A STUDY OF THE EFFECTS OF ALKALINITY AND TOTAL HARDNESS ON POSTLARVAE AND JUVENILES OF THE GIANT FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN).

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TO

THOSE

WHO PRODUCE PRAWNS

DECLARATION

I declare that this thesis has been composed by myself and that it embodies the results of my own research. It has neither been submitted nor accepted for any other degree.

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ABSTRACT

The effects of environmental water hardness and alkalinity on the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) were investigated.

Preliminary studies indicated significant interaction effects between alkalinity and hardness and also that the ratio of calcium to magnesium ions in the water was important for growth. At moderate alkalinity a Ca:Mg ratio of 1:1 allowed better growth than ratios of 4:1 and 1:4.

In a series of experiments made with postlarval and juvenile prawns, the levels of alkalinity and hardness in the test waters were adjusted separately so that the influence of each factor could be determined independently as well as in combination. Data on aspects of survival, growth and carapace mineralization were recorded.

The results showed that, in contrast to some reports, high water hardness did not necessarily constrain performance provided alkalinity was low (25mg l^{-1} as $CaCO_3$). On the other hand, high alkalinity (>100mg l^{-1}) caused a number of problems. These included increased mortalities (particularly when hardness was low, 20mg l^{-1}), an increased incidence of a pathological condition called "white muscle syndrome", a reduced size increment achieved at moult while, paradoxically, increasing the moulting frequency, and an enhanced calcium deposition in carapaces during intermoult. In all experiments, postlarvae were more sensitive than juveniles to

adverse combinations of alkalinity and hardness. This feature is discussed in relation to Asian prawn farming practises.

The activity of dephosphorylating enzymes in gills from immediate postmoult and from intermoult prawns exposed to low and high alkalinity was measured. The activity was maximal at both low and high alkalinity in postmoult prawns i.e. during the time of expected maximum calcium uptake, and significantly lower in intermoult prawns but only when alkalinity was low. At high alkalinity, activity in intermoult prawns remained at postmoult levels suggesting that this activity was associated with the increased calcium deposition observed in intact animals held in high alkalinity waters.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Distribution

The genus *Macrobrachium*, a member of the Palaemonidae, comprises approximately 125 species which are found in tropical and sub-tropical environments worldwide (Holthuis 1949, 1950a, 1950b, 1980). There is a wide interspecific variation in maximum size and growth rate, and *Macrobrachium rosenbergii* (de Man) is one of the largest species known (New and Singholka 1982). *M. rosenbergii*, also called the giant freshwater prawn, is widely distributed in most of the tropical and sub-tropical areas of the Indo-Pacific Region, including Bangladesh, Burma, Cambodia, India, Indonesia, Malaysia, Pakistan, Philippines, Thailand and Vietnam (Ling 1969). It lives in a wide variety of habitats like rivers, lakes, water reservoirs, irrigation canals and even some paddy fields which have direct or indirect access to the rivers. It can be found at least 200 Km from the coast but it is also found in brackish waters where breeding females release their eggs. Postlarvae may be more abundant in the lower reaches of rivers which are influenced by tides.

1.2 Life Cycle

There are four distinct phases in the life cycle of *M. rosenbergii*, namely egg, larva, postlarva and juvenile/adult. Under natural conditions mating of *M. rosenbergii* occurs throughout the year, though peak periods relate to local environmental conditions. Females spawn eggs which are fertilized immediately by the sperm released and deposited externally by the male during copulation. The fertilized eggs are incubated on the female's abdomen where they remain for about 19-21 days. Eggs will hatch both in fresh and brackish waters. The larvae which hatch in freshwaters, however, will die unless they reach brackish water within a few days. Larvae pass through 8 (Ling 1969) to 11 (Uno and Soo 1969) distinct stages before metamorphosis. Stage I larvae can survive for five days in freshwater and live on yolk reserves until they moult into stage II larvae and start to feed on planktonic food (Ling 1969). In a natural brackish water environment the larvae feed principally on zooplankton, very small worms and the larvae of other aquatic invertebrates (New and Singholka 1982). On completion of their larval life (20-40 days) metamorphosis into postlarvae occurs and the prawns begin to migrate upstream into freshwater. These postlarvae are omnivorous and feed on aquatic worms, insects, insect larvae, small molluscs and crustaceans, flesh and offal of fish and other animals, grain, seeds, nuts, fruits, algae, tender leaves and stems of aquatic plants (Ling 1969). They will live and mature in freshwater and berried females will migrate to estuarine water to ensure better survival of their offspring.

1.3 Importance as an Aquaculture Species

Following the elucidation and control of the life cycle of *M. rosenbergii* (Ling 1969) and the subsequent development of mass rearing techniques for larval production (Fujimura and Okamoto 1972), interest in *M. rosenbergii* culture became wide-spread and this species is now farmed in significant quantities in many countries outside its natural habitat range (Table 1.1).

The expansion of freshwater prawn farming started in Asia and later, in Latin America. The lion's share of the global production figures of freshwater prawns comes from the Asian countries. The following production figures were taken from New (1990). World production of *M. rosenbergii* is about 27000 metric tonnes year^{-1}, of which Thailand 44%, Vietnam 32%, and Taiwan 17% are the main producers. Apart from these three countries only Brazil, the Dominican Republic, Mexico, the French Overseas Territories, and perhaps Puerto Rico each produce more than 100 tonnes annually. Production of *M. rosenbergii* in other countries are Japan 55 tonnes, Mauritius 35t, Jamaica 20t, and Polynesia 20t annually while many other countries produces less than 20t in a year.

M. rosenbergii possesses a number of attributes which contribute to its importance as an aquaculture species. Sexual maturation and gonadal developments readily take place in captivity throughout the year and artificial breeding is thus made easier. It has a larger size and better growth compared to other freshwater prawns and

Table 1.1 Countries where production of *M. rosenbergii* were reported (Source, New 1990).

Country	Annual production (metric tonnes)	Year	Remarks
Africa:			
La Reunion	7	1987	
Malawi	8	1987	
Mauritius	35	1987	
Zimbabwe	12	1987	
Total in Africa	62		
Asia:			
Bangladesh	+		production data from Bangladesh and India are not separated from other crustacea
India	+		
Israel	10	1987	
Japan	55	1987	
Malaysia	5	1987	
Taiwan	4500	1988	
Thailand	11839	1987	
Vietnam	8600	1987	
Total in Asia	25009		
Latin America and the Caribbean:			
Brazil	1000	1987	
Costa Rica	6	1987	

Country	Annual production	Year	Remarks
Dominican Republic	186	1987	
El Salvador	1	1987	
Guadaloupe	52	1988	
Guatemala	13	1987	
Guyanne	63	1988	
Honduras	10	1987	
Jamaica	20	1987	
Martinique	52	1988	
Mexico	361	1987	
Panama	7	1987	
Peru	10	1987	
Puerto Rico	93	1987	
Venezuela	1	1987	
Total in L.A. + Carib.	1875		
North America:			
U.S.A. + Hawaii	91	1987	
Total in North America	91		
Oceeania and Pacific:			
New Caledonia	1	1988	
Polynesia	20	1988	
Solomon Islands	6	1987	
Total in Oceania & Pacific	27		
GLOBAL TOTAL	27064		

is omnivorous in its feeding habit. It is extremely tolerant of a wide range of water conditions and has considerable disease resistance. *M. rosenbergii* can be farmed in ponds, lakes, canals and even in integrated systems with rice farming. Polyculture with other species such as carp, tilapia and catfish has also been attempted (Rouse and Stickney 1982, Behrends *et al.* 1985 and Pavel *et al.* 1985). Thus *M. rosenbergii* has become a popular candidate for aquaculture in tropical and sub-tropical countries which have large freshwater resources, and where commercial production of healthy postlarvae and juveniles exists.

1.4 Culture Methods

1.4.1 Hatchery Operation

Most hatcheries rely on berried females captured from farm ponds rather than from the wild (New 1990) and separate brood stocks are rarely maintained except in Taiwan (Hsieh *et al.* 1989). Many Hawaiian hatcheries stock first-stage larvae at 60 l^{-1} (although some practise two-stage rearing with an initial stocking density of 160 l^{-1}). Thai hatchery management results in a stocking density of 30-50 l^{-1}. Hawaiian hatcheries expect 50-70% survival to achieve a postlarval production of 30 l^{-1}. Intensive prawn hatcheries both in French Overseas Territories and in French-inspired hatcheries elsewhere stock 100 l^{-1} (Palanisamy 1989). Taiwanese inland hatcheries often use a multistage hatchery system with initial stocking rates of 300-1000 l^{-1} in

500 litre tanks, and transfer the larvae to larger tanks as the larvae grow (Hsieh *et al.* 1989). Larvae are generally reared at a salinity range of 10-15‰. Optimum temperature range is 26-31°C; temperatures below 24°C or above 35°C cause retarded development or mortality. Taiwanese hatcheries are reported to rear larvae at 30-33°C (Hsieh *et al.* 1989). Other desirable water quality criteria include pH 7.0-8.5, maximum nitrite (NO_2-N) and nitrate (NO_3-N) levels of 0.1 and 20mg l^{-1} respectively, maximum total hardness of 100mg l^{-1} as $CaCO_3$ and low iron and manganese content (New 1990).

Coastal hatcheries usually mix filtered seawater with well water or dechlorinated tap water. Inland hatcheries in Thailand operate by trucking seawater, brine solution or rock salt to the site and then diluting with freshwater (Tunsutapanich 1981).

Ammonia toxicity levels in larval rearing waters can be reduced by daily water change (50% day $^{-1}$). Recirculation systems for prawn hatcheries have also been devised (Singholka and Sukapunt 1982, Justo and Ochoa 1983, Ong 1983, Soe 1989, Tien *et al.* 1989) but are normally only found in commercial scale operations in French-owned or designed hatcheries (Griessinger *et al.* 1989).

Most commercial hatcheries combine feeding with a "green-water" or a "clear-water" rearing system. In the "green-water" system an algal bloom is encouraged to maintain good water quality, but mortalities are often greater in the green-water systems particularly due to the high pH associated with the algal bloom (Hummel *et*

al. 1989, Hudon *et al.* 1989). Few Thai and French hatcheries use this system (New 1990) but Taiwanese hatcheries maintain a balanced ecosystem of culture water which includes algae (Hsieh *et al.* 1989). The clear water system frequently relies on recirculating water through a water treatment facility.

Prawn larvae must eat continuously to survive but do not actively search for food (Moller 1978). Various live and inert feeds are used in different hatcheries. Newly hatched brine shrimp nauplii (*Artemia*), fish flesh, mussel flesh, squid flesh and egg custard are frequently used. *Artemia* nauplii are maintained at 1 nauplii ml^{-1} and increased to about 5 ml^{-1} while feeding in Thailand but Hawaiian hatcheries tend to use 5-15 nauplii ml^{-1} (New 1990). Modifying the fatty acid content of *Artemia* nauplii has proved effective (Sorgeloos *et al.* 1986) and has led to specific *Artemia* enrichment diets suitable for prawn, shrimp and fish larvae.

Postlarval production rates have been reported as 10-20 l^{-1} in Thailand, 30 l^{-1} in Hawaii (New 1990) and by using intensive, antibiotic-aided techniques in French hatcheries production of up to 50 l^{-1} has been achieved (AQUACOP 1977).

1.4.2 Nursery and Grow-out System

Many farmers stock 1-4 week-old postlarvae directly into rearing ponds while others use an intermediate 1-3 month nursery phase with stocking densities from 350

$sq.m^{-1}$ (Alston 1989) to 1500 $sq.m^{-1}$ (New 1990). Nursery reared juveniles (0.5-2.0g) are then stocked into rearing ponds. The use of a nursery phase is particularly important where climatic conditions or intermittent water availability prevents continuous culture to market size.

The heterogeneous growth exhibited by populations of *M. rosenbergii* has led to two basically different stocking and harvesting strategies being used; a) batch stocking and harvesting. b) continuous stocking and cull harvesting. In Thailand initial stocking rates vary from 5-10 prawns $sq.m^{-1}$ for ponds to be batch-harvested within 8 months to 16-22 $sq.m^{-1}$ for ponds operated within a continuous cull-harvesting system (New 1990). Similar stocking rates for continuous culture in Martinique (IFREMER 1989) have been reported where a stocking rate of 18 prawns $sq.m^{-1}$ was split into 9:4:5 four-monthly batches in the first and 6:6:6 in the second year. D'Abramo *et al.* (1989) stated that stocking rates of <4 prawns $sq.m^{-1}$ were the most economically attractive in temperate zones where mean final weight would be greater while reducing stocking and feeding costs. New (1990) reported that some Thai farms use a 2.5-3 months nursery phase, stocked at 20-25 prawns $sq.m^{-1}$ followed by restocking at 3-5 prawns $sq.m^{-1}$ in a 3-month grow-out phase. Cull-harvesting starts 5.5-6 months after initial stocking and is repeated every 2-4 weeks in continuous systems. Restocking happens once a year in Thailand and the number restocked is normally twice the number harvested.

The diet of *Macrobrachium* includes insects and larvae, algae, nuts, grains, seeds, fruits, small molluscs and crustaceans, fish flesh and offal of fish and other animals. Enzyme studies have confirmed their omnivorous feeding habit (Lee 1980). They will readily accept compound pelleted feeds but will also be cannibalistic. The protein content of commercial prawn pellets have been reported as 23.8-38.5% in Hawaii (Corbin *et al.* 1983), 22-30% in Thailand (New 1990), 28-36% in Taiwan (Hsieh *et al* 1989) and 25-30% in French Guiana (IFREMER 1989). However, studies on concrete ponds (Boonyaratpalin and New 1982), asbestos asphalt or earthen bottom ponds (Bartlett and Enkerlin 1983) and aquaria (Antiporda 1986) have produced satisfactory growth at protein levels as low as 14%. However, under laboratory conditions, optimum dietary protein levels of 30-35% have been demonstrated (D'Abramo and Reed 1988, Fruechtenicht *et al.* 1988). New (1976) suggested that prawns are able to utilize complex carbohydrates better than simple ones like glucose.

Prawn production in earthen ponds in Thailand averaged more than 1006kg ha^{-1} in 1987 (New 1990) but average productivity was greater in Hawaii at 1449kg ha^{-1} in 1984 (Lam and Wang 1986). The growth rate of individual *M. rosenbergii* is rapid in tropical environments. Increases of 100g in stagnant earthen ponds in 9 months, 120g in 7 months in running-water cement ponds in India (Panicker and Kadri 1981); and average weights of 142g after one year have been reported in Bangladeshi ponds (Khan *et al.* 1980). Experiments in U.S.A. (Willis and Berrigan 1977) showed that, in earthen ponds at 27°C (20.5-30.5°C), 0.8g juveniles reached 43g in 167 days while 0.06g postlarvae reached 28g in 170 days at 79% and 88% survival rates, respectively.

Postlarval *M. rosenbergii* are tolerant of a wide range of salinity and temperature (Smith *et al.* 1982); despite this, nearly all commercial farming of *M. rosenbergii* takes place in freshwater environments. Temperatures below 14°C or above 35°C are generally lethal, 29-31°C being optimal (New 1990). Rearing temperatures of 18-22°C have been reported to markedly stunt growth and predispose prawns to idiopathic muscle necrosis (Akiyama *et al.* 1982). Dissolved oxygen levels below 1.0mg l^{-1} can be tolerated for short periods (Avault 1987) but levels below 25-30% saturation cause visible distress; therefore pond managers prefer to maintain 6-8mg l^{-1} dissolved oxygen.

