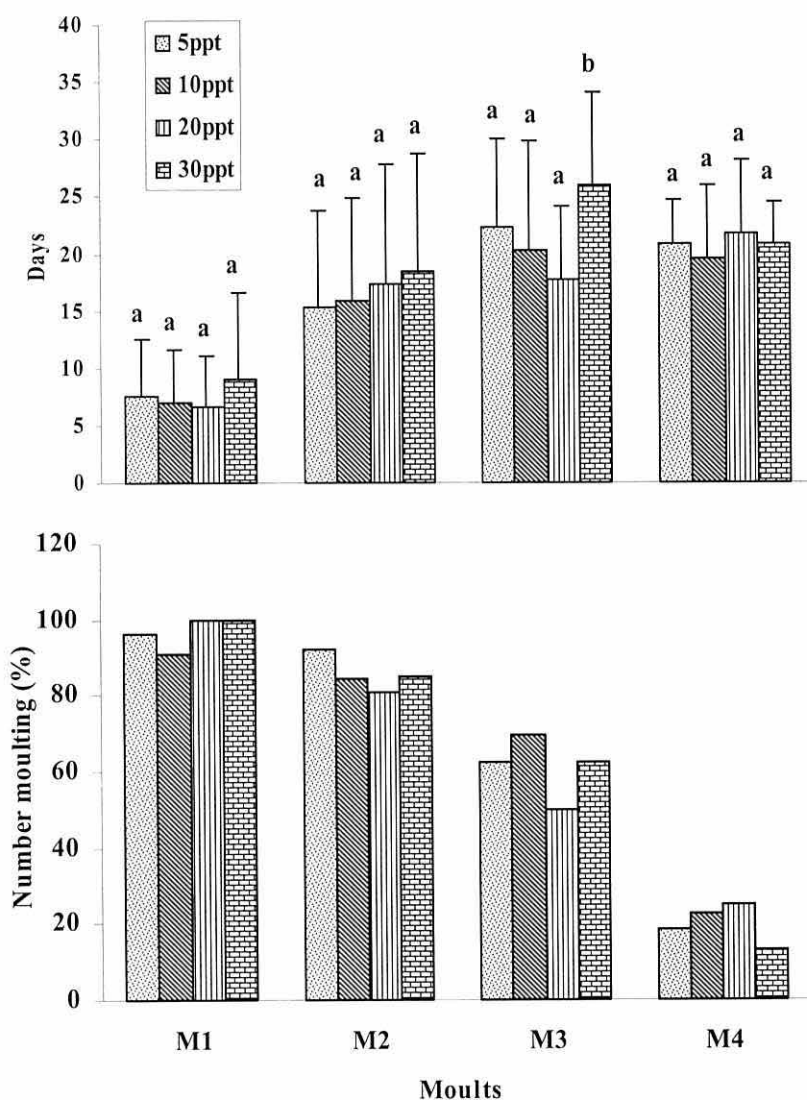
Figure 16: Experiment 3: Moult duration and number of crabs moulted at each moult for wild *S. paramamosain* tested in different salinities in a recirculation system. Within each moult stage, treatments marked with different letter are significantly different from each other ($p < 0.05$).
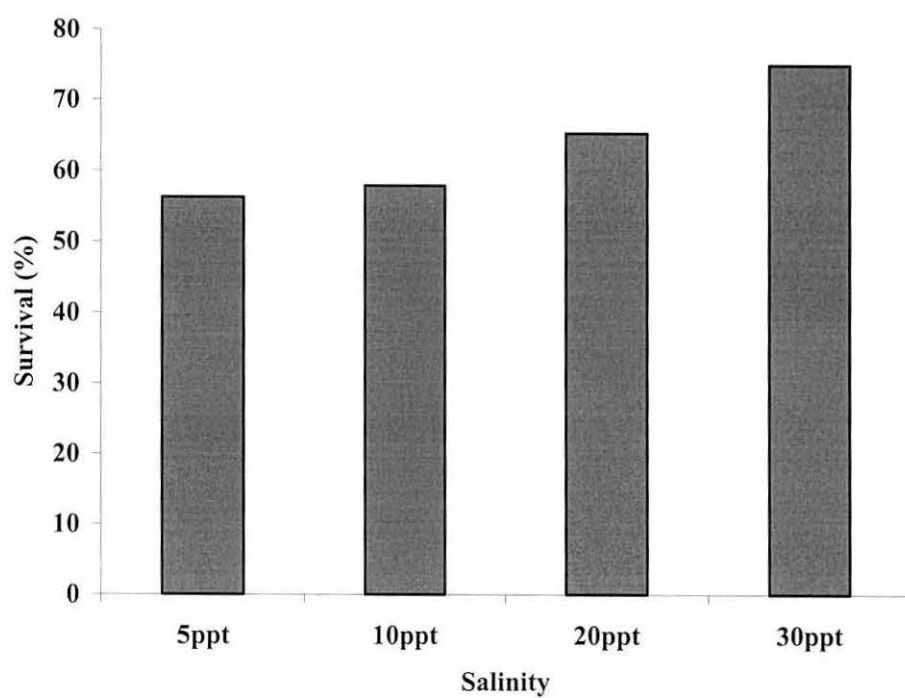
Figure 17: Experiment 3: Survival of wild *S. paramamosain* tested in 5ppt, 10ppt, 20ppt and 30ppt in a recirculation system.
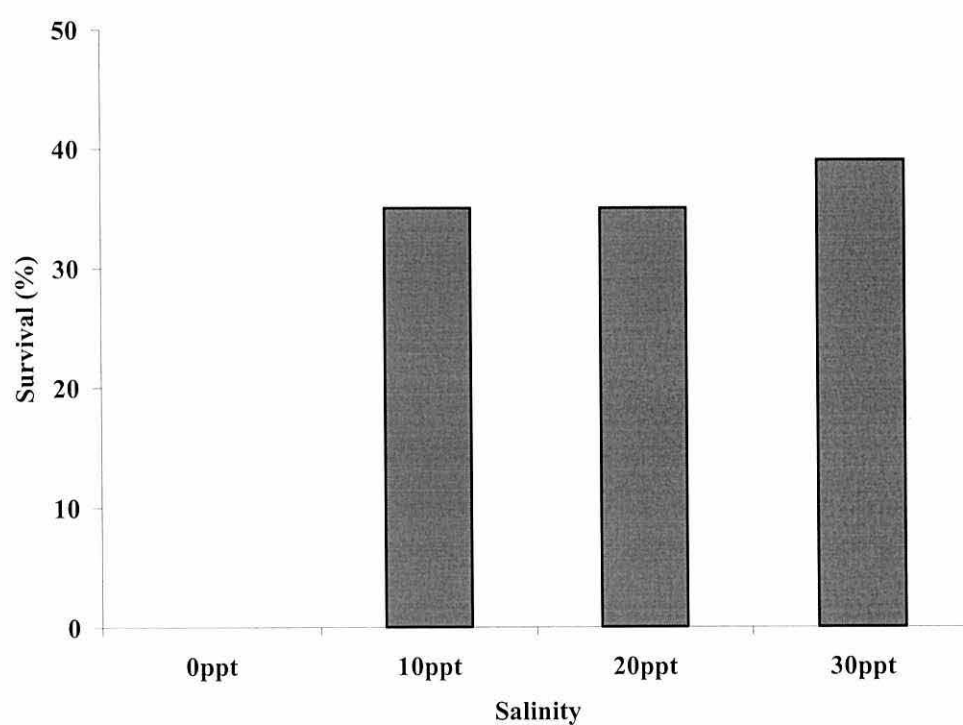
Figure 18: Experiment 4: Survival of hatchery-reared crabs, *S. paramamosain* (CW 4-11 mm)tested in 0ppt, 10ppt, 20ppt and 30ppt in a recirculation system.
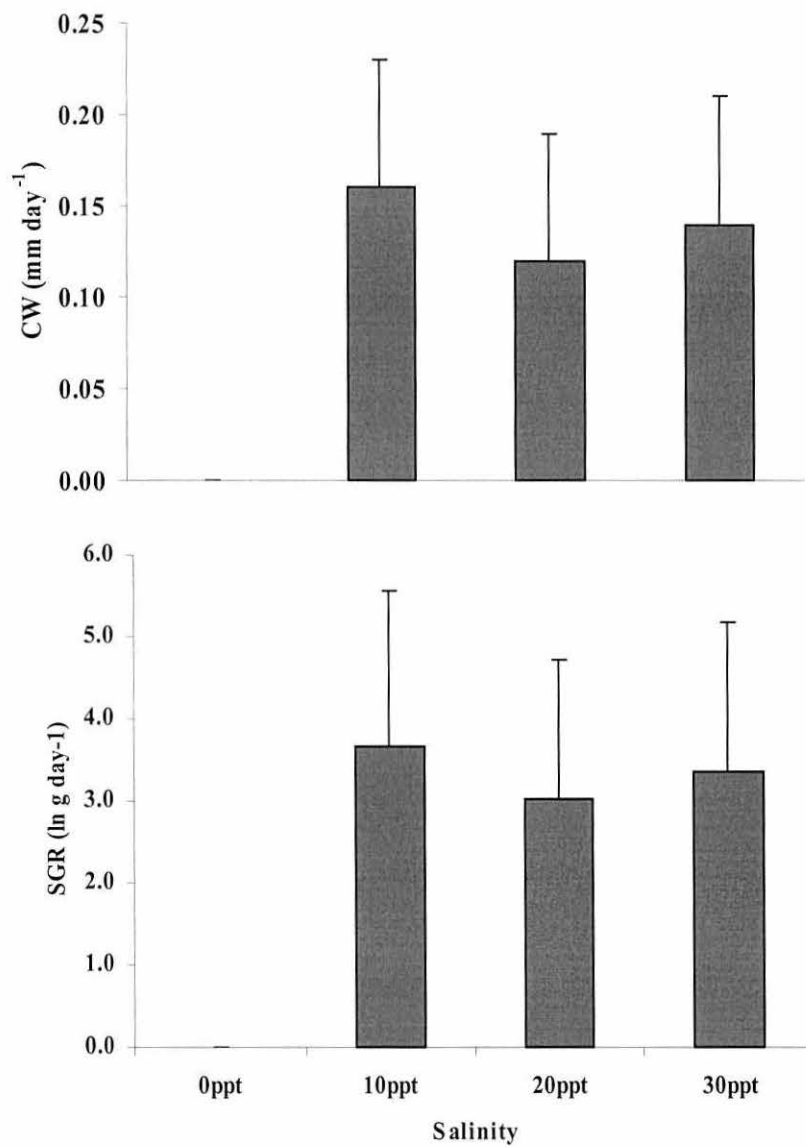
Figure 19: Experiment 4: Overall growth rate (CW and weight) of hatchery-reared crabs, *S. paramamosain* reared in 0ppt, 10ppt, 20ppt and 30ppt. There was no significant difference between treatments (p>0.05).
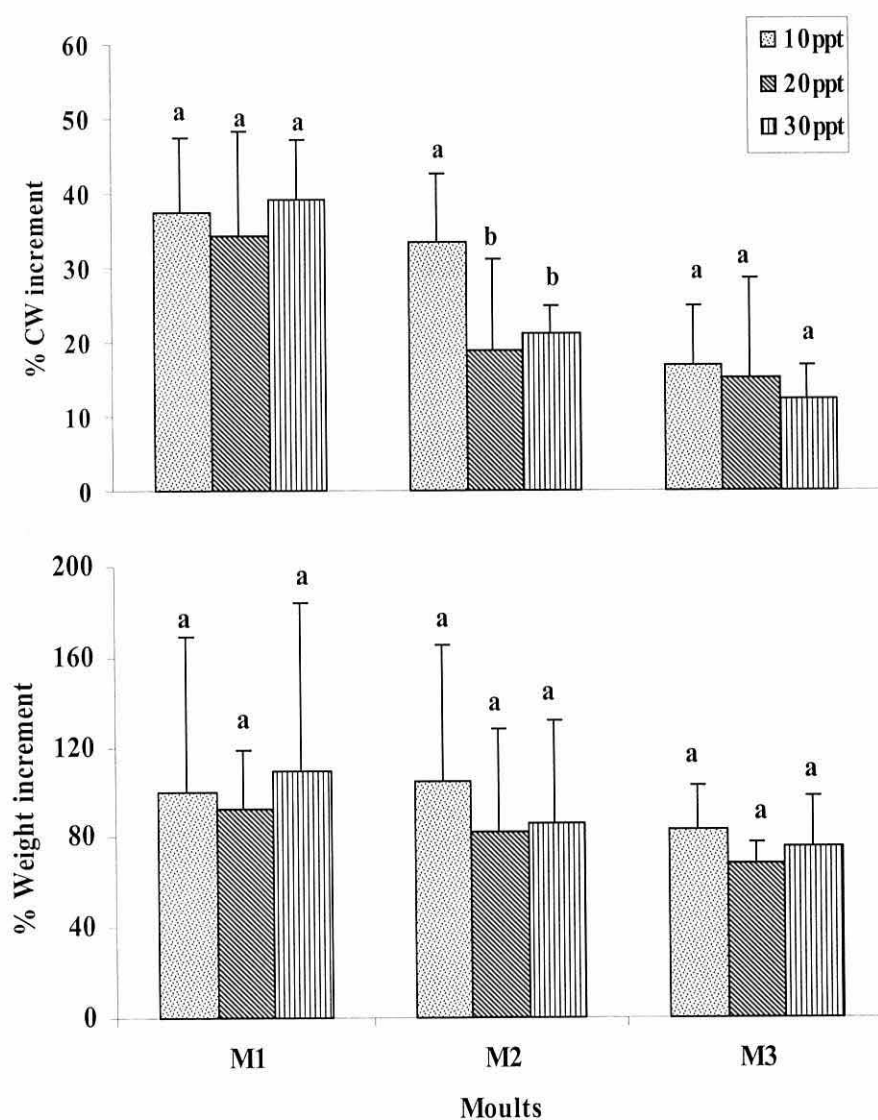
Figure 20: Experiment 4: Percentage increment in CW and weight of hatchery-reared crabs, *S. paramamosain* tested in 10ppt, 20ppt and 30ppt in a recirculation system. Within each moult stage, treatments marked with different letter are significantly different from each other ($p < 0.05$).
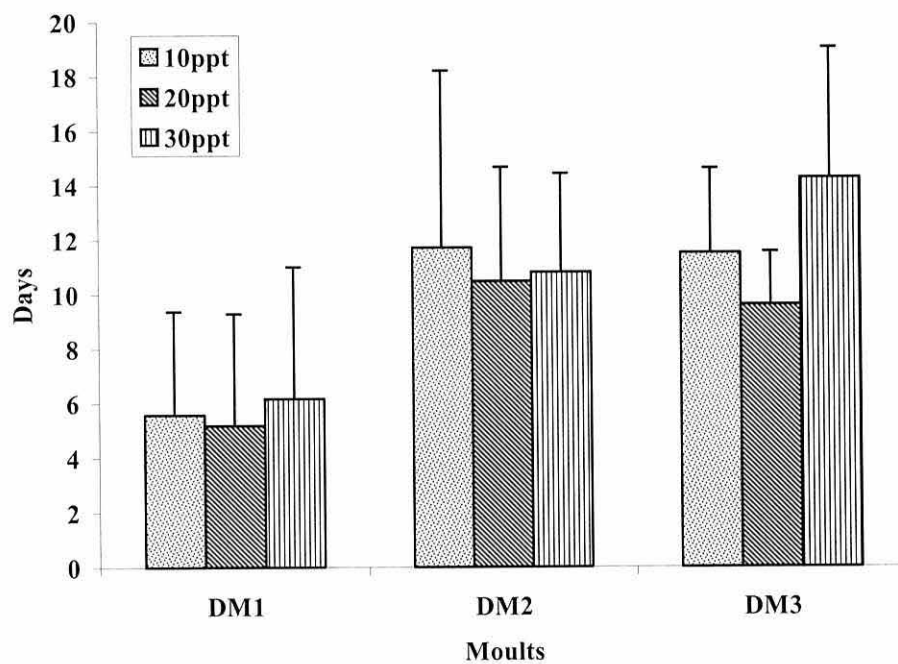
Figure 21: Experiment 4: Moult intervals of hatchery-reared crabs, *S. paramamosain* tested in 10ppt, 20ppt and 30ppt in a recirculation system. There was no significant difference between treatments ($p>0.05$).
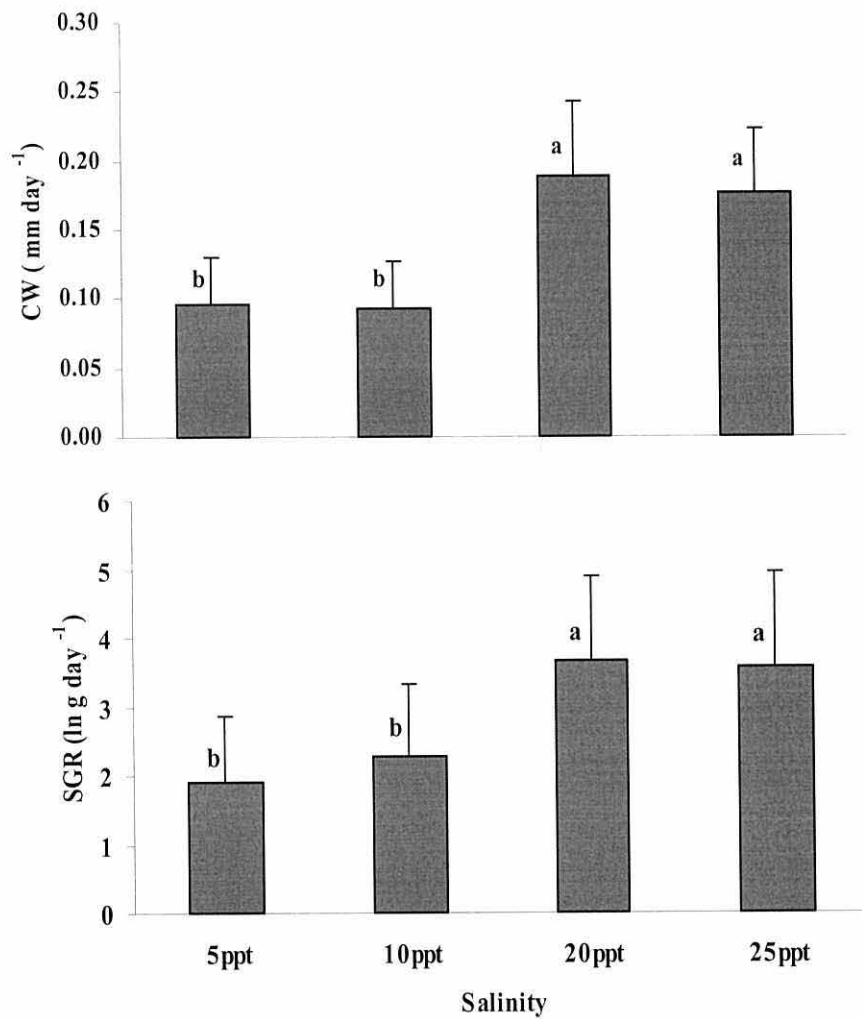
Figure 22: Experiment 5: Growth rate (CW and weight) of wild crabs, *S. paramamosain* in 5ppt, 10ppt, 20ppt and 25ppt in a batch water exchange system. Treatments marked with different letter are significantly different from each other (p<0.0001).
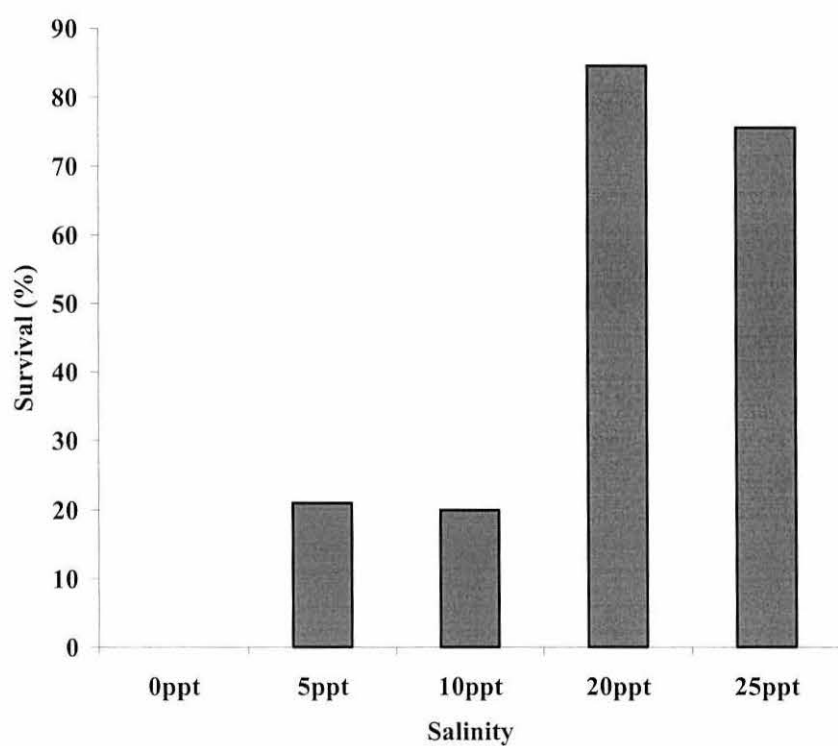
Figure 23: Survival of wild crabs, *S. paramamosain* in 0ppt, 5ppt, 10ppt, 20ppt and 25ppt in a batch water exchange system.
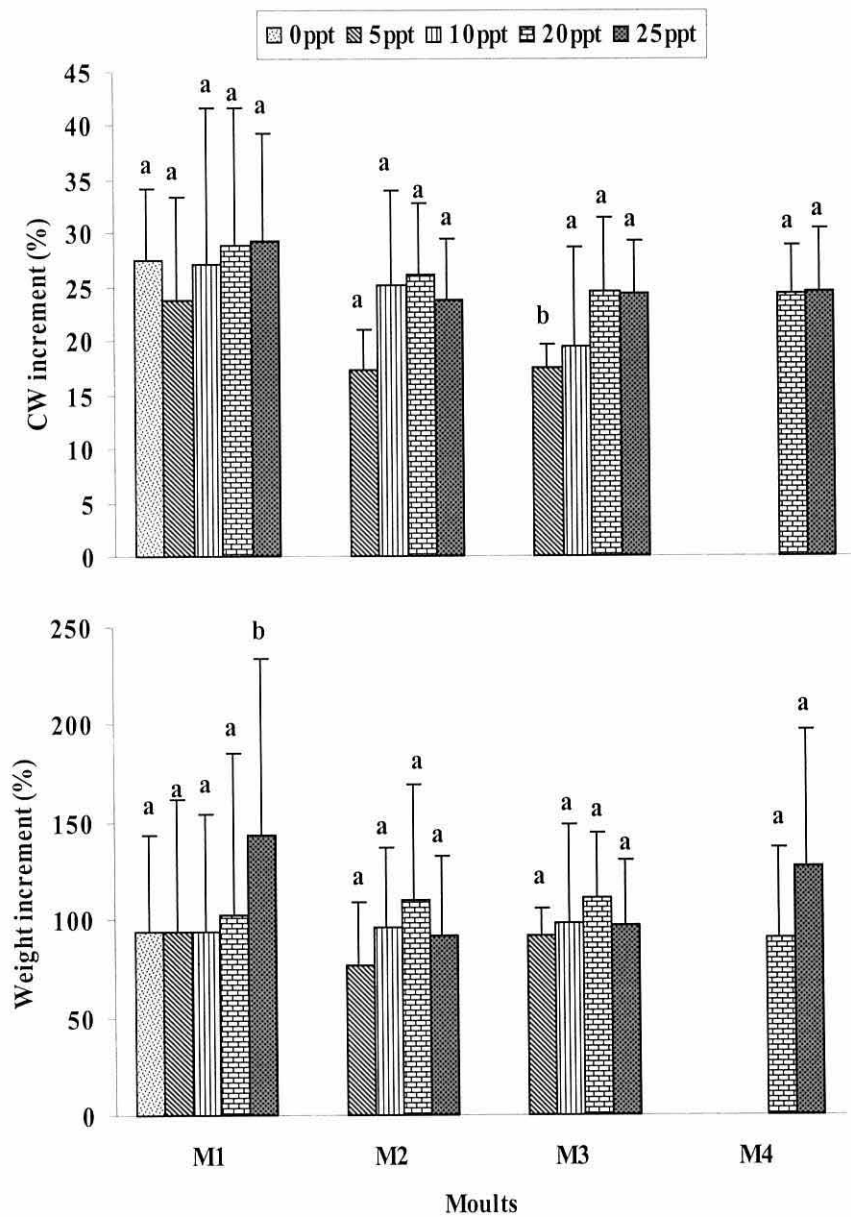
Figure 24: Experiment 5: Increment in percentage of CW and weight of small wild crabs, *S. paramamosain* tested in 0ppt, 5ppt, 10ppt, 20ppt and 25ppt in a batch water exchange system. Treatments marked with different letter are significantly different from each other (p<0.05).
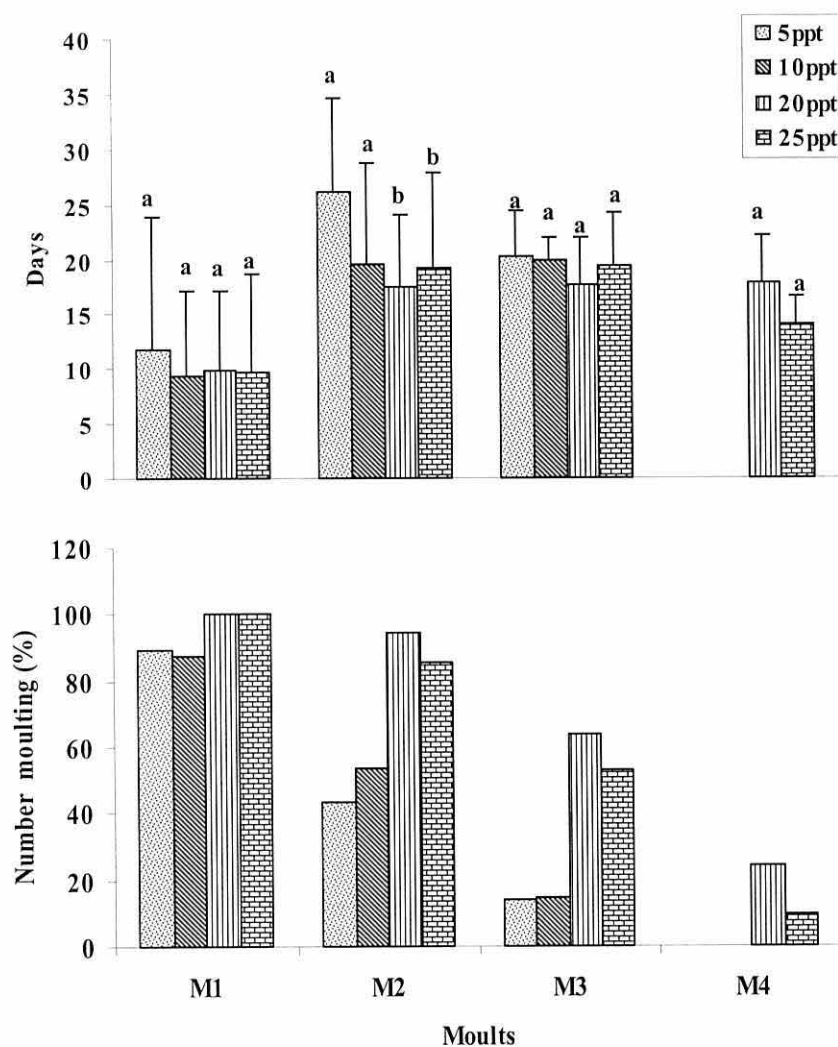
Figure 25: Experiment 5: Moult intervals and number of moulted crabs (%) of wild *S. paramamosain* tested in 5ppt, 10ppt, 20ppt and 25ppt in a batch water exchange system. Treatments marked with different letter are significantly different from each other (p<0.05).
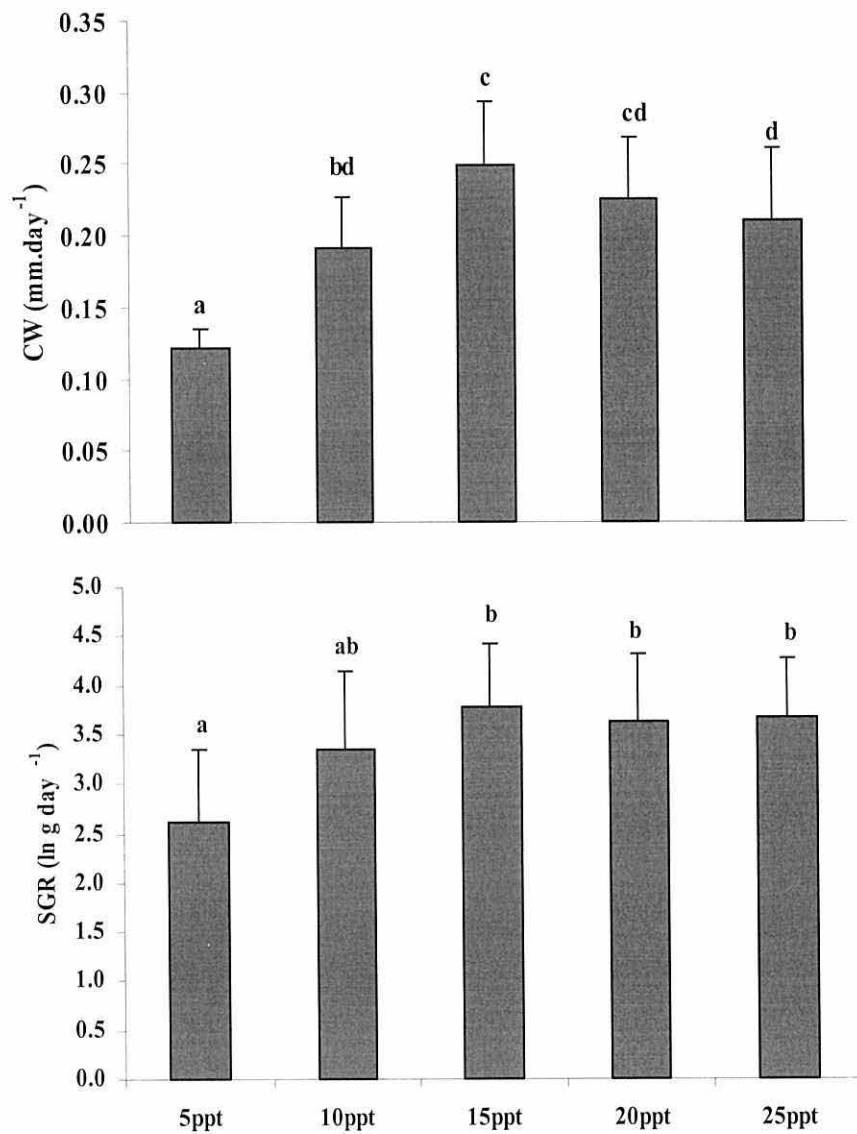
Figure 26: Experiment 6: Growth rate (CW and weight) of hatchery-reared *S paramamosain* tested in 5ppt, 10ppt, 15ppt, 20ppt and 25ppt in a batch water exchange system. Treatments marked with different letter are significantly different from each other ($p < 0.05$).
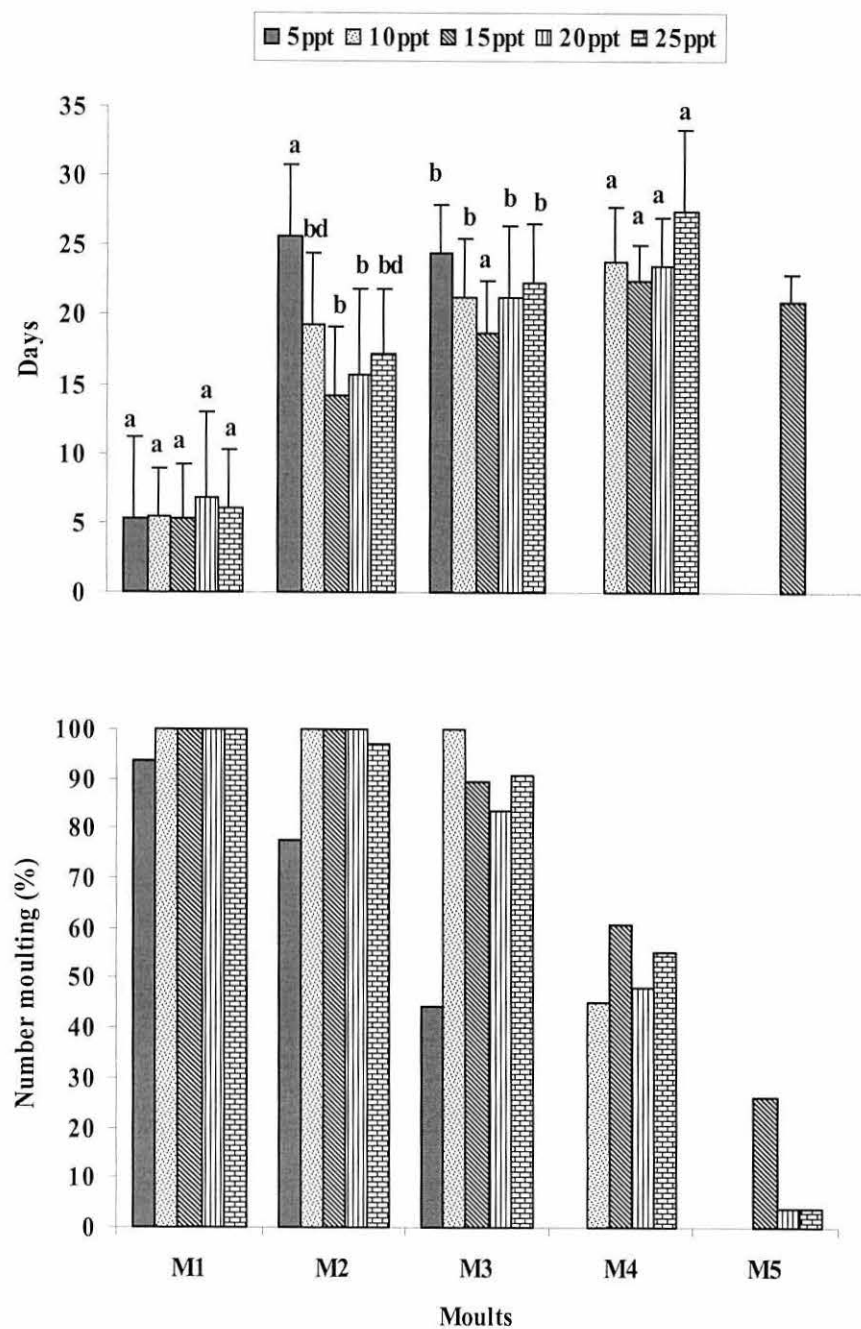
Figure 27: Experiment 6: Moult intervals and numbers of moults of hatchery-reared *S. paramamosain* tested in 5ppt, 10ppt, 15ppt, 20ppt and 25ppt in a batch water exchange system. Within each moult stage, treatments marked with different letter are significantly different from each other (p<0.05).
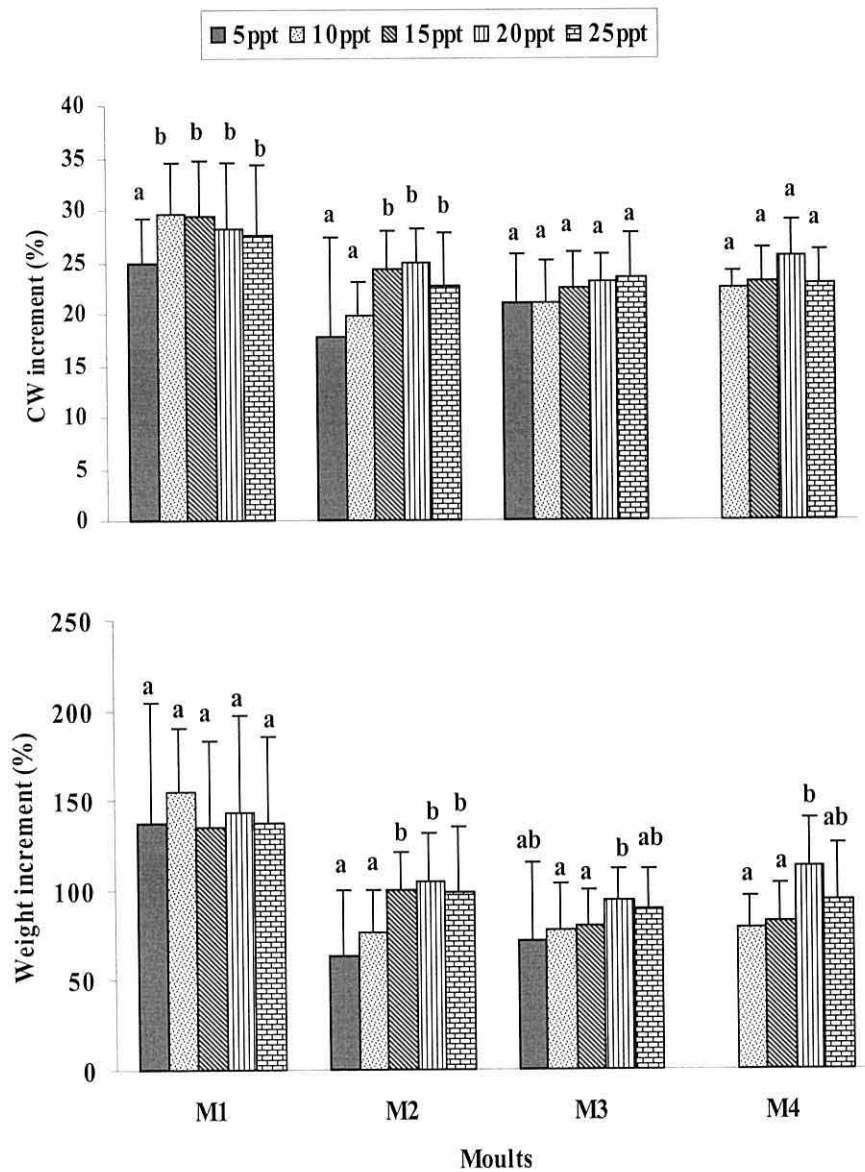
Figure 28: Experiment 6: Moult increment in percentage of hatchery-reared *S. paramamosain* tested in 5ppt, 10ppt, 15ppt, 20ppt and 25ppt in a batch water exchange system. Within each moult stage, treatments marked with different letter are significantly different from each other (p<0.05).
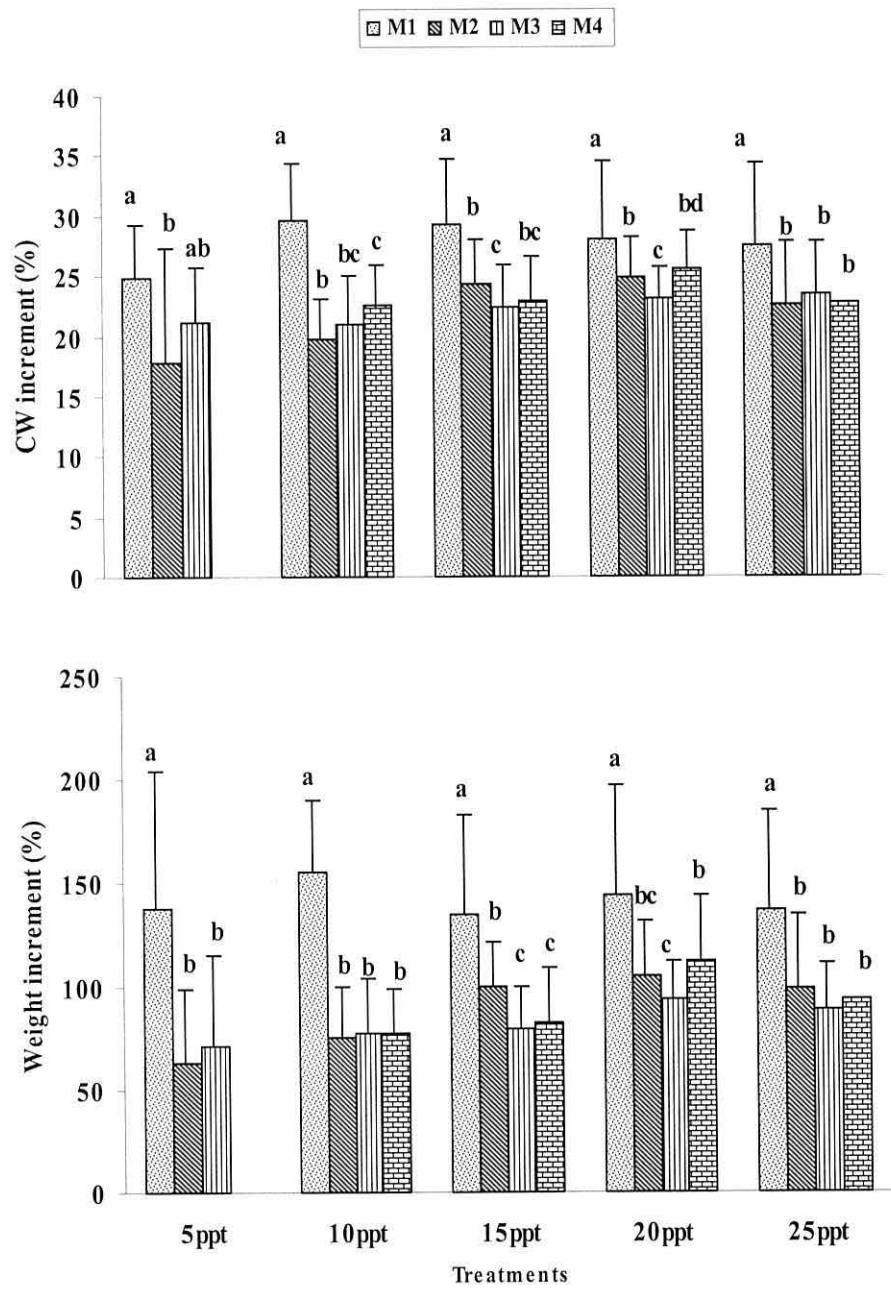
Figure 29: Experiment 6: Differences in CW and weight percentage increment at each moult in each treatment in hatchery-reared *S. paramamosain* tested in 5ppt, 10ppt, 15ppt, 20ppt and 25ppt in a batch water exchange system. Within treatment, each moult stage marked with different letter are significantly different from each other (p<0.05).
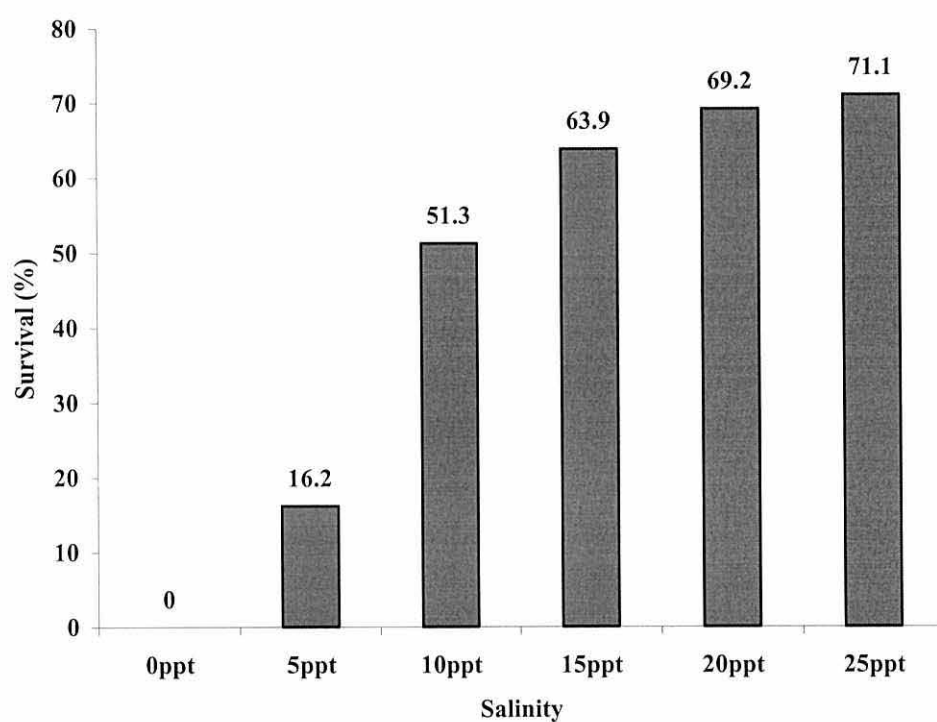
Figure 30: Experiment 6: Survival of hatchery-reared *S. paramamosain* tested in 0ppt, 5ppt, 10ppt, 15ppt, 20ppt and 25ppt in a batch water exchange system.
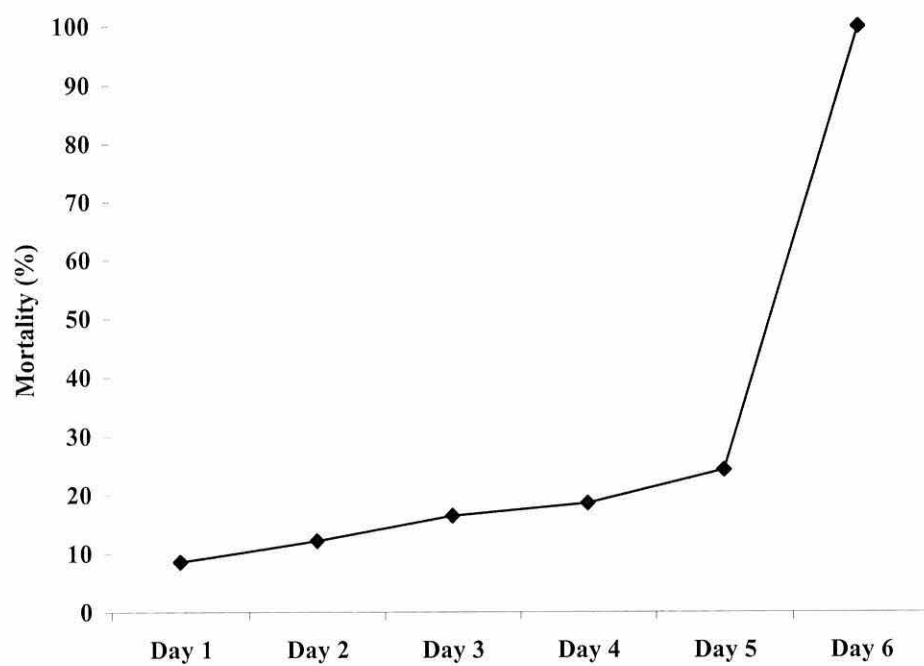
Figure 31: Experiment 7: Cumulative mortality (%) of wild *S. paramamosain* exposed to 0ppt.
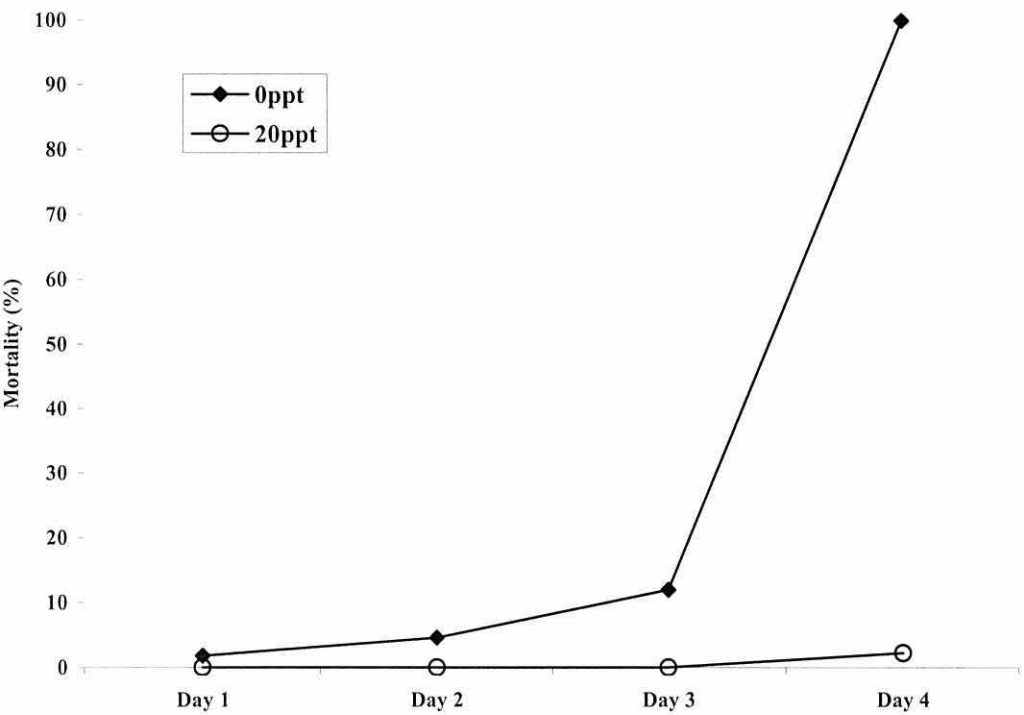
Figure 32: Experiment 8: Cumulative mortality (%) of wild *S. paramamosain* exposed to 0ppt compared with the control treatment (20ppt).