A pH range of 7.0-8.5 is reported to be optimal for prawn culture (New 1990). In some areas, for example parts of Malaysia, water hardness is low and lime is applied to prawn ponds. Many farmers lime their ponds principally to disinfect the pond bottom and release the nutrients from the bottom soil. Even so, liming increases the total hardness and alkalinity of water. There are few reports of scientific experiments to determine optimum liming rates in prawn farms. Kongkeo (1990) suggested treating marine shrimp ponds thoroughly with dissolved lime at the rate of 200-300kg ha^{-1} for intensive culture of *Penaeus monodon* in Thailand. In Malaysia liming is practised at the rate of 700kg ha^{-1} in typical prawn and fish ponds (Ang Kok Jee, Personal communication). In Bangladesh lime is used in an equivalent quantity for carp nursery/rearing i.e. at the rate of 220-230kg ha^{-1} (Personal experience).

Despite the widespread practise of liming (which increases hardness and alkalinity of water), reports on the effects of water hardness on *M. rosenbergii* are controversial. Growth of *M. rosenbergii* have been reported to be inhibited by increased levels of water hardness (Cripps and Nakamura 1979, Howlader and Turjoman 1984, Vasquez *et al.* 1989, Brown *et al.* 1991); while, others have reported that growth of *M. rosenbergii* were not inhibited even by very high levels of water hardness (Heinen 1977, Bartlett and Enkerlin 1983). In the study of Heinen (1977) the survival of postlarval *M. rosenbergii* was not affected by water hardness (10-310mg l^{-1} as $CaCO_3$) while Brown *et al.* (1991) reported adverse effects of increased water hardness on the survival of *M. rosenbergii* (hardness range was 9-326mg l^{-1} as $CaCO_3$). Thus the matter remained unresolved. However Bartlett and Enkerlin (1983) indicated that the higher carbonate in the water of Cripps and Nakamura (1979) was probably responsible for reduced growth rate of prawns, and until recently, almost nothing was reported of the effects of alkalinity on prawns. It is thus necessary to investigate whether the alkalinity of water was responsible for the apparent discrepancies of the effects of water hardness.

1.5 Constraints on *M. rosenbergii* Culture

1.5.1 Hatchery Phase

The larvae of *M. rosenbergii* require brackish water for their survival (Ling 1969)

and the larval life lasts from 18 to 40 days until they metamorphose into postlarvae. While this obligatory period of culture in brackish water is a constraint to the expansion of *M. rosenbergii* (Lee and Wickins 1992), establishment of hatcheries in the coastal belts, and the introduction of recirculation systems in inland hatcheries (where access to brackish water is difficult) using sea salts or concentrated brine, can substantially reduce the problem.

1.5.2 Heterogeneous Growth

At metamorphosis postlarvae are normally of similar size. At each subsequent moult there may be considerable variation in the growth increment that ultimately leads to large size variations in many crustacean species (Hartnoll and Dalley 1981). In *M. rosenbergii* distinct male morphotypes occur and enhance this heterogenous growth pattern through hierarchical, aggressive behaviours. This has caused the development of special stocking and harvesting strategies without which many of the animals harvested would be stunted growers worth little on the market. Stocking of larger juveniles rather than postlarvae facilitates the removal of the slow growers and thus would probably reduce the gap between sizes of animals at harvest. Another way to reduce the heterogeneous growth pattern might involve genetic manipulation but this is a matter of research for the future.

1.5.3 Marketing

While the interest in crustacean farming is usually stimulated by the desire to exploit markets for higher price, the 20% less tail meat in *M. rosenbergii* than penaeid shrimp might be a marketing and economic constraint to *M. rosenbergii* farming. Although markets for the head-on freshwater prawns are long established as a desirable food in many Asian countries (New 1990), the major markets for crustaceans such as Japan and U.S.A prefer penaeids to *Macrobrachium*. The economics of *Macrobrachium* farming would improve if freshwater prawns could be sold on a market which would not differentiate between shrimp and prawns (New 1990), for example, the existing market in Belgium where freshwater prawns from Bangladesh are imported because of their similarity in taste to the preferred cold-water species *Crangon* (Nierentz and Josupeit 1988). Liao and Smith (1981) reported most South Carolina consumers are said to evaluate prawns as similar to marine shrimp, which seems to an encouraging signal for *M. rosenbergii* marketing.

1.5.4 Water Quality

While *M. rosenbergii* is generally very tolerant of a wide range of water quality factors, the effects of adverse conditions causing stress and disease have been reported. For example, idiopathic muscle necrosis due to environmental stressors has resulted in stunted growth in *M. rosenbergii* (Akiyama *et al.* 1982), and has been

reported to have caused sudden mortality of up to 60% in 28-day-old prawn postlarvae in intensive rearing systems in Thailand (Nash *et al.* 1987). It is not known whether postlarval *M. rosenbergii* will be more affected by adverse environmental conditions than juveniles. The few studies that exist on the effects of water hardness on prawn growth and survival had discrepancies in their results (section 1.4.2), and little precise information relevant to such water quality factors desirable at a potential farm or nursery site is available.

1.6 Physiology

1.6.1 Composition of the Exoskeleton

In many decapod crustacean exoskeletons, calcium carbonate is the major mineral component representing up to 99% of the inorganic components (Dall 1965a, Huner *et al.* 1979). Magnesium is the second most prevalent cation in the exoskeleton, present largely as carbonate. Dall (1965a) found that in *Metapenaeus* sp., calcium carbonate, chitin and protein were the major constituents of the exoskeleton, with protein being the most abundant. The degree of exoskeletal calcification depends on species, diet and environment, and on the location on the exoskeleton itself, being highest where greatest strength is required (Stevenson 1974, Mills and Lake 1976, Barnes 1987). At the hard intermoult stage when the cuticle is fully hardened ,the bulk of the total body calcium is in the exoskeleton and the proportion in the soft

tissues and hemolymph will be very small (Greenaway 1985). Fieber and Lutz (1985) reported that in intermoult stage the exoskeleton of *M. rosenbergii* contained 53% of the total calcium and 32% of the total magnesium content of the prawn.

1.6.2 Moulting and Mineralization

Moulting is one of the most common but stressful physiological processes in a crustacean's life. Momot (1976) indicated that disturbances in moulting can increase the mortality of animals. Casting the exoskeleton itself is energy expensive; moreover immediately after moult the animals are soft, hardly able to move and therefore exposed to predation, cannibalism, disease and parasitic attack.

Wright (1980) reported newly moulted *Gammarus pulex* contained only 4% of the calcium of the intermoult stage, while *Austropotamobius pallipes* had only 10% of the intermoult stage (Chasemartin 1967, Cited in Greenaway 1974a). Greenaway (1985) suggested that aquatic crustaceans lose most of their body calcium during premoult and in the exuviae, and up to 20% may be retained for postmoult calcification. Therefore, the pressing need for all postmoult aquatic crustaceans is to harden the cuticle for its protection and to regain mobility. This would probably require rapid initial calcium uptake from the environment followed by a lower, perhaps more constant, uptake over the remainder of the moult cycle (Huner *et al.* 1979, Cameron and Wood 1985).

Calcium concentrations in the water vary considerably between environments. In seawater, calcium is present at a concentration around 400mg l^{-1}, in freshwater environments it varies from zero to 120mg l^{-1} depending on the geological conditions of the area. Thus to a achieve a similar rate of calcium uptake to that of marine crustaceans, freshwater crustaceans would require an uptake mechanism with greater affinity for calcium. Cameron (1985) presented an electrically balanced model for calcium uptake in postmoult marine crab *Callinectes sapidus*. He proposed that calcium uptake is accompanied by bicarbonate uptake and balanced by loss of hydrogen ions, the carbonate required for mineralization being also partly derived from metabolic CO_2. Malley (1980) found uptake of calcium in postmoult freshwater crayfish was inhibited below pH 5.75 and considered possible causes to be the scarcity of HCO_3^- below pH 6.0, high external concentrations of H^+ interfering with exchange of internal H^+ or the direct effects of H^+ on the active ion transport systems. Rates of calcium uptake have been shown to increase when dietary calcium levels are low (Deshimaru *et al.* 1978) and it is generally accepted that at least some of the minerals required are met from nutrition.

The calcium to magnesium ratio in water may also play a significant role in the physiology of prawns. Robertson (1953) indicated that the balance between calcium and magnesium in the hemolymph was important. There is some evidence that these two ions may act antagonistically, magnesium inducing anaesthesia and calcium inducing excitability (Bethe 1929, Cited in Robertson 1953). Walters and Uglow (1981) studied the relationship between the heart activity and hemolymph magnesium

concentrations in some marine crustaceans. They concluded that species with a high heart activity had the lowest hemolymph magnesium concentrations. Tentori and Lockwood (1990), however, found little evidence to support the theory of magnesium affecting activity in the oceanic crustaceans they studied.

Winkler (1986) demonstrated the effect of an altered calcium to magnesium ratio on Na/K ATPase activity in the gills of *Carcinus maenas*. At ratios corresponding to those found in seawater the ATPase functioned maximally and at ratios with low magnesium and high calcium there was almost complete inhibition of enzyme activity.

While it is believed that the major site of calcium transport in crustaceans is the gill (Dall 1965b, Greenaway 1972, 1974) little is known with any certainty concerning calcium transport mechanisms. A high affinity, calmodulin-dependent, Ca^{2+}-ATPase is associated with active transport of calcium ions across the fish gill membrane (Flik *et al.* 1983, 1984, 1985) but the enzyme had not been looked for in crustacean gills (Greenaway 1985) until recently (Cameron 1989). A high affinity Ca^{2+}-ATPase activity has now been identified in marine crab gills (*Leptograpsus variegatus*) but its activity did not change during the moult cycle and it was judged not to contribute substantially to the observed increased rate of calcium influx from the water during postmoult (Morris and Greenaway 1992). An alternative mechanism which responds to higher intracellular levels of calcium and has a much greater translocating capacity (Philipson 1985) must therefore be operating. In eel gills the activity of alkaline phosphatase appears to reflect the rate of transepithelial calcium transport (Fenwick

1976, So and Fenwick 1977) and Roer (1980) proposed that high affinity Ca^{2+}-ATPase in conjunction with a Na^{+}/Ca^{2+} exchanger were responsible for calcium movements across *Carcinus* hypodermis.

1.7 Purpose of the Study

The purposes of the study were in two fields. Firstly to contribute knowledge fundamental to the understanding of mineral ion uptake in freshwater prawns with particular reference to the hardness and alkalinity of the surrounding waters, and secondly, to provide data vital for the selection of suitable sites for prawn farms and for rational management of water quality in farm ponds. While the first objective may be considered as a scientifically justifiable entity in itself, the second aim can not be achieved in isolation. The quality of the advice given to the farming industry will clearly be dependent upon the quality and relevance of the results from the physiological investigations.

It is intended that this study will resolve the apparent discrepancies concerning the effect of high hardness levels on growth and survival of *M. rosenbergii* that have been reported in the scientific literature. Attention is given to differences between postlarvae and older juveniles in their tolerance to hardness and alkalinity levels in order to provide data which has relevance to commercial nursery practises undertaken prior to on-growing. Finally an attempt is made to understand the possible

mechanisms responsible for calcium and bicarbonate translocation in prawns during immediate postmoult when calcium uptake is high and in intermoult when uptake is normally much less.

The study was undertaken in three phases to investigate :

1) the effect of the calcium to magnesium ion ratio in water on survival, growth, moulting and mineralization in juvenile *M. rosenbergii*;

2) the independent and interaction effects of environmental alkalinity and hardness on survival, growth, moulting and carapace mineralization in postlarval and juvenile *M. rosenbergii*;

3) the possible mechanisms through which mineral ion translocation rate in gill tissue is altered by external alkalinity levels. This involved experiments to find if;

a) calcium-activated dephosphorylating enzymes are present in prawn gills,

b) the enzyme activity is influenced by environmental alkalinity and moult stage, and

c) the enzyme could be involved in calcium transport through gills of *M. rosenbergii*.

1.8 Summary of Potential Applications of the Results in Aquaculture

In addition to increasing the fundamental understanding of prawn physiology (moulting, mineralization and calcium transport mechanisms), it was intended that the results demonstrated in this study would have some direct impact on *M. rosenbergii* farming both in Bangladesh and elsewhere in the World where tropical and subtropical environments prevail. The areas of most immediate application would be:

a) the specification of alkalinity, hardness and Ca:Mg ion ratio requirements to assist selection of sites for freshwater prawn farming;

b) whether lime should be used in nursery and grow-out ponds for *M. rosenbergii*, in order to maintain culture water quality within prescribed limits.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Experiment Location

The experiments were conducted in the tropical prawn unit at the Institute of Aquaculture, University of Stirling. The prawn unit maintains a tropical environment of air temperature 28°C ± 1°C for 24 hours. This controlled environment also provided a photoperiod of 12 hours light and 12 hours dark.

2.2 Source of Experimental Animals

2.2.1 Broodstock Origin and Maintenance

The original *Macrobrachium rosenbergii* were imported from Thailand and maintained in a recirculatory system at the Institute of Aquaculture, Stirling. To avoid territorial aggression and cannibalistic behaviour they were housed individually in separate tanks and fed on a formulated pellet, plus chopped fish, liver, fresh mussels, green beans and spinach on an *ad libitum* basis.

2.2.2 Breeding

When a female with ripe orange-coloured ovaries completed the prespawning moult, a hard shelled male was introduced into her tank, left undisturbed for about 4-5 hours, and then the male was removed. The female spawned and transferred the orange coloured eggs to beneath her abdomen on the same day. The eggs were then incubated for about 20 days. When the eggs became dark-brown in colour and almost ready to hatch, the female was transferred into a brackishwater (12‰) tank where the eggs were hatched. After hatching the spent female was returned to her original tank.

2.2.3 Larval Rearing

The newly hatched larvae were reared in a recirculatory system containing brackishwater (12‰) and fed on newly hatched nauplii of *Artemia*. Metamorphosis began around day 20 and generally continued up to 35 days. Metamorphosed postlarvae were transferred to tanks in a freshwater recirculatory system.

2.2.4 Postlarvae and Juveniles for Experiments

Initially the postlarvae were fed on flake fish food and gradually weaned onto a pelleted diet (section 2.5.1). Postlarvae and juveniles of the desired size were taken

from such stocks for the experiments.

2.3 Experimental Water System

2.3.1 Deionized Water

Deionized water (conductance <0.2μS cm^{-1}) was produced every 48 hours using a Permutit Deionizer (Model No. 3c) and piped into a 250 L black polypropylene overhead reservoir tank. The resin of the deionizer was usually changed when the conductance of water rose to between 4 and 5μS cm^{-1} and was never used if the level exceeded 5μS cm^{-1}. The overhead tank was provided with 3 thermostatically controlled water heaters to warm the water to the desired temperature (28°C). Two airstones each 15 x 2.5cm wide continuously aerated the water in the reservoir tank to ensure uniform heat distribution and proper oxygenation.

2.3.2 Stock Solution

The chemicals and concentrations used in stock solutions are presented in Table 2.1. Throughout this study concentrations of stock solutions were the same with the exception of the early experiment discussed in Chapter 3 where different stock solutions were used to prepare the culture media. Stock solutions were kept in glass bottles and sealed tightly to prevent any evaporation.

Table 2.1 The chemical composition and concentrations of stock solutions. All cheminals were of AnalaR grade (BDH, Poole, Dorset).