DISCUSSION

Growth

Observations on the post-larval life history of *Scylla serrata* reared in the laboratory were first undertaken by Ong (1966), who studied the first crab stage up to the 18th instar, but only the first two instars were described in detail. Their mean carapace widths were recorded to be 3.4 mm and 5.1 mm, respectively. For *Scylla paramamosain*, there is very little information about life history, except a description of larval development by O'Kelly (1998). In the present study, collection of detailed information on life history through different moult stages was not attempted, however, growth over the first 8-9 crab stages was precisely recorded for the first time. The sizes obtained for these stages were similar to those as recorded by Ong (1966). This may indicate that the specimens observed by Ong might have not been *S. serrata* (Keenan *et al.*, 1998; O' Kelly, 1998), but *S. olivacea* (Quinitio, pers. com).

Growth of an organism can be expressed as the increase with time of length, volume, wet weight or dry weight. However, crustacean growth is discontinuous due to the exoskeleton which is shed and renewed through moults. Thus, growth in crustacea has two components, moult increment (increase in size occurring at a moult) and moult interval (the time between two successive moults) (Hartnoll, 1982). Moult frequency and moult increment may be highly different between individuals reared under similar conditions (Hartnoll, 1982), as observed in *Scylla* sp. from the 6th instar onwards (Ong, 1966). In the present study the same phenomenon was also observed for *S. paramamosain*. As a consequence, the fast growers were at a more advanced stage than the slower growers. The decreasing percentage moult increment through development found in the current study was also in agreement with Ong (1966) and Thomas *et al.* (1987) who indicated that the percentage moult increment was greater for the early crab stages and decreased in the later stages in *Scylla* sp. Hartnoll (1982) also stated that the percentage increment is extremely variable, both within and between species but generally decreases with size. In the grapsid crabs, *Cyrtograpsus angulatua* and *C. altimarus* the same trend of decreased moult increment and

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increased moult interval with age were also reported (Spivak, 1999). Guerin and Stickle (1997) also concluded that the percentage moult increment decreased with increasing size class for two species of *Callinectes*. A moult increment of 25% was reported in both *Scylla* sp. and *Thalamita crenata* by Thomas *et al.* (1987). However, higher values have been obtained in the present study (29.8% and 31.3%) for crabs in recirculation and batch exchange systems, respectively.

Environmental parameters have well documented effects on moult interval and moult increment in crustaceans. Chang (1995) has reviewed the environmental influences on moulting in crustaceans and pointed out that photoperiod, food supply and space may influence the moult interval and moult increment. He also mentioned the probable effect of various stressors, such as pollutants on moulting. Hartnoll (1982) reviewed the effect of temperature, food supply, light, salinity and loss of appendages on moult increment and duration in crustaceans. In addition, Hartnoll (2001) described in detail the role of the moult inhibiting hormone (MIH) and hyperglycaemic hormone (CHH) in moulting regulation. He indicated that levels of CHH can rise in response to stress and this raised CHH level is a possible factor in extending the moult interval. In the present study, food was supplied to excess, so that the crabs received more than they could eat on a daily basis. Growth therefore would not be affected by limited food supply (Guerin and Stickle, 1997). However, in terms of quality, crabs which were fed live *Artemia* biomass in the batch exchange system may gain more benefit than those supplied with the frozen *Artemia* in the recirculation system. Moreover, the highly variable level of nitrite recorded in the recirculation system (Table 3) indicates that the biofilter used was likely to be unreliable. Such stress may have raised levels of CHH, affecting moulting of the crabs in the closed system and resulting in the differences in sizes and moult intervals for crabs between the two systems. Furthermore, higher salinity (30 ppt) may have also been an additional cause resulting in a lower growth observed in the recirculation system.

However, in both cases growth of crabs may have been limited by confined conditions and may not reflect the actual growth of the animals under natural conditions. Ong (1966) concluded that although the conditions in the laboratory may be favourable for

growth of an organism, to some extent they are different to those in nature. Growth in the confined environment may be slower than in natural conditions (Manjulatha and Babu, 1998). Hartnoll (1982) also emphasized that growth observed in captivity may not be indiscriminately applied to populations in the wild as both moult increment and moult interval vary so greatly with environment.

Salinity

Salinity tolerance of estuarine crabs has been reported to be variable with development stage, as a function of ontogenetic changes and phylogenetic adaptation of the species to breeding in a physically unstable environment (Anger, 1996). In invertebrates, Kinne (1971) pointed out that the early ontogenetic stages exhibit less tolerance to salinity than the later stages or adults. Anger (1996) and Anger & Charmantier (2000) found an increasing tolerance to low salinities in the grapsids, *Armases miersii* and *Sesarma curacaoense* during their successive developmental stages. Hill (1974) reported that the first stage zoea of *Scylla serrata* was not tolerant in low salinities (i.e. 17ppt). However, together with the results from Ong (1964) and Du Plessis (1971) Hill indicated that later larval and especially postlarval stages would be expected to have greater tolerance to reduced salinity. In further experiments, Ong (1966) also observed improved tolerance and performance of crab juveniles reared in reduced salinity water. A similar trend was also recorded for postlarvae of shrimp (*Penaeus*), which may be attracted towards lower salinities, this adaptation being an important factor in the recruitment of postlarvae to lagoons (Mair, 1980). However, in the extreme low or high salinities, negative effects may be expected (Anger, 1996; Spivak, 1999).

Poor performances of crabs in low salinities have been demonstrated for a number of species. Zhongli *et al.* (2001) claimed that the metamorphosis of *Scylla serrata* will be prolonged and lower survival predicted at salinity under 10ppt. Poor feeding and reduced growth were also observed in *Scylla oceanica* (species now uncertain, Keenan *et al.*, 1998) at salinity below 10ppt (Manjulatha and Babu, 1998). In *Callinectes*, growth is significantly lower in crabs subjected to salinity of 5ppt (Guerin and Stickle,

1997). Similar results have also been recorded for the grapsid crabs with poor growth and extended intermoult period in salinity as low as 3ppt (Spivak, 1999). In addition, Anger (1996), also found a prolonged development and increased mortality rates in the grapsid crab, *Armases miersii* in 5ppt. In the present study, performance of wild and hatchery-reared juveniles was significantly reduced in 5ppt (Experiments 3 and 4, batch system) with lower growth rate, higher intermoult duration and poor survival. In contrast, better performance of crabs was observed in higher salinities, 15-25ppt. Based on the higher growth rate and shorter moult intervals recorded, optimal salinities for growth of juvenile *Scylla paramamosain* are in the range 15-20ppt. A similar salinity preference was also reported for *Scylla serrata*, with a faster growth rate at 21-22ppt (Ong, 1966). Anger (1996) also indicated that the most suitable salinity for the grapsid crab, *Armases miersii* is 15-25ppt yielding higher survival and faster development. He also mentioned that extreme salinities (45-55ppt) extended development and lowered survival rates. Although mortality of crabs in the present study did not significantly increase, their growth tended to decrease at higher salinities (25-30ppt). However, further study is needed to determine the upper salinity threshold for this species.

In most of the cases, the percentage moult increment (both CW and weight) and moult duration were not always significantly different between salinities, only at the low (5ppt) and high (30ppt) salinities. This observation is consistent with observations by Hartnoll (1982) that the effects on moult increment are generally minimal over a wide range of salinity, and the effect of salinity on intermoult duration is minimal except for a lengthening of the intermoult at extremely high or low salinities.

Scylla sp have been reported to be euryhaline, hyper/hypo-osmotic osmoregulator (Davenport and Wong, 1987; Chen and Chia, 1997) and they should be tolerant of a wide range of salinity. Davenport and Wong (1987) stated that they do not discriminate between salinities and Hill (1979) found *Scylla* could survive in estuaries with salinity as low as 2ppt. In addition, a recent study on abundance of *Scylla paramamosain* (Chapter 2) also indicated their occurrence in freshwater (0ppt) during the monsoon season. However, in the laboratory, crabs only survived in freshwater for

3-5 days, in the present study. Results from analysis of hemolymph ammonia and urea and nitrogenous excretions of *Scylla* sp (species is uncertain according to Keenan *et al.*, 1998) revealed that excretion of ammonia-N, organic-N, nitrite-N, and nitrate-N and total nitrogen excretion increased with decreased salinity (Chen and Chia, 1996). In crustaceans, increased ammonia-N excretion is considered an indicator of a stressful environment (Nelson *et al.*, 1977 cited by Chen and Chia, 1996). They suggested that higher ammonia-N excretion at low salinity (10ppt) is a consequence of the catabolism of amino acid required for reducing osmolality. This process may consume resources reserved for growth and more importantly nitrate formation may serve in the detoxication of ammonia and the maintenance of electro-neutrality inside the crabs (Spaargaren, 1985 cited by Chen and Chia, 1996). Consequently, crabs reared in low salinity (5ppt) in the present study were likely to be subjected to a more toxic environment, as a result of adapting to the salinity. Moreover, in a stressful situation, production of CHH (Hartnoll, 2001) may further reduce growth and survival. In natural conditions (i.e. in estuaries) the increase in nitrogen excretion would be diluted. With a powerful osmoregulation capacity, crabs therefore can survive and grow in highly variable low salinities in the estuaries. However, there may be other factors that may have contributed to the existence of crabs rather than only salinity in the estuarine environment (see below) as Davenport and Wong (1987) suspected that other factors (e.g. subtratum type or food availability) rather than salinity determine the species distribution in estuaries, mangroves and lagoons.

The complete mortality of crabs reared in 0ppt in the present study indicates that *S. paramamosain* are unable to survive in freshwater for long periods in captivity at all tested size classes. Since the ontogenetic changes in most euryhaline species indicates that hyper-hyporegulation is normally found only in juveniles and adults (Anger and Charmantier, 2000) they might have been expected to be able to osmoregulate in different salinity environments. There is no information for *Scylla* indicating the development of ability to osmoregulate with age, as yet. However, in blue crabs, *Callinectes sapidus*, Tagatz (1971) has tested osmoregulatory capacity in various size categories and between sexes. She found no significant differences in haemolymph osmotic concentration regardless of sex or size. However, her results revealed a

significant difference in regulatory ability between males and females, but not between sizes. In a detailed study on ontogeny of osmoregulation of the grapsid crabs, Charmantier *et al.* (1998) suggested that osmoregulation changes during postembryonic development, with hyper- and hypo-osmoregulatory capacities increasing from juveniles to adult. They also mention a pattern of osmoregulation linked with metamorphosis. The inability to tolerate 0ppt for a wide size range (2-5cm CW) of *S. paramamosain* in the present study may be due to the incomplete size range selected for the study (i.e. juvenile vs adult sizes), or differences may only be expected between sizes for crabs exposed to higher salinities (Guerin and Stickle, 1997). The fact that crabs were not able to survive a short time in 0ppt in the laboratory conditions may indicate that the persistence of crabs in freshwater in the wild may be the result of burying into the mud, where salinity may be sufficient for hyper-osmoregulation. Atkinson and Taylor (1988) stated that many decapods have adopted a burrowing mode as a physiological adaptation to cope with the problem of aerial respiration and salt and water balance. In addition, McGaw and Naylor (1992) confirmed the role of shelter in low salinity tolerance of the shore crab, *Carcinus maenas*. They pointed out that the presence of suitable shelters may permit the colonization of estuaries beyond the limit predicted from responses to salinity alone. They found the crabs, in laboratory conditions, remained longer in low salinities with shelters provided than when there were no shelters. Barnes (1974), (cited by McGaw and Naylor, 1992) suspected that shelter might provide microhabitats of slightly raised salinity. Although it was uncertain what probable mechanism is behind this observation, McGaw and Naylor (1992) concluded that "salinity does not wholly account for distributional differences of *Carcinus maenas* in estuaries and that the availability of suitable areas of shelter may affect their distribution and retention". The muddy tidal flat and mangrove in the estuary in the present study (Chapter 2) may similarly influence the distribution of *Scylla paramamosain* as for the shore crab (McGaw and Naylor, 1992). It seems likely that the growth rate observed in crabs in the estuary in the mark-recapture study (Chapter 5), may represent optimal growth in the dry season, with lower growth in the low salinity conditions of the rainy season.

In summary, growth of *S. paramamosain* juveniles is comparable to the results of the previous study by Ong (1966). Juveniles of 30 mm CW can be obtained in two months by rearing in tanks run with a batch tank system, prior to stocking in ponds. Crabs cannot grow and survive well in 5ppt. This may be related to the osmotic problem associated with stress caused by the increased nitrogen-excretion concentration in the captive environment. A hypothesis that may arise from this is that if crabs were stocked in the systems with water exchange to reduce as much as possible the accumulation of nitrogen excretion, they may adapt to low salinities. The preferable salinities for juvenile *S. paramamosain* are from 15-25ppt, the most suitable range is 15-20ppt. Crabs are not able to survive in 0ppt regardless of size in captivity conditions, despite their survival in the wild in low salinity or freshwater conditions. Further studies are required to determine responses at higher salinities.

CHAPTER IV

APPLICATION OF MICROWIRE TAGGING
IN *SCYLLA PARAMAMOSAIN*

INTRODUCTION

Marking or tagging is considered to be the most useful and important tool for fisheries biologists studying migration, growth, population size, mortality and recruitment of both finfish and shellfish. Farmer (1981) reviewed a range of methods which are applicable to crustaceans. These include staining, immersion, injection or feeding with stains. The main disadvantage of stains is that they fade after a short period following administration making it impossible to obtain long-term data on growth and migration of tagged targets. High mortality occurred in shrimp during the administration of stains (e.g. trypan blue) and staining is less applicable for crabs as the development of the opaque exoskeleton obscures marks with time.

Mutilation such as tail clipping or eyestalk ablation has also been developed for short-term marking of shrimps (Kurata *et al.*, 1971). Although these methods are simple to use, they are likely to be limited in application as there are many disadvantages. With the tail clipping method, the mark is eventually lost through gradual regeneration resulting in difficulties in individual identification. Eyestalk ablation can cause high mortality after marking. Linnane and Mercer (1998) tagged juvenile lobster *Homarus gammarus* using rostrum ablation and concluded that this is a poor external mark, as regeneration of the rostrum occurred after 3 moults making identification of the tagged animals impossible. However, clipping has been successfully applied in crabs by mutilating the anterior carapace spines of adult shore crabs (*Carcinus maenas*), which only moult once a year (Munch-Petersen *et al.* 1982).

External tags have been widely applied in crustaceans. Their advantage is that the tagged animals can be obviously recognized at recapture. In addition, by using these tags individual identification is possible. External tags used to mark shrimp, described by Farmer (1981), are the Petersen disc (Linder and Anderson, 1956); wire tags (Kourist *et al.*, 1964; Meyer-Waarden and Tiews, 1965; Tiews, 1968), dart and loop type tags (Melville-Smith, 1987), anchor tags (Hill, 1975; Hyland *et al.*, 1984), "T-bar" tags (Taylor, 1982). More recently, modified "T-bar" anchor tags (McPherson, 2002) or polyethylene streamer tags have been used together with branding (Linnane and Mercer, 1998). When using a polyethylene streamer, Linnane and Mercer (1998)

suspected that the tags prolonged the moulting process of the lobster as during ecdysis the cast exoskeleton frequently became entangled with the tag. Modified "T bar" anchor tags were assessed for marking the blue swimmer crab *Portunus pelagicus* and appeared to be superior for use with this species (McPherson, 2002).

A variety of internal tagging methods have been investigated for fish and crustaceans. Caceci *et al.* (1999) applied microchip transponders inserted into the body of the prawn, *Macrobrachium rosenbergii* for individual identification. Results indicated that this is a reliable method for studies on selective breeding in prawns as it did not affect growth, behaviour or life span of the animals. Visual implant fluorescent elastomer (VIFE) tags have been widely used in a number of species. They were evaluated with the crayfish, *Cherax destructor* as a method to identify juveniles stocked in the same ponds (Jerry *et al.*, 2001). Visible implant fluorescent elastomer tags (VIFE) were also used to tag the lobster *Homarus gammarus* (Uglem *et al.*, 1996; Linnane and Mercer, 1998) and adult shrimp *Litopenaeus vannamei* (Godin *et al.*, 1996). Results from the study by Uglem *et al.* (1996) indicated that use of VIFE tags is a promising method for marking juveniles in both controlled experiments and in large-scale stock enhancement release. The authors also mention that tag retention was even higher than that seen in juveniles marked with internal microtags (Wickins *et al.*, 1986; Uglem & Grimsen, 1995). After injecting intramuscular fluorescent elastomer tags on the ventral side of the sixth tail segment of *Litopenaeus vannamei*, Godin *et al.* (1996) concluded that there was no major detrimental impact to the general health of the shrimp. Linnane and Mercer (1998) also found positive results with tag retention, growth and survival of juvenile lobster *Homarus gammarus* tagged with VIFE. In all cases tagging with elastomer tags has been reported to be one of the most suitable options for marking crustaceans as they are readable and remain visible for identification during the study periods.

Interest in using internal coded wire tags in aquatic animals has recently increased. Micro-wire tags were first developed by Jefferts *et al.* (1963) and have been widely used for tagging fish. Microwire tags can be used to identify individuals or groups, which is not possible with VIFE. They have been widely applied in salmon and trout (Habicht *et al.*, 1998; Hale and Gray, 1998; Thedinga *et al.*, 2000; Nass (ed.), 2001; Wertheimer *et al.*, 2002;) and in other fish species such as red snapper, *Lutjanus*

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campechanus (Brennan *et al.*, 2001), Barramundi, *Lates calcarifer* (Rimmer and Russell, 2001), shortnose sturgeon, *Acipenser brevirostrum* (Isely and Fontenot, 2000), European eel, *Anguilla anguilla* (Thomassen *et al.*, 2000), and herring (Morrison and MacDonald, 1986). Miller and Able (2002) were successful in using internal sequential coded micro-wire tags for study of movement and growth of the Atlantic croaker (*Micropogonias undulatus*), while Courtney *et al.* (2000) succeeded with juvenile Pacific salmon. Microwire tags have been also used in marking studies for shrimps, lobsters and crabs. The advantage of internal tags over external tags with crustaceans is reduced tag loss through ecdysis. Wickins *et al.* (1986) and Uglem and Grimsen (1995) applied these tags in lobsters, *Homarus gammarus*, for stock enhancement studies. Sharp *et al.* (2000) used them in juvenile Caribbean spiny lobster, *Panulirus argus* for mark-recapture studies. Bailey and Dufour (1987) used micro-wire tags for mark-recapture studies of the snow crab (*Chinoecetes opilio*) and found no unfavorable reactions in tagged crabs kept in captivity. Tags were also utilized for detailed studies of population dynamics in the portunid crab, *Callinectes sapidus* by van Montfrans *et al.* (1991) and Fitz & Wiegert (1992). Recently, Kneib and Huggler (2001) applied binary-coded wire tags to study the effects of tagging on growth, survival and mark retention in juvenile white shrimp, *Litopenaeus setiferus*. Coded micro-wire tags have not only been used in fish and crustaceans but also have been applied in molluscs. Lim and Nobuo (1999) tagged the short necked clam *Ruditapes philippinarum* and found no significant difference between tagged clams and controls in terms of survival and growth with a high tag retention.

In crabs, tagging techniques have been developed and practiced for a number of species and variety of studies. A tagging method was introduced by Fannaly (1978) using internally anchored external spaghetti-type tags on the blue crab, *Callinectes sapidus* but found there was a slight impediment to moulting. Tagging was used in this species to study population dynamics and migration (Steele, 1991), abundance, recruitment and loss (Fitz and Wiegert, 1992). Tagging methods have also been developed for population, growth and migration studies in shore crab, *Carcinus maenas* (Gomes, 1988, 1991); red king crab, *Paralithodes camtschaiticus* (Takeshita *et al.*, 1973; Donaldson *et al.*, 1992); snow crab, *Chionoecetes japonicus* (Ryo and Yasuyuki, 1999), *Chionoecetes opilio* (Taylor, 1982; Bailey and Dufour, 1987; Taylor

and Hoenig, 1991) and the portunid crabs, *Portunus pelagicus* (Cheng *et al.*, 2001), and *Portunus trituberculatus* (Kim *et al.*, 1986 and Kazutoshi, 1999)

The position and procedure for tagging or marking animals using microwire tags is reported to be of importance to minimize damage, mortality or reduce any effect on growth of the animals (Wickins, *et al.* 1986). The most suitable position for injection of microwire tags in lobsters, according to Wickins *et al.* (1986) and Linnane and Mercer (1998) is the muscle block between the fifth pereiopods. Kneib and Huggler (2001) found the ideal site to place tags in *Litopenaeus setiferus* to be in the abdominal musculature. They also mention that inserting tags into other positions such as the coxa, uropod or telson is unsuitable as it causes difficulty during moulting, exposing shrimp to a greater risk of cannibalism. In crabs, microwire tags are either injected into the basal muscle of the fifth pereiopod (van Montfrans *et al.* 1991; Fitz & Wiegert, 1992; Kazutoshi, 1999), or into the dactylus of the walking legs (Bailey & Dufour, 1987).

In most cases, tagging appears to have no negative effect on growth or survival of the tagged animals and the loss rate of tags is acceptable. Applying microwire tags in lobsters, Wickins *et al.* (1986) found that tag retention was 85-100% as the animal grew through 22-29 moults (90-102 mm carapace width) in captivity. Using micro-wire tags in *Litopenaeus setiferus*, Kneib and Huggler (2001) confirmed that there was no effect of tags on growth and that tag retention was high, especially when tags were injected in the first abdominal segment (95.8-100%). Similarly, Linnane and Mercer (1998) also found that micro-tagging was the most successful means of marking, resulting in high survival (82-97%) and tag retention (97%) in juvenile lobsters, *Homarus gammarus*. Kazutoshi (1999) working with *Portunus trituberculatus* confirmed that there was no significant difference in survival and growth between tagged and non-tagged crabs. Tag retention was high (90%) in larger animals (more than 20mm carapace width). However, in contrast, Cheng *et al.* (2001) found survival and growth of tagged *Portunus pelagicus* were lower than for the controls although tag retention was 100% after several moults.

To date, tagging studies with mud crabs *Scylla* spp. are few, although Hill (1975) and Hyland *et al.* (1984) used anchor tags to mark *Scylla serrata* for abundance, breeding,

growth and movement studies. In the present study a tagging technique for the mud crab *Scylla paramamosain* is evaluated. Before attempting to investigate population dynamics, growth and migration in this species, sequential binary coded micro-wire tags were tested to ascertain their effects on growth and survival of the crabs under study.

MATERIALS AND METHODS

Experiment 1:

Juvenile *Scylla paramamosain* were purchased from an agency in Long Phu, Soc Trang Province, where they had been collected by hand from intertidal mangrove areas of the Hau River estuary. The crabs were transported to the laboratory, and separated into individual plastic jars (200ml), to avoid cannibalism or other aggressive damage prior to the experiment.

The carapace width (CW) of the crabs was measured using a caliper and the crabs weighed with an electronic balance. They were randomly divided for tagging and control treatments. Twenty-one crabs, with a size range of 28.8- 39.8 mm CW were allocated to the tagging treatment and 22 of 29.6 - 42.0 mm CW to the control. Prior to tagging, crabs were anaesthetized with 500ppm chloroform for 5 min. Microwire tags 1mm long and 0.25mm in diameter were used. Insertion of tags into the crabs was conducted using a hand-held multishot injector (Northwest Marine Technologies Inc.). The crabs were held on their backs and tags were injected at a joint into the coxal muscle of the left 4th pereiopod (Fig. 1). Successful tagging was checked with a hand-held wand detector. The crabs were then placed in individual plastic baskets (25 x 20 x 20 cm, mesh size 0.8cm) floating in a 2m x 2m concrete tank with recirculated, biofiltered seawater (Fig. 2). Crabs in the control treatment were allocated to identical baskets held in the same tank. Monitoring for new moults was maintained daily. Any newly moulted crab was measured and checked for tag retention, where appropriate. The crabs were fed daily to excess with clam or blood cockle. The left-over food was removed the following day. Temperature, salinity and pH were measured daily and nitrite was monitored every 3 days. When the nitrite concentration increased above 0.5ppm, or when necessary, 50 -100% of water was renewed.

Experiment 2:

The objective of this experiment was to test the effectiveness of tagging in juvenile crabs of a smaller size range, which were not available for the previous experiment, so

as to extend the size range tested for tagging down to a lower limit which might be appropriate to hatchery seed production.

Crabs were purchased from the same agency as in Experiment 1. The number of crabs was limited by availability at the experimental start time. Only 22 crabs ranging from 25-32.4mm CW were obtained. The crabs were measured and randomly allocated with 12 crabs (25-31.4mm CW) and 10 (25-32.4mm CW) to the tagged and control treatments, respectively. The same tagging method was applied but anaesthesia was not used. Crabs were maintained individually in round baskets (15cm diameter and 10 cm high) floating in the same recirculating tank. The crabs were fed daily to satiation with clam or blood cockle flesh. Monitoring for moults was maintained as in Experiment 1.

Experiment 3:

The aim of this experiment was to repeat the previous experiments, but under pond culture conditions. The objective was to test the tagging system and to gather growth data for *Scylla paramamosain* under more typical culture conditions. This was also a step towards developing a method for reliable tagging and release of crabs into the wild, with individual or batch identification using sequentially coded microwire tags.

Wild crab juveniles were obtained from the same agency as for the previous experiments. They were transported to the Vinh Chau field station and acclimatized to the salinity in ponds. A total of 147 crabs were individually measured and held in small labelled jars. They were randomly divided into 2 groups. The tagged group consisted of 74 crabs of 23.4 - 41 mm CW (mean 34.7 ± 4.37 mm). Tagged crabs were injected with 1mm sequentially coded microwire tags in the coxal muscle of the 4th pereiopod. Archived tags were kept immediately before and after the injected crabs. With this procedure when tags are retrieved from adult crabs, it should be possible to use the archived tags to identify the individual animal, so that its growth history can be recorded. A second group of 73 crabs with a size range of 24.1-42.1mm CW (mean 34.7 ± 4.16 mm) was allocated as the non-tagged control.

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A wooden frame cage system was constructed, which allowed the crabs to be held individually in the pond. The cage was 2 x 1 x 0.7 m, containing 18 compartments (0.3 x 0.3 x 0.7 m each). A group of 36 tagged and 36 non-tagged crabs were randomly distributed in the cages. The remaining crabs were released into the pond around the cages, so that the growth of crabs in cages might be compared to that of free-ranging animals. The crabs were fed daily to excess with eviscerated chopped Tilapia. Moults and tag retention were monitored daily, but only for the crabs kept in cages. The cages were lifted up once every morning to facilitate handling for checking or measuring.