No. of solution	Chemical	Concentrations (g l^{-1} deionized water)
1	$CaCl_2.6H_2O$	380
2	$MgCl_2.6H_2O$	360
3	$NaHCO_3$	60
4	Na_2SO_4	175
5	KCl	70

2.3.3 Preparation of Culture Media

Oxygenated, warm (28°C), deionized water flowed from the reservoir tank by gravity through a 1cm diameter clean PVC pipe into the experimental tanks. Artificial culture media were made up as required using the appropriate volumes of the different stock solutions. Stock solutions were always dosed into the media using automatic pipettes (Finpipette). This procedure was accurate and rapid. To maintain the purity of stock solutions a different pipette tip was used for each different stock solution. Variations in procedure and details of the different concentrations of chemicals used in specific rearing media are explained and presented in other Tables in the relevant Chapters.

2.4 Experimental Tanks and Holding Facility

Prawns were housed individually in cylindrical mesh pots (holders) of 11cm diameter and 17cm height with sides of 0.1cm nylon mesh and 0.25cm mesh bottoms. The holders were placed in identical plastic tanks each of 39.5 x 21.5 x 25.5cm deep. Each tank had 9 litres of treatment water, 3 holders and one airstone. The holders were covered with 0.1cm nylon mesh to prevent escapes. Each tank was again covered with a black polythene sheet in order to: a) reduce evaporative loss, and b) protect the prawns from excessive illumination. All the experimental tanks were placed in 203.5 x 47.0 x 16.5cm deep fibre-glass troughs in a recirculation system so

that a uniform temperature could be maintained (28°C ± 1°C).

2.5 Husbandry

2.5.1 Feeding

The prawns were fed twice a day on a pelleted diet produced in the Institute of Aquaculture (40% protein based on squid, shrimp and fish meal). Prior to giving feed to the prawns, the leftover feed of the previous meal was removed by siphoning carefully to minimize loss of water.

2.5.2 Water Change

The experimental waters were static but aerated. They were completely renewed every 48 hours with minimum disturbance to the prawns. During changes, water was siphoned out into an empty tank. The prawn holders were taken out slowly and very carefully placed in to the siphoned water. Tanks were cleaned using a sponge and deionized water, replaced into their original positions. Fresh media prepared using deionized water and stock solutions was added and aerated vigorously, the prawns in their holders were then replaced into the fresh media. This was done quickly and very carefully to minimize stress.

2.5.3 Aeration

Aeration was provided from a high volume blower (BVC YP3/100, Class E, Gosport, England) but was liberated to each tank gently through a cylindrical airstone diffuser (2cm length and 1.5cm diameter). The level of aeration was adjusted empirically and care was taken to avoid variations from one tank to another. Periodic spot measurements of dissolved oxygen at this level of aeration showed the water always to be air saturated.

2.6 Water Quality Measurements

2.6.1 Alkalinity and Hardness

The total alkalinity refers to the total concentrations of bases in water expressed in mg l^{-1} of equivalent $CaCO_3$, and the total concentrations of divalent cations (Ca^{2+} and Mg^{2+}) expressed in mg l^{-1} of equivalent $CaCO_3$ is termed the total hardness of the water. Total alkalinity and total hardness of the rearing media were measured as described by Stirling (1985). They were checked once a week at 0 hour and 48 hours after water changes.

2.6.2 Ammonia

Total ammonia and nitrite nitrogen were checked twice daily using 'Tetra' Ammonia and Nitrite test kit respectively (Animal House, U.K. Ltd.). Total ammonia levels <0.25mg l^{-1} and nitrite levels <0.30mg l^{-1} were considered to be acceptable (Wickins 1976) and did not exceed these levels.

2.6.3 pH

The pH of the culture media was measured daily using a Corning pH Meter (Model No. 225) and no major fluctuations were observed between the period of water changes (Table 5.1).

2.6.4 Water Temperature

The temperature of the rearing waters was checked each morning and evening using a thermometer and did not vary from 28 ± 1°C in any experiment.

2.7 Weighing and Measurements

2.7.1 Weighing of Prawns

Prawns of similar size for each experiment were initially sorted into a bucket.

Care was taken to select the experimental animals within the narrowest possible size range. At the beginning and end of each trial they were blotted dry and weighed individually to the nearest 1.0mg on a Mettler AC 100 balance.

2.7.2 Carapace Collection and Measurements

Prawns were checked for moults and cast carapaces were collected twice a day while feeding the prawns. At the end of each experiment the prawns were harvested at the mid intermoult stage which was estimated as being half of the previous intermoult period. Prawns were killed by rapid chilling at 4°C for half an hour. The intermoult carapaces were dissected and cleaned of all soft tissues. Cast and intermoult carapace lengths (posterior margin of orbit to mid dorsal posterior margin of carapace) were measured to the nearest 0.1mm using a Dial Caliper. Immediately after length measurements, carapaces were rinsed thoroughly in distilled water and dried in an oven at 105°C for 24 hours. From the oven they were placed into a vacuum desiccator and kept for 20 minutes to cool to room temperature. After weighing they were stored in plastic vials for later chemical analysis.

2.8 Calcium and Magnesium Analysis of Carapaces

The stored carapaces were placed individually into digestion tubes and 1ml of

concentrated nitric acid (Aristar) was added to each tube. Acid digestion was carried out at 110°C for one hour in a Tecator Digester (Model No. 1016). Each digested sample was diluted to 5ml by adding distilled water and mixing. For calcium and magnesium measurements 10-100µl samples (depending on the weight of carapace) were placed in a separate set of test tubes and diluted further to 5ml with 1% nitric acid and 5% lanthanum chloride solution and mixed further using a vortex mixer. Measurements were carried out using a Perkin Elmer Atomic Absorption Spectrophotometer (Model No. 2280) against calcium standards of 4.0 and 2.0mg l^{-1}, and magnesium standards of 0.30 and 0.15mg l^{-1}. The 5% lanthanum chloride and 1% nitric acid solutions were also used in standards. Calcium was measured at 423nm and magnesium at 285nm wavelengths. To prevent any possible ionic contamination, the digestion tubes and other test tubes were immersed before use in 20% nitric acid solution for at least 48 hours and subsequently in distilled water for another 24 hours. Finally the tubes were rinsed individually 4-5 times again with distilled water and finally dried in an oven.

2.9 Presentation of Results and Terminology

Data have been presented as arithmetic means ± standard error (SE). The terminology used for the presentation of results were as follows:

Growth rate = $\frac{\text{final weight (mg) - initial weight (mg)}}{\text{no. of rearing days for each prawn}}$

Carapace length increment (% moult^{-1}) = $\frac{CL_{n+1} - CL_n}{CL_n} \times 100$

where, CL = carapace length, n+1 = present moult, n = previous moult.

Length specific dry weight of carapace = $\frac{\text{dry weight of carapace (mg)}}{\text{carapace length (mm)}}$

Cation concentration in carapace = $\frac{\text{weight of cation in carapace (mg)}}{\text{dry weight of carapace (g)}}$

Length specific calcium content of carapace = $\frac{\text{weight of calcium in carapace (mg)}}{\text{carapace length (mm)}}$

2.10 Statistical Analyses

The statistical analyses were carried out by using the Statgraphic Computer Package (Version 3.0, Statistical Graphic Corporation, STSC, Inc.). Before performing any statistical analysis, the normality of data and homogeneity of variances were checked by Distributions and Fittings (Statgraphics Package). When required, appropriate transformations were carried out to normalize data and ensure homogeneity of variances. One-way analysis of variance (One-way ANOVA) was

used to assess the overall effect of different levels of one factor. To detect interactions as well as overall and independent effects of two factors (alkalinity and hardness), two-way analysis of variance (Two-way ANOVA) was used. The two-sample t-test was used to discern the significant differences between the means of two sets of data.

When F-statistics indicated a significant difference ($P \leq 0.05$), multiple range analysis was used to discern specific differences between treatment groups.

CHAPTER 3

PRELIMINARY EXPERIMENT ON THE EFFECT OF WATER HARDNESS ON POSTLARVAL AND JUVENILE *MACROBRACHIUM ROSENBERGII.*

3.1 Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* is widely distributed from brackish to freshwater and again from running river waters to impounded paddy fields. The larvae of *M. rosenbergii* can not survive without brackishwater while in general, the juvenile and adults live in freshwater. The intrinsic behavioural patterns exhibited during their life cycle suggests that their responses or sensitivity to critical water quality factors (such as alkalinity or hardness) may also vary between postlarvae and juveniles according to their age or size (Brown *et al.* 1991).

Aquatic crustaceans lose most of their body calcium during premoult, and in the exuviae, although up to 20% may be retained and reused in postmoult calcification (Greenaway 1985). Wright (1980) reported that adult *Gammarus pulex* lost about 42% of body calcium into the environment over a 2-3 day period preceding moulting and a further 54% body calcium was lost with the exuviae, leaving only 4% in the newly moulted animals. To replenish these losses as well as for quick hardening of

their exoskeletons such animals rely mainly on available environmental calcium but also on sources in the food. Immediately after moulting, a crustacean's body is soft, and some large specimens may be barely able to move and indeed may be unable to eat for some time. In *M. rosenbergii* however the new shell hardens rapidly over 2-6 hours (Ling and Merican 1961), mainly as a result of uptake of calcium from the environment.

Reports on water hardness (Ca^{2+} and Mg^{2+}) requirements by different size groups of *M. rosenbergii* are rare. Moreover, previous studies on water hardness requirements produced some conflicting results. For example Cripps and Nakamura (1979), Howlader and Turjoman (1984), Vasquez *et al.* (1989) and Brown *et al.* (1991) indicated that survival and growth were reduced by increased water hardness while others, (Heinen 1977; and Bartlett and Enkerlin 1983) reported that survival and growth were not reduced.

As larval *M. rosenbergii* live in brackishwater containing relatively large amounts of Ca^{2+} and Mg^{2+} (i.e the water is of high hardness), it might be expected that the immediate postlarval stages of prawns would be tolerant enough to survive and grow well in high hardness freshwaters. This preliminary experiment was therefore conducted to :

a) compare the effect of water hardness on postlarval and juvenile *M. rosenbergii*;

b) identify the possible factor(s) responsible for the conflicting results on the effect of water hardness in the studies mentioned above.

3.2 Materials and Methods

3.2.1 Experiment Location, Facility and Source of Animals

The experiment location, environment and the experimental tanks, prawn holders and water system were as described in Chapter 2. Full sibling postlarval (mean wt 0.12g ± 0.003) and juvenile (0.97g ± 0.03) *M. rosenbergii* were selected from the laboratory stock (section 2.2).

3.2.2 Design of Experiment

Four treatments of water hardness (20, 80, 160 and 320mg l^{-1} expressed as $CaCO_3$) were tested both with postlarval and juvenile prawns in which each treatment was replicated three times. Each tank representing one replicate, a total of 24 tanks were used in this experiment where 72 prawns (36 postlarvae + 36 juveniles) were exposed to different test media. The treatment tanks were arranged in a randomised design in 4 troughs as described in Chapter 2 (section 2.4).

3.2.3 Stock Solutions and Preparation of Culture Media

The chemical compositions of stock solutions is shown in Table 3.1 which was adapted from HMSO (1969). Culture media of different water hardnesses were prepared using appropriate quantities of the stock solutions. The measured alkalinity of each medium is given in Tables 3.2 and 3.3.

3.2.4 Prawn Husbandry

Prawn husbandry and water quality measurements were carried out as described in Chapter 2 (sections 2.5 and 2.6 respectively).

3.2.5 Measurements and Analysis of Carapaces

Cast and intermoult carapace collection, preservation and analyses of calcium and magnesium were also conducted as described in Chapter 2 (sections 2.7 and 2.8 respectively).

Table 3.1 Chemical compositions of stock solutions. All chemicals used were of AnalaR grade (BDH, Poole, Dorset).

Stock solution No.	Chemical	Quantity ($g\ l^{-1}$)
1	$CaCl_2.6H_2O$	320
	$NaCl_2$	29
	$NaNO_3$	9
2	$MgSO_4.7H_2O$	151
	$Na2SO_4$	79
3	$NaHCO_3$	27.5

Media of different water hardness were prepared according to HMSO (1969) and the methods were as follows:

Medium of 20mg l^{-1} as $CaCO_3$ water hardness: One ml each of solution 1 and 2 and 10.0 ml of solution 3 were added to each 10 litres of deionized water.

Medium of 80mg l^{-1} : 4.0 ml of solution 1 and 2 and 40.0 ml of solution 3 were added to each 10 litres of deionized water.

Medium of 160mg l^{-1} : 8.0 ml of solution 1 and 2 and 80.0 ml of solution 3 were added to each 10 litres of deionized water.

Medium of 320mg l^{-1} : 16.0 ml of solution 1 and 2 and 160.0 ml of solution 3 were added to each 10 litres of deionized water.

3.2.6 Presentation of Results and Terminology

Results are presented as arithmetic means ± standard error (SE). The terms of growth rate, carapace length increment (% moult^{-1}) and calcium concentration in carapaces are explained in Chapter 2 (section 2.9).

3.2.7 Statistical Analyses

The normality of data was checked before applying the analysis of variance. Where possible one-way analysis of variance (One-way ANOVA) was applied to determine the overall effect of water hardness. When the significance level was ≤ 0.05, multiple range analysis at 95% LSD intervals was further used to discern specific differences between treatment groups. Statistical analysis of the results from postlarval prawns in this experiment was not possible due to lack of data in two treatments where all postlarvae died before harvest. In juveniles One-way ANOVA was used to analyze the data from only 3 treatments because of the death of all prawns in one of the treatments. The t-test was used to compare the calcium concentrations between cast and intermoult carapaces.

3.3 Results and Discussion

Although it was expected initially that the effects of water hardness on postlarvae and juveniles would be compared in this experiment, the results obtained in this study were of limited value because of the unexpectedly high mortality that occurred in the high hardness treatments (section 3.4).

3.3.1 Physical Appearance and Behavioural Response of Prawns

During daily routine procedures (e.g. feeding and moult checking) the physical appearance and behavioural differences among the prawns of the different treatment groups were recorded. In the media of hardness levels 160 and 320mg l^{-1} as $CaCO_3$ (which also had alkalinity levels of 135 and 285mg l^{-1} as $CaCO_3$ respectively), both postlarval and juvenile prawns looked severely stressed, lost appetite and suffered from "white muscle syndrome" (milky-white abdomen and opaque abdominal muscles). A higher incidence of the syndrome occurred in postlarval prawns than in juveniles particularly at hardness levels of 80 and 160mg l^{-1} (Tables 3.2 and 3.3). Prawns at the lowest water hardness (20mg l^{-1}) and alkalinity (17mg l^{-1}) did not show any signs of white muscle syndrome. Further details of the incidence of white muscle syndrome in relation to water quality factors in other experiments are discussed in Chapter 5 (section 5.5.2).

Table 3.2 The effects of increasing water hardness and alkalinity on the occurrence of white muscle syndrome (%), survival (%), growth rate (mg day^{-1}), intermoult period (days) and carapace length increment (% $moult^{-1}$) in postlarval *M. rosenbergii*. Values are mean ± SE. Values within parenthesis indicate number of observations.