At the end of the experiment, all the surviving crabs were measured. In the tagged crabs, tags were retrieved by making a small opening in the thoracic sternite and removing the tag from the muscle cavity by using forceps. Tags were glued on a book with transparent tapes for later reading in the laboratory. Prior to reading, tags were rubbed using the thumb and forefinger in a clean water bowl to remove the glue. Care was taken to avoid dropping or losing the tags. They were then read under a dissecting binocular microscope and interpreted with the help of sequential tag conversion (GR) software (Northwest Marine Technology, Inc).

Statistical analysis

Differences between treatments were compared either by t-test or one-way ANOVA as indicated in the results. All data were tested for normality and homogeneity of variance. Data not conforming were square root transformed before ANOVA using the Minitab 13 statistical package.
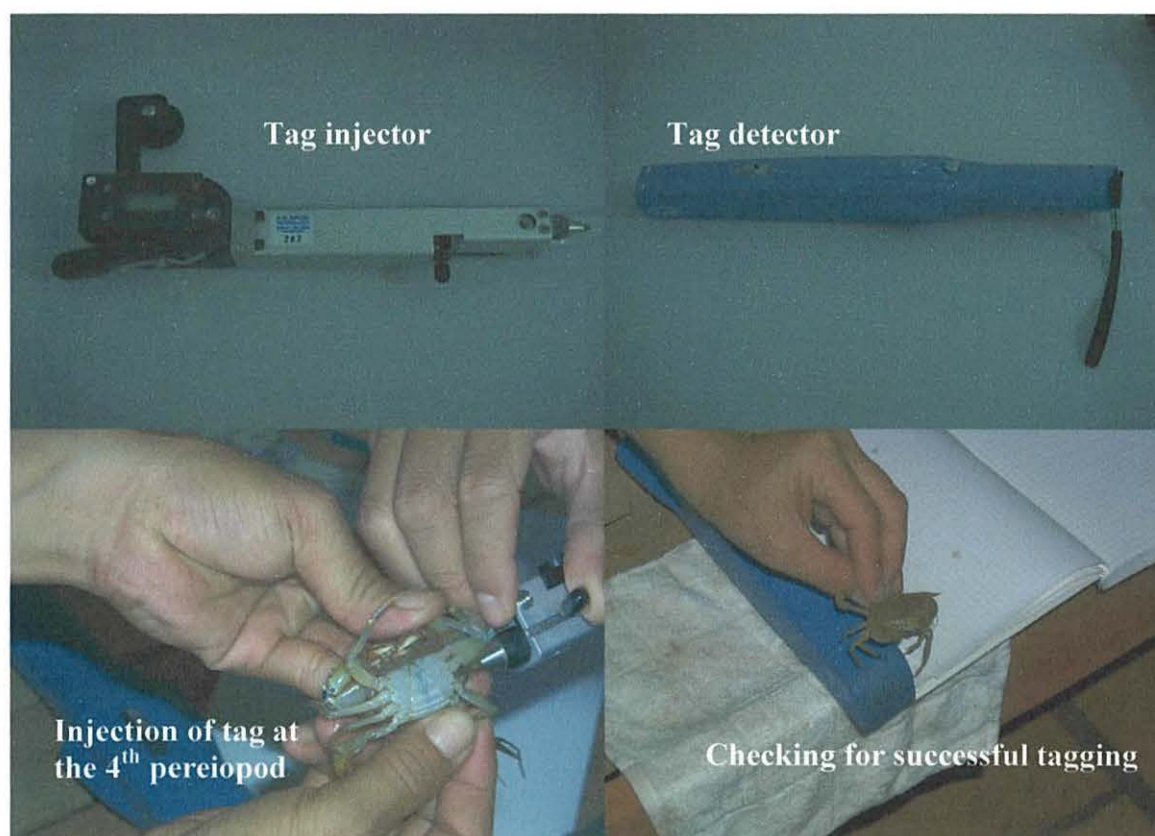
Figure 1: Equipment and procedure applied for tagging experiments in tanks and ponds.

Figure 2: A 4 m^3 concrete tank connected to a bio-recirculation system was used to contain individual crabs held in baskets in Experiment 1 and 2.

RESULTS

Experiment 1

The first experiment ran for 147 days. Survival rate was 68% (15/22) for tagged crabs and 71% (15/21) for the control. The number of crabs that moulted four times was higher in the tagged treatment than in the control. The intermoult period was 31-38 days (mean 33.4 ± 4.1) and 34-39 days (mean 36.6 ± 2.5) for tagged and control crabs, respectively (Fig. 3). There was no significant difference in intermoult duration between tagged and control crabs (f= 1.14, p=0.345). Growth in carapace width and weight is shown in Figs. 4 & 5. Of the 15 surviving tagged crabs, two lost their tags, one at the third moult, the other at the fourth.

Experiment 2

Experiment 2 ran for 120 days and 2 out of 10 control crabs and 2 out of 12 tagged crabs died. The number of crabs that moulted 4 times differed between tagged and non-tagged crabs. Among the surviving crabs in the control group, 5 had moulted four times, compared to 8 for the tagged crabs. Intermoult period and growth of crabs is presented in Figs. 4, 5 & 6. No significant difference in intermoult duration between tagged and control crabs was found (f=0.83, p= 0.414). All tags were retained (100%) in the surviving crabs.

Experiment 3

The experiment was terminated after 83 days. 13 tagged crabs were recaptured from the pond at the end of the experiment. Their specific growth rate (SGR), calculated from initial and final weights was substantially and significantly higher than that of caged crabs (F=9.91, p<0.001, Table 1). However, there was no significant difference in SGR between tagged and control crabs held in cages (F=0.07, p=0.792), and no significant difference in final weight (F=0.01, p=0.929) between free-ranging tagged and control crabs. Most of caged crabs had moulted 3 times and a few moulted for a fourth time. As in previous experiments, the number of crabs accomplishing a third

moult was higher in the tagged group than in the control. The intermoult duration of the tagged crabs was significantly shorter than that for the control animals (f=19.6, p=0.01), and caged crabs moulted significantly faster than those kept in tanks (Experiment 1) (f=45.24, p<0.001). Comparison of intermoult duration between tagged and control stocked tank and in pond is shown in Fig. 6.

Survival of free-ranging crabs in the pond was significantly lower than for those held in the cages (36% to 77%, respectively). Of the caged crabs, four tagged animals lost their tags and two control animals escaped. A similar rate of tag loss might account for the difference in numbers of tagged (13) and non-tagged (26) crabs in the free-ranging group, as any crab that lost its tag and any caged control group animal which escaped would be counted in the control group. This would result in a more realistic estimate for survival of 19 tagged animals and 20 non-tagged individuals.

Table 1: Comparison of carapace width, weight and growth rate in tagged and control *S. paramamosain* juveniles in Experiment 3, reared individually in cages and free-ranging in ponds.

	Weight (g)		C.W. (mm)		Number		Specific Growth Rate
	Initial (sd)	Final (sd)	Initial (sd)	Final (sd)	Initial	Final	(ln g/d ×100) (sd)
Cage Tagged	7.2 (2.8)	55.6 (22.0)	36.6 (5.0)	65.0 (4.9)	36	26	2.46 (0.5) n=26
Cage Control	6.9 (2.7)	53.7 (23.2)	37.7 (4.7)	66.4 (11.8)	36	21	2.47 (0.6) n=21
Pond Tagged	8.2 (2.5)	133 (51.4)	35.8 (4.4)	87.3 (9.2)	38	13	3.36 (0.6) n=13
Pond Control	8.3 (2.5)	137 (57.2)	37.7 (5.4)	87.9 (11.5)	37	26	3.38 *

Legend: * no (sd) standard deviation calculable as no individual growth rates were measured.
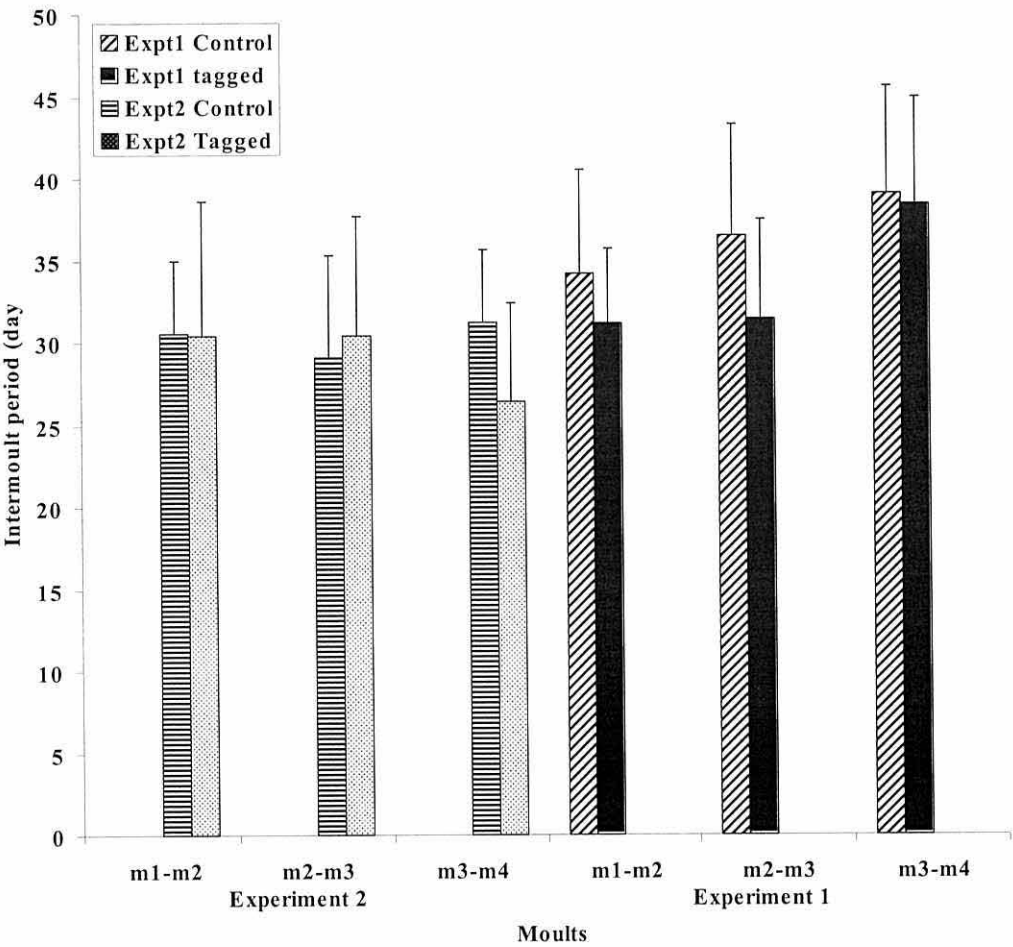
Figure 3: Mean intermoult duration (days) and standard deviations at moult number of tagged and control *S. paramamosai*n juveniles kept individually in baskets in Experiment 1 for 147 days and in Experiment 2 for 120 days. A one-way ANOVA found no significant differences in intermoult period between tagged and control crab in either experiment (Experiment 1; f=3.11, p=0.082. Experiment 2; F=0.45, p=0.509).
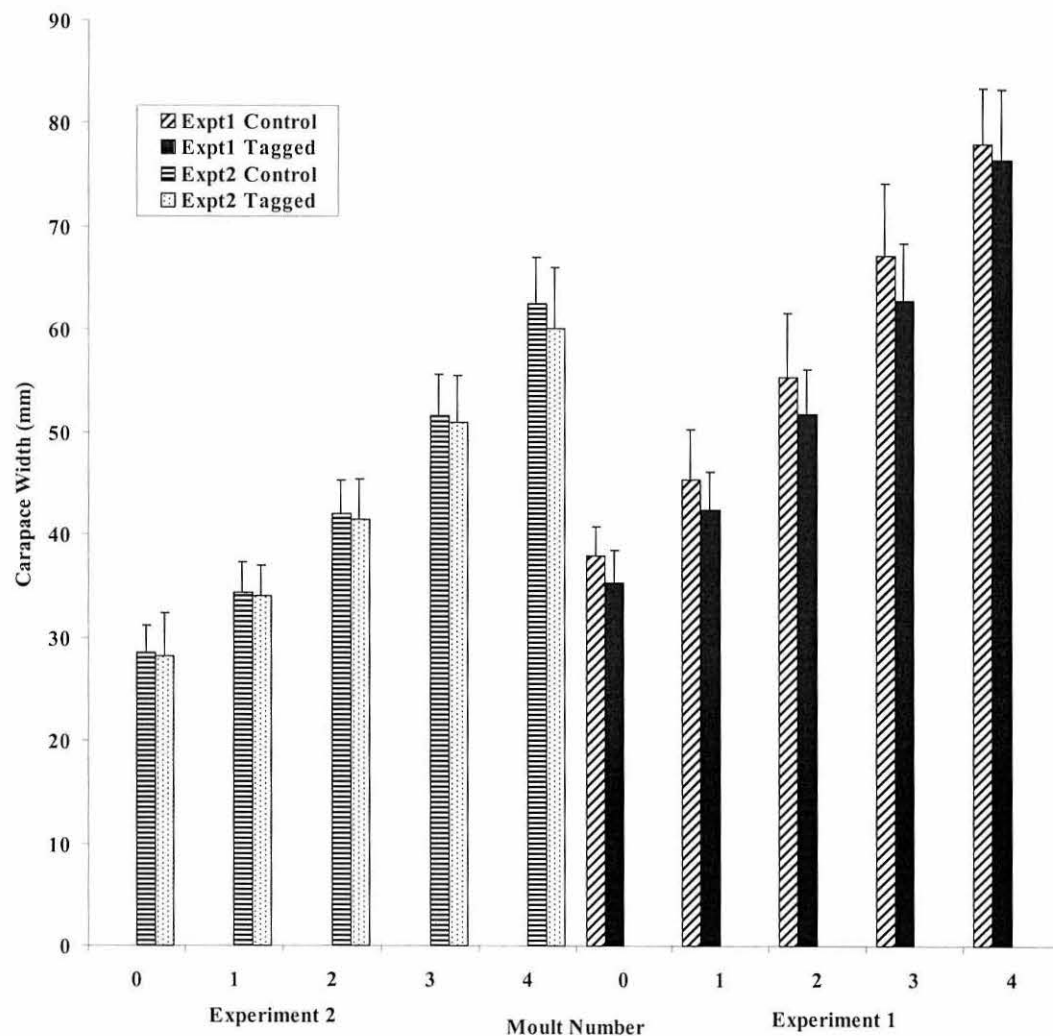
Figure 4: Mean carapace width (mm) and standard deviations at moult number of tagged and control *S. paramamosain* juveniles kept individually in baskets in Experiment 1 for 147 days and in Experiment 2 for 120 days. A one-way ANOVA found no significant differences in CW between tagged and control crab in either experiment (Experiment 1: f=0.69, p=0.409. Experiment 2: F=1.67, p=0.199).
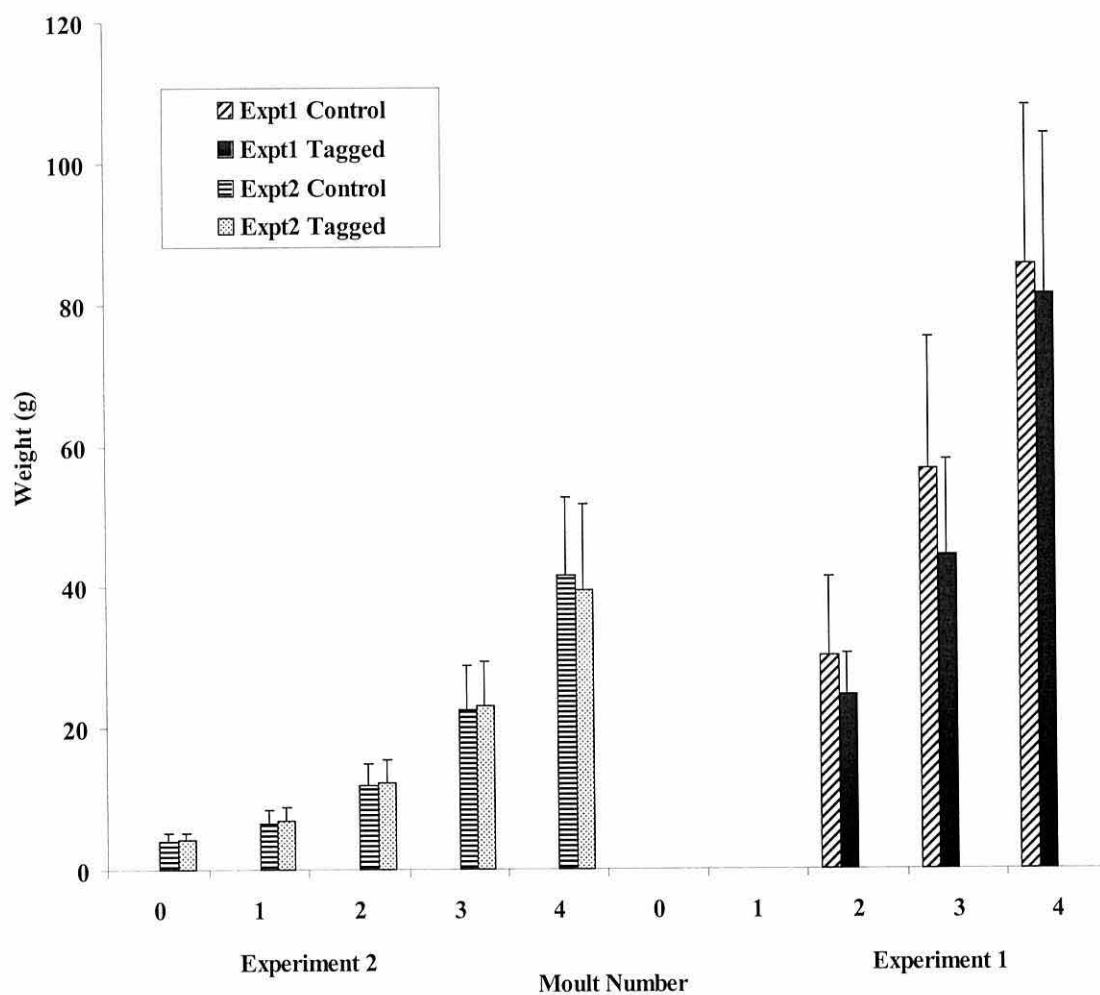
Figure 5: Mean weight (g) and standard deviations at moult number of tagged and control *S. paramamosai*n juveniles kept individually in baskets in Experiment 1 for 147 days and in Experiment 2 for 120 days. A one-way ANOVA found no significant differences in weight between tagged and control crabs in either experiment (Experiment 1; f=2.15, p=0.149. Experiment 2; Square root transformed; F< 0.001, p=0.955).
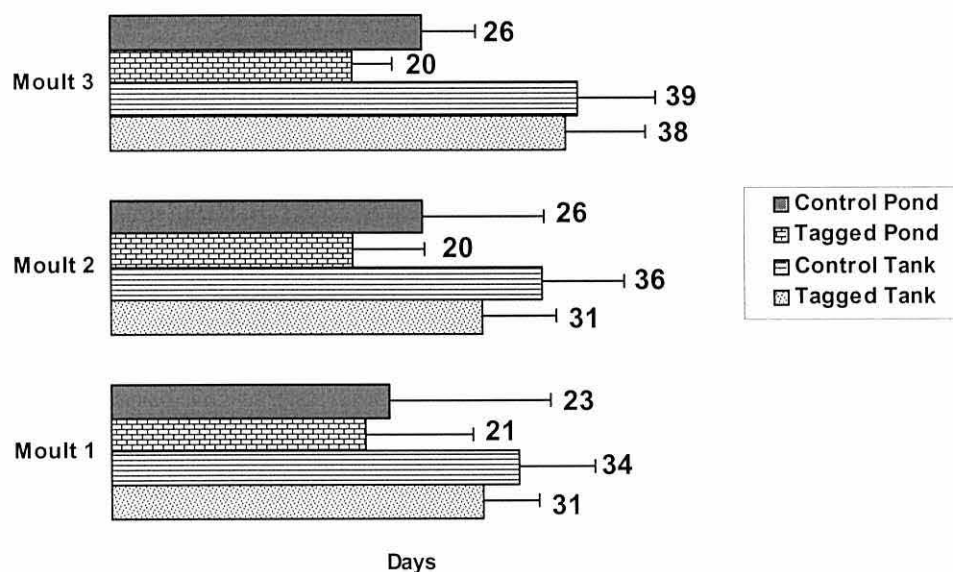
Figure 6: Comparison of intermoult duration of tagged and non-tagged crabs within Experiment 1 (in tank) and Experiment 3 (in pond), and between tanks and ponds. A one-way ANOVA found no significant difference on intermoult duration between tagged and control crabs in tanks (Exp 1, f=1.19, p=0.336) but a significant difference for crabs in ponds (f=19.6, p=0.011). There was a significant difference in intermoult duration between tank and pond for both tagged and control crabs (f=30.42, p=0.005 and f=41.29, p=0.003, respectively).

DISCUSSION

Results from tank-based experiments demonstrate that tagging has no effect on growth or survival of juvenile *S. paramamosain*. Crabs with a carapace width above 20mm can be tagged successfully without any affect on growth and survival. This is in agreement with earlier studies on other genera showing microwire tagging to be an effective tool for marking *Callinectes sapidus* (Fitz & Wiegert, 1991, Rugolo et al, 1998). Kazutoshi (1999) also concluded that there was no significant difference in survival and growth between tagged and non-tagged juvenile swimming crabs (*P. trituberculatus*). Interestingly, the tagged crabs in all three experiments were found to have a higher number of individuals completing the last moult and a shorter intermoult duration than controls, although no significant difference could be determined. In one experiment, tagged white shrimp (*L. setiferus*) were also reported to grow more rapidly than controls (Kneib and Hugger, 2001). However, van Montfrans *et al.* (1986) found that tagging small blue crabs (25mm carapace width) impaired growth and increased mortality. Cheng *et al.* (2001) also found lower survival and growth in juvenile *Portunus pelagicus* tagged with microwire tags. In the present study, the coxal muscle of the fourth pereiopod under the thoracic sternite was found to be an ideal site for injecting the tags. In the other studies, tags were mostly inserted into the muscle of the fifth pereiopod, and damage caused by injection may hinder an individual's swimming ability.

Similarly, in ponds, growth was unaffected by tagging, although mortality appeared to be higher amongst tagged individuals. However, non-tagged escapees from cages constructed within the pond, and the loss of tags on the part of pond animals may have resulted in an over-estimation of the survival rate of non-tagged individuals, thereby explaining apparent differences in mortality.

The improved tag retention noted with increasing tagging experience suggests that good technique and experienced technicians may be the key to successful tagging rather than differences in the tagging method. Kazutoshi (1999) reported a 90% tag loss in 13mm CW crabs, but only 10% loss was recorded in 20mm CW crabs, while all tags were retained in juveniles after several moults (Cheng *et al.*, 2001). Jerry *et al.*

(2001) when tagging juvenile freshwater crayfish suggested that tags tend to be retained in the animal during the subsequent moults if successfully retained at the first moult, although some movement of the tags may occur. Gradual movement of the tags out of the body muscle from moult to moult is a possible explanation for tag loss at the third and the fourth moult in a number of crabs of the present study.

Archive tags allowed the identification of free-ranging crabs, providing valuable information on the specific growth rate of known crabs. However, this requires sacrifice of the animals and is time consuming. The present tagging study suggests that tags can be placed close to the surface of muscle under the thoracic sternite. Manipulation of shallow tags in the muscle enables retrieval of the tags with ease and without damaging the animals. Linnane and Mercer (1998) handled microwire tags in the lobster *Homarus gammarus* and also suggested that microwire tags can be placed shallowly in muscle tissue without significant loss or migration allowing them to be removed surgically without harming the animal. The present tagging method also negates the need for replicates that may cause problems due to pond effects (e.g. differences in pond bacteria, algae and water quality). A similar conclusion was also drawn from a tagging study on crayfish where marking and rearing of numerous families collectively in the same environment reduced the number of rearing facilities, and importantly eliminated possible differences in growth or survival due to rearing facility effects (Jerry *et al.*, 2001). Individual tag recognition is expensive and time consuming and so, for large-scale experiments batch releases are recommended, keeping an archive reference tag at the beginning and end of a sequence to identify the batch of crabs. However, the use of new commercially available numeric-coded tags is likely to facilitate the reading process compared with binary-coded ones. Results of the present study also reveal that tagging on different sides of the crabs allows identification of different tagged groups during interim sampling without dissecting the animals.

Although caged crabs were fed daily to satiation, their growth was significantly lower than the free-ranging animals in the pond, whose weight was almost 2½ times greater. This is in agreement with Manjulatha and Babu (1998) who reported that the growth of *Scylla* sp. in ponds is significantly higher than in the laboratory, in spite of good water quality. Furthermore, Ong (1966) suggested that the average growth of *Scylla*

under natural conditions is probably faster than even the fastest observed under laboratory rearing conditions. The possible effects of natural supplementation, food from cannibalism and scavenging on growth of free-ranging animals remain unclear. However, Christensen *et al.* (2001) reported low growth rates in pond-raised *S. paramamosain* and *S. olivacea* relying only on natural food (i.e. without supplemental feeding). Stress has been considered to be a major influence on growth rate (Tort *et al.*, 2001) and the restricted movement and lack of substrate for burying may have resulted in increased stress in the caged animals. An escape response under confined conditions (Courtney *et al.*, 2001) is likely to contribute further stress on crabs. Repetitive additional stress may have also occurred when the animals were lifted up and down daily with the cages during the monitoring periods.

In summary the microwire tagging method has proved a useful tool in marking juvenile mud crabs with high (91.5%) retention rates and without any adverse effect on either growth or survival, in crabs as small as 20 mm CW. Promising results from tagging in terms of growth and survival in *S. paramamosain* will support a further study of mark-recapture to investigate population dynamics of this species in the wild. However, crabs stocked in confined conditions suffered more stresses and consequently grew more slowly than the free-ranging animals, and further studies are required on growth in a range of culture conditions.

CHAPTER V

A MARK-RECAPTURE STUDY OF AN
ESTUARINE POPULATION OF
SCYLLA PARAMAMOSAIN
IN THE MEKONG DELTA

INTRODUCTION

Stock enhancement in marine and coastal fisheries has been attempted in over 25 countries, with success reported for twelve species (New, 2001). Lobsters have been a target species for stock enhancement in the United Kingdom (Bannister *et al*, 1994; Wickins, 1998), Norway (Meeren *et al*., 1998), France (Latrouite, 1998) and Ireland (Browne and Mercer, 1998). A variety of fish species have also been restocked, such as sea bass (*Lates calcarifer*) in Australia (Russel and Rimmer, 1997), commercial mullet (*Mugil cephalus*) in Hawaii (Leber and Arce, 1996), cod in Norway (Svasand *et al*., 2000) and Japanese flounder in Japan (Murai and Koshiishi, 1998). A number of mollusc species including scallop (Aoyama, 1989; Heath, 1999), abalone (Kojima, 1995) and giant clams (Heslinga *et al*., 1984) have also been considered for stock enhancement. Recently, stock enhancement of penaeid shrimp has also been attempted, especially in Asian countries (Liu, 1990; Davenport *et al*., 1999; Ye *et al*., 1999). As yet, no attempts have been made to attempt stock enhancement for mud crabs.

There have been many studies of population dynamics for crabs, often using mark-release-recapture techniques (Hill, 1975; Hill *et al*., 1982; Williams and Hill, 1982; Hyland *et al*., 1984; Robertson and Piper, 1991; Barnes *et al*., 2002). These techniques have also been applied for many other crab species. Kim *et al*. (1986) investigated the migration route of blue crab, *Portunus trituberculatus* in the Yellow Sea off Korea. Fitz and Wiegert (1992) and van Montfrans *et al*. (1991) studied population dynamics of the blue crab, *Callinectes sapidus* in the Chesapeake Bay. Munch-Petersen *et al*. (1982) estimated abundance of the shore crab, *Carcinus maenas* and Gomes (1991) also assessed seasonal migration, population and size at maturity in this species. Similar studies have also been undertaken for many other species including spanner crabs, *Ranina ranina* (Chen and Kennelly, 1999), hair crab, *Erimacrus isenbeckii* (Eiji and Masayoshi, 1999), red crab *Chaceon maritae* (Beyers, 1994), snow crab, *Chionoecetes japonicus* (Ryo and Yasuyuki, 1999), blue crab, *Portunus pelagicus* (Potter *et al*., 1991), red crab, *Geryon maritae* (Melville-Smith, 1988), horseshoe crab, *Tachypleus gigas* (Debnath and Choudhury, 1988), European

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edible crab, *Cancer pagurus* (Bennett and Brown, 1983) and stone crab, *Menippe mercenaria* (Ehrhardt, 1990). In addition, mark-recapture studies have also been reported to be a powerful tool for studying population dynamics and stock assessment of lobsters, *Homarus americanus* (Robichaud and Campbell, 1995), *Homarus gammarus* (Bannister *et al.*, 1994), scyllarid lobster, *Thenus* spp (Courtney *et al.*, 2001) and spiny lobsters, *Panulirus argus* (Sharp, *et al.*, 2000), *Palinurus delagoae* (Groeneveld, 2000).

Generally, mark-recapture studies are carried out by means of tagging using a variety of tags. For example, coded microwire tags were used in population studies of the blue crab, *Callinectes sapidus* (van Montfrans *et al.*, 1991; Fitz and Wiegert, 1992) and spiny lobster, *Panulirus argus* (Sharp *et al.*, 2000). Floy anchor tags were used to study growth in *Portunus pelagicus* (Potter *et al.*, 1991) and population dynamics in *Scylla serrata* (Hill, 1975; Williams and Hill, 1982, Hyland *et al.*, 1984). In most cases, Floy anchor tags have been successfully used to tag *Scylla serrata*. However, Potter *et al.* (1991) reported that the tags had a negative effect on survival of *P. pelagicus* and concluded that they are not suitable for field growth studies of this species. T-bar tags were applied in population studies in *Scylla serrata* (Robertson and Piper, 1991) and in scyllarid lobster, *Thenus* spp. (Courtney *et al.*, 2001). Suture tags have been employed in long term growth and migration studies in *Cancer pagurus* (Bennett and Brown, 1983) and for the first time coloured cable ties were used by Barnes *et al.* (2002) to study abundance in *Scylla serrata*.

In mark-recapture studies, crabs are tagged and released into the sea and finally recaptured. Depending on the objectives of the studies, long term or short term recapture intervals are applied. Long term studies are typically designed to estimate growth (Hill, 1975, Bennett and Brown, 1983), migration (Fujita *et al.*, 1973; Hyland *et al.*, 1984; Eiji and Masayoshi, 1999) and recruitment (Fitz and Wiegert, 1992). In contrast, population estimate or abundance studies are usually undertaken over short term periods (Robertson and Piper, 1991; Barnes *et al.*, 2002).

Tagged crabs may be recaptured by various methods, but catchability may be different for different recapture methods. Pots (Williams and Hill, 1982; Hyland *et al.*, 1984)

and traps (Hill, 1975; Robertson and Piper, 1991) have been used as the recapture means for population estimates of *Scylla* sp. However, they were reported to cause bias in capture probability between crabs due to the effects of animal size, moulting and gear setting. Furthermore, trap responses (i.e. trap-happy or trap-shyness) are also major problems resulting in unreliable catchability (Greenwood, 1996). Using a trawl net for recapture of *C. sapidus* was considered appropriate but was not efficient, especially for smaller size class crabs (Fitz and Wiegert, 1992). Potter *et al.* (1991) comparing three recapture methods, namely pot fishers, recreational fishers and trawl fishers, suggested that recapture rates were highly variable due to the heterogeneity of fishing effort. Heterogeneity of catch probability may introduce inaccuracies into population estimates.

The typical models most often used in crab population estimations include Jolly-Seber (Fitz and Wiegert, 1992), Jackson's capture-recapture method (Debnath and Choudhury (1988), Burnham & Overton and Petersen models (Robertson and Piper, 1991). Validation of the assumptions for different methods is highly recommended before application, to minimize probable biases in the estimates (Seber, 1982; Gulland, 1983; King, 1995 and Greenwood, 1996).

Valuable data obtained from the mark-recapture studies of mud crabs includes information on abundance, age-at-maturity and growth (Hill, 1975; Robertson and Piper, 1991; Barnes *et al.*, 2002); migration (Hyland *et al.*, 1984); distribution (Hill *et al.*, 1982) and population estimates (Williams and Hill, 1982; Robertson and Piper, 1991; Barnes *et al.*, 2002). All these are considered important baseline information for management and protection of fishery resources.

In the present study, coded microwire tags were used in a mark-recapture study of the population dynamics of *S. paramamosain* in an estuarine mangrove system. Hand fishing was employed as the recapture method. The aim of the study was to estimate recruitment, mortality, growth, mobility and abundance of the species, as part of an assessment of the theoretical feasibility of stock enhancement.

MATERIALS AND METHODS

Study area

The study area is described in Chapter 2. It is an island surrounded with more than 1000 ha of mangrove. Fishing activities in this area are controlled by licence, including intertidal hand-fishing in the mangrove by day and fishing on the mud flats at night. For the night fishery, small crabs (20-60 mm CW) are the main target. Fishermen use torches and walk along the mud flat to collect all crabs encountered. The activities are terminated once the tide rises. Small crabs are transported and sold to crab agencies located on the opposite side of the Tran De estuary, as mentioned in Chapter 2.

Marking procedure and monitoring of tag returns

Wild night-caught juveniles were purchased from the agency in Long Phu and transferred to the research station (landing point) in the State Farm. The crabs were sorted into 0.5 cm CW size classes between 2 cm to 6 cm CW. Males and females were separated. Damaged and moribund animals were discarded. All crabs were injected with sequentially coded microwire tags into the coxal muscle of the fourth pereiopod, using a handheld "multishot" injector (Northwest Marine Technology, Inc). Crabs were tagged in batches. Archive reference tags were stored immediately before and after each batch for later identification. Application of batch tagging allowed identification of date, size classes, sex and release point. After injection crabs were checked with a tag detector for successful tagging (see chapter 4 for more details on the tagging method). The tagged animals were not released at once after tagging, but were placed in seawater for a while to encourage clotting of the haemolymph over the insertion point to prevent tag loss.

Crabs were transported by boat and released on the afternoon high tide of the same day. A total of 6114 crabs were tagged in four sessions of 3-4 days each in February to March 2000. The size distribution of crabs released is shown in Fig. 1 and also

represents the size-frequency distribution for juveniles crabs fished at night. The tagged crabs were released at 13 positions around the study area, on the fringe of the mangroves and in creeks through to the seaward fringe (Table 1). The release positions were marked using a hand GPS around the leased study area (Fig. 2).

Fishing activity was continuous during the period of tagging and recapture. Scanning for tag returns was carried out every night at the agency in Long Phu and every day at the research station. The tagged recaptured crabs were measured (carapace width) and sex recorded. All tags were retrieved following the method described in Chapter 4. Fishing gears used and positions where crabs were caught were also noted.

Growth

Tag retrieval allowed batch identification and determination of growth for individual crabs. Growth rate was calculated using only data from crabs recaptured more than 10 days after release in order to eliminate (i) any effects of trauma caused by the tag injection and (ii) overestimation or underestimation of growth rate, as some of crabs might have not moulted prior to recapture and some might have moulted immediately after release. Based on the results obtained from previous experiments, an estimated moult interval for crabs circa 20 mm CW was estimated at 10 days. This agreed with Ong (1966), who indicated that juveniles of *Scylla* sp with CW >20 mm required about 11 days to moult into the next stage. Growth rate was calculated by regression analysis of individual growth (from the initial and final carapace width data) plotted against recapture intervals. Growth rates for different groups such as males, females, juveniles of 2-5 cm CW and total population were calculated similarly, but separately.

Population estimate

The intention was to use the Jolly-Seber method for a population estimate, as it is appropriate for open populations. However, data of total crabs captured in the fishery over the study period were incompletely recorded by the buying agency and therefore it was not possible to complete the calculations. Instead, the Petersen model (known

as the Lincoln Index) was used. The Petersen model assumes that population size (N) is constant during the mark-recapture period (no recruitment, mortality, emigration or immigration) and that the marked animals mix completely with the rest of the population. However, the study area is an island in an open estuary and clearly may fail in some of these assumptions. In order to minimize potential deviation from the assumptions, only release and recovery data from a limited period was used (14-23 February and 24-29 February). The aim was to reduce the potential for population turnover, but also allow sufficient time for the marked crabs to disperse within the population.

Using the Petersen equation, estimation of the population size is calculated as

$$N = \frac{(m+1)(n+1)}{(r+1)} - 1$$ (Equation 1)

Where N: population size estimate

m: number of marked and released crabs

n: total number of individuals caught in the second sample

r: number of recaptured crabs

The standard deviation for N can be calculated as

$$S.E. = \sqrt{\frac{m^2 n(n-r)}{r^3}}$$ (Equation 2)

The 95% confidence interval is calculated as S.E. x 1.96 (Fowler *et al.*, 1998)

Estimation of mortality from tag-return data

As reliable total landings data were not available for a Jolly-Seber analysis, the time series of tag recovery has been used to estimate mortality using the method outlined by Gulland (1983) and King (1995). Total mortality (Z) is calculated as the slope of the regression of the logged decline in tag recoveries over time. If N_0 is the number of crabs initially tagged, then the number of tagged crabs, N_t at time t would be:

$$N_t = N_0 \, e^{-Zt}$$

Thus, $\ln N_t = \ln N_0 - Zt$

This equation is in the form of a straight line, and suggests that a regression of the natural logarithms of the numbers recaptured against time will have a slope which is an estimate of the instantaneous mortality Z; that is Z = -b.

In order to fit the model, all releases were considered as being on a single day. Thus the release and recovery dates for each individual were adjusted and all recoveries noted as days from release, rather than actual dates. The adjustment should not influence the accuracy of the mortality estimate, as it depends on the rate of decline in tag recoveries.

The tag returns were pooled monthly (=30 days) and separated for males and females. In addition, to examine the effect of size on mortality, the data were also divided by initial size classes (< and > 35 mm CW). Ln number of tag recoveries per month was plotted against time for each case and total mortality Z estimated as the slope of the regression fitted to the transformed return data.