Water hardness ($mg.l^{-1}$ as $CaCO_3$)	20	80	160	320
No. of prawns	9	9	9	9
Initial wt (g)	0.13 ± 0.004	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Measured alkalinity ($mg.l^{-1}$ as $CaCO_3$)	17 ± 0.33 (2)	84 ± 0.60 (2)	135 ± 1.25 (2)	285 ± 2.8 (2)
White muscle syndrome (%)	0	55	100	100
Survival (%)	100	44	0	0
Growth rate (mg day^{-1})	1.54 ± 0.40 (9)	0.65 ± 0.26 (6)	-	-
Intermoult period (days)	5.64 ± 0.28 (9)	5.03 ± 0.13 (6)	4.87 ± 0.43	-
Carapace length increment (% $moult^{-1}$)	3.40 ± 0.40 (9)	2.09 ± 0.55 (6)	-	-
Range of rearing period (days)	24 - 32	15 - 27	8 - 15	3 - 7

Table 3.3 The effects of increasing water hardness and alkalinity on the occurrence of white muscle syndrome (%), survival (%), growth rate (mg day^{-1}), intermoult period (days), carapace length increment (% moult^{-1}), and calcium concentrations (mg.g^{-1}) in intermoult and cast carapaces of juvenile *M. rosenbergii*. Values are mean ± SE. Values within parenthesis indicate number of observations. Mean values with common superscript letters along the horizontal rows did not show significant differences at 95% LSD intervals.

Water hardness (mg.l^{-1} as $CaCO_3$)	20	80	160	320
No. of prawns	9	9	9	9
Initial wt (g)	1.00 ± 0.06	0.97 ± 0.06	0.91 ± 0.06	1.01 ± 0.04
Measured alkalinity (mg.l^{-1} as $CaCO_3$)	17 ± 0.33 (2)	84 ± 0.60 (2)	135 ± 1.25 (2)	285 ± 2.8 (2)
White muscle syndrome (%)	0	22	44	100
Survival (%)	100	78	56	0
Growth rate (mg day^{-1})	7.23^{a} ± 1.15 (9)	4.19^{b} ± 2.19 (7)	0.16^{c} ± 1.52 (7)	-
Intermoult period (days)	12.34^{a} ± 0.83 (9)	9.82^{b} ± 0.48 (7)	8.75^{b} ± 0.38 (7)	-
Carapace length increment (% moult^{-1})	4.00^{a} ± 0.35 (9)	2.77^{a} ± 0.60 (7)	1.10^{b} ± 0.39 (7)	-
Calcium in intermoult carapaces (mg.g^{-1})	184^{a} ± 5.04 (9)	191^{a} ± 6.60 (5)	192^{a} ± 10.20 (4)	-
Calcium in cast carapaces (mg.g^{-1})	209^{a} ± 8.25 (9)	217^{a} ± 7.12 (5)	220^{a} ± 4.33 (4)	-
Range of rearing period (days)	45 - 62	32 - 47	30 - 41	14 - 29

3.3.2 Survival

Prawns of both size groups suffered a very high mortality in the media of high hardness and high alkalinity but all prawns survived in the lowest hardness (20mg l^{-1}) where alkalinity (17mg l^{-1}) was also low. No postlarval prawns survived through 5 moults in hardness levels of 160 and 320mg l^{-1} where alkalinity was 135 and 285mg l^{-1} respectively (Table 3.2). Juveniles also did not survive in the rearing medium of hardness level 320mg l^{-1} with alkalinity of 285mg l^{-1} (Table 3.3). At the highest hardness and alkalinity, postlarvae did not survive more than one moult although some juveniles survived up to 3 moults. In these stressful environments most of the prawns died at the premoult stage or while moulting. Some prawns were also observed to die within one day after moulting. It was noticed that the prawns which died during ecdysis were not able to cast either carapace or anterior appendages or sometimes telson. Brock (1983) reported an exuvial entrapment disease in some hatchery cultures of *M. rosenbergii* where mortality rates reached 20-30%, and several reports of moult deaths in cultured crustaceans exist (Wickins 1972, Bowser and Rosemark 1981, Conklin 1990).

These results confirm the findings of Brown *et al.* (1991) who used culture media prepared in a similar way, and who also reported that all juvenile prawns died at 320mg l^{-1} water hardness. In contrast, Smith *et al.* (1982) and, Howlader and Turjoman (1984) observed good survival of *M. rosenbergii* at hardness levels >900mg l^{-1} as $CaCO_3$. The death of all the prawns in this study and that of Brown *et al.*

(1991) at a hardness level of 320mg l^{-1} may not, therefore, have been due entirely to water hardness. It is proposed that the mortality was due to the effects of either the increased alkalinity, other accompanying ions, interactions among them or interactions between alkalinity and hardness.

3.3.3 Growth Rate and Intermoult Period

The growth rates of postlarvae and juveniles of *M. rosenbergii* were inhibited in the high hardness media but interestingly it was noticed that the intermoult periods in such media were shorter than in the low hardness (and thus low alkalinity) waters (Tables 3.2 and 3.3). Similar observations were made by Brown *et al.* (1991). The slow growth rate in conjunction with a higher moulting frequency is abnormal and provides further evidence that the metabolism of mineralization or ion uptake and deposition were adversely affected by the ionic composition of the rearing waters. It is suggested that the more rapid moulting in this study may have been a defensive response of the prawns to the adverse ionic environment. This hypothesis was investigated further in the experiments described in Chapters 4 and 5.

3.3.4 Carapace Length Increment (% $moult^{-1}$)

Carapace length increments followed a similar pattern to the growth rate of

prawns. The data from two treatments in postlarvae (20 and 80mg l^{-1} water hardness) and three treatments in juveniles (20, 80 and 160mg l^{-1} water hardness) indicated that the carapace length increment per moult declined with increased hardness and alkalinity (Tables 3.2 and 3.3). The reduction in size increment at moult suggested that the prawn's energetic resources were being diverted from growth in order to speed up moulting and that this process was enhanced in the high hardness media.

3.3.5 Calcium Concentrations in Carapaces

Calcium concentrations in intermoult and cast carapaces were recorded only from juvenile prawns and are presented in Table 3.3. Although survival and growth were affected in the high hardness media, it was surprising that the prawns were able to regulate the calcium concentrations in their intermoult carapaces without showing any significant differences between treatments, though the values were a little higher in high hardness water. The calcium concentrations in cast carapaces also did not vary significantly between the three treatment groups. The two-sample t-test demonstrated that the calcium concentrations were significantly higher ($P<0.05$) in cast than in intermoult carapaces. This result contrasts with the results of Brown *et al.* (1991) but is in agreement with the results of Dall (1965a) for *Metapenaeus* sp., Wickins (1984) for *Penaeus monodon* and Huner (1985) for crayfish. Although the media and diet used in this experiment and of Brown *et al.* (1991) were similar, no explanation can be offered for the apparent discrepancy.

3.4 Conclusion

It appeared that the influence of water hardness in this experiment was confounded by other water quality parameters particularly the alkalinity of the media which also increased with increasing water hardness due to the way the rearing media were made up (Table 3.1). The other accompanying ions like sodium, nitrate, chloride and sulphate also increased with increasing water hardness. In these circumstances and because of the consequential high mortality in some treatments, it was difficult to confirm the actual effect of water hardness on the test animals. However the results obtained provided the stimulus to formulate a better recipe for the culture media for all subsequent studies so that the effects of water hardness could be seperated from the effects of alkalinity.

The results did indicate however that the postlarval prawns were more severely affected than the juveniles by the high hardness media. This therefore emphasized the need to conduct further research to:

a) determine a Ca:Mg ion ratio in the culture media suitable for *M. rosenbergii* (Chapter 4);

b) formulate suitable culture media in which hardness and alkalinity could be varied independently (Chapter 5);

c) study the independent and interaction effects of environmental alkalinity and hardness levels (with a Ca:Mg ratio as determined in Chapter 4) on both postlarval and juvenile *M. rosenbergii* (Chapter 5).

CHAPTER 4

EFFECTS OF CALCIUM/MAGNESIUM ION RATIO IN WATERS OF DIFFERENT HARDNESSES ON *M. ROSENBERGII*.

4.1 Introduction

The high mortality encountered at high hardness levels in the experiment described in Chapter 3 made it necessary to investigate further the role of magnesium particularly in relation to calcium ions in the water. The main purpose of this study was to identify a calcium/magnesium ion ratio in the rearing media that would improve the survival and growth of *M. rosenbergii* especially at high hardness levels. There was also an intrinsic value in investigating the influence of the calcium:magnesium ratio on prawns in view of the differences encountered in natural waters. In this respect, it was also thought that the findings of this experiment would be relevant to the selection of sites for freshwater prawn farms.

In seawater the calcium/magnesium ion ratio is almost constant while that in freshwater varies from place to place. Table 4.1 contains calcium/magnesium ion ratios in some natural marine, brackish and fresh waters. All the ratios in the following table (except that of Boyd and Walley 1975) have been re-calculated from

Table 4.1 Calcium/magnesium ion ratio in natural waters.

Type of water	Calcium/magnesium ratio	Source
Sea water	0.19	Lyman and Fleming (1940)
	0.19	Barnes (1954)
	0.19	Culkin (1965)
	0.18	Goldberg (1965)
	0.19	Morcos (1973)
	0.19	Stern *et al.* (1987)
Brackishwater:		
Conwy, Wales	0.66	Wickins & Helm (1981)
Yahel, Israel	1.36	Stern *et al.* (1987)
Elat, Israel	1.41	,, ,, ,, ,,
Yotvetah, Israel	0.45	,, ,, ,, ,,
Freshwater:		
Tapwater, Jerusalem	1.18	Stern *et al.* (1987)
Mevo Hamma, Israel	1.69	,, ,, ,, ,,
Alabama and Mississippi, U.S.A	2-6	Boyd and Walley (1975)
Scotland (5 lochs)	1-4	Watts and Duncan (1981)

the calcium and magnesium ion measurements by different authors so that they may be compared to the ratios used in this experiment.

From Table 4.1 it appears that the magnesium concentrations in sea water are about 5 times higher than the calcium concentrations whereas in freshwater environments the reverse is often true. In natural fresh water, calcium concentrations are always higher than the magnesium concentrations but the variation among ratios may also be considerable.

Since the calcium/magnesium ion ratio in freshwater environments varies in accordance with the geology of the area, it is possible that the changes in the ionic ratio might also affect the survival, growth and exoskeletal mineralization in the freshwater prawn *M. rosenbergii*. Although a few reports are available on the effect of environmental Ca/Mg atom ratios on exoskeletal mineralization in marine decapod crustaceans (Gibbs and Bryan 1972, and Wickins 1984b), no information could be found for *M. rosenbergii* which inhabits freshwater. This experiment was therefore designed to investigate the effects of three widely different calcium to magnesium ion ratios in waters of three different total hardnesses on survival, growth, moulting and carapace mineralization in juveniles of the giant freshwater prawn *M. rosenbergii*. The ion ratios and total hardness levels in the water were adjusted independently.

4.2 Materials and methods

4.2.1 Artificial Freshwater Formulation

Five chemical salts ($CaCl_2.6H_2O$, $MgCl_2.6H_2O$, KCl, Na_2SO_4 and $NaHCO_3$) and deionized water were used to prepare test media of different ion ratios (Ca:Mg) and hardnesses. The salts recommended as a freshwater source of Ca, Mg and SO_4 ions for toxicity testing were calcium and magnesium sulphates (HMSO 1969, APHA-AWWA-WPCF 1989). In the present study however calcium and magnesium chlorides were used instead of the sulphate-salts because preliminary trials on stock solution preparation revealed that the solubility of the sulphate-salts of calcium and magnesium was very poor. The possibility of using carbonate-salts of calcium and magnesium was considered (Chapter 3) and was rejected in this experiment since these would cause an increase in the alkalinity of the water as water hardness was increased. The remaining candidates were nitrate and chloride-salts. Previous studies on aluminium toxicity on fish involving assessment of the influence of calcium (where calcium was a variable) used the chloride-salt on the assumption that any ameliorative effect of calcium was a function of calcium rather than chloride (Witters 1986, Dalziel *et al.* 1986, Reader *et al.* 1988). On the other hand, studies concerned with the effect of external calcium concentrations on ion regulation and gill function employed the nitrate-salt of calcium and were probably based on the supposition that accompanying increases in chloride ions with increased calcium levels may be an additional variable (McDonald *et al.* 1980, McDonald *et al.* 1983, Perry and Wood 1985). However,

McDonald and Milligan (1988) reported that they found no differences in the action of chloride and nitrate salts of calcium on the toxicity of aluminium. Moreover, chloride ions are more abundant than any other anions in dilute brackishwater (Funge-Smith 1991) where *M. rosenbergii* can survive, grow and reproduce. In view of these circumstances and the overriding need to be able to alter hardness and alkalinity levels independently, chloride salts of calcium and magnesium were used to prepare the test media.

The stock solutions for this study were prepared as described in section 2.3.2 (Table 2.1) and the amount of each stock solution used to prepare the rearing media of different hardness and ratios is shown in Table 4.2, and the actual concentrations of calcium and magnesium in media of different ratios and hardnesses is presented in Table 4.3.

4.2.2 Experiment Location, Facility, and Source, Size and Number of Animals, and Duration of the Experiment

Experiment location, environment, experimental tanks, prawn holders and water system were as described in Chapter 2. A total of 81 full sibling juvenile *M. rosenbergii* (0.48g ± 0.01) were selected from the laboratory stock (section 2.2). The rearing period of prawns in this experiment continued upto 51 days.

Table 4.2 Preparation of culture media of different Ca:Mg ion ratio using stock solutions described in section 2.3.2 (Table 2.1)

Total hardness ($mg.l^{-1}$ $CaCO_3$)	Stock solution	Inclusion $ml.l^{-1}$ of culture media		
		Calcium : magnesium		
		4:1	1:1	1:4
20	$CaCl_2.6H_2O$	0.0922	0.0577	0.0230
	$MgCl_2.6H_2O$	0.0226	0.0564	0.0903
70	$CaCl_2.6H_2O$	0.3222	0.2011	0.0807
	$MgCl_2.6H_2O$	0.0791	0.1976	0.3162
120	$CaCl_2.6H_2O$	0.5522	0.3454	0.1378
	$MgCl_2.6H_2O$	0.1353	0.3384	0.5411

The amount of $NaHCO_3$, Na_2SO_4 and KCl were constant in all media and the quantities of each stock solution used per litre of media were 2.83, 1.02 and 0.10ml respectively. The measured alkalinity was 110 (± SE 1.23, n=6) mgl^{-1} as $CaCO_3$, and the pH range was 8.3-8.4.

Table 4.3 Concentrations of calcium and magnesium ions ($mmol.l^{-1}$) in culture media of different Ca:Mg ratio and hardness.

Hardness ($mg\ l^{-1}\ CaCO_3$)	Ions or atoms	Concentrations ($mmol.l^{-1}$) in the culture media		
		Ca : Mg ratio		
		4:1	1:1	1:4
20	Ca	0.16	0.10	0.04
	Mg	0.04	0.10	0.16
70	Ca	0.56	0.35	0.14
	Mg	0.14	0.35	0.56
120	Ca	0.96	0.60	0.24
	Mg	0.24	0.60	0.96

4.2.3 Design of Experiment

The experiment was designed to assess the influence of Ca/Mg ion ratio and hardness in water each at 3 levels, in a 3x3 factorial experiment. The 3 ratios of Ca:Mg were 4:1, 1:1 and 1:4, and the 3 hardness levels were 20, 70 and 120mg l^{-1} expressed as $CaCO_3$. The total combinations of the two factors each at 3 levels were therefore 9. Each combination had 3 replicates, thus with each tank representing one replicate containing 3 prawns held in individual holders, a total of 27 tanks and 81 prawns were used in the experiment. The treatment combinations were randomly distributed in 4 troughs as described in Chapter 2 (section 2.4).