Estimation of mortality from age-catch curves

The growth data obtained from the mark-recapture study allows the conversion of CW frequency data collected during the same period into age-catch curves. Total instantaneous mortality (Z) can be estimated as the slope of the regression of the natural logarithm of number of recaptured crabs against time, for the declining size age classes in the population (Robson and Chapman, 1961).

Separation of natural and fishing mortality

The estimates of total mortality (Z) from the tag-return data can be used to estimate fishing mortality (F) and hence natural mortality M. The relationship between tag returns and mortality can be defined in Equation (3) (Gulland, 1983; King, 1995) as:

$$C_{tp} = Zt + \frac{\ln(N_o.F)}{Z} + \ln(1 - e^{-Ztp})$$

(Equation 3)

C_{tp} = Number of tags returned in time period **tp**

N_o = Number of tagged animals initially released.

F = Fishing mortality

tp = t_2-t_1 (in this case using one month = 30 days)

The two right hand terms of Equation (3) are a constant, **a,** which can be estimated as the intercept on the plots of ln tag returns against t-1 (as used in Fig. 10).

$$a = \frac{\ln(N_o.F)}{Z} + \ln(1 - e^{-Ztp})$$

(Equation 4)

From the estimate of **a,** F can be calculated by re-arranging Equation (4)

$$F = \frac{e^a}{(\frac{N_o}{Z} - \frac{N_o.e^{-Ztp}}{Z})}$$

Equation (5)

Once *F* is known, natural mortality *M* and *X* can be estimated from *Z*.

$$Z = F + M + X$$

Equation (6) (Gulland, 1983)

F= fishing mortality

M = natural mortality + emigration

X = mortality caused by tagging.
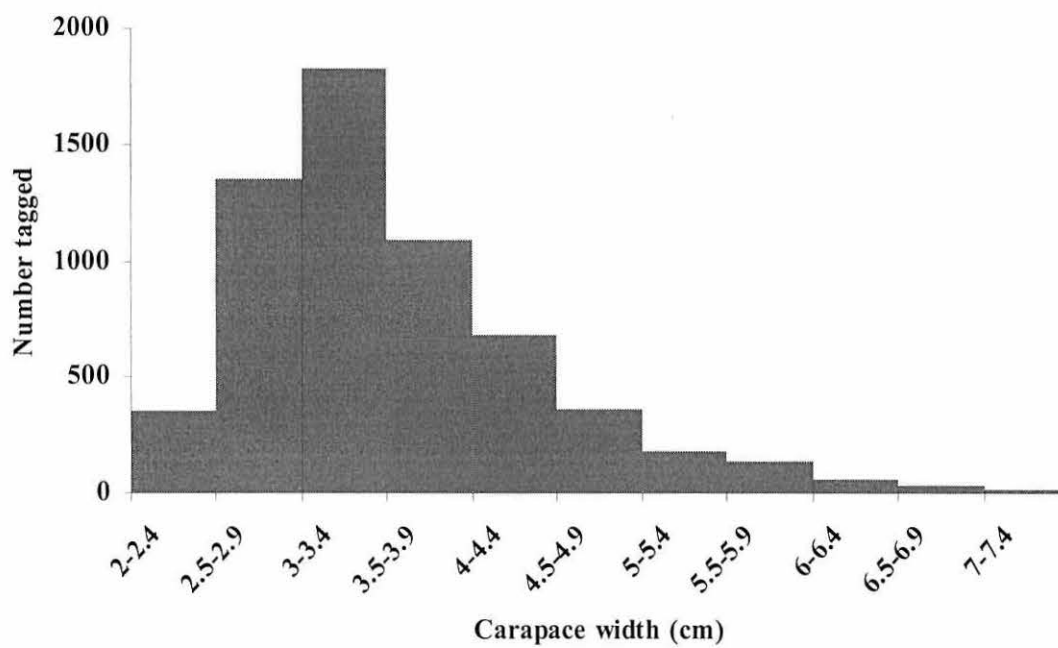
Figure 1: Overall size-distribution of tagged crabs released in February-March 2000
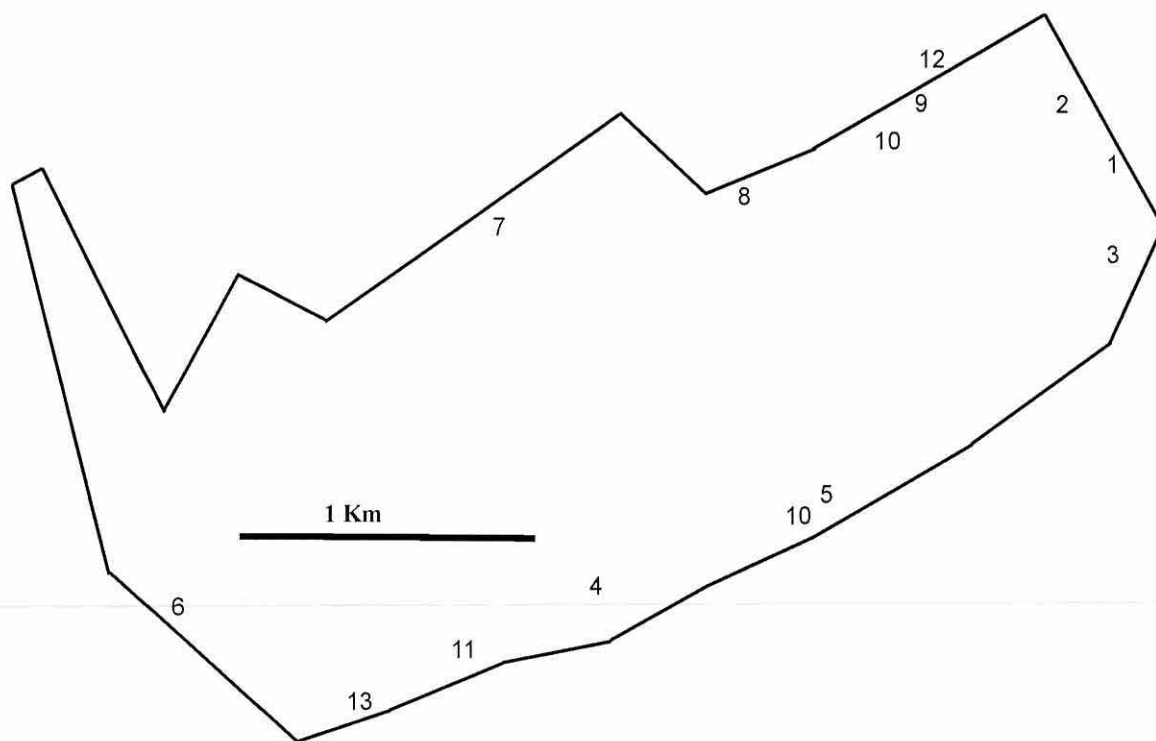
Figure 2: Position of release sites within the leased mangrove area marked with a hand GPS.

RESULTS

Location of tag returns

Monitoring for tag returns was maintained until the end of July 2000. The time interval between mark and recapture varied between 0 to 144 days, with a mean of 26 days. Three crabs were recaptured immediately the night following the afternoon release (recapture interval = 0 day) and seven recaptured in the following night (recapture interval = 1 day). A total of 285 tagged crabs were recaptured, representing 5% of 6114 released animals (allowing for 6.3% tag loss, Chapter 4). From the total crabs recovered for which recapture positions are known, 79% were from the night fishery on the mud flat, 14% from hand fishing at daytime inside the mangrove and 7% from gillnets set at least 1 km offshore from the mangrove edges. This suggests that the majority of crabs remained in the study area. There was an exception; two crabs were recovered from the west shore of the Tran De Estuary (distance from release points 5.8-12.3 km). A summary of release and recapture numbers corresponding to fishing areas is presented in Table 2. From the results of batch identification, it is likely that crabs released at the positions along the fringe and near to the mud flat were recaptured mainly from the night fishery, on the mud flat. Similarly, those introduced inside the mangrove contributed to a higher percentage of the catch in the daytime fishing, in the mangrove. However, a high number of crabs were found intermixed from both areas (i.e. inside and outside mangrove). In addition, 25% of crabs caught in gillnets were originally released inside the mangrove (see Table 2) and all crabs recaptured on the mud flat at 0 and 1 days after release were also from the batch released inside the mangrove. This suggests a mobility of tagged crabs over a large area and may indicate their even dispersal within the study area. The distance moved by the three crabs mentioned above is estimated as 500-1000 m over a time period of 5-10 h.

Growth

A total of 180 tagged crabs recaptured over a time period of 10-144 days were used for calculating the growth rate using regression analysis. The relationship

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Growth (mm CW) = 7.6 + 0.45 D (days=30) (Fig. 3)

has yielded a mean growth of 21.1 ± 0.9 mm CW month^{-1} for all crabs. For juvenile crabs of 2-5 cm CW that were recaptured between 10-30 days, there was linear growth over this short period of time with a rate of 24.1 ± 3.0 mm CW month^{-1} (Fig. 4). An ANOVA general linear model showed there was no significant difference between the growth rate of males and females for both groups, juveniles (20-50 mm CW) recaptured between 10-30 days (F = 0.26, p = 0.608) and all crabs recaptured from 10-144 days after release (F = 2.71, p = 0.102) (Table 3).

Compared to growth of tagged crabs stocked in ponds (experiment 1, Chapter 7), the growth rate of the recaptured animals is significantly lower (26.0 ± 5.7 mm CW month^{-1} compared to 21.1± 0.9 mm CW month^{-1}, respectively) (H=21.9, df=1, p<0.0001). However, in comparison with growth of tagged crabs stocked in ponds in other experiment (20.4 ± 4.2 mm CW month^{-1}) (Chapter 7), growth of recaptured crabs was similar (H=3.33, df=1, p=0.07) (Fig. 5).

Sex ratio and maturation

Of the tagged crabs, 46.7% were males and 53.3% females. However, of 285 recaptured crabs, 161 were males and 124 were females representing a male to female ratio of 1.3 to 1. The results also indicated that number of recaptured adult males was higher than adult females, 23 (60.5%) compared to 15 (39.5%). This may suggest offshore migration of some females. Among the recaptured females, 4.1 % were assessed to be mature, with a size range of 83-105 mm CW (mean 93 ± 10 mm CW). The time taken for females to become mature ranged from 84 to 144 days from release (mean 110 ± 28 days). Characteristics used to identify maturity in females have been indicated in Chapter 2.

Population estimates

A Petersen population estimate for juvenile crabs was calculated for the release period 14th -23rd February and recapture period 24th – 29th February, using the data:

m = 2326

n = 16,567

r = 23

95% confidence limits for the estimate of N are calculated using Equation 2 (Fowler, *et al.*, 1998).

$$N = \frac{(2326+1)(16567+1)}{(23+1)} - 1 = 1,606,405 \quad (95\% \quad \text{confidence} \quad \text{limits} \quad 922,152 - 2,290,657).$$

The rapid growth (24.1 mm CW month^{-1}) of crabs of 20-50 mm (Fig. 6) indicates that during the mark recapture period a population of the juveniles will have grown beyond the size range studied, to be replaced by recruitment of small crabs reaching the size of first capture. The average recapture time for crabs used in the Petersen estimate was 9.2 days. Based on the growth rate obtained, 77, 68% of the size range studied will be replaced by growth over 30 days (see Fig. 1). Hence 23.82% of the population will have been replaced over 9.2 days. Tag dilution through recruitment, therefore will be of the same order. Tag loss has been estimated as 6.3% (see Chapter 4). Following Ricker (1975) r should be adjusted for tag dilution and tag loss during the recapture period:

r = 23 x 23.8 x 6.3% = 34

Therefore:

$$N = \frac{(2326+1)(16567+1)}{(34+1)} - 1 = 1,101,534 \text{ (95\% confidence limits } 417,282 - 1,785,787)$$

The total area of the fishing ground including mangrove fringe and mud flat for night collectors is approximately 2010 ha. Therefore, the density of the crabs is estimated as 0.05 crabs m^{-2} or one crab per 20 m^2.

From the size-frequency data for crabs that were purchased for tagging (Fig. 1), the number of juveniles by size class can be estimated as proportions of N (Fig. 6). This gives values of 176,170; 510,040 and 148,229 for crabs of CW of 20-29 mm; 30-39 mm and 40-44 mm, respectively. Taken together, these size classes represent the standing stock of new recruits into the crab population in the study area. With the estimated growth rate of 24.1 mm CW month^{-1} obtained from the recovery data, these size classes can be expected to be replaced once every month. Consequently, the monthly recruitment to the crab population during February was estimated to be 834,438. Using the CPUE as a relative index of abundance (Fig. 7), monthly recruitment during 2000 can be extrapolated from the abundance estimates for February (Fig. 8). Mean monthly recruitment during year 2000 (January-December) was estimated as 505,614 per month, with the total recruitment over the one year CPUE sampling period of 6,067,370 juveniles.

Estimation of mortality from tag-return data

The decline in tag returns was plotted monthly and used to estimate mortality (Z). Raw data for monthly returns is shown in Fig. 9 and adjusted data after correction to a single release date is illustrated in Fig. 10. Total mortality (Z) of the estimated population based on the naturally logged number of recaptured tagged crabs regressed against time (months = 30 days) is 1.11 month^{-1} (Fig. 10). Using the raw return data, Z is lower (0.84 month^{-1}) compared to when data is adjusted to the single release day (Fig. 9). Mortality for males and females, and for crabs greater and smaller than 35 mm CW was calculated by the same method, with values shown in Table 5.

Estimation of mortality from age-catch curves

Size-frequency data obtained from May and June 2000 were used to calculate mortality, as these were very large samples and covered the same period as the tagging

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study. However, these data sets followed a period of high recruitment in February-March (see Fig. 8). With an estimated growth rate of 24.1 mm CW month $^{-1}$, this peak is likely to have introduced a biased increase in cohorts in the modal size classes 5-7 cm CW in the May and June sampling, and hence an overestimate of mortality (Ricker 1975, Gulland 1984).

For comparison, mortality for all size classes during a period of more constant recruitment (August) was also examined. The total mortality estimate (Z) from age-catch curves for August (Fig. 11) is 1.35 month^{-1} which compares well with the estimate from tag returns. However, the estimate for May- June is much higher at 2.51 month^{-1} (Fig. 12). Periodic episodes of recruitment tend to result in overestimation of Z using the catch curve method, as the slope of the regression is increased by the high abundance of younger age groups (Ricker 1975, Gulland 1984). Similarly, the relatively high Z value for 20-70 mm CW juveniles in February-March (Z = 3.12, Fig. 13) may reflect high recruitment during the sampling period (Feb-March), in addition to higher mortality in juveniles. Total mortality estimated for females was higher than for males in both two periods (May-June and August) possibly indicating greater losses through emigration by breeding age females. A summary of the total mortality for total population, juveniles and males and females in different periods is presented in Table 4.

Separation of fishing and natural mortality

From Equation (6), fishing and natural mortality are separated from the total mortality for all groups (i.e. males and females, greater and smaller 35 mm CW). Tagging mortality in tank and pond studies was reported to be low (see Chapter 4) and therefore X may be considered negligible. However, this may not be the case given the handling time of tagged animals in the field release and the potential for predation on release. Estimates of total mortality (Z), fishing mortality (F) and natural mortality (M) from tag recoveries for females, males and different size classes are shown in Table 5. In overall, the results indicate that the crab population has been subjected to a much higher natural mortality (M) than fishing mortality (F) (0.96 month^{-1} >0.15 month^{-1}, respectively).

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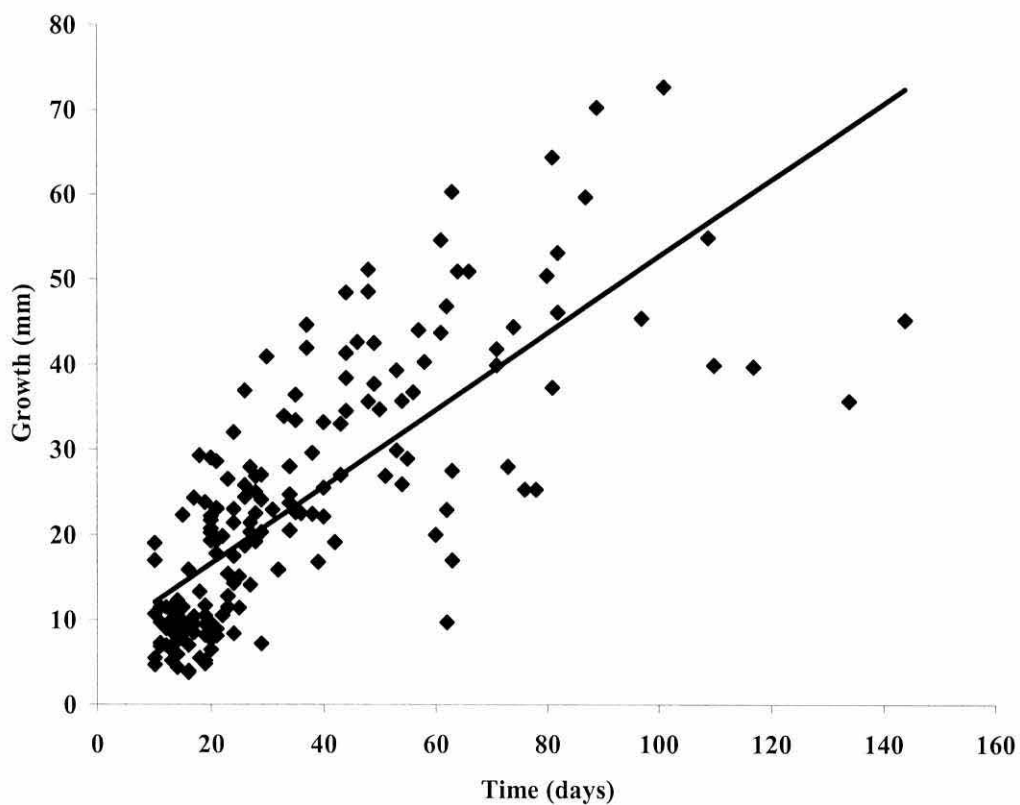
Figure 3: CW increase (mm) against days since release for all crabs recorded between 10-144 days after release. Regression equation, CW increase (mm) = 7.6 + 0.45 (days) r^2 = 58.5% (p < 0.001).
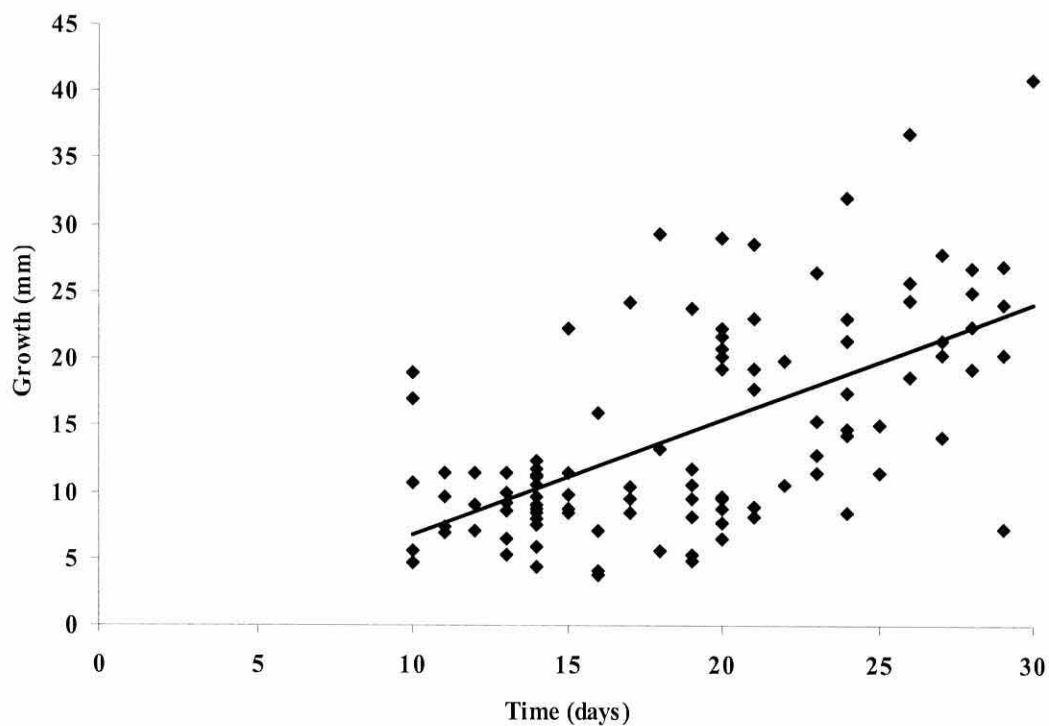
Figure 4: CW increase (mm) against time (days) for crab juveniles 20-50 mm CW that were recovered 10-30 days after release. Regression equation is CW increase (mm) = -1.98 + 0.87 (days). r^2 = 36.9% (p < 0.001).
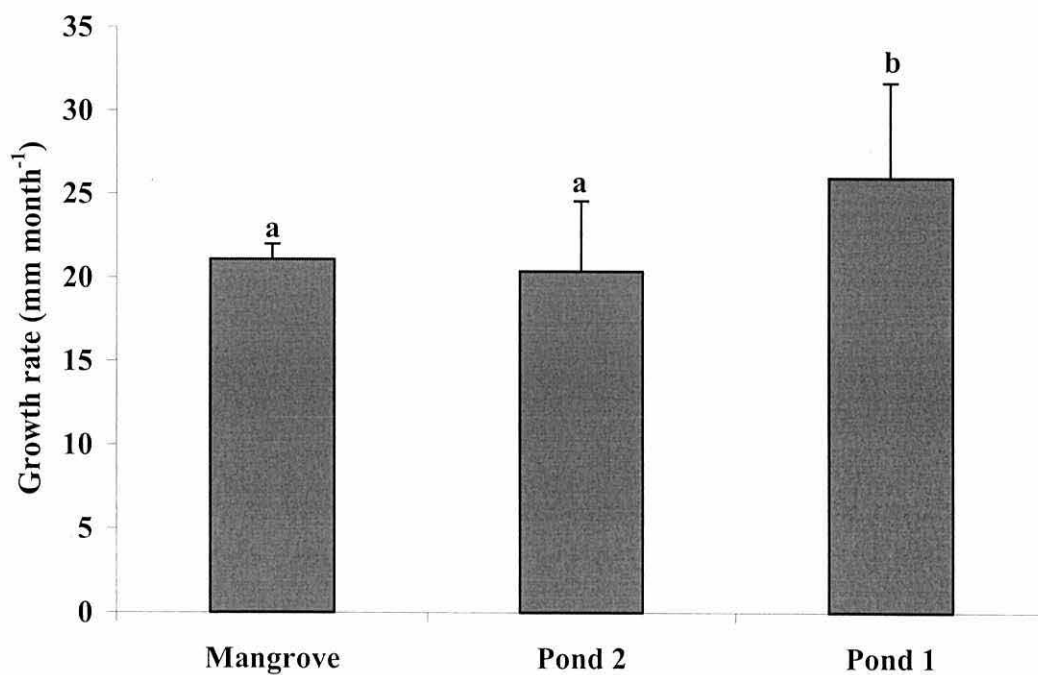
Figure 5: Comparison of growth rates (mm CW month $^{-1}$) for *Scylla paramamosain* under pond conditions and in the wild (data from tagged crabs in ponds presented in Chapter 7). Crabs stocked in pond 2 were caught from Long Phu where growth was studied by mark-recapture method, while crabs stocked in pond 1 were locally from Vinh Chau. Different letters indicate significant difference (Kruskal-Wallis test).
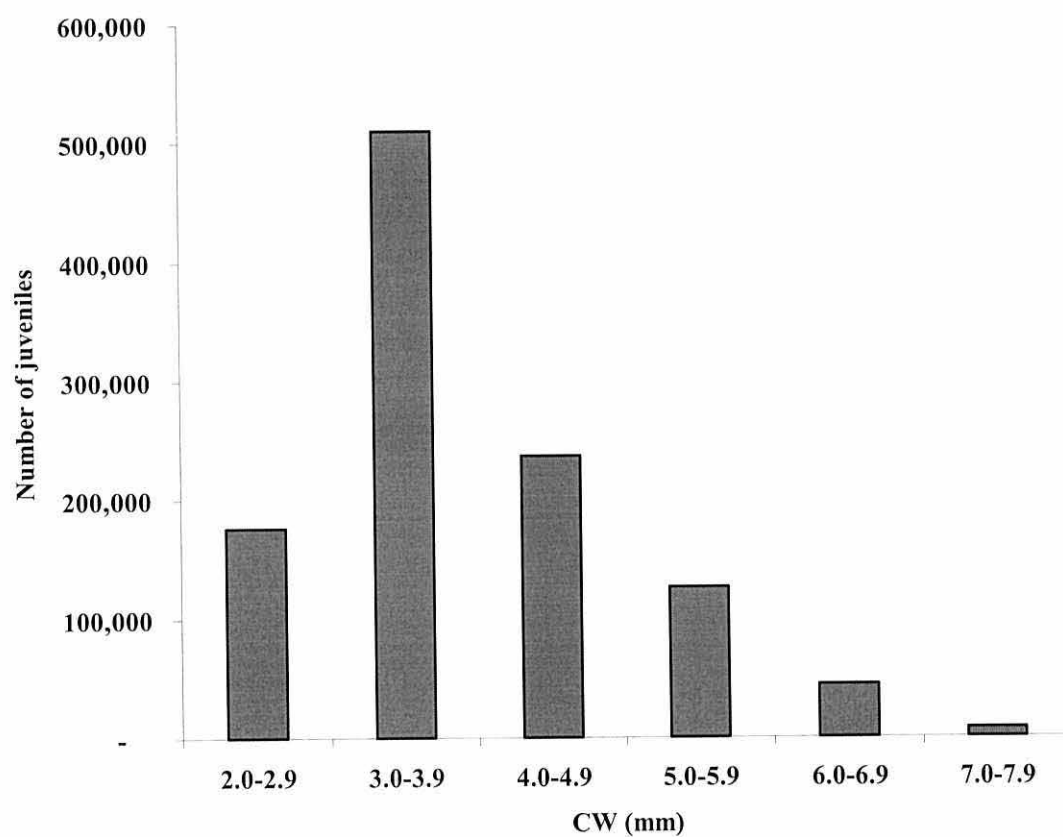
Figure 6: Abundance of 1 cm size classes of juveniles *S. paramamosain* subject to the night time fishery on mangrove fringes and associated mud flats of the study area, February 2000.

Table 1: Sites and released number of crabs during the period of marking in Feb-March.

Release date	Positions	Number tagged/released
14/2	09.30.54 N; 106.15.65 E	
15/2	09.30.64 N; 106.15.54 E	1262
16/2	09.30.46 N; 106.15.64 E	
21/2	09.29.61 N; 106.14.30 E	
22/2	09.30.68 N; 106.15.18 E	1064
23/2	09.29.77 N; 106.13.94 E	
6/3	09.30.40 N; 106.14.11 E	
7/3	09.31.57 N; 106.14.99 E	2164
8/3	09.30.71 N; 106.15.25 E	
9/3	09.30.68 N; 106.15.18 E	
20/3	09.29.65 N; 106.14.45 E	
21/3	09.30.72 N; 106.15.30 E	1624
22/3	09.29.56 N, 106.14.25 E	
Total		**6114**

Table 2: Summary of recoveries by release sites and different fishing areas (i.e. day, night and from gillnets) to show mobility of crabs between sites, especially between inside and outside the mangrove. (*) indicates the sites inside the mangrove.

Release date	Description	Number released	Number recaptured				
			Total	Return (% of released)	Day	Night	Gillnet
14-Feb	Channel close to seaward fringe (1)	356	8	2.2	1	7	0
15-Feb	Inside channel (1)	561	13	2.3	1	12	0
16-Feb	Mouth of channel (1)	345	17	4.9	0	17	0
21-Feb	Seaward fringe	389	28	7.2	2	26	0
22-Feb	Seaward fringe	436	31	7.1	2	27	2
23-Feb	Channel behind mangrove (2)*	239	9	3.8	0	9	0
06-Mar	Inside mangrove *	482	20	4.1	11	4	5
07-Mar	150m inside mangrove from channel (2)*	552	23	4.2	4	19	0
08-Mar	Channel behind mangrove (2)*	506	20	4	6	13	1
09-Mar	150m inside mangrove from seaward fringe*	624	20	3.2	4	16	0
20-Mar	Seaward fringe	497	39	7.8	2	25	12
21-Mar	Channel behind mangrove (3)*	574	16	2.8	0	15	1
22-Mar	Seaward fringe	553	16	2.9	3	10	3

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Table 3: Growth rate of male and female juvenile *S. paramamosain* (20-50 mm CW) recaptured between 10-30 days after release and of all crabs recaptured over a period of 10-144 days after release.

Groups	Growth rate (mm CW month^{-1})	
	Males	**Females**
Juveniles (20-50 mm CW), recaptured between 10-30 days	24.6 ± 4.5	28.1 ± 4.8
All crabs recaptured between 10—144 days	21.1 ± 0.1	20.5 ± 0.9

Table 4: Estimates of total mortality from age-catch curve conversion. All values are monthly (i.e ln N month^{-1}, one month = 30 days).

Group	Z
Pooled males and females in May-June	2.51
Pooled males and females in August	1.35
Males in May-June	2.30
Females in May-June	2.80
Males in August	1.17
Females in August	1.88
Juveniles in February	3.12

Table 5: Estimates of total mortality (Z), fishing mortality (F) and natural mortality + emigration (M) from tag recoveries. All values are monthly (i.e $\ln N$ month^{-1} , one month = 30 days).

Group	Z	F	M
All crabs	1.11	0.15	0.96
Males only	1.15	0.19	0.97
Females only	1.15	0.25	1.15
Initial CW < 35mm	1.06	0.14	0.93
Initial CW > 35 mm	1.40	0.26	1.15

Table 6: Comparison of growth rate recorded in wild and pond/pen conditions for *Scylla paramamosain* and *S. serrata*.

	Wild	Pond/pen culture			Cited
	CW	**CW**	**Weight**		
	Growth rate (CW mm month^{-1})	**Growth rate** (CW mm month^{-1})	**Growth rate** (g month^{-1})	**SGR** (ln g day^{-1})	
S. paramamosain	21.1 ± 0.9	26 ± 5.7	60.8 ± 29.9	5.0 ± 0.7	The present study
S. serrata	15.2 ± 0.6	11.8 ± 0.2			Hill (1975)
			54.5 ± 2.7	1.76 ± 0.04	Triño & Rodriguez (2002)
				1.85 ± 0.01	Triño *et al.* (2001)

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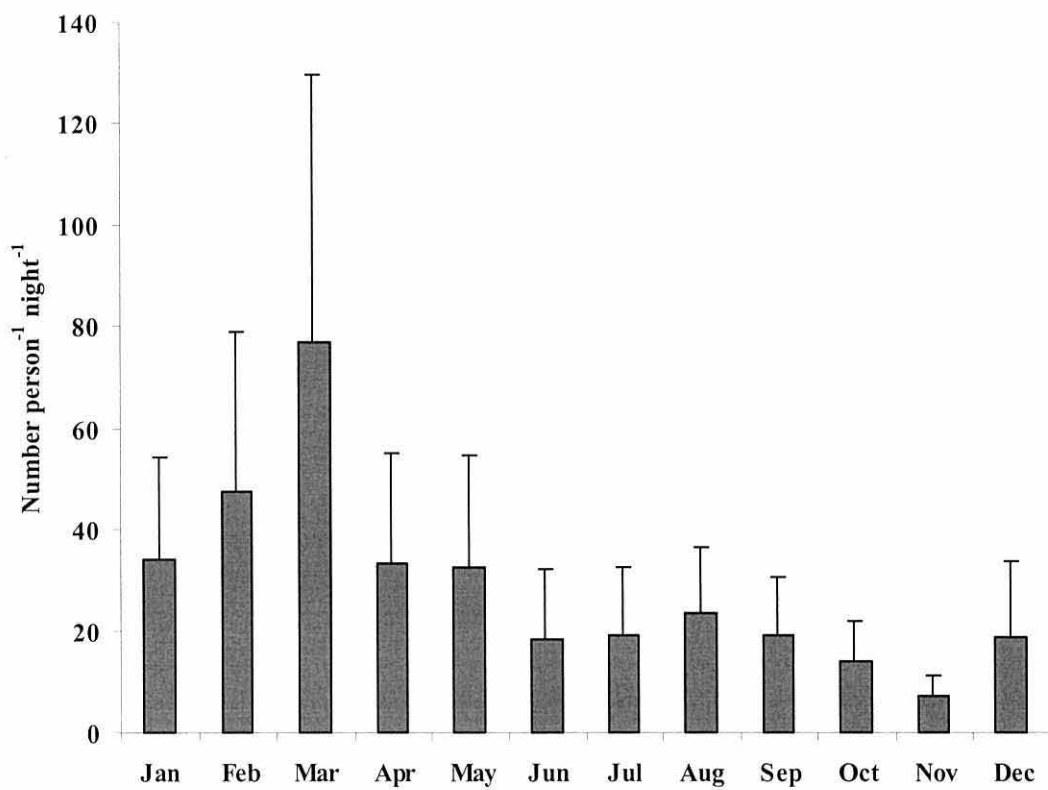
Figure 7: CPUE for crab juveniles, predominantly *S. paramamosain* collected at night on the intertidal mud flat of the mangrove study area during 2000.
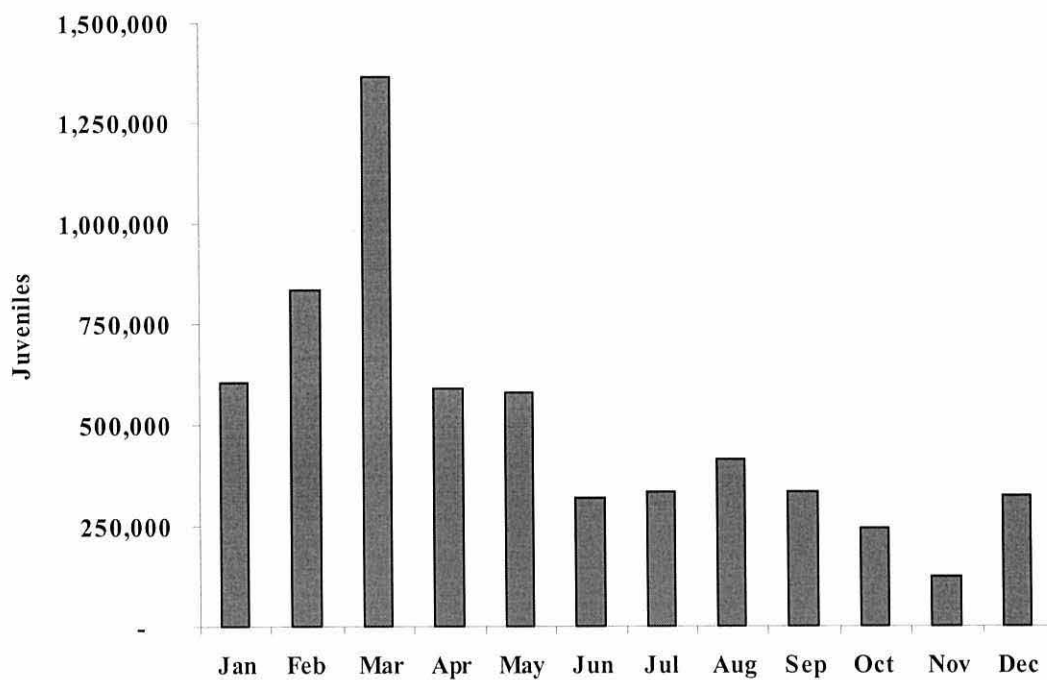
Figure 8: Estimated numbers of recruits (2.0-4.47 cm CW) to the juvenile population subject to the night time fishery on mangrove fringes and associated mud flats of the study area Jan-Dec 2000 (calculated from abundance in February and monthly relative juvenile abundance from the CPUE analysis).
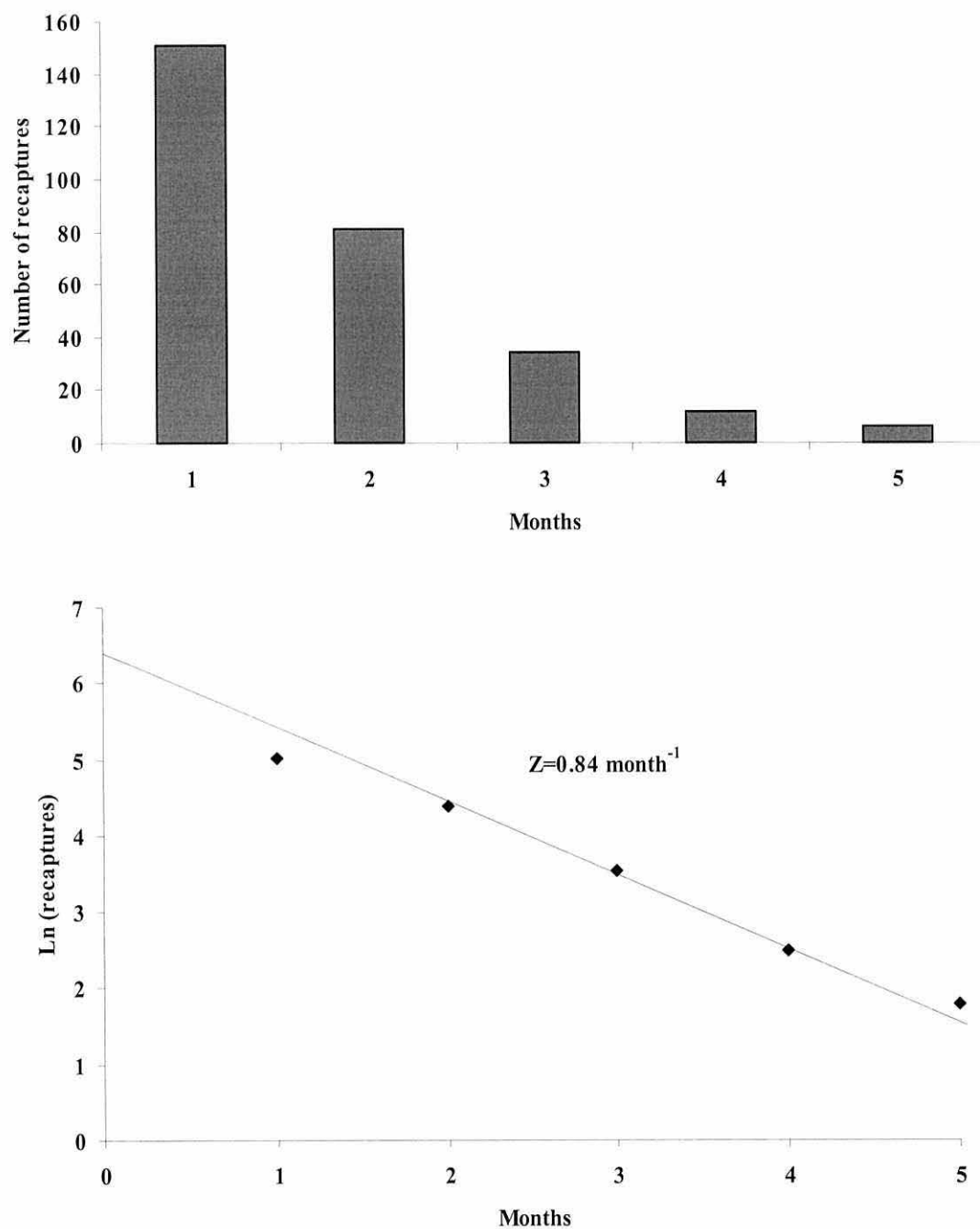
Figure 9: Raw data of pooled monthly tag returns for the hand-fishery for *S. paramamosain* juveniles released into the 30th April State Farm mangrove study area, and estimate of mortality from the logged number of recaptured tagged juvenile *S. paramamosain* (C) versus actual recapture time in months. Regression equation is Ln C = 5.95 − 0.84 months, r^2 = 99.4%.
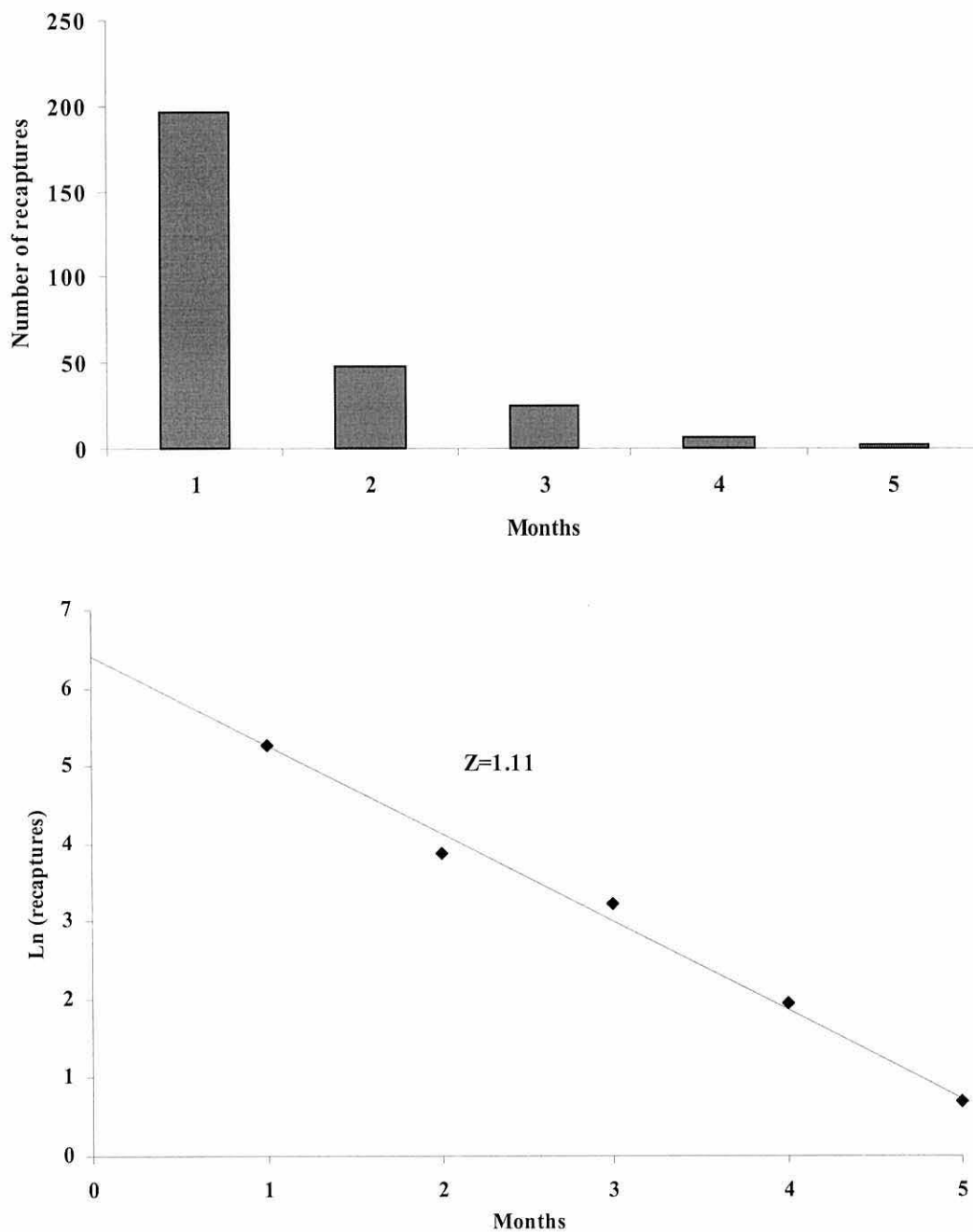
Figure 10: Pooled monthly tag returns of tagged crabs *S. paramamosain* released into the 30th April State farm mangrove study area, and estimate of mortality from the logged number of recaptured tagged juveniles *S. paramamosain* (C) against recapture time in months. Regression equation is Ln C = 6.33 – 1.11 months, r^2 = 99%.
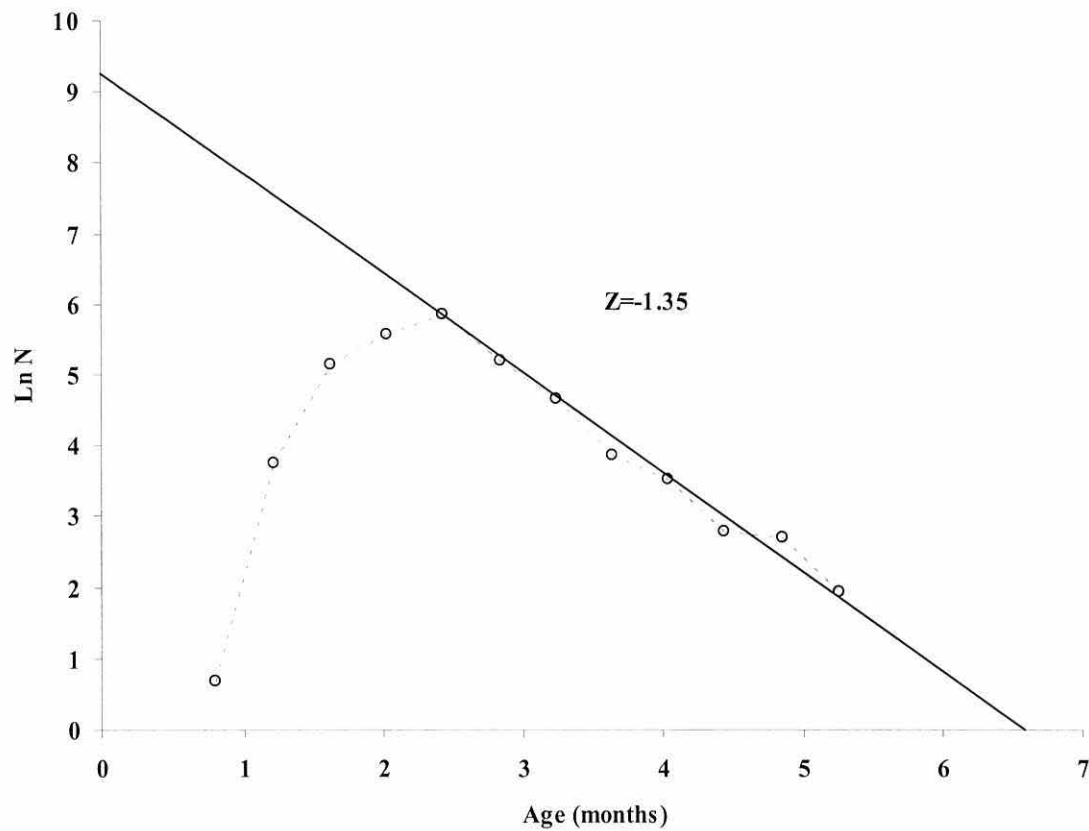
Figure 11: Age converted catch data for August 2000 for the intertidal hand fishery of total population *S. paramamosain* in the 30th April State Farm study site. Mortality (Z, Ln numbers of crabs month[-1]) is estimated as regression slope for the component of the populations indicated. Regression equation is Ln C = 9.20 – 1.35 age (months).
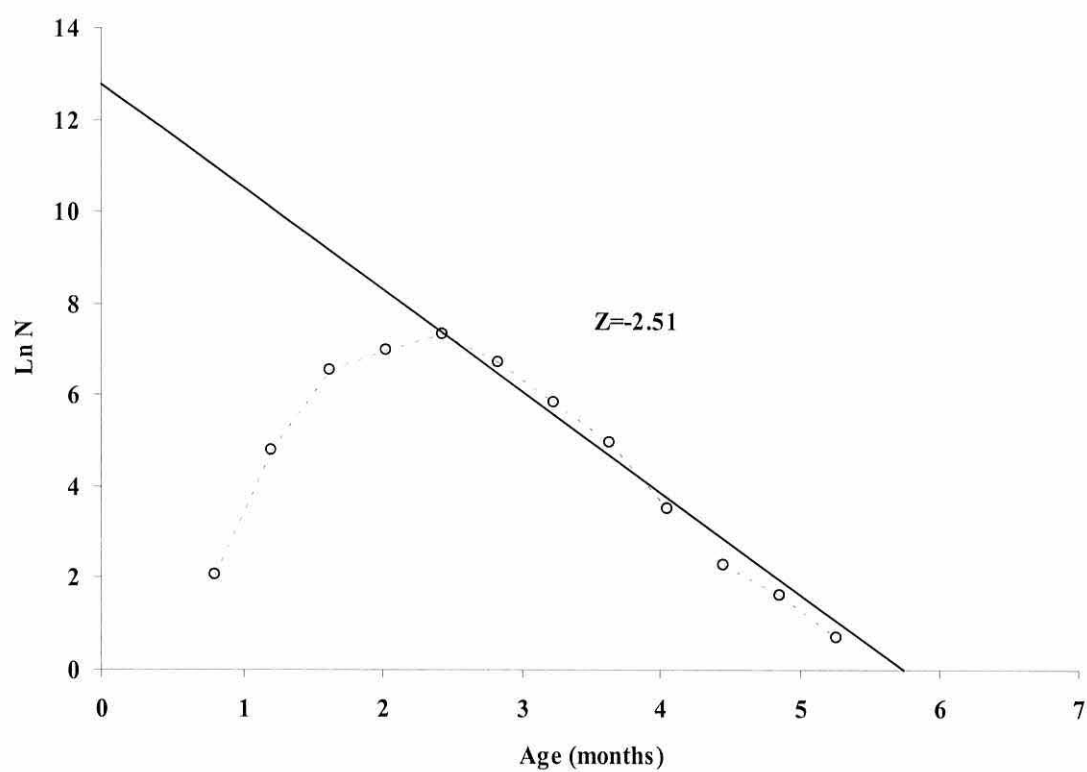
Figure 12: Age converted catch data for May-June 2000 for the intertidal hand fishery of total population *S. paramamosain* in the 30th April State Farm study site. Mortality (Z, Ln numbers of crabs month^{-1}) is estimated as regression slope for the component of the populations indicated.
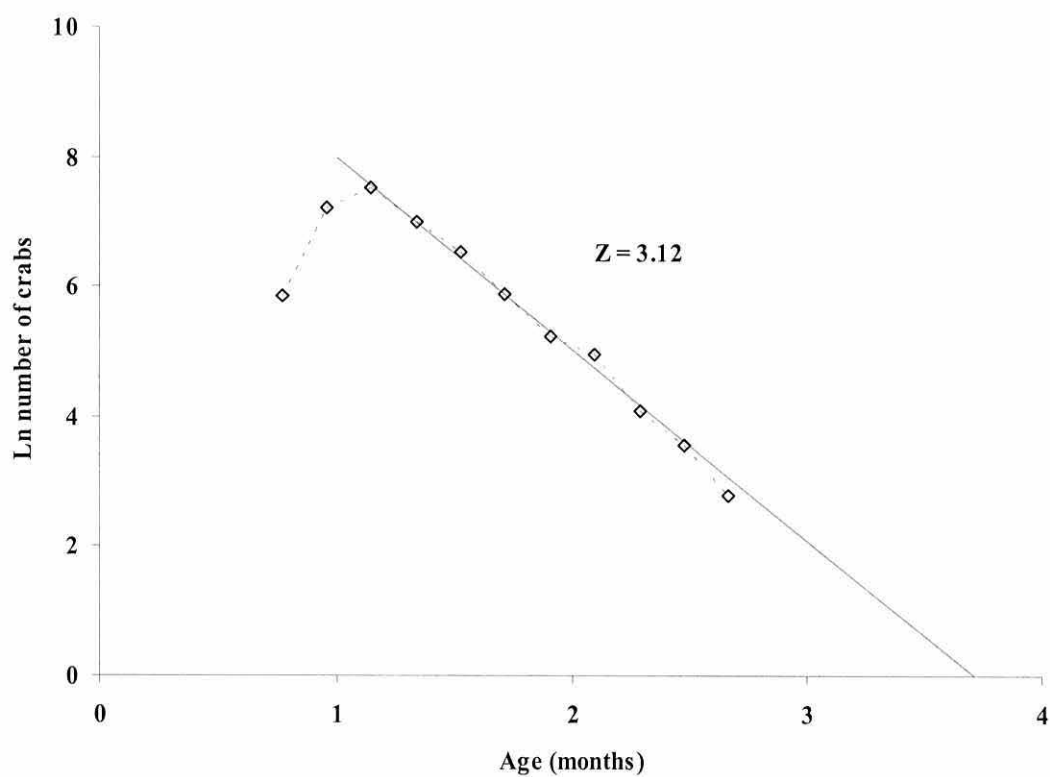
Figure 13: Age converted catch data for juvenile *S. paramamosain* in February-March 2000 subject to intertidal hand fishery in the 30th April State Farm study site. Mortality (Z, Ln numbers of crabs month[-1]) is estimated as regression slope for the component of the populations indicated. Regression equation is Ln C = 11.3 – 3.12 age (months).

DISCUSSION

Growth

The present mark-recapture study has for the first time produced valuable data on natural growth, abundance and mortality of *S. paramamosain*. As indicated by Hartnoll (2001) determination of growth or age in crustaceans under natural conditions has always been a major problem, as there are no persistent skeletal structures to retain a record of the age and growth of an individual. Mark-recapture studies have been accepted as the most effective method for direct determination of growth in crustaceans (Hartnoll, 1982; Chen and Kennelly, 1999; Sharp *et al.*, 2000). In the present study, long term monitoring of crab recoveries of *Scylla paramamosain* that were internally tagged, has enabled determination for the first time of the growth rate of this species under natural conditions. In contrast, studies with short periods before recapture, where growth rate of *Scylla* has not been possible to estimate (Robertson and Piper, 1991; Barnes *et al.*, 2002). Although growth of crabs in the wild was significantly lower than those in ponds, with a rate of 21 mm CW month^{-1}, it is considered a fast growth rate. In addition, this growth was found similarly to that of crabs also stocked in ponds in other experiments. High growth rate of recaptured crabs suggests that foraging time and food availability are not limiting factors on growth in free-ranging animals in the wild. This is encouraging for future potential restocking projects, as it indicates that *S. paramamosain* crabs released into natural habitats at 2-3 cm CW may be expected to reach adult size within only 3-4 months. Growth of males was reported to be faster than that of females for *Scylla serrata* under pond conditions (Manganpa *et al.,* 1987; Triño *et al.*, 1999). However, in the present study similar growth was observed in both *S. paramamosain* males and females. This may reflect the fact that few crabs reached maturity in the present sampling. As indicated by Van Engel (1958) (cited by Ong, 1966) female portunids are known to complete growth at the time they become sexually mature. However, results from Chapter 4 also indicate no significant difference in growth rate between males and females for tagged crabs stocked in ponds. Similarity in growth of males and females crabs has

also been described for other crab species (Donaldson *et al.*, 1981; Wolf & Soto, 1992).

Growth has also been recorded for *Scylla serrata* in the wild and pond/pen conditions (Hill, 1975; Triño *et al.*, 1999; Triño *et al.*, 2001; Triño and Rodriguez, 2002). From Table 6 growth of *S. paramamosain* in the present study was surprisingly higher than that observed in *S. serrata*, both in the ponds and wild. The growth rate in CW (mm CW month^{-1}) was double for *S. paramamosain* to that in *S. serrata*. Growth of *Scylla* is reported to be affected by temperature (Luo and Wei, 1986; Lee, 1992). Low temperature recorded in the Kleinemond estuary in the winter (13-15°C) may result in the low growth rate of *S. serrata* observed during this study period (Hill, 1975). However, despite high temperature (27-32°C) (Triño and Rodriguez, 2002) a similar growth rate for *S. serrata* was obtained (Table 6). This may indicate other factors affect growth than simply temperature.

Sex ratio and size at maturity

The male to female ratio found in the present study is consistent with that already observed in Chapter 2. This finding has reconfirmed that males were more abundant in the *S. paramamosain* population in the study area, and may indicate offshore migration of females, as also indicated in Chapter 2. Results from a mark-recapture study in the crab *Erimacrus isenbeckii* also indicated a higher recapture rate for males (2.13%) than for females (0.35%) (Eiji and Masayoshi, 1999). A similar ratio was also observed in the snow crab, *Chionoecetes japonicus*, with recapture rates of 6% for males and only 1% for females (Ryo and Yasuyuki, 1999). However, the sex ratio in juvenile portunid populations may be slightly different. Fitz and Wiegert (1992) studied the population dynamics of the blue crab (*Callinectes sapidus*) and reported that the sex ratio was close to unity when the population was mostly small juveniles, but became heavily biased towards males when the population structure shifted towards the larger sizes. This might also be the case for *S. paramamosain* in the present study. However, probable errors of distinguishing sex between small males

and females of less than 35 mm CW might also have resulted in the different sex ratio observed for the juvenile population, as a proportion of males might have been recorded as females. Catacutan (2002) stated that the abdominal flap, as sex indicator, of *Scylla* juveniles at about 9 g looks similar for males and females, hence sex is not possible to be determined at this stage.

The characteristics used to determine maturity of females by Ong (1966) coupled with ageing by individual tag retrieval suggest that size at puberty of female *S. paramamosain* is smaller than in the *Scylla* species studied by Ong (1966) and time to reach maturity is shorter, accordingly. Heasman (1980) reported the size at which female *S. serrata* (Queensland, Australia) reach sexual maturity is greater than 80mm CW. In the South Africa *Scylla serrata* population, while size at 50% maturity for females in Natal was estimated to be 123 mm CW (Robertson and Kruger, 1994), in Kowie, Hill (1975) recorded the smallest size of maturity for females as 137 mm CW. Prasad and Neelakantan (1989) found the size at 50% maturity for female *Scylla* sp (species uncertain) was 91-100 mm CW. Similarly, size at 50% maturity for female crabs is 120 mm CW in Sri Lanka (Jayamanna and Jinadasa, 1993) and 70—100 mm CW in Thailand (Tongdee, 2001). A recent study, carried out by Overton and Macintosh (2002), on sexual maturity in *S. paramamosain* and *S. olivacea* revealed that sizes at 50% maturity for female *S. paramamosain* and *S. olivacea* are 110.5 mm CW and 91.2 mm CW, respectively. Maturation of mud crabs is therefore geographically variable (Barnes *et al.*, 2002) and may be species-specific.

Population estimate

The Petersen population estimate relies on the fact that the population is closed and that catchability does not change with time and is the same for all individuals. In addition, it requires that the proportion of tagged individuals in any sample is the same as that in the population studied (Ricker, 1975). Any variable that affects the proportion of recaptured individuals may directly violate the population estimate. Previous tagging studies (Chapter 4) have shown there is no negative effect of tagging

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on the animals, suggesting no difference in mortality of tagged animals in either the long or short term. However, in the present study, there is no guarantee that the tagged crabs were not more susceptible to predation immediately following their re-introduction. However, use of internal coded microwire tags should have no effect on movement or behaviour of the animals. There should not be interference in either swimming or walking ability of the crabs and therefore tagging should not increase their chances of capture relative to the rest of the population. Fitz and Wiegert (1992) have also confirmed that tagging did not affect growth or survival rates of *Callinectes sapidus* in the field. They also indicated that tag presence or absence most likely did not affect the probability of capture.

In the present study, using tag loss data obtained from the previous study (Chapter 4) to compensate for any loss in the field is likely to be appropriate due to the similarity in tagging methodology and animal size. With a non-selective fishing method as already described (see Chapter 2), all individuals are assumed to have the same catchability and capture probabilities may not change during the study period. This is an advantage over using traps, for which unequal probability of capture between individuals may result in biased Petersen estimates (Otis *et al.*, 1978, cited by Robertson and Piper, 1991) when the trapping method may be size-selective; trap catchability is known to increase with size in *Scylla serrata* (Hill, 1975; Williams and Hill, 1982). Furthermore, Robertson and Piper (1991) also pointed out that fixed trap positions and the practice of crabs returning to the water in the vicinity of the trap in which they were caught could result in different catchability among crabs. Such trap responses (trap-happiness or trap-shyness) are known to be major biases in capture probability (Sutherland, 1996). This suggests the advantages of the fishing method used in the present study, in reducing bias in the population estimate. Errors resulting from the incomplete reporting of tag returns are likely to be negligible as every crab caught on the island was scanned for tags at the landing point and the agency.

The short period applied in the population estimate should have allowed sufficient time for tagged crabs to disperse through the population, but also to minimize gain or

loss from the population. Although there was no specific analysis undertaken on movement of crabs in the current study, release and recapture positions have indicated high mobility of crabs within the study area but with most crabs recaptured within its boundaries. The fact that a few crabs were recaptured far from their release points over a short period of time (5-10 hours) indicates high mobility. This mobility suggests the likelihood of rapid random distribution of tags over the population. Similar substantial movement in *Scylla serrata* was observed in areas with large intertidal flats bare of mangrove (Hyland *et al.*, 1984). The maximum recorded distance of movement (5.8-12.3 km) obtained from two recaptured crabs during 20 weeks in the present study is consistent with the observation of Hyland *et al.*, (1984), who reported that there were only 2 out of 73 recaptures more than 10 km from their release site.

A lowest density estimated by Robertson and Piper (1991) was one crab per 355-357 m^2 and one crab per 124-5000 m^2 (by Hill, 1975 & 1982) for *Scylla serrata* in South Africa and Australia, respectively. However, in all their cases traps were used as the fishing method and the size targets were larger, 40->150 mm CW and fished subtidally. In the present study, the size class studies were of 30-35 mm CW and sampled in the intertidal area, which may explain higher densities of juveniles observed. With an estimated density of 0.05 crabs m^{-2} or 1 crab per 20m^2 coupled with high monthly recruitment, this suggests that the study area is a healthy mangrove system with high abundance of associated fauna.

Mortality

The estimate of instantaneous mortality (Z) from the raw data of tag return was slightly lower than that of adjusted data to a single day. This may be a result of lower number of tag returns obtained during the first fortnight. However, the reason remains unknown. Monthly pooled data of tag recoveries yielded an instantaneous mortality of 1.11 month^{-1} which is close to the estimate of 1.35 month^{-1} from the age converted catch curve in August. There was no difference in total mortality between males and

females in the present study. However, separation into fishing and natural mortality shows that females are subjected to a higher natural mortality. This may suggest losses through spawning emigration of mature females. Similarly, Melville-Smith (1988) also found red crab *Geryon maritae* females were subjected to higher mortality (0.41 year^{-1}) than males (0.24 year^{-1}). However, mortality estimated on an annual basis for *S. paramamosain* in the present study was 30 times higher than that of the red crabs or blue crabs. This may be a consequence of a shorter life span in the tropical species than temperate species, with additional loss through emigration also possible. Hill (1982) suggested that sub-adult *Scylla serrata* were more abundant in subtidal areas and were only found in the intertidal zone during high tide. Fitz and Wiegert (1992) argued that small juvenile crabs moult more frequently than their larger counterparts and are more vulnerable to predation, and therefore mortality may decrease with size to some extent. However, the authors also drew the conclusion that emigration of adult males and females may increase their apparent mortality in the study area.

From the separation of components of total mortality, it is clear that fishing pressure is relatively low. This indicates that over-fishing of juveniles does not occur in the study area and that the crab population is not limited by fishing pressure. A fishing mortality $F < 0.32$ year^{-1} for the population of blue crab (*Callinectes sapidus*) in Chesapeake Bay was considered a moderate level of exploitation (Miller, 2001). However, fishing and natural mortality act on a population simultaneously (Melville-Smith, 1988) and sustainability of fisheries is dependent upon the balance between the two factors (Miller, 2001). With the model used to predict level of exploitation for *Callinectes sapidus* population, Miller (2001) indicated that if a natural mortality approximates to 0.4 year^{-1}, exploitation rates should be reduced so that they do not exceed 0.3. Following this calculation, fishing in the present study area should be undertaken with caution to maintain a stable population. Natural mortality includes both mortality due to disease and predation and emigration by larger individuals out of the fishery area. Hill *et al.* (1982), Hyland *et al.* (1984) indicate that *Scylla serrata* are subtidal species with sub-adults and adults migrating into the intertidal zone at high tide to feed. It is

likely that the same behaviour is seen in *S. paramamosain*. The Z values generated from tag recoveries are likely to be underestimates, due to known loss of tags in marked crabs (see Chapter 4) and possible mortality immediately post-release. The first factor can be corrected for, but the second is unknown.

Recruitment

Recruitment of mud crabs has been well documented in the literature (Hill, 1979; Robertson, 1987; Forbes and Hay, 1988; Robertson and Kruger, 1994; Chandrasekaran and Natarajan, 1994; Tongdee, 2001). However, these estimations of recruitment were based on the abundance observed in the population. In the present study, for the first time tag return data were combined with CPUE data (Chapter 2) to estimate recruitment of the mud crabs. These reliable data have reflected a peak recruitment of *S. paramamosain* juveniles occurring during the dry season coinciding with the high salinity period.

In summary, the mark-recapture study in the mangrove system at the 30th April State Farm has for the first time enabled estimation of the growth rate of *S. paramamosain* under natural conditions. This growth rate proved to be as fast as that of the animals reared in ponds and indicates that a restocking enhancement program might be feasible. However, abundance and recruitment in the study area indicate a healthy crab population, with high levels of natural recruitment throughout the year. Consequently, the area is not considered a suitable site for further stock enhancement trial. The fairly low fishing mortality suggests that exploitation of the stock is not at critical levels but caution is still needed and monitoring of future changes in population due to habitat loss and increased fishing effort. The study is a useful baseline, as the east coast of the Lower Mekong is undergoing substantial changes in mangrove management and the data generated in this study will allow effective monitoring of long-term effects on fisheries.

CHAPTER VI

EFFECTS OF SUBSTRATES/SHELTERS AND DIETS ON GROWTH AND SURVIVAL OF JUVENILE *SCYLLA PARAMAMOSAIN* DURING NURSERY PHASES

INTRODUCTION

The strong cannibalistic behaviour of juvenile crabs may lead to low survival during post-settlement in the wild and in the nursery phase of aquaculture of many species. In natural crab populations at higher densities, conspecific encounter rates and cannibalism are significant causes of mortality (Perkins-Visser *et al.*, 1996). Moksnes *et al.* (1998) found that juvenile shore crabs (*Carcinus* sp.) were extremely efficient predators on small conspecifics. Cannibalism has been widely reported in a number of crabs species including blue crab, *Callinectes sapidus* (Perkins-Visser *et al.*, 1996; Moksness *et al.*, 1997), snow crab, *Chionoecetes opilio* (Lovrich and Sainte-Marie, 1997; Dutil *et al.*, 1997); shore crab, *Carcinus maenas* (Moksnes *et al.*, 1998) and the grapsid crabs, *Hemigrapsus penicillatus* (Kurihara and Okamoto, 1987), *Chasmagnathus granulate* and *Cyrtograpsus angulatus* (Luppi *et al.*, 2001). Strong cannibalism is also found in crayfish, *Procambarus clarkia* (Figler *et al.*, 1999), lobster, *Homarus americanus* (Richards and Cobb, 1986), spiny lobster, *Panulirus argus* (Lipcius and Herrnkind, 1982) and rock lobster, *Jasus edwardsii* (Thomas *et al.*, 2003). The intensity of cannibalism in crustaceans can be affected by several factors including availability of a refuge/shelter (Richards and Cobb, 1986; Kurihara and Okamoto, 1987; Moksnes *et al.*, 1998; Luppi *et al.*, 2001), substrate/shelter preference (Pottle and Elner, 1982; Day and Lawton, 1988), predator size (Figler *et al.*, 1999), sex (Figler *et al.*,. 1995), prey size and prey availability (Dutil *et al.*, 1997; Thomas *et al.*, 2003), predator density (Perkins-Visser, 1996; Moksnes *et al.*, 1997; Lovrich and Sainte-Marie, 1997), light and photoperiod (Gardner and Maguire, 1998) or moulting status (Lipcius and Herrnkind, 1982).

However, the availability of a refuge/shelter has been considered the most crucial factor affecting cannibalism in crabs (Moksnes *et al.*, 1998; Luppi *et al.*, 2001; Catacutan, 2002). Structured habitats, such as seagrass, blue mussels, and filamentous algae provide significant shelter from predation and cannibalism in crabs in the wild (Heck and Thoman, 1981; Perkins-Visser *et al.*, 1996; Moksnes *et al.*, 1998). Triño *et al.*, (1999) used seaweeds (such as *Gracilariopsis*) and bamboo as shelters for the mud crab, *Scylla serrata*, in grow-out ponds. Catacutan (2002) has also noted that addition of substrate may prevent cannibalism in *S. serrata* during rearing in the laboratory. Providing physical substrates/shelters, adequate feeding, and reducing stocking density in nursery systems are all factors which could mitigate

the high level of cannibalism that is typical of the post-larval culture of mud crabs.

In the present study, a series of experiments was designed to investigate the effects of stocking densities, provision of sand substrate, 3-dimensional shelters, and feeding regimes on the survival of juvenile *Scylla paramamosain* during the nursery stage. The objective was to reduce cannibalism in small crabs and to enhance their survival throughout the nursery period.

MATERIALS AND METHODS

Experiment 1

The aim of this experiment was to determine suitable densities for crabs stocked during the nursery phase using sand as substrate. Hatchery-reared crabs, carapace width 4.4 ± 1.1 mm, were placed in twelve flat bottomed 15 l PVC tanks. All tanks were connected to a recirculation system. The tank bottoms were covered with a layer of sand 2 cm deep. Three different stocking densities (110, 175 and 230 crabs m^{-2}) were tested. Crabs were fed to excess with *Artemia* biomass until day 3 and supplemented with chopped peeled shrimp. Survival and growth (CW and weight) were evaluated at the end of the experiment (15 days).

Experiment 2

In the second experiment the use of clay bricks was compared with the sand substrate. The bricks were 17cm long and 7cm wide and each had four circular holes with a diameter of 2.5cm that ran the length of the brick. The experiment was set up in $4m^2$ cement tanks connected to a bio-recirculation system (Fig. 1). Three replicates per treatment were used. The initial size of the crabs was 3.7 ± 0.1 mm CW and crabs were stocked at 110 crabs m^{-2}. In the sand substrate tanks a layer of 2 cm sand was spread over the complete tank bottom, while in the other tanks a number of bricks was placed at fixed distances. Crabs were fed *ad libidum* with peeled shrimp.

Experiment 3

Two tests were simultaneously carried out using the same batch of crabs. First, the brick and sand substrate comparison was repeated. Crabs were fed peeled shrimp. Sand and brick were set up as in the previous experiment. In the second test different nursing diets were used (fish flesh, peeled shrimp and the mixture of the two), using only the brick refuge. The length of the bricks used was reduced to 8cm (half length of the ones used in the Experiment 2). Each treatment had 3 replicates and was set up in the same system used for Experiment 2. 100 bricks were spread on the bottom of each tank (Fig. 2). 400 crabs were stocked in each tank

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with sizes of 3.65 ± 0.12 mm CW. In all cases crabs were fed once a day in the afternoon. The experiments were set up indoors.

An identical set of treatments was simultaneously set up, but in outdoor tanks, without roof covering. The aim was to detect if there is any difference on growth or survival of crabs between the 2 systems.

Survival and growth (CW and weight) were calculated and measured at the end of experiments and statistically analysed (one-way ANOVA).

Figure 1: Tank recirculation system used for testing effect of shelter/substrate including sand vs bricks in Experiment 2.
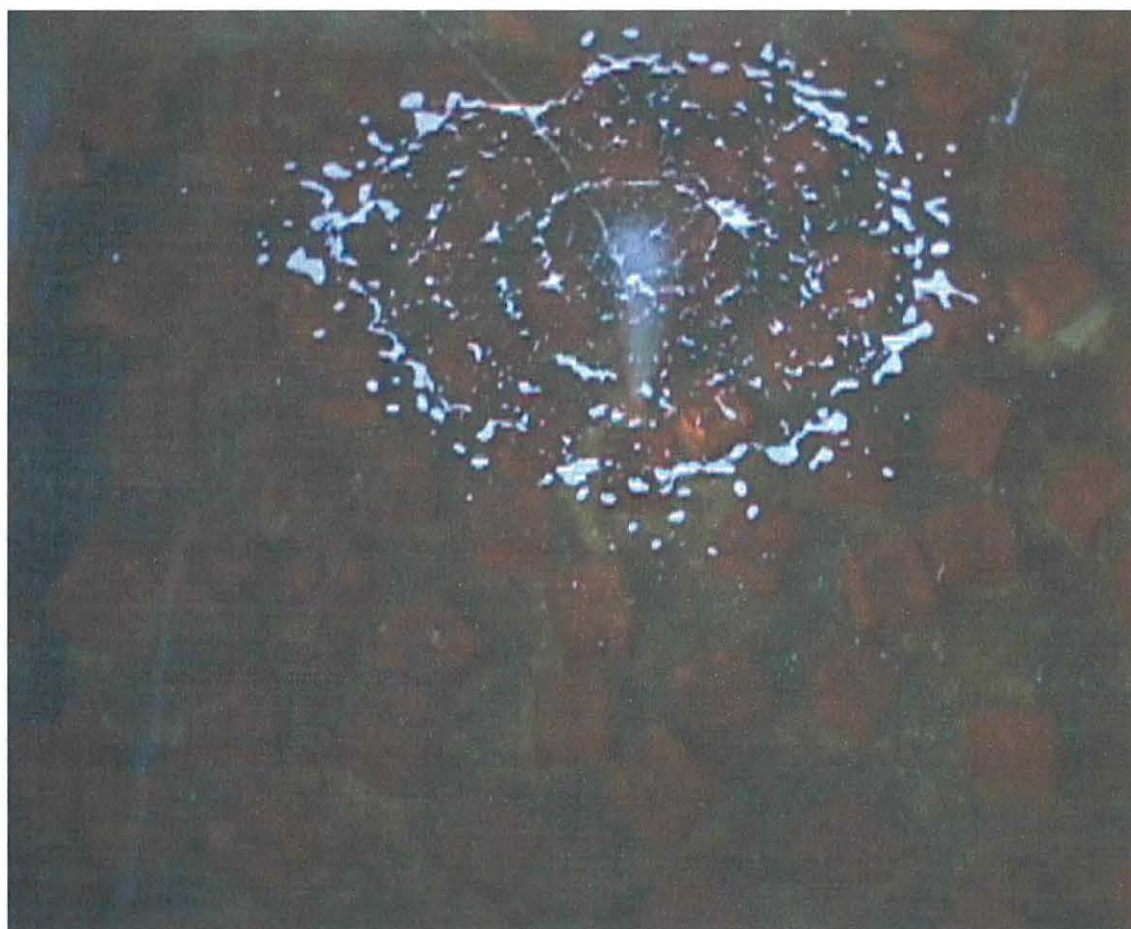
Figure 2: Bricks (8 cm long) were spread over the tank bottom with a number of 100 equivalents to 400 holes as shelters for 400 crabs in Experiment 3.

RESULTS

Experiment 1: Stocking densities

After 15 days of stocking, survival of crabs stocked at low density was higher compared to crabs stocked at higher densities (71.3, 61.7 and 57.5 %, respectively) although no significant difference between treatments could be found (Fig. 3). This indicates cannibalism may be stronger with higher densities. There was also no significant difference on growth of crabs at different densities (Fig. 4).

Experiment 2: Shelters/substrates

The experiment lasted for 17 days. A significant difference ($P < 0.05$) in survival was found between sand and brick treatments. Survival was significantly higher in tanks having bricks than sand as shelter ($25.3 \pm 2.7\%$ compared to $13.5 \pm 2.3\%$, respectively) (Fig. 5). However, survival of crabs in both treatments was significantly lower (13.5 and 25.3% for sand and brick, respectively) compared to that of the previous experiment. There was no significant difference in growth between the treatments for both CW and weight (Fig. 6).