4.2.4 Prawn Husbandry and Water Quality Measurements

Prawn husbandry was managed as described in Chapter 2 (section 2.5). In this experiment, for greater clarity, the ionic ratio, calcium and magnesium concentrations in waters were determined separately using a Perkin Elmer Atomic Absorption Flame Spectrophotometer (Model No. 2280) as described in section 2.8. Total hardness of water was calculated using the following equation:

Total hardness (mg l^{-1} as $CaCO_3$) = 2.497 (Ca^{2+} mg l^{-1}) + 4.118 (Mg^{2+} mg l^{-1})

Other water quality parameters such as alkalinity, ammonia, pH and temperature of water were measured as described in section 2.6.

4.2.5 Prawn Harvesting, Weighing, Carapace Collection and Measurements

The prawns were weighed at the beginning and at the end of the experiment as described in section 2.7.1. They were harvested after 4 moults at the mid intermoult stage which was determined as being half the duration of the previous intermoult period. Carapace (cast and intermoult) collection, measurements, weighing, preservation and analysis of calcium and magnesium concentrations were carried out as described in sections 2.7 and 2.8.

4.2.6 Presentation of Results and Terminology

Data are presented as arithmetic means ± standard error. The terminology used in the presentation of results are explained in section 2.9.

4.2.7 Statistical Analyses

The normality of data was checked before the statistical analysis. In this experiment all data were found to be normally distributed. Two-way ANOVA was used to detect the interactions between Ca:Mg ratio and hardness as well as the overall independent effects of each of the factors. When F-statistics indicated a significant difference ($P \leq 0.05$) multiple range analysis at 95% LSD intervals was used to discern specific differences between treatment groups. The two-sample t-test was used to compare the length specific dry weights and calcium and magnesium concentrations of intermoult and cast carapaces.

4.3 Results

4.3.1 White Muscle Syndrome and Survival

Prawns showing signs of the milky-white opaque abdominal muscle previously described as white muscle syndrome (section 3.3.1) also occurred in this experiment. The incidence of white muscle syndrome was significantly higher at hardness levels of 70 and 120mg l^{-1} when the Ca:Mg ratio was 4:1 than in all other treatment combinations (Table 4.4a).

Table 4.4 Effect of Ca:Mg ratio and hardness in water on a) white muscle syndrome (%) and b) survival of juvenile *M. rosenbergii*. Values are means ± SE (n=9 and 3 for white muscle syndrome and survival respectively). Mean values (n=27 and 9 for white muscle syndrome and survival respectively) along the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	11 ± 11	0	0	4^{a} ± 4
70	44 ± 29	11 ± 11	11 ± 11	22^{b} ± 11
120	77 ± 11	11 ± 11	11 ± 11	33^{b} ± 21
Mean	44^{x} ± 14	7^{y} ± 5	7^{y} ± 5	

b)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	100	100	100	100^{a}
70	100	100	100	100^{a}
120	89 ± 11	100	100	99^{a} ± 2
Mean	99^{x} ± 2	100^{x}	100^{x}	

Survival of prawns in all the ratios and hardness levels tested was good (Table 4.4b). However the death of one animal in the treatment with the highest incidence of white muscle disease indicated that longer exposure to this treatment may have increased mortality.

4.3.2 Growth

Growth in terms of mean final weight (g), daily weight increment (mg day^{-1}), intermoult period (days) and carapace length increment (% $moult^{-1}$) all suggested poorer performance ($P<0.001$) at high (120mg l^{-1}) hardness than at 20 and 70mg l^{-1} water hardnesses (Tables 4.5 a, b; and 4.6 a, b respectively). Although the effects of Ca:Mg ratio on any of the growth parameters were not significant, it was noticed that in 3 of the 4 attributes worst performance again occurred at 120 mgl^{-1} water hardness when the Ca:Mg ratio was 4:1.

Increased water hardness significantly ($P<0.001$) reduced the intermoult period of prawns i.e. moulting occurred more frequently at high hardnesses particularly in the presence of Ca:Mg ratios 4:1 and 1:1 (Table 4.6a). Prawns in waters of low Ca:Mg ratio, 1:4, had significantly ($P<0.001$) longer intermoult periods than those in other treatment combinations. At low (20mg l^{-1}) water hardness, excess magnesium (Ca:Mg=1:4) was related to the longest intermoult period.

Table 4.5 Effect of Ca:Mg ratio and hardness in water on a) mean final weight (g), and b) growth rate (mg day^{-1}) of juvenile *M. rosenbergii*. Values are means ± SE (n=9). Mean values (n=27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	1.00 ± 0.14	1.05 ± 0.08	1.01 ± 0.08	1.02^{a} ± 0.06
70	0.89 ± 0.11	1.05 ± 0.08	1.13 ± 0.07	1.01^{a} ± 0.06
120	0.60 ± 0.08	0.63 ± 0.13	0.87 ± 0.11	0.70^{b} ± 0.06
Mean	0.86^{x}± 0.08	0.88^{x} ± 0.06	0.98^{x} ± 0.06	

b)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	16.61 ± 2.68	15.48 ± 2.13	11.81 ± 1.74	14.63^{a}± 1.29
70	10.48 ± 1.29	15.51 ± 2.12	14.73 ± 1.04	13.43^{a}± 1.05
120	5.06 ± 1.15	9.19 ± 2.68	8.37 ± 1.46	7.26^{b}± 1.09
Mean	10.72^{x}± 1.38	13.11^{x} ± 1.45	11.25^{x} ± 1.00	

Table 4.6 Effect of Ca:Mg ratio and hardness in water on a) intermoult period (days), and b) carapace length increment (% moult $^{-1}$) of juvenile *M. rosenbergii*. Values are means ± SE (n=9). Mean values (n=27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	9.45 ± 0.30	9.15 ± 0.39	11.48 ± 0.34	9.77^{a} ± 0.27
70	8.52 ± 0.52	8.38 ± 0.42	9.56 ± 0.31	8.78^{b} ± 0.27
120	7.63 ± 0.43	7.65 ± 0.28	9.82 ± 0.69	8.57^{b} ± 0.33
Mean	8.58^{x}± 0.25	8.41^{x}± 0.23	10.23^{y}± 0.30	

b)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	6.82 ± 0.51	7.47 ± 0.59	6.14 ± 0.59	6.81^{a} ± 0.33
70	6.18 ± 0.45	6.67 ± 0.50	7.77 ± 0.59	6.77^{a} ± 0.31
120	4.59 ± 0.84	5.15 ± 0.65	5.10 ± 0.58	4.95^{b} ± 0.39
Mean	5.86^{x}± 0.39	6.43^{x}± 0.37	6.16^{x} ± 0.39	

4.3.3 Length Specific Dry Weights of Carapaces

The length specific dry weights of intermoult carapaces were significantly higher ($P<0.001$) at 20 and 70mg l^{-1} water hardnesses than in 120mg l^{-1}, and again were heavier ($P<0.001$) in the media of Ca:Mg ratios 1:1 and 1:4 than in 4:1 (Table 4.7a, Two-way ANOVA). The length specific dry weights of cast carapaces were affected neither by hardness nor Ca:Mg ratios (Table 4.7b)

The t-test demonstrated that intermoult carapaces were heavier ($P<0.001$) than the cast carapaces. Two-way ANOVA again demonstrated that the differences in length specific dry weights between intermoult and cast carapaces decreased progressively ($P<0.001$) with increasing water hardness and on the other hand the differences were significantly ($P<0.001$) higher in the media of Ca:Mg ratios of 1:1 and 1:4 than in 4:1 (Table 4.7c).

4.3.3 Length Specific Dry Weights of Carapaces

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Table 4.7 Effect of Ca:Mg ratio and hardness in water on the length specific dry weights of a) intermoult, b) cast and c) differences between intermoult and cast carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=8-9). Mean values (n=26-27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	1.72 ± 0.23	1.87 ± 0.14	1.65 ± 0.12	1.74^{a} ± 0.10
70	1.19 ± 0.14	1.67 ± 0.16	1.88 ± 0.13	1.54^{a} ± 0.10
120	0.63 ± 0.06	0.94 ± 0.20	1.39 ± 0.22	1.02^{b} ± 0.10
Mean	1.22^{x}± 0.13	1.51^{y} ± 0.12	1.61^{y} ± 0.10	

b)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	0.66 ± 0.06	0.50 ± 0.04	0.45 ± 0.05	0.53^{a} ± 0.03
70	0.58 ± 0.06	0.67 ± 0.06	0.64 ± 0.05	0.61^{a} ± 0.03
120	0.41 ± 0.07	0.44 ± 0.11	0.66 ± 0.12	0.52^{a} ± 0.06
Mean	0.54^{x} ± 0.03	0.53^{x}± 0.04	0.58^{x} ± 0.05	

c)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	1.06 ± 0.06	1.36 ± 0.12	1.19 ± 0.10	1.22^{a} ± 0.08
70	0.61 ± 0.14	1.00 ± 0.14	1.24 ± 0.08	0.92^{b} ± 0.09
120	0.22 ± 0.06	0.49 ± 0.15	0.73 ± 0.19	0.52^{c} ± 0.09
Mean	0.69^{x} ± 0.11	0.97^{y} ± 0.10	1.03^{y} ± 0.09	

4.3.4 Calcium Concentrations in Carapaces

Calcium concentrations in intermoult carapaces tended to rise slowly with increasing water hardness but the increase was not statistically significant (Table 4.8a). Calcium concentrations in cast carapaces were not affected by water hardness (Table 4.8b). On the other hand the Ca:Mg ion ratio in the media had a significant ($P<0.01$) influence on calcium concentrations in intermoult and cast carapaces, where calcium concentrations increased progressively with increased environmental calcium concentrations.

In cast carapaces calcium concentrations were a little higher than in intermoult carapaces but did not vary significantly. The differences in calcium concentrations between cast and intermoult carapaces also did not differ either by ratio or hardness of the waters (Table 4.8c).

4.3.5 Magnesium Concentrations in Carapaces

Compared to calcium, the magnesium concentrations in carapaces were less well regulated and increased significantly ($P<0.001$) in intermoult and cast carapaces as the Ca:Mg ratio decreased from 4:1 to 1:4 i.e. as the level of magnesium in the medium rose (Tables 4.9 a and b; Fig 1).

Table 4.8 Effect of Ca:Mg ratio and hardness in water on calcium concentrations (mg.g dry wt^{-1}) in a) intermoult, b) cast and c) their differences between cast and intermoult carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=8-9). Mean values (n=26-27) along the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	181.25 ± 9.22	159.20 ± 4.81	140.35 ± 1.20	160.40^{a} ± 6.68
70	168.02 ± 2.01	168.42 ± 2.01	167.22 ± 2.41	168.02^{a} ± 1.17
120	175.24 ± 4.01	169.62 ± 5.61	162.81 ± 6.42	169.22^{a} ± 3.75
Mean	174.84^{x} ± 3.66	165.61^{xy} ± 3.08	156.79^{y} ± 4.77	

b)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	182.05 ± 1.60	176.84 ± 7.62	151.58 ± 4.41	170.02^{a} ± 3.57
70	192.08 ± 5.61	170.83 ± 6.42	174.44 ± 0.80	179.25^{a} ± 3.57
120	175.64 ± 6.02	171.63 ± 8.02	165.21 ± 3.61	170.83^{a} ± 3.57
Mean	183.26^{x} ± 3.81	173.23^{xy} ± 4.09	163.61^{y} ± 3.91	

c)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	0.80 ±10.83	17.64 ±12.43	11.23 ±6.42	10.03^{a} ±5.64
70	24.06 ±7.22	2.41 ±4.41	7.22 ±2.81	11.23^{a} ±4.13
120	0.40 ±10.03	2.01 ±2.41	2.41 ±10.03	1.60^{a} ±4.17
Mean	8.42^{x} ±6.16	7.23^{x} ±4.60	6.82^{x} ±3.71	

Table 4.9 Effect of Ca:Mg ratio and hardness in water on magnesium concentrations (mg.g dry wt^{-1}) in a) intermoult, b) cast and c) differences between cast and intermoult carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=8-9). Mean values (n=26-27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	3.03 ± 0.09	3.89 ± 0.58	5.94 ± 0.61	4.29^{a}±0.52
70	2.98 ± 0.61	3.43 ± 0.45	4.41 ± 0.71	3.61^{a}±0.42
120	1.99 ± 0.37	2.72 ± 0.17	6.02 ± 0.71	3.50^{a}±0.71
Mean	2.58^{x}±0.32	3.35^{x}±0.35	5.46^{y}±0.70	

b)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	3.04 ± 0.13	4.71 ± 0.91	7.54 ± 0.41	5.10^{a}±0.75
70	2.98 ± 0.59	3.38 ± 0.31	5.32 ± 0.39	3.89^{b}±0.44
120	2.27 ± 0.17	3.32 ± 0.15	6.65 ± 0.64	4.08^{b}±0.70
Mean	2.76^{x}±0.25	3.80^{y}±0.41	6.50^{z}±0.43	

c)

Hardness	Calcium : Magnesium 4:1	1:1	1:4	Mean
20	0.01 ± 0.20	0.83 ± 1.38	1.59 ± 0.68	0.81^{a}±0.51
70	0.01 ± 0.89	0.95 ± 0.36	2.88 ± 0.27	1.27^{a}±0.50
120	0.52 ± 0.33	0.59 ± 0.22	0.62 ± 0.38	0.58^{a}±0.16
Mean	0.17^{x} ±0.29	0.79^{xy}±0.42	1.70^{y} ± 0.40	

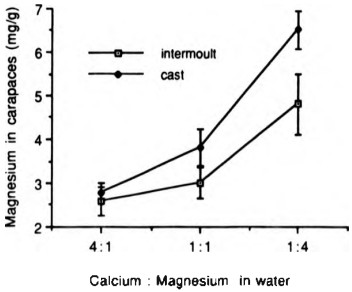


Figure 1 Effect of environmental Ca:Mg ratio on the magnesium concentrations in intermoult and cast carapaces of juvenile *M. rosenbergii*. Vertical bars are ± SE (n=26-27).

Table 4.10 Effect of environmental ratio (Ca:Mg) on the Ca:Mg ratio in intermoult and cast carapaces of juvenile *M. rosenbergii*. The atom ratio was calculated from seperate measurements of Ca and Mg ($mmol.g^{-1}$).

Ca:Mg ratio in culture media	Ca:Mg ratio in intermoult carapaces	Ca:Mg ratio in cast carapaces
4 : 1	4.36 : 0.11 = 39.64	4.57 : 0.11 = 41.55
1 : 1	4.13 : 0.12 = 34.42	4.32 : 0.17 = 27.00
1 : 4	3.91 : 0.20 = 19.55	4.08 : 0.27 = 15.11

Like calcium, the magnesium levels in cast carapaces were higher than in intermoult but again the differences were not significant ($P>0.05$, t-test). However the differences in magnesium concentrations between cast and intermoult carapaces showed a significant increase ($P<0.05$) as Ca:Mg ratio decreased from 4:1 to 1:4 (Two-way ANOVA, Table 4.9c), but water hardness did not influence this result significantly.

4.3.6 Ca/Mg Ratio in Carapaces

The increased Ca/Mg ratio in water resulted in an increased atom ratio both in intermoult and cast carapaces (Table 4.10).