Experiment 3: Shelters/substrates and diets

Environmental parameters recorded showed that temperature and salinity were fairly constant during the period of the experiment in the indoor system. However, due to rainfall, salinity in the outdoor system dropped sometimes. Temperature in this system was also higher than in the indoor system, especially in the afternoon (Fig. 7).

The results indicated a highly significant difference in survival ($p < 0.001$) between brick and sand substrate (Fig. 8). Crabs stocked in brick had higher survival than those in sand. However, growth of crabs stocked in brick was significantly lower ($p < 0.001$) than those in the sand substrate.

A highly significant difference was also found in survival ($p < 0.001$) between different diets,

with best results using shrimp and worst using fish. The mixed diet supported intermediate survival. Crabs fed shrimp exhibited lower growth in terms of weight gain than those fed either the fish or the mixed diet. Crabs fed peeled shrimp had a significant lower growth rate ($p < 0.05$) in terms of weight than other treatments, but not in terms of CW (Fig. 9).

Results of proximate analysis in the fish and shrimp flesh used during the experiments show that fish contained a higher percentage of lipid than shrimp meat (Table 1), while protein contents are not different between the two feeds.

Comparison of growth between indoor and outdoor tanks showed a significant difference ($p < 0.05$) in weight in treatments with brick refuges fed the shrimp diet. Crabs in the outdoor system had a significant higher growth in weight than those indoors. However, there was no significant difference ($p > 0.05$) in growth of crabs in the sand substrate between the two systems (Fig. 10). There was no significant difference in CW and weight between the other treatments. Survival of crabs stocked indoors was slightly lower than that in outdoors, though not significantly (Fig. 11).

Table 1: Proximate analysis of fish and shrimp meat used as daily diet for crab juveniles, *S. paramamosain* during the nursery experiments.

Components (%)	Fish flesh	Shrimp flesh
Protein	66.4	69.5
Lipid	18.2	3.3
Ash	3.6	5.5
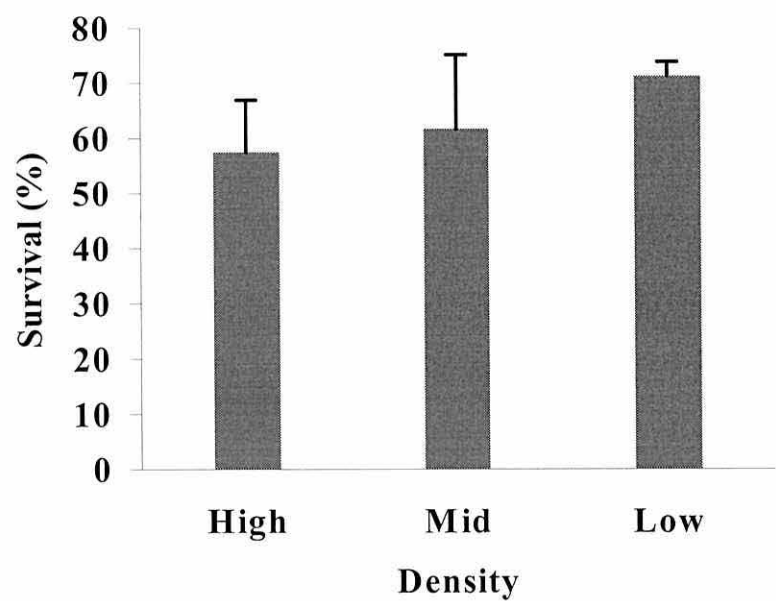
Figure 3: Experiment 1: Survival of crabs after 15 days at three stocking densities, high (230m^{-2}), mid (175m^{-2}) and low (110m^{-2}) on a sand substrate. No significant difference between treatments (p>0.05).
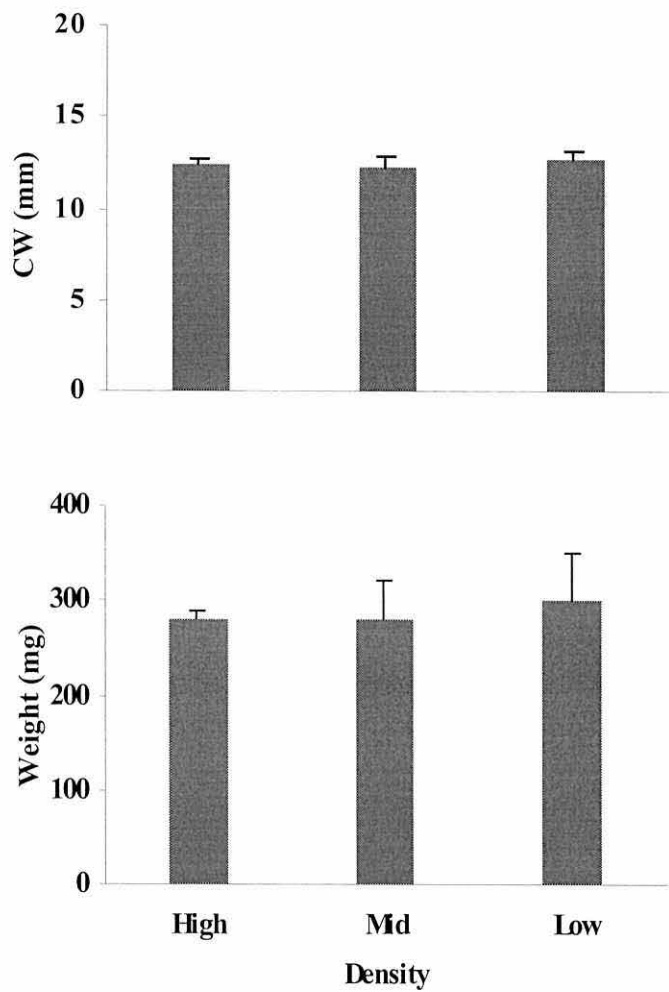
Figure 4: Experiment 1: Growth (CW and weight) of crabs after 15 days at three stocking densities, high (230m^{-2}), mid (175m^{-2}) and low (110m^{-2}) on a sand substrate. No significant difference between treatments (p>0.05).
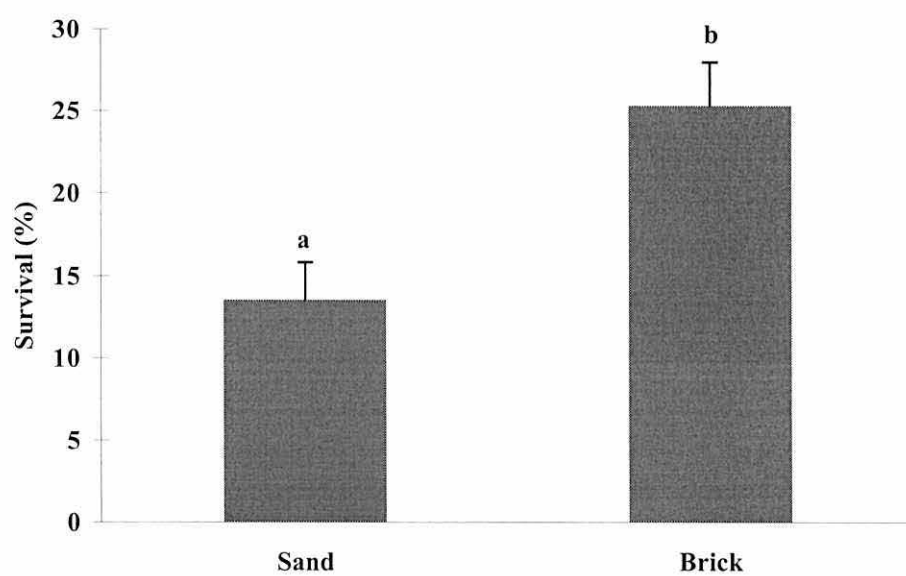
Figure 5: Experiment 2: Survival of crabs stocked on sand and brick. Significantly higher survival with brick than with sand (p<0.05).
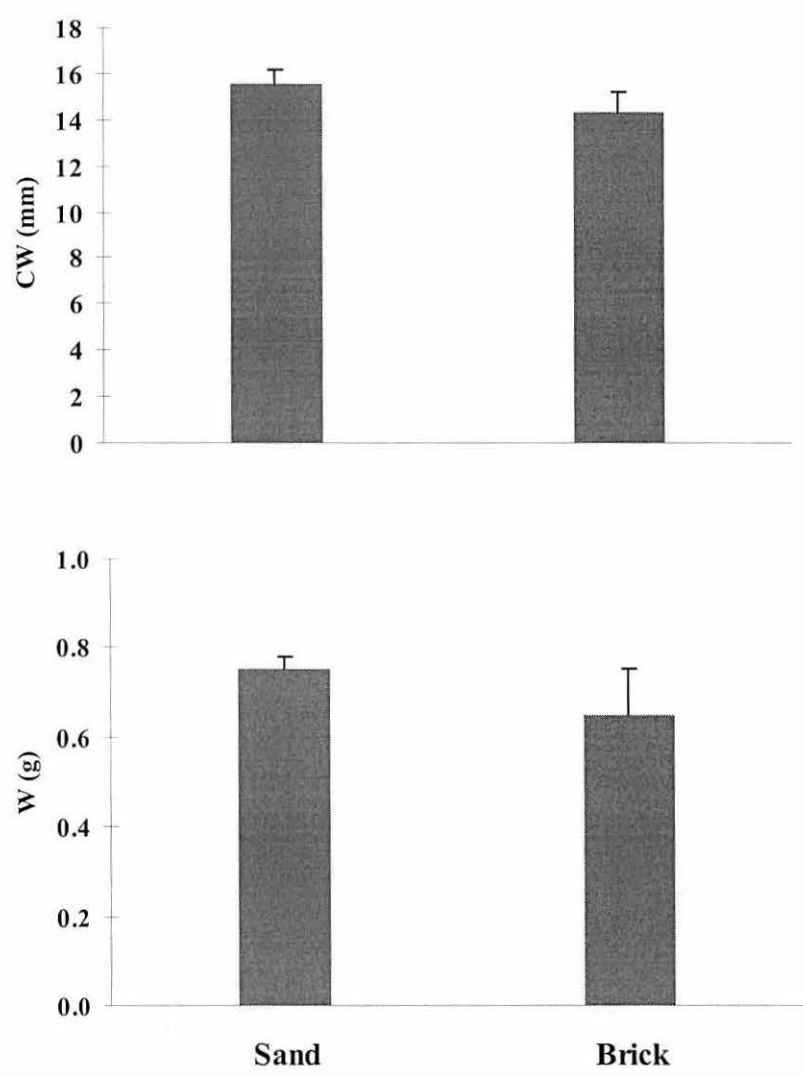
Figure 6: Experiment 2: Growth (CW and weight) of crabs stocked in sand and brick. No significant difference between treatments (p>0.05).
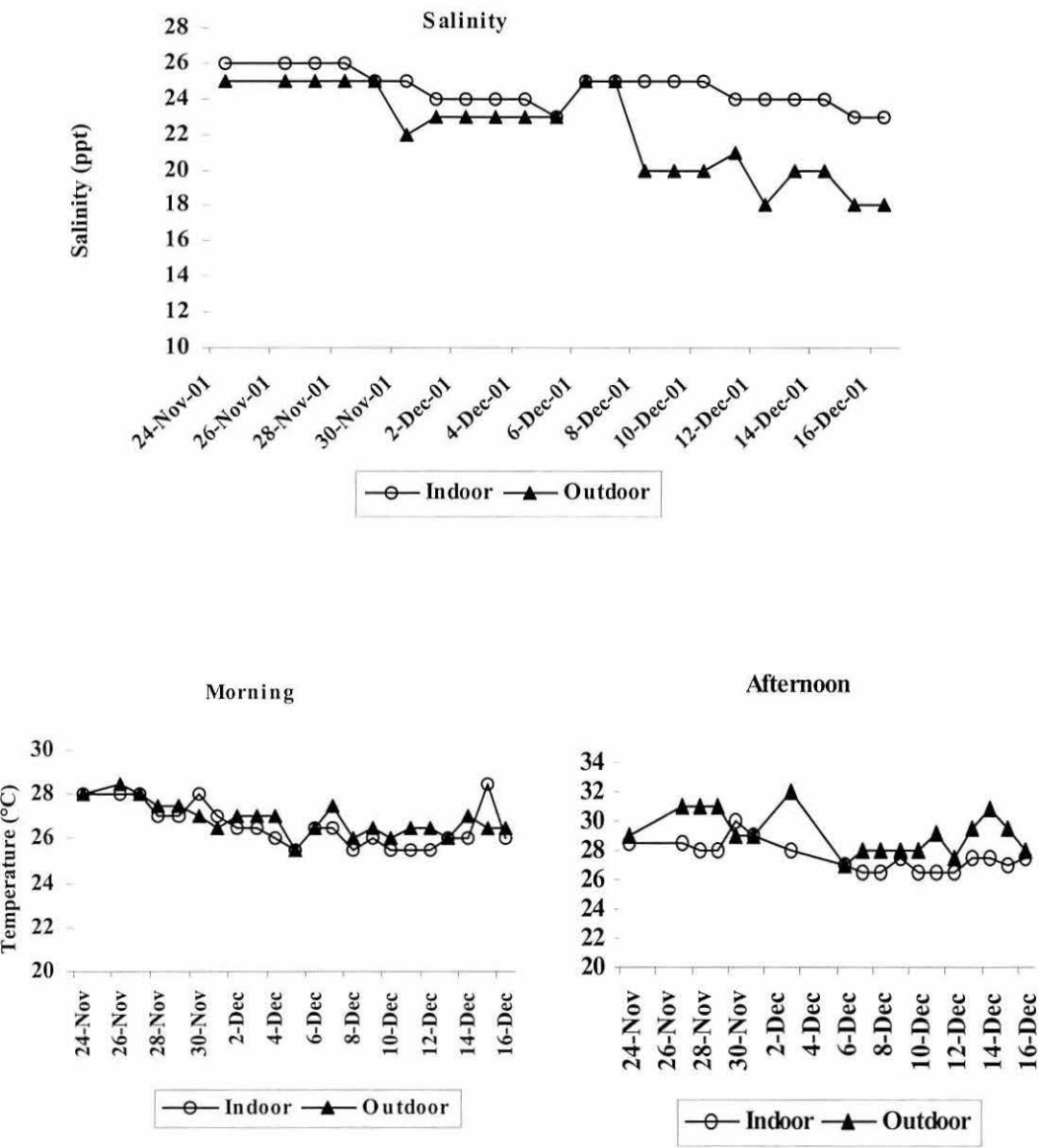
Figure 7: Experiment 3: Salinity and temperature (for morning and afternoon) recorded in the crab systems (indoor and outdoor) during the nursery period.
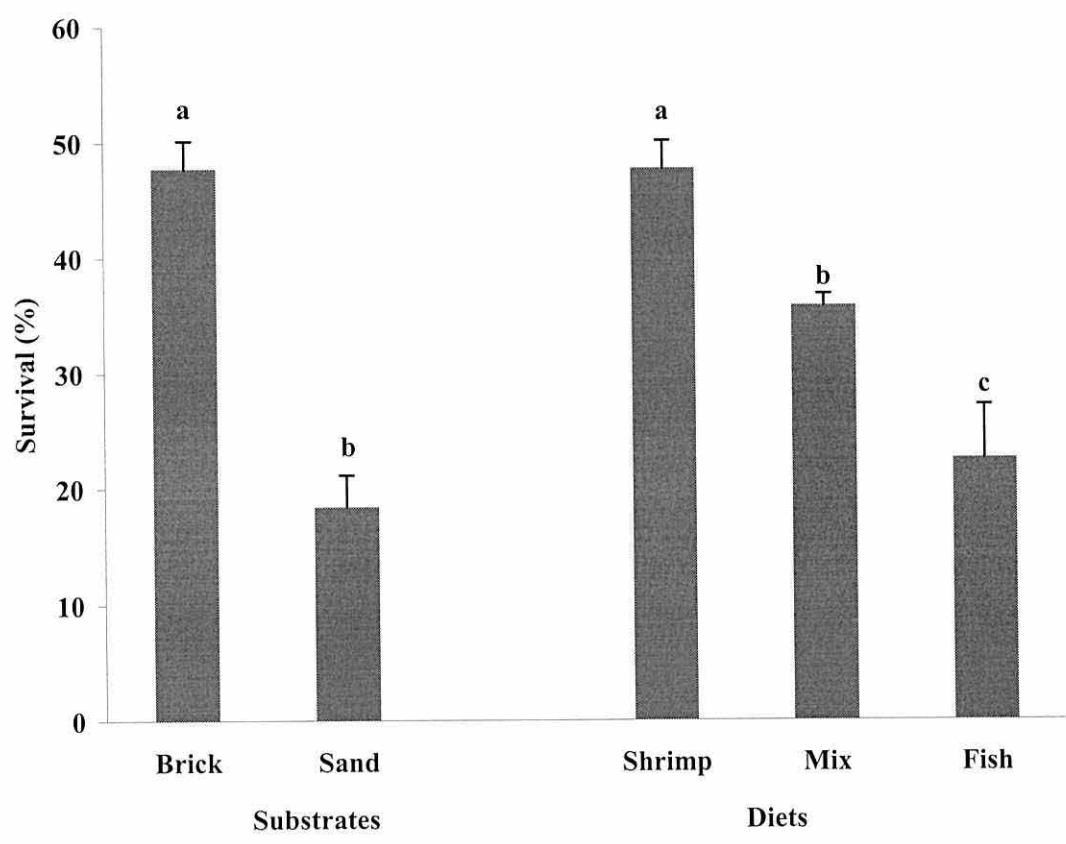
Figure 8: Experiment 3: Survival of crabs stocked on sand and brick, and of crabs fed with different diets in brick substrate. Different letters indicate significantly differences between treatments.
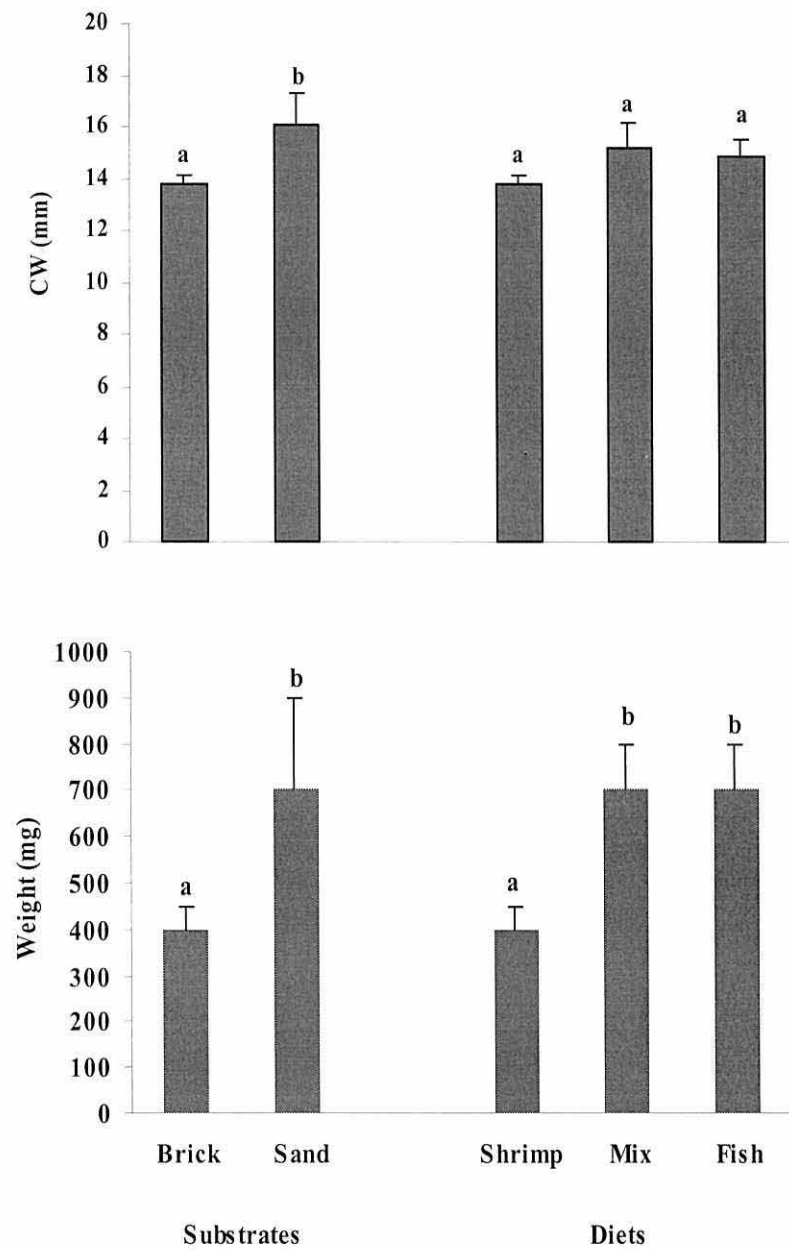
Figure 9: Experiment 3: Growth of crabs stocked on different substrates fed with peeled shrimp, and in bricks only fed with different diets. Within substrates and diets treatments, different letters indicate significant differences between treatments.
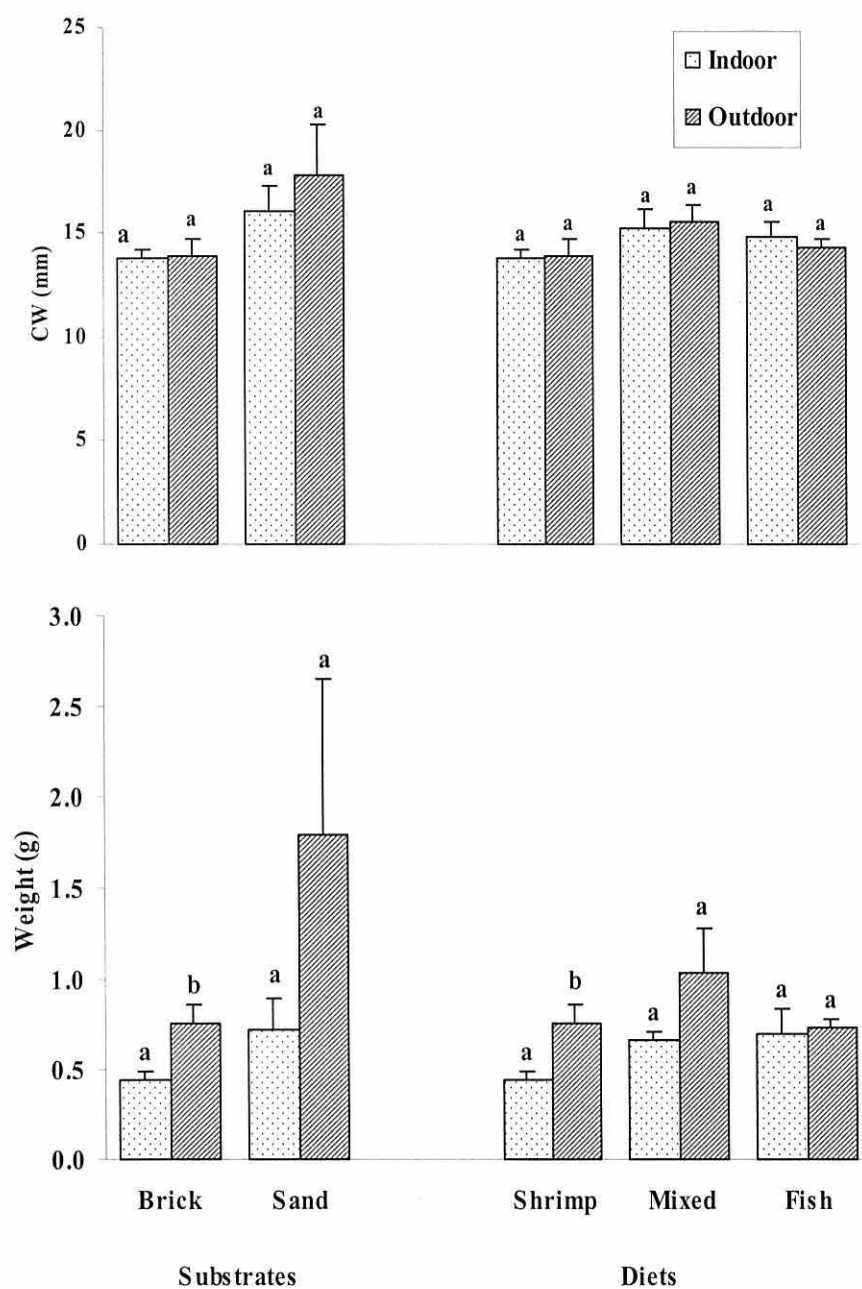
Figure 10: Experiment 3: Comparison of growth (CW and weight) of crabs stocked indoor and outdoor on all substrates and diets. Statistical comparison between indoor and outdoor from each treatment; different letters indicate significant differences (p<0.05) (n=3).
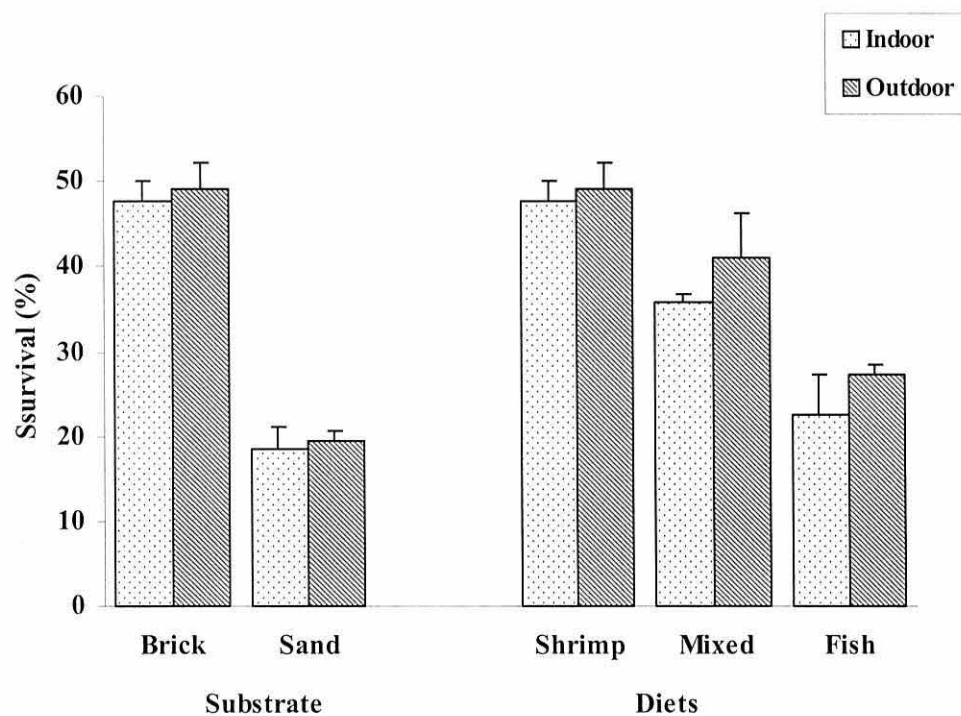
Figure 11: Experiment 3: Survival of crabs stocked outdoors and indoors on different substrates and diets. No significant difference between outdoors and indoors (p>0.05).

DISCUSSION

Shelters/substrates

Cannibalism usually causes high mortality during settling in a number of crab species, and survival during settlement is dependent on finding shelter (Moksnes *et al.*, 1998). Substrates and shelters are known to be important in reducing cannibalism in mud crabs in pond grow-out, where seaweeds (such as *Gracilariopsis*) and bamboo shelters have been used (Triño *et al.*, 1999). The presence of artificial refuges reduced cannibalism in juveniles of *C. angulatus* and *C. granulata* (Luppi *et al.*, 2001). In the present study, in comparison between two types of shelters/substrates, bricks were a better shelter for mud crabs than sand. The higher mortality in sand may have resulted from high cannibalism in this treatment. This may indicate that the possibility for crabs to encounter each other is higher in sand than in brick, especially after moulting. Dittel *et al.* (1996) reported that mortality in mud crabs, *Panopeus herbstii* in a sand bottom by predation was close to 100%, while a shell bottom provided refuge from predation. Barshaw *et al.* (1994) found that for postlarval lobsters, *Homarus americanus*, cobble provided better shelter than peat or sand. The reason for choice of gravel substrate over silt-clay observed in juvenile *H. americanus* was elucidated by Pottle and Elner (1982) and is due to the latter lacking readily available shelter. Crabs may protect themselves better when inside any type of shelter with holes/cracks rather than being totally exposed. In nature, Nandi and Roy (1990) suggested that crabs construct burrows to serve as the shelter for at least a part of their life, perhaps to spend the days during moulting. Atkinson and Taylor (1988) also pointed out that burrows besides offering the crabs opportunities to protect against extremes of environmental conditions, help reduce the risk of predation. In an experiment to determine shelter preferences of three xanthid mud crabs species (*Neopanope sayi*, *Panopeus herbstii* and *Eurypanopeus depressus*), Day and Lawton (1988) observed all three species preferred broken oyster shell much more than sand. These examples support the results of the present study, that brick may be considered a readily available shelter which is absent in sand. However, Richards and Cobb (1986) pointed out that Jonah crab, *Cancer borealis* was able to escape predation by rapid burrowing into sand. However, soft postmoult crabs were observed not to be able to bury themselves, so when they are most vulnerable they cannot find refuge in sand. Ecdysis may be the most critical time for crabs, with high risk of cannibalism. Juvenile

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S. paramamosain maintained with a sand substrate remained buried for most of the day, but emerge to moult on the sand surface (Le Vay, pers. com.). Moksnes *et al.* (1998) indicated that cannibalism between crabs of the same size appeared to occur only during ecdysis. The refuge inside the bricks has apparently resulted in a higher survival for crabs, while higher cannibalism in sand has resulted in lower survival.

Effect of diets

Inclusion of shrimp in the diet (either alone or mixed with fish) supported higher survival, probably reflecting greater attractiveness and palatability, as the shrimp was more rapidly consumed than the fish. Fish seems to be a less preferable diet for juvenile crabs and feeding on the conspecific juveniles was still high in the treatment with brick shelters with fish as diet. Jayamanne (1992) has pointed out that in *Scylla* feed preference may change with age and that the juveniles feed mainly on crustaceans. Luppi *et al.* (2001) pointed out that the protecting effect of refuges varies according to size and nutritional status of the predator. They observed that starved juvenile *C. angulatus* caused high conspecific settler mortality even in the presence of refuges. The higher mortality may result in higher growth rate due to cannibalism among crabs providing the survivors with a dietary supplement supporting growth in larger individuals (Perkins-Visser *et al.*, 1996). They also noted that mean growth of survivors was greatest where survival was lowest. In the present study, growth was significantly better in the crabs fed fish, probably as the consequence of increased cannibalism and reduced densities, rather than any direct dietary effect. In addition, Cuzon and Guillaume (1997) cited by Catacutan (2002) mentioned that crustaceans are not able to tolerate more than 10% of dietary lipid, and its inefficient utilization causes reduced growth. The high lipid content (18.3%) in fish flesh used in the present study may have resulted in a negative growth response and low survival. If this was the case, the high cannibalism in the treatments fed with fish, and higher growth rate obtained may have been resulted from the dietary supplement from conspecific juveniles.

Stocking densities

At higher population densities, conspecific encounter rates and opportunity for cannibalism

would be greater (Perkins-Visser *et al.*, 1996), especially in the nursery as Moksnes *et al.* (1998) found that juvenile shore crabs were extremely efficient predators on small conspecifics, more efficient than adults. The results of the present study agree with this finding. Although there was no significant difference between the three densities, higher survival observed in the lower density may be a result of lower cannibalism as crabs may have had less encounters at the lower density. As there was no significant difference in survival between stocking densities, it is possible that higher stocking densities could be used without impairing yields.

Effect of rearing systems

Temperatures were higher in the outdoor than indoor system. Effects of temperature on growth of crabs have been widely documented (Lou and Wei, 1986; Lee, 1992). Luo and Wei reported that juveniles of *Scylla* sp. moult more frequently and grow rapidly with a rise of water temperature within the suitable temperature range. Moreover, light intensity may have an important effect on growth and survival of crabs. This effect has been confirmed in the larvae of the Australian giant crab, *Pseudocarcinus gigas* (Gardner and Maguire, 1998). They pointed out that larvae reared in a high light intensity (bright light) had a shorter intermoult than those in a dim light system. In addition, in dimmer light intensity a shift to larger prey may occur and result in more cannibalism. As a result, the authors drew a conclusion that larvae grew more rapidly and suffered less cannibalism at the brighter light intensity. The results from the present study may be consistent with this observation as juveniles *S. paramamosain* were observed to have lower growth and survival in the indoor system, which was shaded and dimmer than the outdoor system.

In summary, low survival of crabs is usually associated with the cannibalism phenomenon during settlement and nursery phases. Addition of shelters/substrates to the rearing environment helps reduce risk of conspecific predation in crab juveniles. The best substrate/shelter in the present study was clay bricks. However, investigation of other substrates should be further carried out in terms of effectiveness and economics. For example use of clam or blood cockle shells which are locally available and cheaper compared to bricks that are more expensive should be tested. Higher survival was obtained in treatments fed with

shrimp, indicating shrimp feed is considered the best feed for small crabs. However further studies of different locally available feed (e.g. low-cost mollusc or home-made pellets) should also be conducted to support economic effectiveness. Stocking densities should be further tested to determine upper threshold densities for small crab nurseries. A combination of increment of number of substrates to increase stocking density should be also further investigated.

CHAPTER VII

COMPARISON OF FITNESS OF HATCHERY-REARED AND WILD *SCYLLA PARAMAMOSAIN*

INTRODUCTION

Stock enhancement aims to replenish wild populations by introduction of hatchery-produced organisms of a certain species, for which natural production has been declining. As hatchery-reared (H-R) organisms are used for restocking in the wild, many important aspects have been taken into consideration prior to commencing a stock enhancement programme. One of the major factors affecting feasibility of stock enhancement is the quality of the hatchery-reared animals. Stock enhancement will be useful only if hatchery-reared and wild stocks are identical in growth and mortality (Stoner and Davis, 1994). Hatchery-reared organisms should be able to survive and perform as well as the wild conspecifics (Stoner, 1994; Kellison *et al.*, 2000), with minimal potential risks of spreading disease into the wild population (Knut *et al.*, 1999), alternation of the genetic structure of the wild population (Busack and Currens 1995; Utter 1998; Murai and Koshiishi 1998), and with economical feasibility (Kellison *et al.*, 2000). In most cases the quality or fitness of hatchery-reared organisms is considered an indispensable major factor in the success of stock enhancement and needs to be assessed (Stoner, 1994; Kellison *et al.*, 2000). The hatchery-reared animals must have vigour and adaptability sufficient not only to grow, but also to survive in the wild (Stoner, 1994).

The fitness of animals produced in hatcheries for release in the wild has been increasingly questioned, as it is not known whether they can compete with their wild conspecifics. A number of studies on growth, survival and behaviour of various hatchery-reared species have been carried out, including lobster (Bannister *et al.*, 1994; Robinson and Tully 1999), flounder (Shinpei, 1998; Kellison *et al.*, 2000), cod (Grant *et al.*, 1998), steelhead trout (Chilcote *et al.*, 1986, Berejikian, 1995; Barry 1995), queen conch (Marshall *et al.*, 1992; Davis, 1992; Stoner & Davis, 1994), sole (Ellis *et al.*, 1997) and salmon (Rhodes and Quinn, 1999; Mork *et al.*, 1999). The results of most studies have demonstrated that H-R animals are more vulnerable to predation than wild conspecifics. Feeding behaviour (Shinpei, 1998; Miyazaki *et al.*, 2000), swimming behaviour (McDonald *et al.*, 1998), rearing technique including stocking densities (Stoner, 1994; McDonald *et al.*, 1998) and hatchery substrata (Kellison *et al.*, 2000) or genetic changes (Berejikian, 1995) are major factors

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reported to affect performance of hatchery-reared animals. However, many of these factors may be modified through rearing practices to improve fitness of the H-R animals, or genetic selection of broodstock.

With a trend in increasing fishing effort, overexploitation may occur in the mud crab, *Scylla paramamosain*, in the Mekong Delta and stock enhancement may potentially play an important role in sustaining the production of this species. Hatchery production of *S. paramamosain* in the Mekong Delta is being developed (Truong Trong Nghia *et al.*, 2001), and the feasibility of stock enhancement in this species may depend upon quality of the H-R animals. The present study aimed to compare performance of H-R and wild juveniles. The objective was to determine how well H-R crabs performed in competitive and non-competitive conditions. Positive results for hatchery-reared *S. paramamosain* would certainly support the use of the animals in stock enhancement programmes. More importantly the hatchery-reared juveniles may replace the wild seed crabs in pond culture, that have been relied on until now and would relieve much pressure on fishery resources.

MATERIALS AND METHODS

Source of crabs

Hatchery-reared crabs

In the first experiment, due to the none-availability of small hatchery-reared *Scylla paramamosain* at the hatchery in Can Tho University, small crabs were obtained from the Research Institute for Aquaculture No 3 in Nha Trang, central Vietnam. The crabs were produced using similar approaches to the hatchery at Can Tho University. Larvae hatched from a naturally berried female were stocked in 250l and 500l cylindroconical tanks at a density of 50 l^{-1}. Water temperature was maintained at $31.1^{0}C$, salinity at 32 ppt, with 30-50% water exchange daily. A twice-daily feeding regime maintained *Tetraselmis* spp. at 100 cells μl^{-1}, and rotifers at 60 ml^{-1}. Non-enriched *Artemia* nauplii were added from day 17 onwards. After metamorphosis, crabs (stages 1 - 3) were maintained in 20 ton concrete tanks with a bottom layer of fine sand and fed twice daily with home made feed (mixture of low cost chopped mussel and shrimp). Four hundred crabs were transported as stage 3-4 (7-9 mm CW) to Can Tho in two 30 l containers with a little ice to keep temperature low. Density maintained during transport was 200 crabs per container. The crabs were acclimatized in Can Tho for 3 days before transfer to Vinh Chau, where they were further reared to reach a size suitable for tagging (20 mm CW). The rearing system consisted of 2 m^{3} composite tanks with aerated seawater at a level of 30-40 cm. Crabs were held individually in containers and fed to excess with peeled shrimp each afternoon and water was completely renewed every 2 days.