4.4 Discussion

4.4.1 White Muscle Syndrome

White muscle syndrome occurred in this experiment as it did in previous trials (Chapter 3). A significant number of prawns reared in hardness levels 70 and 120mg l^{-1} were affected by the syndrome, particularly when Ca:Mg ratio was 4:1. At similar hardnesses when the Ca:Mg ratios were 1:1 and 1:4 only one prawn (out of 9 prawns) in each medium showed signs of the syndrome. It appears that at high hardness

levels the 4:1 ratio of calcium to magnesium was responsible for the condition. It should however be noted that the alkalinity level in this experiment was 110mg l^{-1}. Since the role of alkalinity at this level on different ratios was not determined, the possible effects of alkalinity or interactions between alkalinity and high calcium levels on the occurrence of white muscle syndrome cannot be ruled out. Detailed discussions of the effect of alkalinity and hardness on the occurrence of white muscle syndrome are presented later (Chapter 5).

All the prawns in this experiment survived except one animal which died while moulting at hardness level 120mg l^{-1} when Ca:Mg ratio was 4:1. Although death of only one animal seemed insignificant, the higher incidence of white muscle syndrome in that particular treatment indicated that prolonged exposure to this medium might cause higher mortality or could make the prawns more vulnerable to disease.

4.4.2 Growth

In this experiment the best growth rate (13.11mg day^{-1}) and carapace length increment (6.43% $moult^{-1}$) were observed when Ca:Mg ratio in culture media was 1:1. Though the water hardness range in this study was not very high (20-120mg l^{-1}), the sharp decline in the growth rate of prawns from 13.43mg to 7.26mg day^{-1} from hardness level 70mg l^{-1} to 120mg l^{-1} respectively, led to the suspicion that the alkalinity of 110mg l^{-1} in conjunction with 120mg l^{-1} water hardness was perhaps

slowing the growth rate of prawns. Indeed the results from experiments done later (Chapter 5) provided support for the hypothesis that the alkalinity of 110mg l^{-1} in conjunction with high hardness had a disproportionately large effect on the growth rate of prawns.

Interestingly it was noticed that the moulting frequency of prawns was enhanced by increased levels of water hardness (>70mg l^{-1}) while the media of high magnesium (Ca:Mg=1:4) reduced the moulting frequency at all hardness levels. This result again raised the suspicion that perhaps alkalinity of 110mg l^{-1} in conjunction with high environmental calcium was stimulating the moulting frequency. Later (Chapter 5) it was revealed that alkalinity levels of >100mg l^{-1} in conjunction with increased hardness (>80mg l^{-1}) significantly increased the moulting frequency of juvenile prawns (Table 5.17a).

4.4.3 Carapace Mineralization

The major constituents of crustacean exoskeletons are inorganic materials mainly calcium carbonate (Dall 1965a and Richards 1951). The measurements of length specific dry weights of carapaces provided an indication of the thickness of carapaces and in this experiment the length specific dry weights of intermoult carapaces increased as the Ca:Mg ratio decreased from 4:1 to 1:4. The increased length specific dry weights of intermoult carapaces might be either due to heavy mineralization or

due to the presence of a higher amount of organic matter in carapaces. Later experiments described in Chapter 5 demonstrated that increased alkalinity (>100mg l^{-1}) enhanced calcium deposition in intermoult carapaces of prawns (Table 5.21 a). In this experiment the alkalinity level of 110mg l^{-1} slightly enhanced the calcium deposition in carapaces specifically when calcium level in the media was high (i.e. Ca:Mg=4:1) (Table 4.8a). Despite the trend of higher calcium mineralization, the length specific dry weights of intermoult carapaces declined as the Ca/Mg ratio increased (i.e. Ca:Mg=4:1, Table 4.7a), thus suggesting that the heavier carapace weights in the media of 1:1 and 1:4 ratios were due to the presence of greater amounts of organic matter.

The length specific dry weights of intermoult carapaces were always higher than cast carapaces, thus indicating possible withdrawal of organic matter and/or minerals from the old exuviae prior to casting. Again the higher concentrations of calcium in cast than in intermoult carapaces (Tables 4.8 a and b) could be the result of the withdrawal of organic matter. The differences in length specific dry weights between intermoult and cast carapaces were higher at low hardness and again at Ca:Mg ratios of 1:1 and 1:4 media (Table 4.7c) suggesting more withdrawal of material from old exuviae prior to ecdysis when prawns were exposed to these media. Further discussion of the withdrawal of organic matter and/or minerals from old exuviae prior to casting is presented later in Chapter 5.

In this experiment magnesium did not appear to be as well regulated as calcium. The Ca/Mg ratio in intermoult and cast carapaces declined as the magnesium level in the rearing media was increased i.e. to a Ca:Mg ratio of 1:4 (Table 4.10). In a study of mineralization in *Penaeus monodon*, Wickins (1984b) found that the Ca/Mg atom ratio in carapaces was increased with the increase in the pH of the media though the Ca/Mg atom ratio in the media remained almost the same. In contrast to the present study, Gibbs and Bryan (1972) found a fairly constant Ca/Mg atom ratio (7.81 - 10.31) in the exoskeleton of marine fiddler crabs *Uca burgersi* while the ratio in water varied from 0.19 to 0.78. In the present study the magnesium concentrations in carapaces increased when the environmental concentrations were higher, thus reducing the Ca/Mg ratio value. Zanders (1980) and Stern *et al.* (1987) also observed elevated concentrations of magnesium in the hemolymph of *Carcinus maenas* and *M. rosenbergii* respectively when they were exposed to waters high in magnesium, thus further supporting the idea that magnesium is only poorly regulated.

Despite better regulation of calcium in *M. rosenbergii*, the concentrations of calcium in the rearing media particularly at the 4:1 (Ca:Mg) ratio created a stressful condition that resulted in a higher incidence of white muscle syndrome in prawns at high hardness levels (with alkalinity level of 110mg l^{-1}). On the other hand similar concentrations of magnesium in the media (at Ca:Mg ratio of 1:4) did not cause any detectable problems even though the regulation of magnesium appeared to be poor (Tables 4.9 a, b and c; Fig I).

4.5 Conclusion

The main objective of this experiment was to find a Ca:Mg ion ratio in water suitable for *M. rosenbergii*. The results obtained suggest that a Ca:Mg ratio of 4:1 created a stressful condition at an alkalinity level of 110mg l^{-1} that resulted in the occurrence of white muscle syndrome in prawns and produced the least growth rate (mg day^{-1}) among the 3 ratios. On the other hand 1:1 and 1:4 ratios of calcium to magnesium at 110mg l^{-1} alkalinity level did not produce the white muscle condition to any significant degree and only one prawn in each treatment was affected. However the 1:1 ratio produced better growth than did 1:4. Again prawns in the media of 1:4 ratio showed a poor regulating ability for magnesium and this high ratio magnesium in the water influenced the Ca/Mg atom ratio in their carapaces. Thus high magnesium in the media apparently did not seem to be a satisfactory environment for *M. rosenbergii*. Stern *et al.* (1987) suggested that high levels of magnesium in the hemolymph affect nervous and muscular activity in crustaceans, rendering animals inactive in some cases. In the lights of these facts, a calcium to magnesium ratio of 1:1 was chosen for all the subsequent studies.

CHAPTER 5

EFFECTS OF ENVIRONMENTAL ALKALINITY AND HARDNESS ON POSTLARVAL AND JUVENILE PRAWNS *M. ROSENBERGII*.

5.1 Introduction

In many natural waters total alkalinity and total hardness values are normally similar in magnitude because calcium, magnesium, bicarbonate and carbonate ions in water are derived in equivalent quantities from the solution of limestone in geological deposits (Boyd and Lichtkoppler 1979). However, in some waters calcium and magnesium concentrations are low and bicarbonate ions are associated largely with sodium and potassium ions whose carbonates are highly soluble in water. Thus large amounts of carbonate may persist in waters where total alkalinity is high and total hardness is low (Mandal and Boyd 1980). Under these conditions the pH of water may rise to extremely high levels particularly during periods of rapid photosynthesis (Boyd and Lichtkoppler 1979). It has also been reported by Boyd *et al.* (1978) that some wells on the coastal plain of the south eastern United States yield water with total alkalinity to total hardness ratios of 5-10:1 ; and when fish ponds were supplied water from these wells, prolonged periods of high pH resulted in poor growth of fish or fish kills (Mandal and Boyd 1980). It is thus clear that the alkalinity and hardness

of water can vary independently from one place to another depending on the geology of the area. The changes in the alkalinity and hardness of water may affect the life of the freshwater prawn *M. rosenbergii*. Moulting is an essential part of crustacean life, and environmental differences in Ca^{2+} and HCO_3^- ions are likely to affect the normal physiology, growth and survival of prawns (particularly since $CaCO_3$ is the major inorganic constituent of the exoskeleton, Dall 1965a).

It is not known whether postlarvae are more or less sensitive to alkalinity and hardness than older juveniles. In previous studies on water hardness there have been apparent conflicting results. Cripps and Nakamura (1979), Howlader and Turjoman (1984), Vasquez *et al.* (1989) and Brown *et al.* (1991) found that growth was adversely affected (and in the latter case, also survival) by increased water hardness. In contrast, Heinen (1977) and Bartlett and Enkerlin (1983) found no inhibition of growth due to water hardness (and in the former case, also survival). Bartlett and Enkerlin (1983) indicated that probably the higher carbonate in the water of Cripps and Nakamura's study was responsible for growth inhibition. Recently, Brown *et al.* (1991) highlighted the need to determine the extent to which prawn growth and shell mineralization are affected by the balance between levels of hardness and alkalinity in water, and it is possible that the postlarval prawns might be more susceptible to variability in alkalinity and hardness of water. This study was therefore designed to investigate the independent and interaction effects of alkalinity and hardness on postlarval and juvenile *M. rosenbergii* by altering the levels of alkalinity and hardness in the water independently. The findings are discussed in relation to comparable

results reported in the literature.

5.2 Materials and Methods

5.2.1 Experiment Location, Facility and Source of Animals

Experiment location, environment, experimental tanks and water system were as described in chapter 2. Full sibling postlarval (30-35 days post metamorphosis) and juvenile (45-50 days post metamorphosis) *Macrobrachium rosenbergii* were selected for each experiment from the laboratory stock (section 2.2).

5.2.2 Experimental Protocol and Design of Experiments

Three experiments were conducted both with postlarvae (mean live weight 0.12-0.13g) and with larger juveniles (0.39-0.56g) in which each treatment was replicated three times.

Experiment 1

This was designed to assess the influence of alkalinity and hardness each of three levels, in a 3 x 3 factorial experiment. The three alkalinity levels were 10, 100 and 250mg l^{-1} expressed as $CaCO_3$, and the three hardness levels were 20, 80 and 160mg

l^{-1} expressed as $CaCO_3$. The total combinations of the two factors each at three levels were 9. Each combination had three replicates, each tank representing one replicate so a total of 27 tanks were used in the experiment. The treatment combinations were randomly distributed in four troughs (section 2.4).

Experiment 2

In this experiment the effect of a wider range of water hardness (20, 80, 160 and 320mg l^{-1} as $CaCO_3$) at a medium level of water alkalinity (100mg l^{-1} as $CaCO_3$) was studied in a randomised design.

Experiment 3

Since results of experiment 2 suggested that postlarval and juvenile prawns might survive and grow well even at a very high level of water hardness provided the alkalinity of water was low, this was investigated in experiment 3 where the effects on juvenile prawns of a wide range of water hardness (20 and 1000mg l^{-1} as $CaCO_3$) at a low level of water alkalinity (25mg l^{-1} as $CaCO_3$) were studied. Postlarval prawns were exposed to hardness levels of 20, 500 and 1000mg l^{-1} at an alkalinity level of 25mg l^{-1}. The additional treatment of 500mg l^{-1} water hardness was tested with postlarval prawns incase they fail to survive at the highest water hardness tested (1000mg l^{-1}).

The treatments and initial prawn weights of all three experiments are shown in Table 5.1.

Table 5.1 The total number and initial weights of postlarval and juvenile *M. rosenbergii*. The expected and observed hardness and alkalinity levels (expressed as mg l^{-1} $CaCO_3$ ± SE, n=3), and pH range in three experiments.

Expt No.	No. of prawns	Initial mean wt (g) of postlarvae	Initial mean wt (g) of juveniles	Expected hardness	Observed hardness	Expected alkalinity	Observed alkalinity	pH
1	81	0.12 ± 0.002	0.39 ± 0.01	20	22 ± 0.0	10	9 ± 0.3	6.8 - 7.2
				80	77 ± 0.3	100	100 ± 0.0	8.1 - 8.3
				160	154 ± 0.2	250	258 ± 2.8	8.5 - 8.6
2	36	0.12 ± 0.003	0.56 ± 0.01	20	28 ± 0.4	100	100 ± 0.0	8.1 - 8.4
				80	90 ± 0.3	100	100 ± 0.0	,, ,,
				160	169 ± 0.5	100	100 ± 0.0	,, ,,
				320	317 ± 0.9	100	100 ± 0.3	,, ,,
3	18	0.13 ± 0.003	0.40 ± 0.02	20	23 ± 0.2	25	25 ± 0.0	7.1 - 7.2
				500	507 ± 2.0	25	25 ± 0.0	,, ,,
				1000	1012 ± 2.8	25	26 ± 0.2	,, ,,

Rearing period: Experiment 1 (Postlarvae=40 days; Juveniles=45 days)
Experiment 2 (Postlarvae=40 days, Juveniles=38 days)
Experiment 3 (Postlarvae=39 days; Juveniles=39 days)

5.2.3 Culture Media

In this study a Ca:Mg (1:1) ratio in the culture media was selected on the basis of the previous experiment (Chapter 4). The chemical composition of the culture media is presented in Table 5.2. Alkalinity of test media was adjusted to 10, 25, 100 and 250mg l^{-1} as $CaCO_3$ using $NaHCO_3$ solution. Total alkalinity and total hardness of water were measured as described in Chapter 2 (section 2.6.1).

5.2.4 Husbandry and Water Quality Measurements

Prawn husbandry and water quality measurements were as described in Chapter 2 (sections 2.5 and 2.6 respectively).

5.2.5 Measurements and Analyses of Carapaces

Cast and intermoult carapace collection, preservation and analyses of calcium and magnesium were carried out as described in Chapter 2 (sections 2.7 and 2.8). Carapace thickness was measured using a micrometer scale.

Table 5.2 Chemical compositions of culture media. All chemicals used were of AnalaR grade (BDH, Poole, Dorset).

No.	Chemical	Inclusion (mgl^{-1}) of culture media					
		Hardnesses (mg l^{-1} as $CaCO_3$)					
		20	80	160	320	500	1000
1	$CaCl_2.6H_2O$	21.9	87.6	175.2	350.4	547.5	1095.0
2	$MgCl_2.6H_2O$	20.3	81.3	162.4	324.8	507.5	1015.0
3	Na_2SO_4	178.5	178.5	178.5	178.5	178.5	178.5
4	KCl	7.1	7.1	7.1	7.1	7.1	7.1
5	$NaHCO_3$*						

*$NaHCO_3$ was used at the rate of 5.3, 36.0, 147.0 and 400.0mg l^{-1} of culture media to produce water of total alkalinity 10, 25, 100 and 250mg l^{-1} as $CaCO_3$.

5.2.6 Presentation of Results and Terminology

Results are presented as arithmetic means ± standard error (SE). The terms used for specific measurements in this study were growth rate, carapace length increment (% moult^{-1}), length specific dry weight of carapace, calcium and magnesium concentrations in carapace, and length specific calcium content in carapace as described in section 2.9. The results for postlarvae are presented in section 5.3 and for juveniles in section 5.4. In some of the figures in section 5.3 results from both postlarvae and juveniles are presented to aid comparison.

5.2.7 Statistical Analyses

The normality of data was checked before applying analysis of variance. In experiment 1, two-way analysis of variance (Two-way ANOVA) was used to detect the interaction effects between alkalinity and hardness as well as the overall independent effects of each of the factors. Although experiment 1 was arranged in a 3 x 3 factorial design, when postlarvae were being studied two-way ANOVA was used at 3 x 2 levels (alkalinity x hardness) for the analyses of length specific dry weight, length specific calcium content, calcium and magnesium concentrations of cast and intermoult carapaces due to the death of all postlarval prawns before 5th moult in one of the treatment combinations. In experiments 2 and 3 one-way ANOVA was used to assess the overall effect of water hardness on the means of

different treatment groups. Effects of the two water hardness treatments in experiment 3 on juveniles were compared by the t-test. When F-statistics indicated a significant difference ($P \leq 0.05$), multiple range analysis at 95% LSD intervals (LSD-test) was further employed to discern specific differences between treatment groups. The t-test was again applied in all three experiments to compare the length specific dry weight, length specific calcium content and, calcium and magnesium concentrations and their differences between cast and intermoult carapaces.

5.3 Results (Postlarvae)

The results presented here demonstrate the independent and interaction effects of environmental alkalinity and hardness on the incidence of white muscle syndrome, survival, growth (in terms of final weight, growth rate, moulting frequency and carapace length increment per moult) and carapace mineralization in postlarval prawns of *M. rosenbergii*. The results for juveniles will be presented seperately.

5.3.1 Water Quality

Measurements of water quality in all three experiments showed little variation between the calculated (expected) and observed values of alkalinity and hardness (Table 5.1). The pH of water remained virtually unchanged within the 48 hours

period between water changes. Ammonia and nitrite nitrogen levels showed a gradual increase from 0.01 to 0.20mg total ammonia N l^{-1} and 0.01 to 0.25mg NO_2-N l^{-1} over the 48 hour period between water changes but these levels were judged unlikely to be harmful to the postlarval prawns (Wickins 1976).

5.3.2 White Muscle Syndrome

The abdomens (tails) of healthy postlarval prawns were transparent. However some changes in the physical appearance and behaviour of the postlarvae were noticed particularly in high alkalinity waters. Numerous postlarvae in high alkalinity water lost their body transparency, and became opaque with a milky-white coloration of abdominal muscles. The incidence of this "white muscle syndrome" started after the second moult. These prawns looked severely stressed, showed sluggish movement and had a low appetite for food. Sometimes they were found to remain in a vertical position clinging to the inner side of the mesh container. During harvest they neither jumped nor struggled to escape but often died within a few minutes of harvest. In experiment 1, two-way ANOVA demonstrated that the incidence of white muscle syndrome increased progressively ($P<0.001$) with increasing alkalinity and hardness (Table 5.3a, Fig IIa). The significant interaction ($P<0.01$) between alkalinity and hardness on the occurrence of white muscle syndrome began when alkalinity and hardness levels were 100mg l^{-1} and 80mg l^{-1} respectively where the incidence tended to increase with the increase in either parameter. Water hardness over 80mg l^{-1} (in

Table 5.3 Effect of alkalinity and hardness on a) white muscle syndrome (%), and b) survival (%) of postlarval *M. rosenbergii*. Values are means ± SE (n= 9 and 3 for white muscle syndrome and survival respectively). Mean values (n=27 and 9 for white muscle syndrome and survival respectively) along the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	0	44 ± 15	56 ± 18	45^{a} ± 10
100	0	67 ± 17	89 ± 11	52^{a} ± 10
250	89 ± 11	89 ± 11	89 ± 11	89^{b} ± 11
Mean	30^{x} ± 9	67^{y} ± 8	78^{y} ± 8	

b)

Alkalinity	Hardness 20	80	160	Mean
10	100	78 ± 11	89 ± 11	89^{a} ± 6
100	44 ± 22	67 ± 19	89 ± 11	67^{b} ± 11
250	0	67 ± 0	100	56^{b} ± 14
Mean	48^{x} ± 16	71^{y} ± 7	93^{z} ± 5	

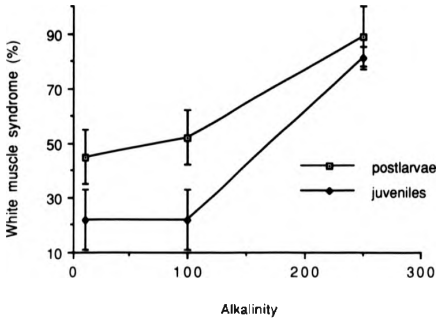


Figure II Effect of alkalinity (mg l^{-1} as $CaCO_3$) on the occurrence of white muscle syndrome (%) in juvenile *M. rosenbergii*. Vertical bars are ± SE (n=27).

combination with alkalinity of 100mg l^{-1}) in experiment 2 significantly increased ($P<0.01$) the incidence of the syndrome (Table 5.4) but no differences in incidence due to water hardness was detected in experiment 3 where alkalinity was low (25mg l^{-1}) (Table 5.5).

5.3.3 Survival

Statistical analyses (Two-way ANOVA) of experiment 1 showed that survival of postlarval prawns declined with increasing alkalinity ($P<0.01$) and decreasing hardness ($P<0.001$). The interaction effect between alkalinity and hardness on prawn survival was also significant ($P<0.01$). In particular, high alkalinity in conjunction with low hardness resulted in poor survival (Table 5.3b). No differences in prawn survival were observed in experiments 2 and 3 where all prawns survived all treatments of water hardness tested (20 to 1000mg l^{-1}) (Tables 5.4 and 5.5 respectively). It is not known why the survival of prawns exposed to 100mg l^{-1} alkalinity and 20mg l^{-1} hardness in experiment 1 was so much less than in experiment 2.

Table 5.4 The effects of increasing hardness at constant moderate alkalinity (100mg l^{-1} as $CaCO_3$) on white muscle syndrome, survival, final weights, growth rate, intermoult period, carapace length increment, length specific dry weights of carapace, length specific calcium content, calcium and magnesium concentrations in carapace of postlarval *M. rosenbergii*. Values are means ± SE (n=9). Mean values along the row with common superscript letters did not show significant differences at 95% LSD intervals.

Alkalinity (mg l^{-1} $CaCO_3$)	100	100	100	100
Hardness (mg l^{-1} $CaCO_3$)	20	80	160	320
White muscle syndrome(%)	0[a]	44[b]	55[b]	55[b]
Survival (%)	100	100	100	100
Final weight of prawns (g)	0.30[a] ± 0.02	0.29[a] ± 0.02	0.22[a] ± 0.02	0.22[a] ± 0.02
Growth rate (mg day^{-1})	5.13[a] ± 0.67	2.96[b] ± 0.39	2.81[b] ± 0.74	2.96[b] ± 0.56
Intermoult period (days)	6.83[a] ± 0.19	6.36[ab]± 0.19	6.14[b] ± 0.15	6.05[b] ± 0.18
Carapace length increment (% $moult^{-1}$)	5.80[a] ± 0.52	3.97[ab]± 0.49	4.39[b] ± 0.80	3.54[b] ± 0.55
Length specific dry weight of intermoult carapaces	0.73[a] ± 0.08	0.43[b] ± 0.04	0.40[b] ± 0.07	0.42[b] ± 0.07
Length specific dry weight of cast carapaces	0.19[a] ± 0.01	0.19[a] ± 0.02	0.19[a] ± 0.02	0.17[a] ± 0.02
Diff. in length specific dry wt between intermoult & cast carapaces	0.51[a] ± 0.06	0.24[b] ± 0.03	0.21[b] ± 0.06	0.25[b] ± 0.05
Calcium concentrations in intermoult carapaces (mg.g^{-1})	181.25[a] ± 2.19	193.28[a] ± 4.81	191.28[a] ± 6.28	190.48[a] ± 4.99
Calcium concentrations in cast carapaces (mg.g^{-1})	196.89[a] ± 5.19	198.09[a] ±10.77	190.07[a] ± 7.18	198.09[a] ± 7.75
Diff.in calcium concentrations between cast & intermoult carapaces	13.23[a] ±4.26	4.81[a] ± 13.44	0.80[a] ± 12.86	0.80[a] ± 9.42
Length specific calcium content in intermoult carapaces	0.1306[a] ±0.0143	0.0829[b] ±0.0070	0.0724[b] ±0.0113	0.0817[b] ±0.0113
Length specific calcium content in cast carapaces	0.0372[a] ±0.0023	0.0369[a] ±0.0033	0.0359[a] ±0.0047	0.0337[b] ±0.0040
Diff.in length sp. calcium content between intermoult & cast carapaces	0.0885[a] ±0.0111	0.0459[b] ±0.0068	0.0408[b] ±0.0106	0.0480[b] ±0.0083
Magnesium concentrations in intermoult carapaces (mg.g^{-1})	3.42[a] ± 0.13	2.18[b] ± 0.11	1.91[b] ± 0.17	2.26[b] ± 0.39
Magnesium concentrations in cast carapaces (mg.g^{-1})	3.74[a] ± 0.14	2.91[b] ± 0.20	2.56[b] ± 0.17	3.41[a] ± 0.34
Diff. in magnesium concentrations between cast & intermoult carapaces	0.29[a] ± 0.20	0.73[a] ± 0.27	0.64[a] ±0.25	1.03[a] ± 0.26

Table 5.5 The effects of low and high hardness with constant, low alkalinity (25mg l^{-1} as $CaCO_3$) on white muscle syndrome, survival, final weights, growth rate, intermoult period, carapace length increment, length specific dry weights of carapace, length specific calcium content, calcium and magnesium concentrations in carapace of postlarval *M. rosenbergii*. Values are means ± SE (n=9). Mean values along the rows with common superscript letters did not show significant differences at 95% LSD intervals.

Alkalinity (mg l^{-1} $CaCO_3$)	25	25	25
Hardness (mg l^{-1} $CaCO_3$)	20	500	1000
White muscle syndrome(%)	22[a]	33[a]	22[a]
Survival (%)	100	100	100
Final weight of prawns (g)	0.21[a] ± 0.02	0.19[a] ± 0.02	0.21[a] ± 0.01
Growth rate (mg day^{-1})	2.85[a] ± 0.45	1.87[a] ± 0.47	2.49[a] ± 0.33
Intermoult period (days)	6.69[a] ± 0.40	6.19[a] ± 0.13	6.75[a] ± 0.29
Carapace length increment (% $moult^{-1}$)	4.47[a] ± 0.82	3.60[a] ± 0.62	5.42[a] ± 0.56
Length specific dry weight of intermoult carapaces	0.50[a] ± 0.07	0.31[a] ± 0.04	0.43[a] ± 0.05
Length specific dry weight of cast carapaces	0.14[a] ± 0.01	0.14[a] ± 0.01	0.16[a] ± 0.01
Diff. in length specific dry wt between intermoult & cast carapaces	0.36[a] ± 0.06	0.19[a] ± 0.04	0.27[a] ± 0.05
Calcium concentrations in intermoult carapaces ($mg.g^{-1}$)	190.48[a] ± 11.63	189.67[a] ± 9.22	181.25[a] ± 10.83
Calcium concentrations in cast carapaces ($mg.g^{-1}$)	201.70[a] ± 2.01	218.95[a] ± 3.61	204.51[a] ± 2.81
Diff.in calcium concentrations between cast & intermoult carapaces	10.93[a] ± 11.24	26.92[a] ± 8.85	15.33[a] ± 10.60
Length specific calcium content in intermoult carapaces	0.0771[a] ± 0.0203	0.0669[a] ± 0.0084	0.0867[a] ± 0.0096
Length specific calcium content in cast carapaces	0.0256[a] ± 0.0041	0.0255[a] ± 0.0023	0.0293[a] ± 0.0024
Diff.in length sp. calcium content between intermoult & cast carapaces	0.0545[a] ± 0.0174	0.0436[a] ± 0.0075	0.0573[a] ± 0.0089
Magnesium concentrations in intermoult carapaces ($mg.g^{-1}$)	2.56[a] ± 0.15	3.18[a] ± 0.34	3.25[a] ± 0.24
Magnesium concentrations in cast carapaces ($mg.g^{-1}$)	3.04[a] ± 0.17	3.54[a] ± 0.32	3.65[a] ± 0.21
Diff. in magnesium concentrations between cast & intermoult carapaces	0.48[a] ± 0.46	0.27[a] ± 0.39	0.41[a] ± 0.30

5.3.4 Growth

5.3.4.1 Mean Final Weight

In experiment 1 mean final weights of postlarvae were significantly ($P<0.05$) less at 250mg l^{-1} alkalinity than in low to moderate alkalinity (10-100mg l^{-1}) while hardness levels >80mg l^{-1} produced smaller prawns than those of 20mg l^{-1} (Two-way ANOVA, Table 5.6 a). The interaction effects of alkalinity and hardness on final weights was also significant ($P<0.05$). In experiment 2, although the highest mean final weight was recorded in the lowest water hardness, it did not differ significantly from the rest (Table 5.4), and the final weights in experiment 3 also did not show any significant differences (Table 5.5) where a wide range of water hardness (20-100mg l^{-1}) was tested at low alkalinity (25mg l^{-1}).

5.3.4.2 Growth Rate

In experiment 1, increased alkalinity and hardness both significantly ($P<0.001$, Two-way ANOVA) inhibited the growth rate of postlarval prawns. Growth rate at low to moderate alkalinity (10-100mg l^{-1}) was significantly higher than in high alkalinity (250 mg l^{-1}) waters, and growth rate at 20mg l^{-1} water hardness was significantly higher than at hardness levels of 80 and 160mg l^{-1} (Table 5.6b). The interaction effects of alkalinity and hardness on growth were also significant ($P<0.05$),

Table 5.6 Effect of alkalinity and hardness on a) mean final weight, and b) growth rate (mg day^{-1}) of postlarval *M. rosenbergii*. Values are means ± SE (n=9). Mean values (n=27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	0.27 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	0.20^{x} ± 0.01
100	0.25 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0.20^{a} ± 0.01
250	0.18 ± 0.02	0.14 ± 0.01	0.19 ± 0.02	0.17^{b} ± 0.01
Mean	0.23^{x}± 0.01	0.18^{y} ± 0.01	0.17^{y} ± 0.01	

b)

Alkalinity	Hardness 20	80	160	Mean
10	4.40 ± 0.76	2.45 ± 0.36	1.78 ± 0.48	2.88^{A} ± 0.40
100	4.00 ± 0.54	2.61 ± 0.59	1.78 ± 0.48	2.80^{a} ± 0.39
250	1.64 ± 0.48	0.86 ± 0.20	1.78 ± 0.43	1.43^{b} ± 0.23
Mean	2.85^{x}± 0.44	1.97^{y}± 0.27	1.78^{y}± 0.26	

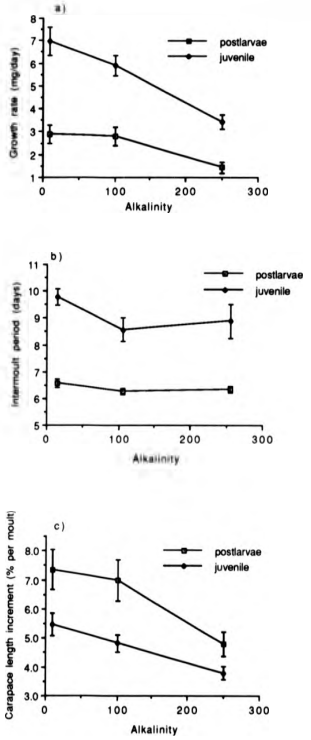


Figure III Effect of alkalinity (mg l^{-1} as $CaCO_3$) on a) growth rate (mg day^{-1}), and b) intermoult period (days) and c) carapace length increment (% $moult^{-1}$) of postlarval and juvenile *M. rosenbergii*. Vertical bars are ± SE (n=19-27 for postlarval and 25-27 for juvenile prawns).

where alkalinity and hardness over 100mg l^{-1} and 80mg l^{-1} respectively were associated with decreasing growth rate. Best growth was obtained at low to moderate alkalinity (10-100mg l^{-1}, Fig IIIa) in conjunction with low hardness (20mg l^{-1}). Increased water hardness in experiment 2 (with alkalinity of 100mg l^{-1}) showed a significant inhibition ($P<0.05$) of prawn growth and again, the highest growth was recorded in the lowest level of water hardness (Table 5.4). Growth was however lower in general but changed little ($P>0.05$) over a wide range of water hardnesses in experiment 3 (Table 5.5) where alkalinity was low (25mg l^{-1}).