In the second experiment, hatchery-reared juveniles were produced at the hatchery in Can Tho University. Crab larvae were hatched from a berried female obtained from a maturation pond in Vinh Hau (coastal area in Bac Lieu). The larvae were reared in 4m^{3} composite tanks connected to a biofilter system. They were fed Selco enriched rotifers twice a day with 30 individual ml^{-1} from day 1 to day 4. From day five onwards enriched *Artemia* nauplii instar 1 were supplemented. The light regime was set at 12 hours light and 12 hours dark. Salinity was maintained at 30 ± 2 ppt and

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temperature was kept stable at 29 ± 0.5 °C. After metamorphosis to crab instar 1, they were fed frozen *Artemia* biomass and peeled shrimp until being transferred to Vinh Chau for rearing in nursery systems.

Crabs were transported for rearing in groups in 30 m^2 lined pond systems with a fibro-cement roof in Vinh Chau. The water depth was maintained at 40-50 cm and changed every 2 days (50-80%). Crabs were fed with peeled shrimp to excess once each afternoon. Clam shells, bricks and shrub stick bunches were placed in each pond to serve as shelters. After a period of 1.5 months, crabs attained a suitable size for tagging and were used for the experiments.

Wild crabs

Wild crabs used in the first experiment were collected by fishermen in the Vinh Chau coastal area, Soc Trang province. They were caught at low tide by scoop-net, trawl-net and by hand. Crabs were selected of a similar size, as near as possible to the hatchery-reared crabs. They were also held individually in containers floating in the composite tanks.

In the second experiment, wild crabs were purchased from an agency in Long Phu, where they were gathered at night on mud flats in the 30th April State Farm, as described in Chapter 2. The crabs were transferred to Vinh Chau and sorted into similar sizes to the hatchery-reared crabs. They were placed individually in jars and baskets, floating in fibreglass tanks with 12 ppt seawater, and then were acclimated to 30ppt over 20 h.

Tagging methodology

Wild and hatchery-reared crabs were measured and sexed prior to tagging. Sequential coded micro-wire tags were used in the first experiment while decimal coded tags were applied in the second experiment. The tagging methodology was as described for previous experiments in Chapter 4. A wet towel was used to wrap the crab, exposing only the injection area to avoid struggling while tagging. Tags were injected into the left and the right 4th pereiopod for hatchery-reared and wild crabs,

respectively. With this procedure, hatchery-reared and wild crabs could be easily distinguished during interim sampling without having to remove the tags. One tag was retained as a sequence reference between each crab, allowing identification of individual animals by tag retrieval at the end of the experiment. After injection, all crabs were individually held overnight in tanks to observe for mortality prior to releasing into the pond.

Experiment 1

A total of 326 tagged crabs were stocked in two earthen ponds surrounded with nylon fences to prevent crabs from escaping. Stocking number, sizes and sex ratio of both H-R and wild crabs in different ponds and tanks are shown in Table 1.

Crabs were fed with eviscerated chopped Tilapia each afternoon. Water was exchanged weekly or whenever necessary. Salinity and temperature were recorded daily. About 10 to 15 animals were sampled monthly by using baited hooks placed along the banks of each pond.

At the end of the experiment, the ponds were drained and surviving crabs collected by hand. All the crabs were tied up and checked for tags with a portable detector. Crabs were separated into 2 groups based on which side they were tagged (right or left). After measuring carapace width and weight tags were retrieved following the method described in Chapter 4.

A number of both H-R and wild crabs were tagged and stocked in tanks as a control to the pond experiment (see Table 1). In the tanks, crabs were contained individually in labelled round plastic baskets (diameter 20 cm and depth 15 cm). Moulting and mortality were checked daily in the early morning and in the afternoon when the feed was added. Carapace width and weight of moulted crabs were measured the day after moulting when the shell had hardened. They were also fed daily with chopped Tilapia and excess food removed when replacing with fresh food.

Experiment 2

Wild and hatchery-reared crabs were stocked in nine 170 m^2 earthen ponds fenced with nylon sheets to prevent escape. Wild and hatchery-reared crabs were not only stocked in the same ponds but were also kept separately. A total of 720 crabs were randomly distributed in three treatments (wild crabs, H-R crabs and both together) with three replicates each, which were randomly allocated in the pond system. Initial numbers, sizes and weights are tabulated in Table 2.

Crabs were fed with peeled shrimp in the first week and chopped eviscerated Tilapia onwards to the end. Water was exchanged every 5 days with 50-80% each time. Salinity, temperature and pH were recorded daily in the morning and in the afternoon. Interim sampling and harvest procedures were carried out similarly to Experiment 1.

Unlike Experiment 1, plastic pipes of 30 cm in length were cut longitudinally in half, together with bunches of sticks placed in each pond as shelters for crabs.

Statistical analysis

Differences between treatments were compared either by t-test or one-way ANOVA as indicated in the results. All data were tested for normality and homogeneity of variance. Data not conforming were square root transformed before ANOVA using the Minitab 13 statistical package. Differences at $p \leq 0.05$, $p < 0.001$ and $p < 0.0001$ were considered slightly or highly significant, respectively.

RESULTS

Experiment 1

The first experiment lasted for 79 days and during this period, salinity gradually dropped from 32-34 ppt at the beginning to 16-19 ppt at the end, as the result of rainfall. Daily temperatures ranged from 25°C (morning) to 33°C (afternoon), with afternoon temperatures rising from 29 to 33°C over the course of the experiment. Changes of salinity and temperature are illustrated in Figs. 1 & 2.

Growth

The initial condition of juvenile crabs (ratio of weight to CW) was evaluated prior to stocking. This indicated that wild juveniles were significantly heavier for their size than H-R crabs as their W/ CW ratio was significantly higher (1.1 ± 0.2 g cm^{-1}, n = 188 and 0.82 ± 0.1 g cm^{-1}, n = 167, respectively) (t-test, p<0.001). Results from two interim samplings are given in Figs. 3 & 4, showing that wild crabs grew significantly faster than H-R crabs, both in carapace width and weight. By the end of the experiment, wild crabs stocked in ponds were bigger than H-R crabs stocked under the same conditions, with a mean final CW of 93.3mm and 67.7mm and weights of 162g and 62.8g, respectively. However, for those animals kept in baskets, both wild and H-R crabs were considerably smaller, with weights of 15.4g and 17.7g and CW of 43.1mm and 43.4mm, respectively at the end of the experiment (see Figs. 3 & 4).

For pond-reared crabs, specific growth rate and daily CW increments of wild crabs was significantly higher than that for H-R crabs (F=7.59, p<0.001 & F=9.28, p<0.001 respectively). Wild crabs gained 0.87 ± 0.2 mm day^{-1} CW compared to 0.56 ± 0.1 mm day^{-1} CW for H-R crabs. There was no significant difference in growth rates between the two ponds or between males and females, for either H-R (F=0.11, P=0.745) or wild crabs (F=1.58, p=0.214).

However, in contrast to the results obtained in ponds, both SGR and CW increment of H-R crabs stocked in baskets were significantly higher (F=10.61, p=0.004 & F=8.86,

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p=0.007 respectively) than those for wild crabs kept under the same conditions (Table 3). Crabs stocked individually in baskets grew more slowly than those in ponds. The mean SGR for wild crabs in baskets was 2.1 ± 0.5 ln g d^{-1} compared to 5.1 ± 0.7 ln g d^{-1} for wild crabs in ponds.

Tag retention

All tagged crabs kept in baskets retained their tags until the end of the experiment. However, 6 crabs were found without tags among the 95 surviving crabs in the ponds, giving a 6.3% tag loss.

Survival

Overall mean survival for all crabs in both ponds over the 79 day experimental period was quite low (29.2%) with H-R crabs exhibiting considerably lower mean survival (18.9%) than wild crabs (39.5%) (see Table 3). Survival rates among crabs individually maintained in baskets were higher than in ponds, with better survival in wild crabs than in H-R (76.5% and 58.8%, respectively).

Experiment 2

The experiment was maintained for a duration of 106 days. Temperature, salinity and pH remained suitable and did not vary within and between treatments (Figs. 5, 6 & 7).

Growth

As in Experiment 1, weight to CW ratio was evaluated in both hatchery-reared and wild crabs. Wild crabs had a significant higher W/CW ratio than that of hatchery-reared crabs (t-test, $p<0.0001$). This also indicates wild crabs were significantly heavier than the hatchery-reared conspecifics (1.6 ± 0.3 g cm^{-1}, n = 388 and 1.4 ± 0.4 g cm^{-1}, n = 410, respectively). However, in contrast to Experiment 1, the hatchery-reared crabs in this experiment grew faster than the wild crabs through interim sampling, in terms of both carapace width and weight, and in both separated and

mixed ponds (Figs. 8 & 9). By the end of the experiment, hatchery-reared crabs displayed significantly higher growth rates for both carapace width (F=51.9, p<0.0001) and weight (F=42.8, p<0.0001) than that of wild crabs in the separate ponds. However, in the mixed ponds, hatchery-reared crabs had significantly higher growth rate in terms of carapace width (Kruskal-Wallis test, H=11.48, p=0.001) than wild crabs, but not in weight (F=1.7, p=0.2) (Fig. 10). There was no significant difference in growth rate between males and females in both H-R and wild crabs neither carapace width (F=2.14, p=0.15; F=0.34, p=0.56, respectively) nor weight (F=0.42, p=0.52; F=0.42, p=0.52, respectively).

Tag retention

Higher tag retention was obtained in this experiment (97.5%). There were only 5 lost tags among 200 surviving crabs, accounting for 2.5% tag loss. This indicates a great improvement in application of the tagging methodology.

Survival rate

As in Experiment 1, overall survival for all crabs in ponds was low (25.3 %). However, this experiment lasted longer than the previous one (106 compared to 79 days, respectively). Estimated survival at 60 days was nearly 50% for both wild and hatchery-reared crabs (Fig. 11). Thus, higher survival would have been expected for crabs in this experiment, over the same duration as for the first experiment (79 days).

There was significant higher survival (F=7.76, p=0.05) in wild crabs in the mixed ponds. In contrast, for the separate ponds, survival of H-R crabs was higher than that of wild crabs, but not significantly (F=0.14, p=0.72) (Fig. 11).

Results from tag retrieval have indicated a high number of crabs intermixing between ponds (Fig. 12). Considerable movement was manifested by 19% of crabs among the total survivors (38 over 200) had moved away their original ponds. This also means that some crabs may have escaped from the ponds. Survival of crabs may therefore be underestimated. High variation in survival for wild crabs compared to hatchery-reared crabs in the separate ponds (see Fig. 11) may have been a result of this movement as

wild crabs in pond number 4 may have escaped to the adjacent ponds (on the other side of the system) which were not checked at harvest.

Table 1: Number at stocking, density, initial sizes and sex ratio of wild and hatchery-reared crabs in earthen ponds and tanks in Experiment 1.

Parameters	Pond 1		Pond 2		Tank	
	Wild	H-R	Wild	H-R	Wild	H-R
Stocking number	101	98	70	53	17	17
Stocking density (crabs m^{-2})	0.5		0.3			
Size (CW mm)	22.9 ± 1.58	22.6 ± 1.70	26.6 ± 1.6	22.3 ± 1.3	25.0 ±2.05	21.4 ±0.77
Male (%)	54.5	53.1	44.3	43.4		
Female (%)	45.5	46.9	55.7	56.6		

Table 2: Number of H-R and wild crabs and their initial sizes (CW and weight) in mixed culture and separate ponds in Experiment 2.

Treatments		Pond No	Stocking number	Initial CW (mm)	Initial weight (g)
Mixed culture	**H-R**	5	40	27.5 ± 3.14	4.07 ± 1.40
	Wild		40	28.7 ± 3.23	4.12 ± 1.34
	H-R	8	40	28.2 ± 2.57	4.43 ± 1.36
	Wild		40	29.2 ± 3.06	4.82 ± 1.52
	H-R	11	40	28.2 ± 3.86	4.43 ± 1.53
	Wild		40	27.4 ± 4.07	4.13 ± 1.62
H-R separate culture		7	80	26.4 ± 2.14	3.56 ± 0.96
		10	80	26.6 ± 2.60	3.59 ± 1.26
		12	80	27.1 ± 3.08	3.60 ± 1.25
Wild separate culture		4	80	29.3 ± 1.28	4.52 ± 0.70
		6	80	30.3 ± 2.75	5.22 ± 1.51
		9	80	30.3 ± 2.96	5.20 ± 1.28

Table 3: Specific growth rates (SGR) and daily carapace width (CW) increments for H-R and wild *S. paramamosain* cultured in earthen ponds and in individual baskets in Experiment 1. Values bearing different superscripts are significantly different (p<0.05).

		CW increment (mm/d) (sd)	SGR (ln g/d × 100) (sd)	Stocking Number	Survival (%)
Pond 1	Wild	0.85 (0.2)[a]	5.1 (0.7)[a]	101	31.7
	H-R	0.59 (0.12)[b]	4.4 (0.6)[b]	98	18.7
Pond 2	Wild	0.89 (0.18)[a]	5.0 (0.6)[a]	70	47.3
	H-R	0.53 (0.06)[b]	4.0 (0.3)[b]	53	19.2
Baskets in tanks	Wild	0.23 (0.05)[c]	2.1 (0.5)[c]	17	76.2
	H-R	0.28 (0.06)[d]	2.9 (0.6)[d]	17	58.8

Table 4: Growth rate of *Scylla paramamosain* recorded in the wild and pond conditions. Natural (mangroves) = growth rate of wild crabs in the wild estimated by mark-recapture (Chapter 3); Ponds (1) = growth rates of wild and H-R crabs in Experiment 1; Ponds (2) = growth rates of wild and hatchery-reared crabs in Experiment 2; Mangrove-ponds = growth rate of wild crabs recorded in replanted mangrove pond (4 years old trees) using tagging methodology stocked at 0.1 crab m^{-2} and fed with chopped eviscerated tilapia.

	Wild crabs		Hatchery-reared crabs	
	CW (mm month^{-1})	Weight (ln g day^{-1})	CW (mm month^{-1})	Weight (ln g day^{-1})
Natural (mangroves)	21.1 ± 0.9			
Ponds (1)	26.0 ± 5.7	5.0 ± 0.7	17.6 ± 3.2	4.4 ± 0.5
Ponds (2)	20.4 ± 4.2	3.4 ± 0.5	22.5 ± 3.2	4.0 ± 0.3
Mangrove-ponds	17.2 ± 4.0	2.8 ± 0.4		
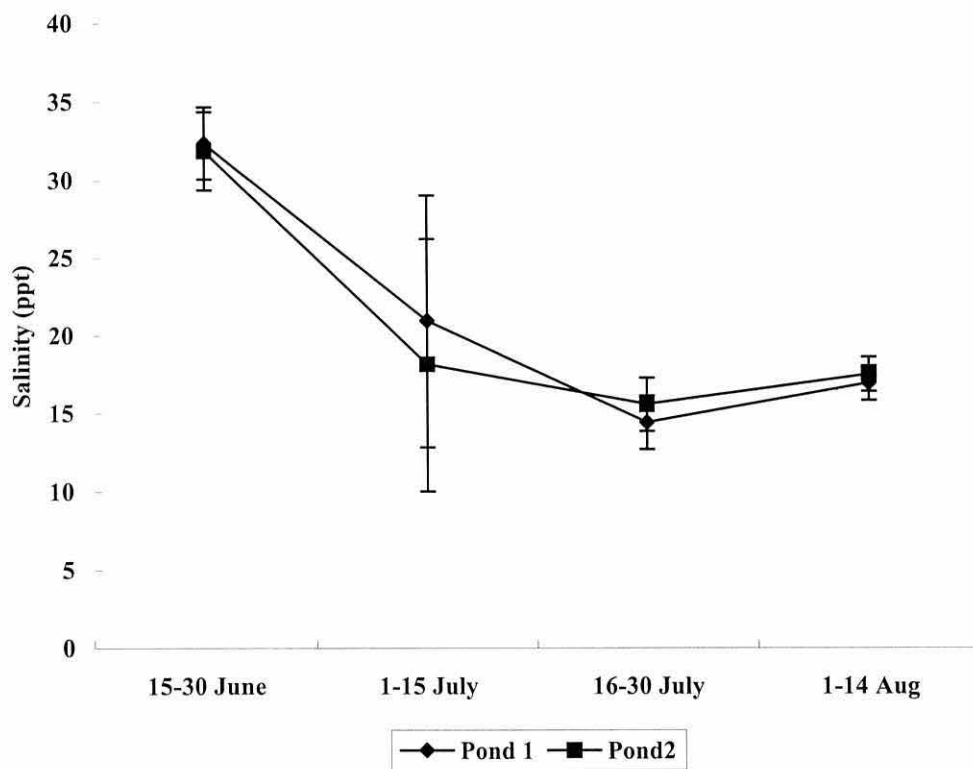
Figure 1: Salinity (ppt) recorded in pond 1 and pond 2 during Experiment 1, mean ± sd.
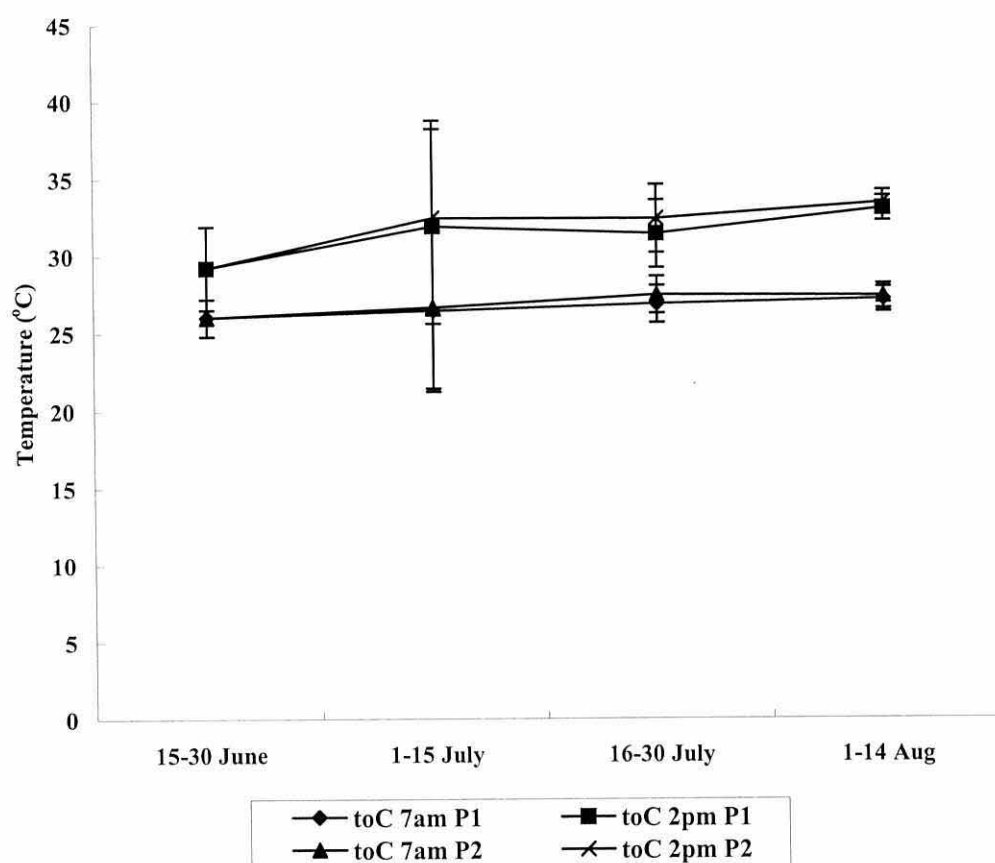
Figure 2: Temperature (^{o}C) in the morning and in the afternoon in ponds 1 & 2 in Experiment 1, mean ± sd.
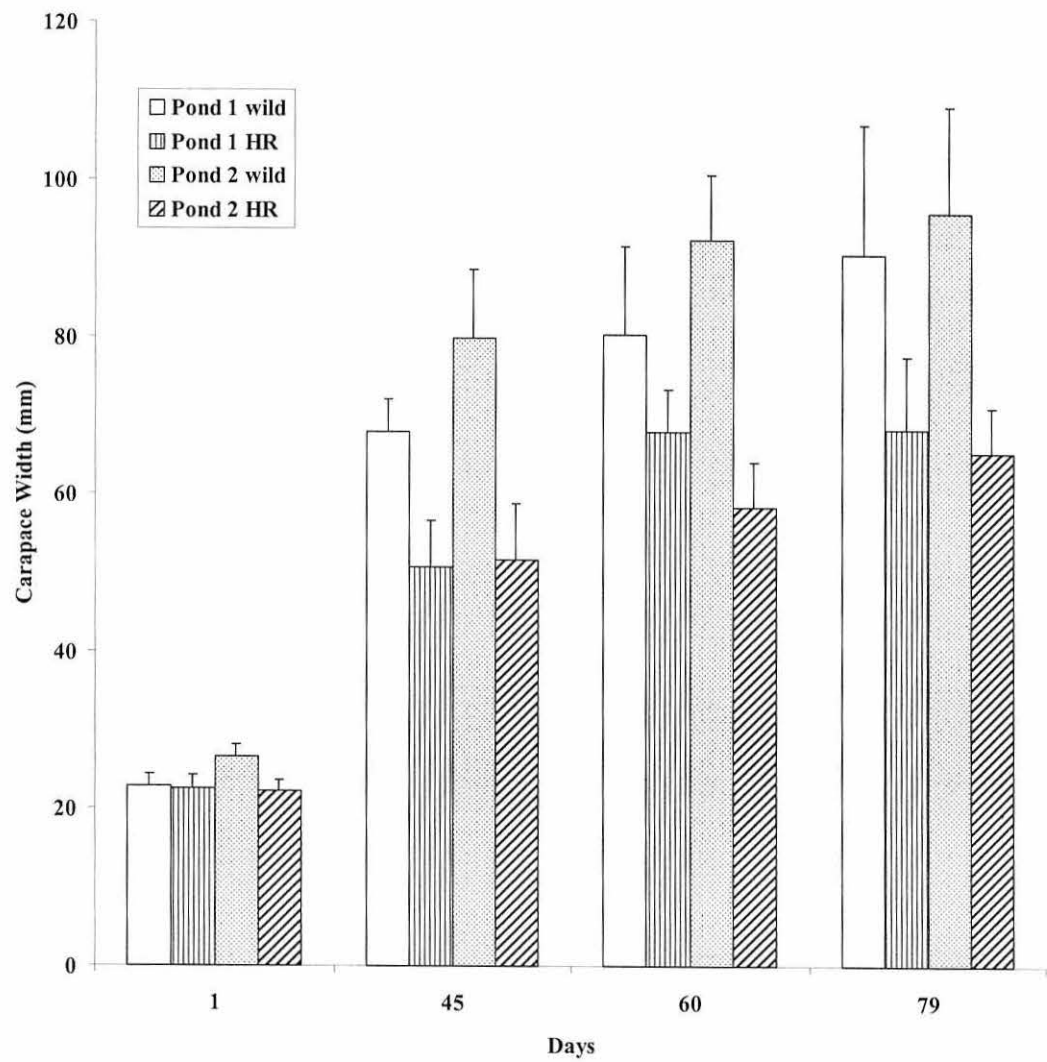
Figure 3: Experiment 1: Initial, sampled and final carapace widths (mm) of mixed wild and hatchery-reared tagged *S. paramamosain* juveniles reared in the two ponds.
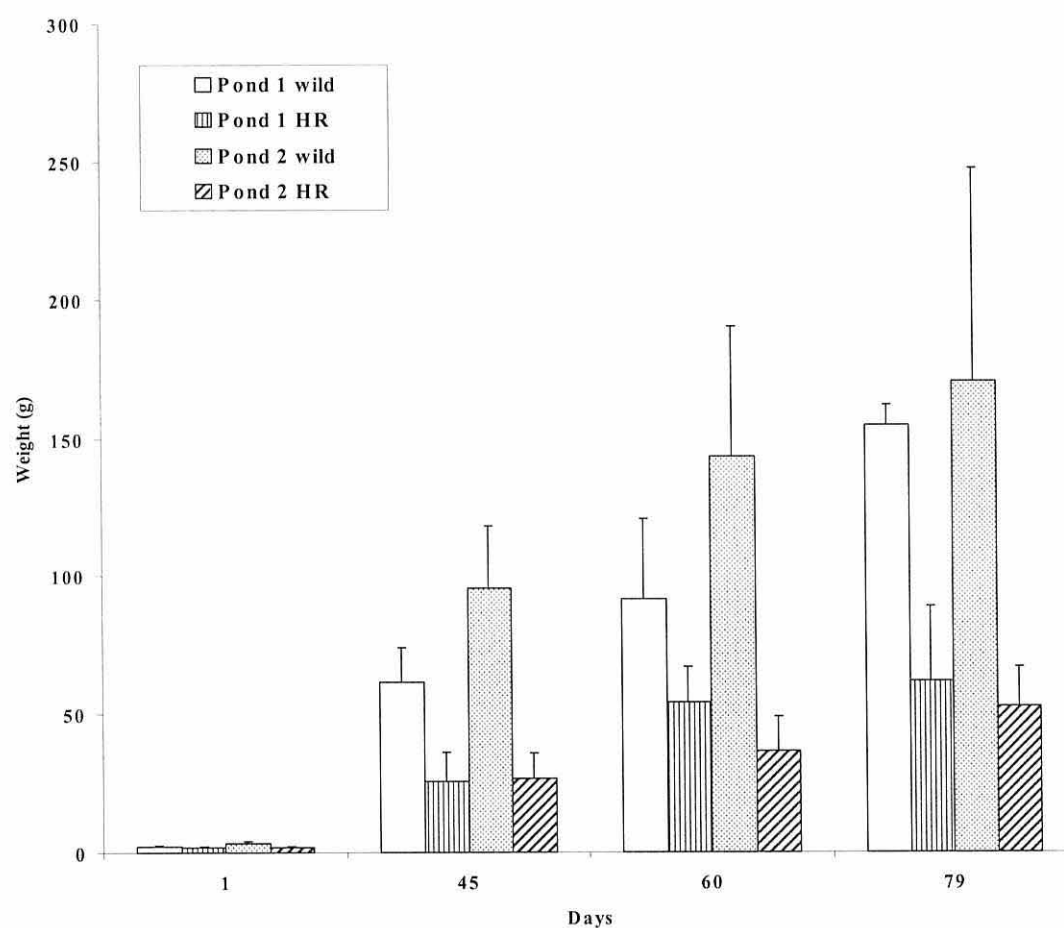
Figure 4: Experiment 1: Initial, sampled and final weight (g) of wild and hatchery reared tagged *S. paramamosain* juveniles reared in the two ponds.
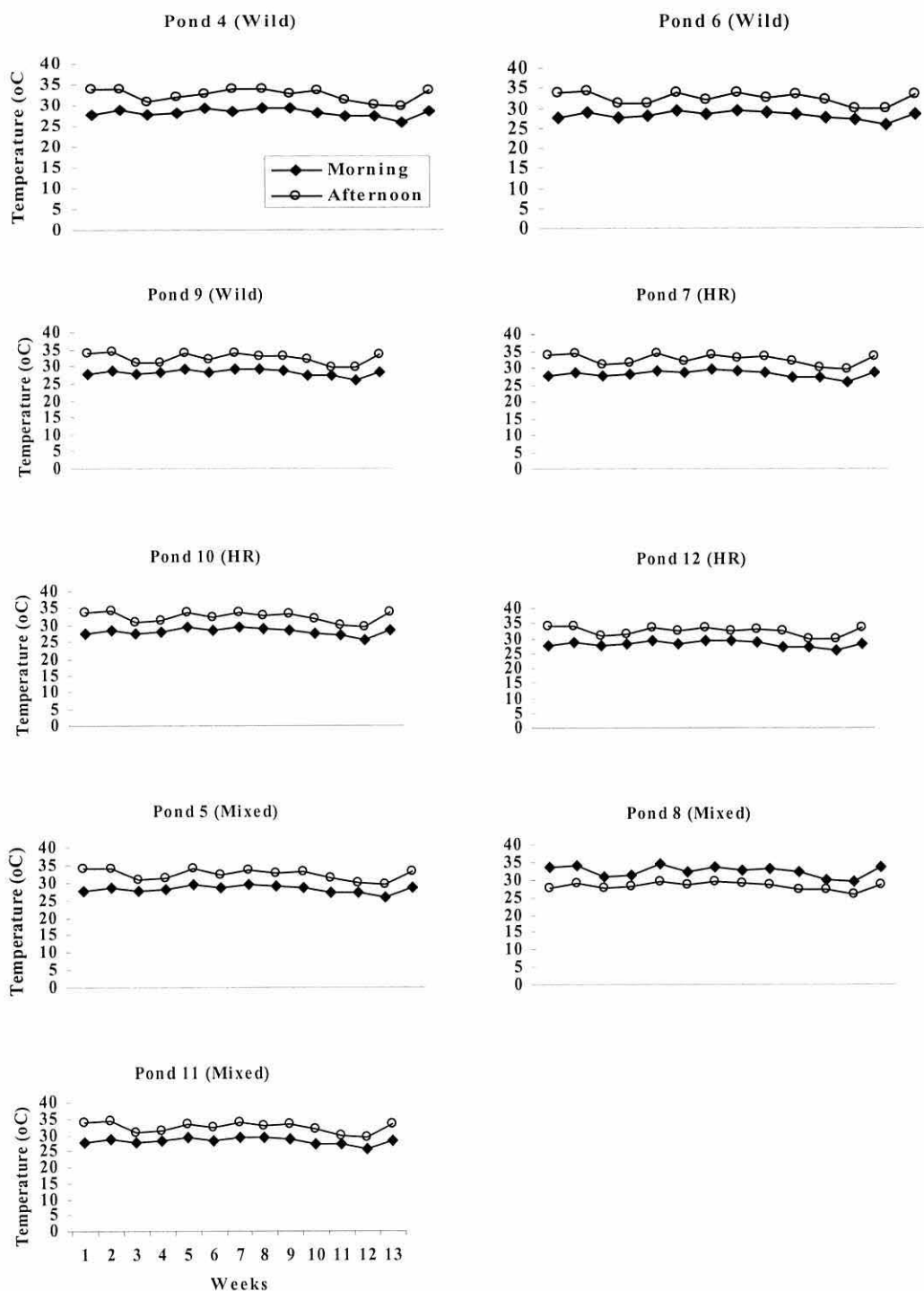
Figure 5: Morning and afternoon temperature recorded in 9 ponds during 13 weeks of Experiment 2.
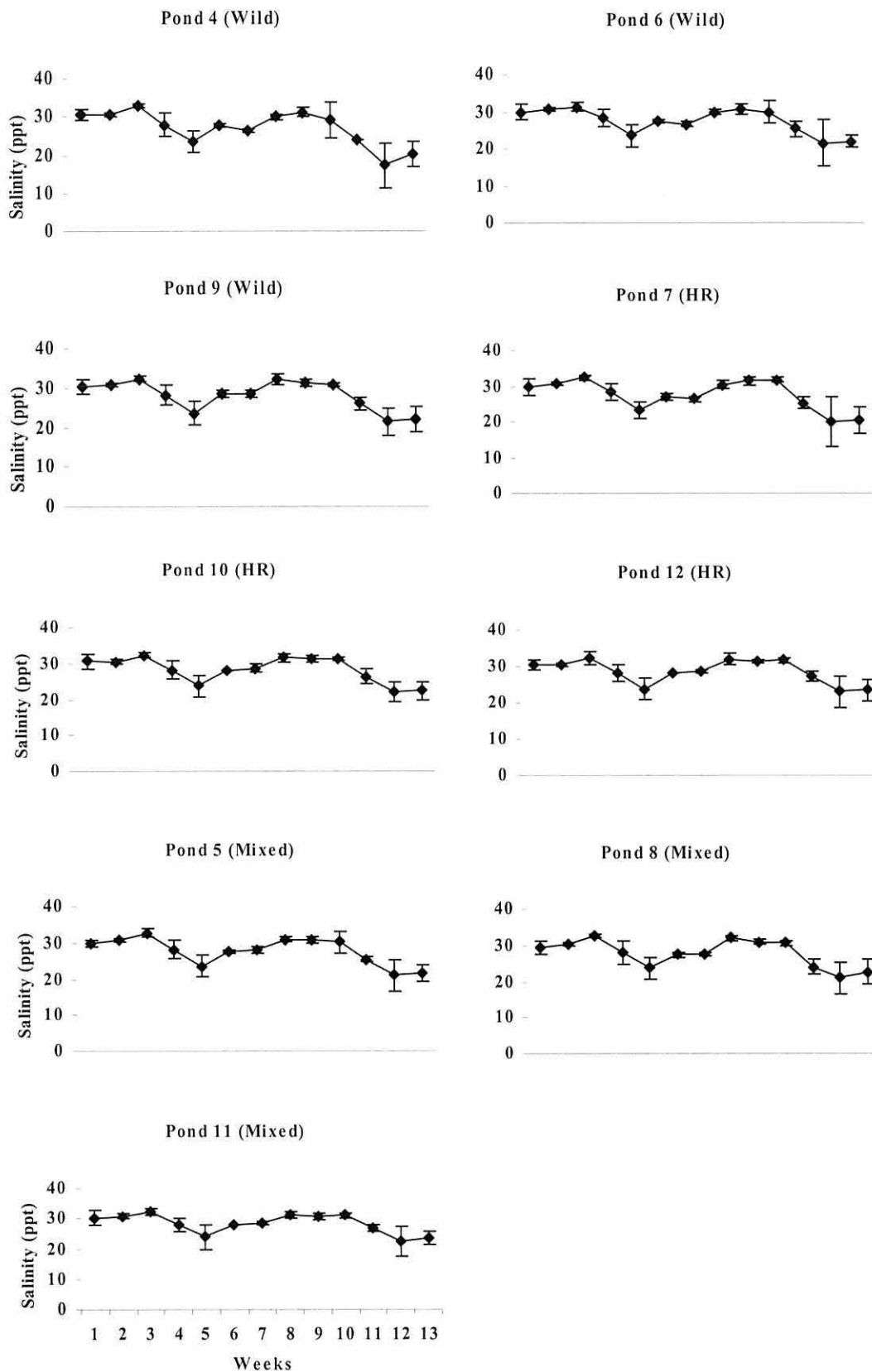
Figure 6: Salinity measured in 9 ponds during 13 weeks of Experiment 2, mean ± sd.
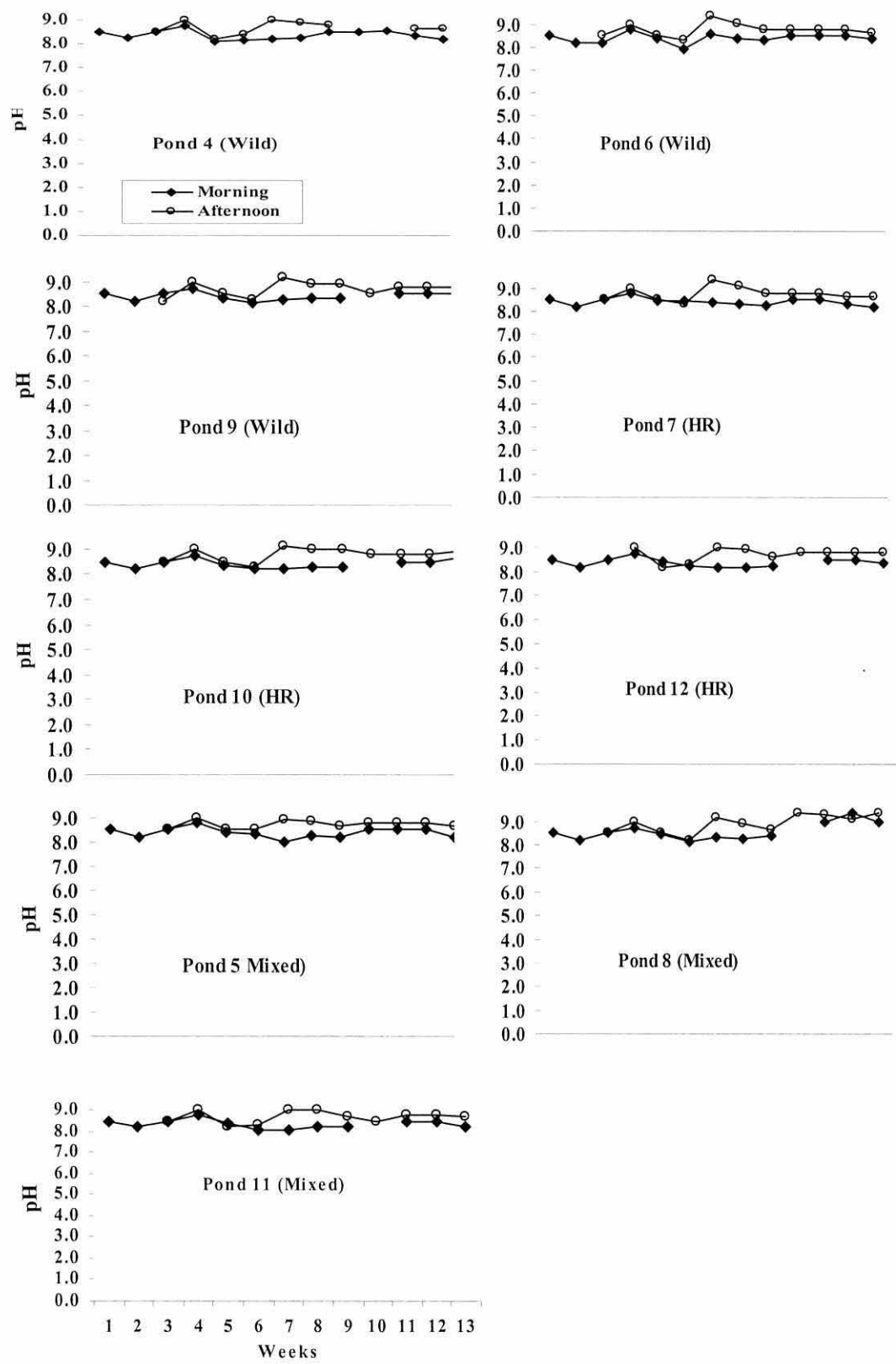
Figure 7: pH recorded in 9 ponds during 13 weeks of Experiment 2.
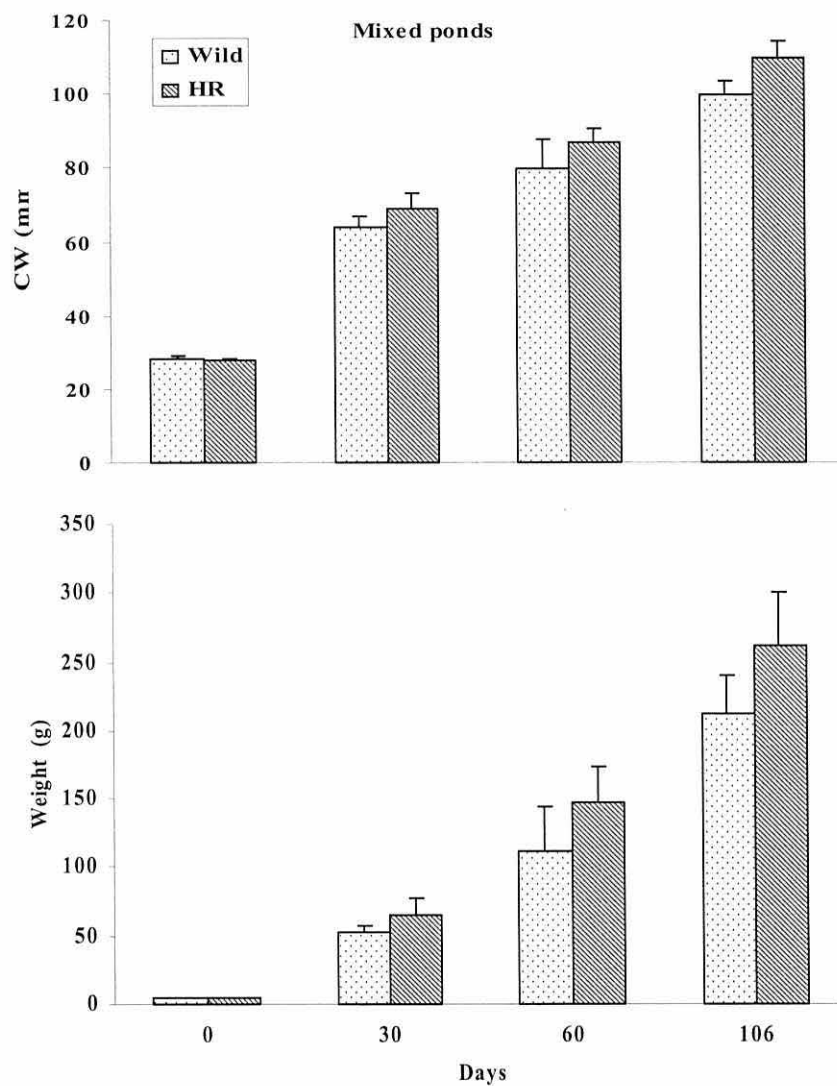
Figure 8: Experiment 2: Mean CW and weight (±SD) of HR and wild crabs in mixed culture through sampling periods.
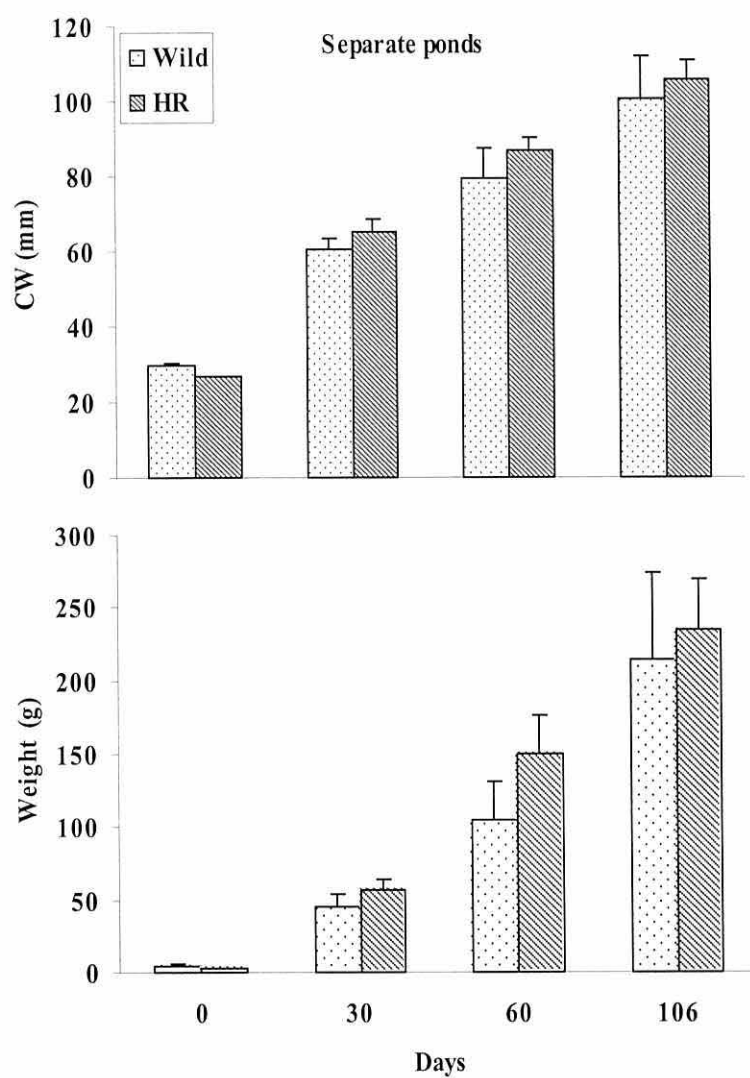
Figure 9: Experiment 2: Mean CW and weight (±SD) of HR and wild crabs in separate culture through sampling periods.
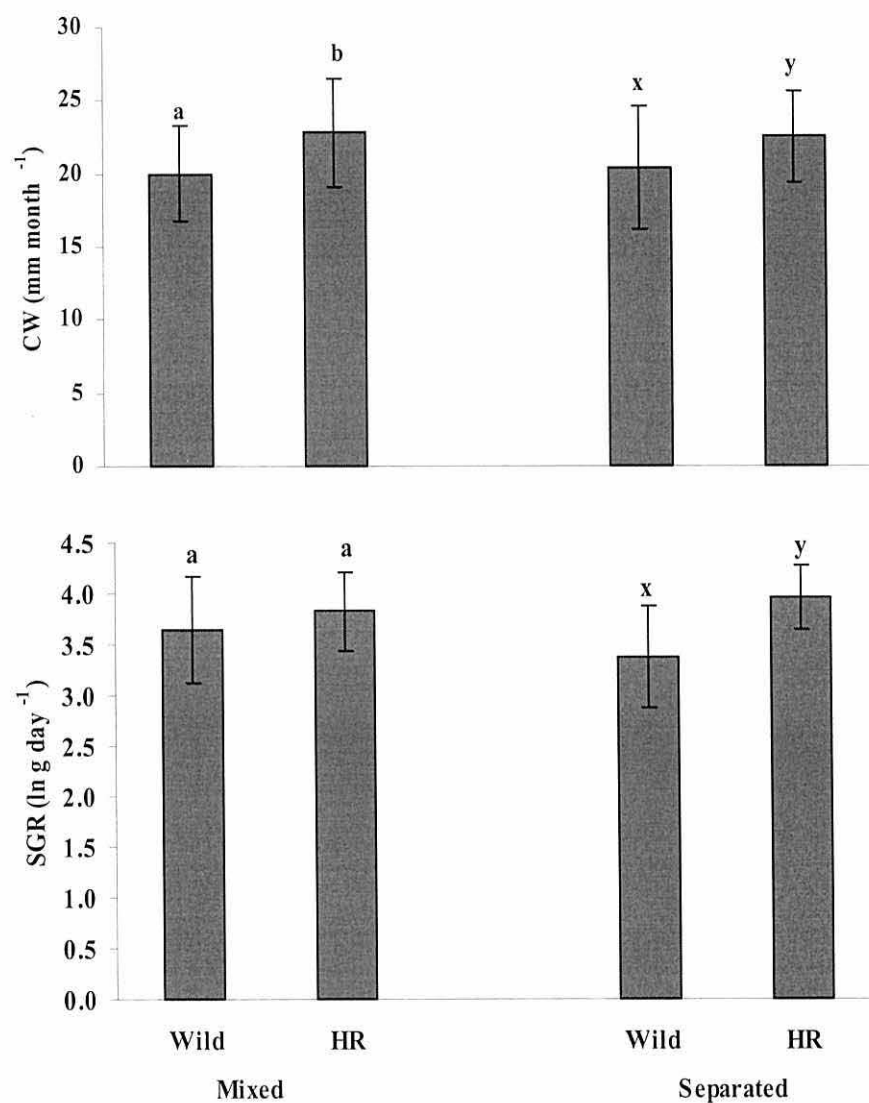
Figure 10: Experiment 2: Mean growth rate (±SD) of H-R and wild crabs in the mixed and separate culture at the end of experiment (after 106 days). Different letters indicate significant difference (p<0.001).
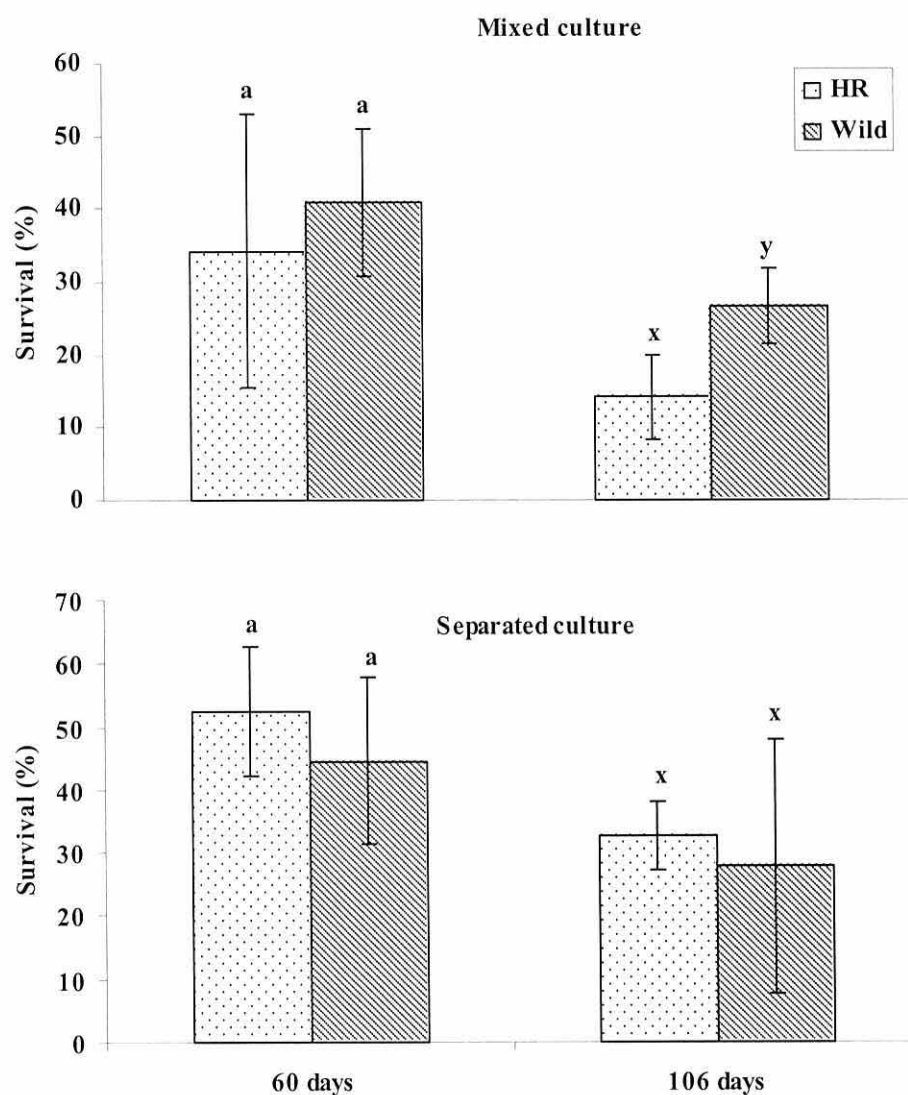
Figure 11: Experiment 2: Mean survival (±SD) of hatchery-reared and wild crabs stocked in mixed and separate ponds. Different letters indicate significant difference (p=0.05).
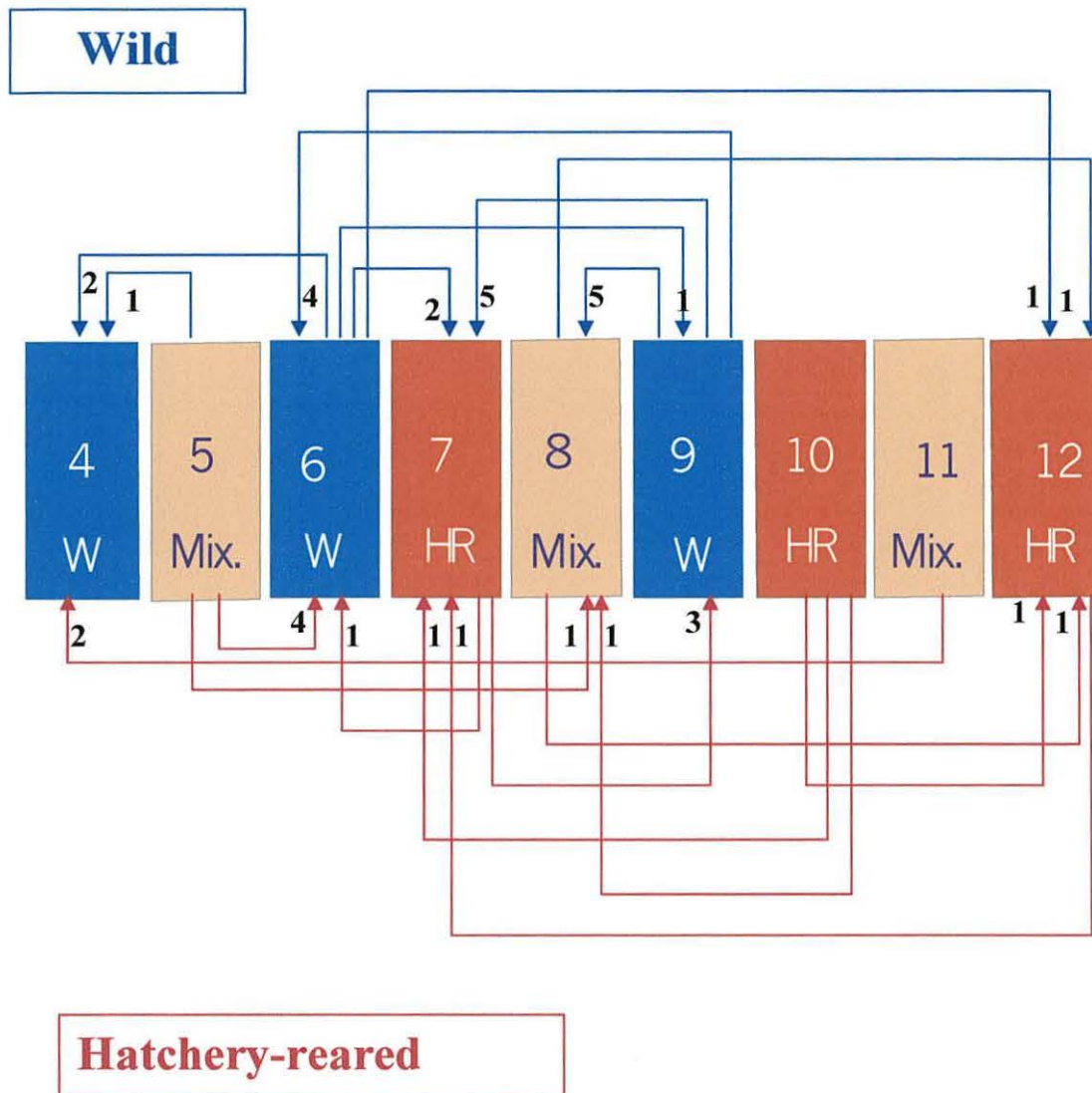
Figure 12: Experiment 2: Diagram showing the intermixing route of crabs in the system. Blue lines on top indicate movement route of wild crabs, red lines underneath show movement route of hatchery-reared crabs. Pond number 4, 6, 9: separated wild crabs; 5, 8, 11: mixture of HR and wild; 7, 10, 12: separated hatchery-reared crabs. Arrow with a number by side indicates number of crabs had moved into that pond.

DISCUSSION

Using the tagging methodology developed previously, growth rate and survival of hatchery-reared crabs have been compared with that of the wild conspecifics stocked in the mixed and separate ponds. However, there is some contradiction in the performance of H-R crabs between the two experiments. In the first experiment, growth rate and survival of hatchery-reared crabs were found to be significantly lower than that of wild conspecifics stocked in the same ponds. In the second experiment, the reverse was true, both in the mixed and in separate ponds, although survival of H-R crabs was slightly lower than that of wild crabs in the mixed culture. This contradiction may be due to several factors including environmental differences, differences in nursery culture, or batch to batch variation in both wild and H-R crabs.

Inferior fitness of H-R animals compared to the wild conspecifics has been recently widely documented (Finstad and Heggberget, 1993; Stoner, 1994; Stoner and Davis, 1994; Berejikian, 1995; Kellison *et al.*, 2000).

Several factors may have resulted in the inferior performance of the H-R crabs observed in the first experiment. The difference in prestocking condition (weight/CW ratio), where H-R crabs of comparable CW were significantly lighter, may be symptomatic of the deficiencies in hatchery-raised animals. Similarly, H-R queen conch were found to have thinner shells, shorter spines and lower survival rates compared to wild conch (Stoner & Davis, 1994). Hence the initial condition of H-R crabs may have had important effects on the ability of these crabs to compete for food and resist cannibalism, which is a common problem in mud crab aquaculture (Triño *et al.*, 1999). Wild *Oncorhynchus mykiss* (Berjikian *et al.*, 1996) are known to be more aggressive than H-R fish, and Mork *et al.* (1999) reported size-related aggression in *Salmo salar*. Olla *et al.* (1994) suggested that H-R fish may be unfit to compete in the natural environment. However, the larger weight/CW ratio noted at the outset in the wild crabs may be due to the fact that they were more advanced in the moult cycle rather than an indication of condition. However, the first moult of H-R crabs was not significantly later, and on average they moulted 7 days earlier. Interestingly, by the end of the experiment (79 days) H-R crabs of CW 68.3mm had attained the same

weight/CW ratio (9.06g/cm) as that attained in 45 days by wild crabs of comparable size (CW 67.9mm) suggesting an improvement in condition. In the second experiment, although starting from a lower W/CW ratio, H-R displayed a better growth rate than wild crabs. Thus, pre-stocking condition (weight/CW ratio) may not be a major problem affecting the performance of H-R crabs.

Another reason for reduced performance of H-R crabs may be that they were certainly not exposed to the diverse diet experienced by the wild conspecifics. Early nutritional history has been shown to be important on the subsequent growth of the lemon sole, *Microstomus kitt* (Howell, 1994). Baxter (1976) suggested that the reduced musculature of H-R animals due to captivity and poorer diet might reduce the efficiency of the escape response when threatened by predators. Furthermore, a reduced ability to recognize foods in the new habitat may have resulted in poor growth in H-R conch (Stoner & Davis, 1994). In addition, once in the natural environment H-R fish are exposed to predation pressure and need to compete against wild conspecifics to grow (Tsukamoto *et al.*, 1989). Hines (1986) also demonstrated that smaller animals are probably weaker competitors for food and refuge, therefore their chances for growth and survival are consequently limited. In the pond, the weaker crabs may have had difficulties competing for food. However, when food competition and predation were eliminated in the basket trials, H-R crabs grew significantly faster than wild crabs, even though not as fast as overall growth of free-ranging animals. In the second experiment, H-R crabs grew significantly faster than wild crabs, when stocked separately. Even in mixed ponds, they were able to compete with wild crabs, and as a result they grew as well (in weight) and even better (slightly significant higher in CW, p=0.03) than wild crabs.

Hatchery conditioning may result in the modification of behavioural patterns due to deprivation in the rearing environment. Stoner & Davis (1994) found reduced burial frequency in H-R conch, while H-R sole showed both reduced burying efficiency and reduced pigmentation adaptation rates (Ellis *et al.*, 1997). The conditions that the H-R topshell, *Trochus niloticus* experience in the hatchery can affect their vulnerability to predators (Crowe *et al.*, 2002). Slower responses to predators in H-R red abalone compared to wild ones was also suspected to be due to hatchery conditions (Schiel and Welden, 1987). Similarly, H-R red drum, *Sciaenops ocellatus*, school in open

water rather than profiting from any available cover like their wild counterparts (Stunz & Minello, 2001). More importantly, Gebauer *et al.* (1999) discovered that metamorphosis of an estuarine grapsid crab larva, *Chasmagnathus granulate*, is highly dependent on chemical and physical cues. In the absence of specific chemical and physical cues from the adult environment and muddy substrate, delayed metamorphosis occurs which also affects the later stages. Consequently, fitness of crab juveniles may be reduced as intermoult duration is longer and due to their smaller size, animals may be more vulnerable to predators.

The improved performance of H-R crabs in the second experiment may have resulted from conditioning during the nursery period, which was different for the two batches of H-R juveniles, prior to stocking in the ponds. Stoner & Davis (1994) and Price (1999) suggested that the problem of H-R crab fitness could be to some extent mitigated by conditioning the animals to natural conditions prior to their release. The different substrates/shelters used during the nursery phase in the two experiments in the present study may have resulted in different behaviour. In the first experiment, crabs were reared for the first few days on a sand substrate, where they may have been subjected to more cannibalism or predation. Dittel *et al.* (1996) found extremely high mortality in the Atlantic mud crab *Panopeus herbstii* settling on sand substrate. A similar phenomenon was also reported for postlarvae of the lobster, *Homarus americanus*, by Barshaw *et al.* (1994). In addition, the crabs were further reared individually in jars, so they were not exposed to the experience in avoiding enemies. Once released into the ponds, without availability of shelters, they may have inevitably been cannibalized by their stronger conspecifics. In contrast, in the second experiment crab juveniles were derived from a batch that was reared in ponds with bricks and shells as shelters. They may have experienced how to avoid encounters with predators. Therefore, in the ponds with shelters used in Experiment 2, H-R animals may have managed to avoid predators to some extent. In addition, during the nursery phase of the second experiment weaker crabs may have been eliminated by the remaining stronger animals. In the first experiment this naturally selective process did not occur as crabs were reared individually in cages. This may be in agreement with Huntingford and Wright (1993) and Fuiman and Magurran (1994) cited by Stunz and Minello (2001) who reported that development of predator-avoidance behaviour in fishes appears to be linked to early life experiences. On the other hand, juvenile

crabs in the second trial were reared in complete pond water, and the potential delay in metamorphosis caused by lack of chemical and physical cues (Gebauer *et al.*, 1999) may be less likely.

Rhodes and Quinn (1999) reported that H-R salmon have been found to be inferior to wild fish in some studies but competitively superior in others. In the recent study on juvenile Coho salmon, Rhodes and Quinn observed the hatchery-reared fish grew at faster rates than wild fish. This finding may support the results of the present study that fitness or performance of H-R crabs was inferior in the first experiment but was superior to the wild conspecifics in the second trial, as a result of batch to batch variability in H-R juveniles. In the first experiment H-R juveniles were obtained originally from central Vietnam, where the environmental conditions may be different to that in the experiment ponds. Although the hatchery technique used was similar for the two batches, exact details are unknown for larval stages of the first batch. The second batch of juveniles was produced from local spawners and the larvae were permanently fed with HUFA-enriched diet during their development stages. An important role of HUFA in performance of larvae and later stages has been widely documented in fish and crustaceans. Hongbo *et al.* (2000) found the highest survival in the larvae of the Chinese crab, *Eriocheir sinensis* fed with HUFA- enriched rotifers. Howell and Baynes (1993) pointed out that use of sub-optimal diets, particularly in relation to lipid, may have an impact on survival of released fish by reducing their tolerance to stress. Differences in hatchery conditioning including nutrition and rearing techniques may have resulted in discrepancies in behaviour and potential competition winners. On the other hand, the different origins of wild crabs may also be possible causes of differences between the experiments. In the first trial, wild crabs were obtained locally from the study area, while H-R crabs originated from an ecologically different environment. In the second trial, the opposite situation was true as wild crabs were caught in an estuary where environmental conditions were different from that in the coastal study area.

The effect of competitive interaction was very obvious in the first trial. Growth in H-R crabs was depressed by the stronger competitive wild counterparts. In the second trial, survival of hatchery-reared crabs was lower, although they grew faster. However, in non-competitive conditions, H-R crabs survived and grew significantly

better than the wild crabs. Genetic effects as reported by Penman & McAndrew (1998) may be an additional factor. Although the H-R stock is from wild broodstock, genetic diversity may be reduced. In addition, wild caught juveniles will have already undergone a process of natural selection, so that survivors are better able to compete for food and avoid predation.

The reason the survival rate for the wild crabs in the second trial was lower than that in the first trial (27.9 % compared to 39.5%, respectively) has been indicated in the results section. In general, this survival rate was much lower than that reported by Triño *et al.* (1999) (98% in *S. serrata* and *S. tranquebarica* raised at similar density). The first trial in the present study used bare earth ponds, unfertilised and without *Gracilaria bailinae* or shelters which Hill *et al.* (1982) regard as important in mud crab culture. However, survival was not improved when using shelters in the second trial. The differences in survival may therefore be related to interspecific differences and future research will concentrate on productivity differences between species. In addition, growth rate is also different between species (see below).

The growth rate observed in *S. paramamosain* in the present study was 3-5 times higher than that of other *Scylla* species studied by Hill (1975), Triño *et al.* (1999), Triño *et al.* (2001) and Triño and Rodriguez (2002). Details of differences in growth rate between these species have been indicated in Chapter 5. Differences in growth rate in *S. paramamosain* cultured in ponds, mangrove, and between wild and hatchery-reared crabs are tabulated in Table 4.

In summary, in the first experiment hatchery-reared crabs were inferior to wild-caught conspecifics in terms of growth and survival. Their condition improved throughout the trial, but it is not clear whether this was due to selection through predation or a general improvement in condition. In the second trial, fitness of H-R crabs proved to be significantly better than the wild conspecifics. Although several factors may be causing the differences in performance between H-R and wild crabs, hatchery and nursery conditioning play an important role in improving fitness of H-R crabs. Further studies are needed to clarify the extent of any effect of spawning batches and ecologically different origins on their performances. The results have made a promising in the production of hatchery-reared crabs for pond culture and stock

enhancement objectives. However, more work is also needed to improve hatchery techniques, nutrition, and broodstock husbandry to ensure reliable production of good quality crabs. In addition, further studies on the effects of broodstock source and nutrition, larval nutrition, nursery nutrition and environment are also required. Investigation of potential genetic effects of use of single spawners to produce each batch of crabs should also be undertaken.

GENERAL DISCUSSIONS AND CONCLUSIONS

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Fishery and seasonal abundance and recruitment of *Scylla paramamosain*

Monthly sampling for size frequency carried out over two years at a crab agency in the estuarine study area has indicated that of the mud crabs, *S. paramamosain* is the predominant species in this area of the Mekong Delta, as has recently been confirmed by Macintosh *et al.* (2002). Although freshwater conditions dominated during the period of the monsoon season, with salinity as low as 0ppt, *S. paramamosain* was still found in high numbers throughout this period. The persistence of *S. paramamosain* recruitment during the rainy season is consistent with some previous studies, but may indicate habitat specificity and environmental tolerance in this species.

The lack of modal progression in the size frequency data recorded during a year has indicated continuous recruitment of young crabs to the population in the mangrove. The discrepancies in male to female ratio in different seasons may refer to spawning migration as the ratio was higher in the dry season, but remained lower during the monsoon period.

The pattern of size-composition recorded over three years shows the difference in population structure between crabs collected in the mangal and the estuary. Abundance of small crabs from intertidal fisheries coupled with continuous loss of large crabs from the intertidal population suggests that the mangrove is acting as a nursery. The standardized, non-selective, hand-fishing method used in the intertidal study area has proved to be a powerful tool in studying seasonal relative abundance and long-term trends in the crab population. It enabled calculation of reliable CPUE data which again indicates the persistence of populations of *S. paramamosain*, despite the large seasonal salinity variation. However, smaller crabs may be less tolerant of low salinity and this needs to be further investigated.

Salinity tolerance of juvenile *S. paramamosain*

Although juvenile crabs were found to be abundant throughout the year, including periods of freshwater, they were able to survive in 0ppt only for about 1 week under laboratory conditions. At low salinity (i.e. 5ppt) growth rate of crabs was retarded and mortality increased. Moult intervals were also increased at decreased salinities. The persistence of crabs during the freshwater periods in estuarine conditions may be related to preference of substrates and adaptation behaviour including burrowing or short-term subtidal migration. The results from nitrogen excretion studies in the laboratory may further elucidate the ability of crabs to tolerate freshwater in the wild. Chen and Chia (1996) found nitrogen excretion increased with decreased salinities and high nitrogen excretion may impair growth and survival of crabs in confined conditions in the laboratory.

The most preferable salinities for growth and survival of mud crab juveniles *S. paramamosain* have been found to be from 15-20ppt. This is consistent with the peak abundance occurring in the wild, when salinities rise up to 15-20ppt during the dry season in March-May. Any stock enhancement of *S. paramamosain* should be carried out during this suitable period of the year.

Tagging technique for *S. paramamosain* for population studies and stock enhancement

Comparison between tagged and non-tagged crabs has indicated that tagging does not affect survival and growth of crabs. Using microwire coded tags, juveniles of mud crabs *S. paramamosain* at a size as small as 20 mm CW can be tagged successfully. The ideal position for inserting the tags has been found to be in the coxal muscle of the fourth pereiopod under the thoracic sternite. Keeping reference or archive tags allowed the identification of individuals, providing valuable information on the specific growth rate of known crabs. The present tagging method also allows use of single replicates in growth studies eliminating possible pond effects as may always occur in multi- replicate experiments. Using this approach the animals are stocked in the same environment and differences in growth or survival caused by environmental factors may be reduced. In conclusion, the microwire tagging technique has been

demonstrated to be suitable for studying population dynamics of *S. paramamosain* in the wild, and for pond growth experiments. The technique can be applied to study growth and survival of H-R crabs under natural conditions. However, crabs should be reared to the size (i.e. 20 mm CW) suitable for tagging through the nursery phase.

Nursery of hatchery-reared crabs

Cannibalism is usually a barrier to production of crabs during the nursery phase. Reducing cannibalism or predation of crabs may be achieved by application of appropriate shelters or substrate in the rearing environments. In the present study, bricks were found to be better shelters than sand as survival of crabs was higher with bricks than in sand. However, further investigation of suitable shelters to support economic effectiveness need to be carried out. Shrimp flesh has been observed to be the more preferable diet for *S. paramamosain* juveniles than fish during the nursery period. Locally cheap diets including snails or home made pellets should be also further tested to reduce production cost. There was no significant difference between three tested stocking densities, indicating there are possibilities for increase in densities during the nursery phases. Investigation of upper threshold stocking densities for juveniles *S. paramamosain* should be further undertaken. The present study has shown that juveniles can reach 20 mm CW within one month in nursery conditions and they are then suitable for tagging.

Mark-recapture study of *S. paramamosain* in an estuarine environment in the Mekong Delta

Using the previously developed tagging method, growth of *S. paramamosain* has been for the first time determined under natural conditions. Growth of crabs in the mangrove was similar to that of crabs stocked in ponds, indicating foraging time and food availability are not limiting factors on growth in free-ranging animals in the wild. Consequently, stock enhancement of this species may be feasible, as crabs of 2-3 cm CW may be expected to reach adult size within only 3-4 months after release into natural habitats.

Using characteristics described by Ong (1966) coupled with ageing by individual tag retrieval, size at puberty of female *S. paramamosain* has been determined as fairly close to that estimated by Overton and Macintosh (2002) in *S. paramamosain* and *S. olivacea* in Ban Don Bay, Thailand. However, size at maturity is smaller and time to reach maturity is shorter than in the *Scylla* species studied by Ong (1966). The results also reconfirmed the higher number of males than females, as a possible consequence of offshore migration of females.

The use of the tagging technique coupled with the standardized, non-selective fishing method has enabled estimation of population of *S. paramamosain* without bias from trapping. The application of a Petersen model yielded an estimate of 0.05 crabs m^{-2} or 1 crab per 20 m^2 with a high annual recruitment in the study area. Recruitment of *S. paramamosain* in the present study was estimated using tag return data in combination with CPUE data, indicating a peak recruitment of juveniles during the dry season coinciding with the high salinity period, reaching over one million juveniles per month. Abundance and recruitment of crab juveniles in the study area indicate a healthy crab population, with high levels of natural recruitment throughout the year.

The high monthly recruitment observed in 2000 with a sharp peak in March of 1,269,809 $month^{-1}$ and falling to 114,512 $month^{-1}$ in November, indicates that a restocking program in this area may be unworkable. Otherwise, a huge number of hatchery-reared crabs should be produced to make a significant difference to the already substantial natural influx of recruits.

Tag return data has also enabled estimation of fishing and natural mortality of the *S. paramamosain* population in the estuarine study area. The results indicate that females were subjected to a higher natural mortality and may suggest losses through spawning emigration of mature females. Fishing mortality was found to be relatively lower than natural mortality indicating the crab population is not limited by fishing pressure. However, fishing should be undertaken with caution to maintain a stable population as the total mortality was estimated to be 30 times higher than that of temperate crab populations studied by Melville-Smith (1988).

Fitness of hatchery-reared crabs

Can hatchery-reared crabs compete with wild crabs in order to grow and survive well in the natural conditions? In the present study, although hatchery-reared crabs in the first experiment were less fit than wild crabs, as seen in lower growth rate and survival, hatchery-reared animals in the second experiment displayed a significantly better performance than that of the wild conspecifics. The inferior performance of hatchery-reared crabs may be related to several factors including hatchery pre-conditioning, nutritional status or genetic effects. However, the different results in two experiments in the present study may be a consequence of different sources of broodstock and rearing techniques. Hatchery-reared crabs in the first experiment originated from a different environment, and they may not have been reared with a nutritionally sufficient diet (enriched HUFA rotifers) as those crabs in the second experiment. HUFA and optimal diet in relation to lipid have been reported to be crucial for larval stages of many species (Howell and Baynes, 1993; Hongbo *et al.*, 2000). Hatchery conditioning may result in the modification of behavioural patterns due to sensory deprivation in the rearing environment and may subject the hatchery-reared animals to higher risks of predation when first released into the natural conditions (Schiel and Welden, 1987; Stoner & Davis, 1994; Stunz & Minello, 2001; Crowe *et al.*, 2002). However, Stoner & Davis (1994) and Price (1999) suggested that the problem of H-R crab fitness could be to some extent mitigated by conditioning the animals to natural conditions prior to their release. In the first experiment hatchery-reared crabs were reared individually in jars, thus they had no experience in protecting themselves or avoiding predation. In the second experiment H-R crabs may have been trained by rearing them in the environment with shelters prior to stocking in ponds. Furthermore, through the nursery phase, hatchery-reared crabs in the second batch were reared in groups, and consequently the surviving stronger animals were chosen for comparison with the wild conspecifics. However, improvement of nutritional aspects and hatchery rearing technique should be also taken into account to produce good quality seed for stock enhancement and for pond culture.

Feasibility of stock enhancement of *S. paramamosain*

Successful stock enhancement of a certain species may depend on several factors, including recruitment of the species, fitness (growth and survival) of H-R animals and their potential for large-scale production. The results from the present study indicate the feasibility for stock enhancement of mud crabs *S. paramamosain* in the Mekong Delta. Growth of crabs, under natural conditions, has been determined to be as fast as those cultured in ponds, so that they can attain marketable size within 3-4 months after release. The nursery systems developed can be used to produce a high number of H-R crabs for stock enhancement. Furthermore, after the nursery phase only the surviving strong crabs remain making stocking more feasible. Fitness of H-R crabs was superior to their wild counterparts, although it was variable from batch to batch. This indicates that H-R crabs may grow as well as wild crabs in natural conditions once released. However, assessment of H-R crab fitness and their competitive capacity with wild conspecifics under natural conditions should be further investigated. Results from salinity tests also indicated that crabs can be released during the dry season when salinity is suitable for their growth in the estuary. Although, the good growth rate observed under natural conditions, coupled with the improved fitness of H-R *S. paramamosain*, and the promising production of crab seeds from the nursery has indicated that stock enhancement of this species in the Mekong Delta is feasible, the high monthly recruitment of this species in the study area has made restocking program unworkable at this site. Selection of an area where crab recruitment is limited may enable a realistic assessment of the feasibility of stock enhancement using release of H-R juveniles. In the future, the tagging method will be used in mark-recapture studies to determine recruitment of *S. paramamosain* in areas where abundance of crabs is considered limited by recruitment, prior to evaluation of stock enhancement by release of tagged H-R juveniles. Tagged juveniles from the same batch should be kept in ponds to monitor survival and growth.

CONCLUSIONS:

> ➢ Stock enhancement of mud crabs is technically feasible, but is unnecessary at the study area as natural recruitment is very high.

> ➢ It could be worthwhile in other coastal sites where the mud crab population has become depleted or where natural recruitment is very poor or doesn't occur. However, the "ownership" of stock enhanced sites would become a potential management issue.

> ➢ Overall, based on the results obtained, it is more feasible to use hatchery-reared crab juveniles for aquaculture because (a) good growth rates in ponds, (b) recovery rate of stocked crabs can be much higher from ponds, and (c) problem of "ownership" would not be the case.

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