5.3.4.3 Intermoult Period

In experiment 1 the mean data of 3 intermoult periods (due to the death of all postlarvae at the combination of the highest alkalinity and the lowest hardness before the 5th moult, data from only 3 intermoult periods was available for statistical analysis) showed that the intermoult period decreased with increase in alkalinity (Fig IIIb) and hardness but statistically, the differences were not significant (Two-way ANOVA, Table 5.7a). In experiment 2 the data from 4 intermoult periods showed a significant decrease ($P<0.05$) with increase in water hardness where alkalinity was 100mg l^{-1} (Table 5.4), but no differences due to water hardness were detected in data from 4 intermoult periods in experiment 3 where alkalinity was 25mg l^{-1} (Table 5.5).

Table 5.7 Effect of alkalinity and hardness on a) intermoult period (days), and b) carapace length increment (% moult^{-1}) of postlarval *M. rosenbergii*. Values are means ± SE (n=9). Mean values (n=27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	6.89 ± 0.30	6.17 ± 0.19	6.22 ± 0.22	6.43^{a} ± 0.15
100	6.04 ± 0.18	6.14 ± 0.28	6.11 ± 0.21	6.10^{a} ± 0.12
250	6.33 ± 0.24	6.11 ± 0.19	6.10 ± 0.22	6.18^{a} ± 0.12
Mean	6.42^{x}± 0.15	6.14^{x}± 0.12	6.15^{x}± 0.12	

b)

Alkalinity	Hardness 20	80	160	Mean
10	8.26 ± 1.16	6.04 ± 0.83	7.59 ± 1.45	7.35^{a} ± 0.69
100	8.69 ± 0.54	6.70 ± 1.58	6.48 ± 0.88	6.98^{a} ± 0.71
250	4.75 ± 1.14	3.58 ± 0.48	5.90 ± 0.52	4.79^{b} ± 0.43
Mean	7.16^{x}± 0.79	5.32^{y}± 0.60	6.67^{xy}± 0.60	

5.3.4.4 Carapace Length Increment (% moult^{-1})

In experiment 1 low to moderate (10-100mg l^{-1}) alkalinity produced significantly ($P<0.01$) higher carapace length increments per moult than in high alkalinity (250mg l^{-1}) waters (Fig IIIc), and the effect of the two water hardnesses (80 and 160mg l^{-1}) was not significant (Two-way ANOVA, Table 5.7 b). No significant interaction between alkalinity and hardness was detected in experiment 1. In experiment 2 the effect of water hardness on carapace length increment was just significant ($P=0.05$) where the highest increment was recorded in the lowest water hardness (Table 5.4), but when alkalinity was low (25mg l^{-1}) the wider range of water hardnesses tested in experiment 3 did not show any significant effect on carapace length increment per moult (Table 5.5).

5.3.5 Carapace Mineralization

5.3.5.1 Length Specific Dry Weight

Length specific dry weight of intermoult carapaces

In experiment 1, neither alkalinity nor hardness had any significant effect on the length specific dry weight of intermoult carapaces (Table 5.8 a). In experiment 2 the length specific dry weight of intermoult carapaces was significantly ($P<0.01$) less at

Table 5.8 Effect of alkalinity and hardness on the length specific dry weights of a) intermoult, b) cast and c) differences between intermoult and cast carapaces of postlarval *M. rosenbergii*. Values are means ± SE (n=6-9). Mean values of the respective columns (n=20-24) and rows (n=12-16) with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 80	Hardness 160	Mean
10	0.1800 ± 0.0422	0.2788 ± 0.0520	0.2327^{ab} ± 0.0354
100	0.3501 ± 0.0258	0.3011 ± 0.0379	0.3215^{a} ± 0.0247
250	0.1627 ± 0.0327	0.2712 ± 0.0499	0.2206^{b} ± 0.0330
Mean	0.2184^{x} ± 0.0271	0.2829^{y} ± 0.0265	

b)

Alkalinity	Hardness 80	Hardness 160	Mean
10	0.0947 ± 0.0166	0.0934 ± 0.0203	0.0939^{a} ± 0.0129
100	0.1697 ± 0.0192	0.1214 ± 0.0210	0.1415^{b} ± 0.0157
250	0.1071 ± 0.0159	0.1561 ± 0.0162	0.1332^{b} ± 0.0128
Mean	0.1190^{x} ± 0.0118	0.1237^{x} ± 0.0120	

c)

Alkalinity	Hardness 80	Hardness 160	Mean
10	0.0853 ± 0.0345	0.1856 ± 0.0363	0.1388^{ab} ± 0.0277
100	0.1805 ± 0.0363	0.1797 ± 0.0319	0.1800^{a} ± 0.0193
250	0.0556 ± 0.0362	0.1164 ± 0.0463	0.0880^{b} ± 0.0300
Mean	0.0994^{x} ± 0.0216	0.1597^{y} ± 0.0227	

hardnesses over 80mg l^{-1} than at 20mg l^{-1} (One-way ANOVA, Table 5.4). The effect of water hardness in experiment 3 was just significant (P=0.05) where again the highest length specific dry weight was recorded in the lowest level of water hardness (Table 5.5).

Length specific dry weight of cast carapaces

In experiment 1, significantly higher ($P<0.05$) length specific dry weights of cast carapaces were recorded from prawns in moderate to high alkalinity (>100mg l^{-1}) than in low alkalinity waters (10mg l^{-1}) (Two-way ANOVA, Table 5.8b). Although alkalinity had a significant interaction ($P<0.05$) with water hardness, the influence of water hardness on the length specific dry weight was not statistically significant. This was confirmed in experiments 2 and 3 where the length specific dry weights of cast carapaces did not show any significant differences due to water hardness (Tables 5.4 and 5.5 respectively) though in general they were higher in experiment 2 (with 100mg l^{-1} alkalinity) than in experiment 3 (with 25mg l^{-1} alkalinity).

Differences in length specific dry weight between intermoult and cast carapaces

In all three experiments, the t-test showed that the length specific dry weights were significantly ($P<0.001$) higher by 130-187% in intermoult than in cast carapaces

Table 5.9 Mean values of carapace length specific dry weights, calcium concentrations, length specific calcium content, magnesium concentrations and their differences between intermoult and cast carapaces of postlarval *M. rosenbergii* in the three experiments.

Expt No	Parameters	Cast carapace	Intermoult carapace	Difference (%)	Sig. level
1	Length specific dry weight	0.1277	0.2934	130	P<0.001
	Calcium (mg.g dry wt^{-1})	232.98	209.32	11	P<0.01
	Length specific calcium content	0.0320	0.0621	94	P<0.001
	Magnesium (mg.g dry wt^{-1})	4.05	3.20	27	P<0.01
2	Length specific dry weight	0.1839	0.5062	175	P<0.001
	Calcium (mg.g dry wt^{-1})	197.79	189.07	4	P>0.05
	Length specific calcium content	0.0359	0.0938	161	P<0.001
	Magnesium (mg.g dry wt^{-1})	3.15	2.44	29	P<0.001
3	Length specific dry weight	0.1438	0.4127	187	P<0.001
	Calcium (mg.g dry wt^{-1})	208.52	189.67	10	P<0.01
	Length specific calcium content	0.0266	0.0769	188	P<0.001
	Magnesium (mg.g dry wt^{-1})	3.50	3.09	13	P>0.05

(Table 5.9). In experiment 2, significantly greater differences ($P<0.001$) in length specific dry weight between intermoult and cast carapaces was recorded in the lowest level of water hardness (One-way ANOVA, Table 5.4). Similarly in experiment 3 the highest differences were recorded at the lowest level of water hardness but did not differ significantly from the other treatment means (One-way ANOVA, Table 5.5).

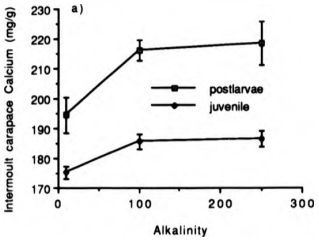
5.3.5.2 Calcium

Calcium concentrations in intermoult carapaces

In experiment 1, statistical analyses demonstrated that calcium concentrations in intermoult carapaces of postlarval prawns were significantly ($P<0.01$) higher in moderate to high (100-250mg l^{-1}, Fig IVa) alkalinity than in low (10mg l^{-1}) alkalinity waters, but water hardness had no significant influence (Two-way ANOVA, Table 5.10a). Water hardnesses in experiments 2 and 3 also did not exert any significant influence on the calcium concentrations in intermoult carapaces (Tables 5.4 and 5.5 respectively).

Calcium concentrations in cast carapaces

Cast carapaces from high alkalinity waters had significantly higher ($P<0.05$)



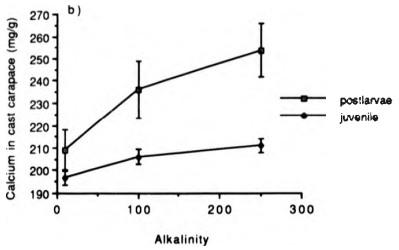


Figure IV Effect of alkalinity (mg l^{-1} as $CaCO_3$) on calcium concentrations (mg.g^{-1}) in a) intermoult and b) cast carapaces of postlarval and juvenile *M. rosenbergii*. Vertical bars are ± SE (n=12-16 for postlarval and 20-27 for juvenile prawns).

Table 5.10 Effect of alkalinity and hardness on the calcium concentrations (mg.g dry wt^{-1}) in a) intermoult, b) cast and c) differences between cast and intermoult carapaces of postlarval *M. rosenbergii*. Values are means ± SE (n=6-9). Mean values of the respective columns (n=20-24) and rows (n=12-16) with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 80	160	Mean
10	190.08 ± 9.62	200.90 ± 2.01	194.49^{a} ± 6.02
100	212.93 ± 2.81	218.55 ± 6.42	216.14^{b} ± 3.61
250	233.38 ± 17.24	211.33 ± 5.61	218.55^{b} ± 7.22
Mean	207.72^{x} ± 7.62	210.93^{x}± 3.21	

b)

Alkalinity	Hardness 80	160	Mean
10	209.72 ± 15.64	209.72 ± 9.22	209.22^{a} ± 9.22
100	214.13 ± 9.62	251.03 ± 18.45	236.19^{ab} ± 12.83
250	267.47 ± 24.86	247.02 ± 14.04	253.83^{b} ± 12.03
Mean	226.16^{x}± 11.63	238.60^{x} ± 9.22	

c)

Alkalinity	Hardness 80	160	Mean
10	19.65 ± 20.45	8.82 ± 8.82	15.24^{a} ± 12.03
100	1.20 ± 11.63	32.48 ± 20.4	20.05^{a} ± 13.63
250	34.09 ± 23.66	35.67 ± 15.64	35.29^{a} ± 12.43
Mean	18.45^{x} ± 11.63	27.67^{x} ± 9.22	

calcium concentrations than from low alkalinity waters, and water hardness again had no significant influence on calcium concentrations in cast carapaces in experiment 1 (Two-way ANOVA, Table 5.10b, Fig IVb). Calcium concentrations in cast carapaces in experiments 2 and 3 were not affected significantly by water hardness (Tables 5.4 and 5.5 respectively).

Differences in calcium concentrations between cast and intermoult carapaces

The t-test demonstrated that calcium concentrations were significantly ($P<0.01$) higher by 10-11% in cast than in intermoult carapaces in experiments 1 and 3, and though in experiment 2 the average calcium concentrations were higher by 4% in cast carapaces they did not differ significantly from intermoult carapaces (Table 5.9). It was noted that in all three experiments some individual prawns, especially those in higher water hardnesses, showed higher calcium concentrations in intermoult than in cast carapaces, however on average, the concentrations were higher in cast than in intermoult carapaces. The differences in calcium concentrations between cast and intermoult carapaces were not affected by treatments either in experiment 1 (Table 5.10 c) or in experiments 2 and 3 (Tables 5.4 and 5.5 respectively).

5.3.5.3 Length Specific Calcium Content of Carapaces

Length specific calcium content of intermoult carapaces

In experiment 1, significantly higher ($P<0.01$) length specific calcium content in intermoult carapaces were obtained from low to moderate alkalinity (10-100mg l^{-1}) than in high alkalinity (250mg l^{-1}) waters and water hardness had no significant effect (Two-way ANOVA, Table 11a). The interaction effect between alkalinity and hardness was however significant ($P<0.05$). High alkalinity (>100mg l^{-1}) in conjunction with 160mg l^{-1} hardness showed a decreasing trend in length specific calcium content in intermoult carapaces and at an alkalinity level of 250mg l^{-1} in conjunction with 80mg l^{-1} water hardness there was a sharp decrease in length specific calcium content. In experiment 2 with an alkalinity level of 100mg l^{-1}, one-way ANOVA demonstrated that a significantly higher ($P<0.01$) length specific calcium content in intermoult carapaces occurred in the lowest level of water hardness (Table 5.4) but water hardness in experiment 3 did not exert any influence on the length specific calcium content in intermoult carapaces where alkalinity was 25mg l^{-1} (Table 5.5).

Length specific calcium content of cast carapaces

In experiment 1, length specific calcium content in cast carapaces was

Table 5.11 Effect of alkalinity and hardness on the length specific calcium content in a) intermoult, b) cast and c) differences between intermoult and cast carapaces of postlarval *M. rosenbergii*. Values are means ± SE (n=6-9). Mean values of the respective columns (n=20-24) and rows (n=12-16) with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 80	Hardness 160	Mean
10	0.0737 ± 0.0076	0.0718 ± 0.0096	0.0718^{a} ± 0.0080
100	0.0785 ± 0.0051	0.0644 ± 0.0104	0.0701^{a} ± 0.0067
250	0.0435 ± 0.0098	0.0556 ± 0.0094	0.0516^{b} ± 0.0070
Mean	0.0615^{x}± 0.0066	0.0627^{x}± 0.0056	

b)

Alkalinity	Hardness 80	Hardness 160	Mean
10	0.0288 ± 0.0096	0.0270 ± 0.0048	0.0273^{a} ± 0.0028
100	0.0412 ± 0.0042	0.0285 ± 0.0047	0.0335^{b} ± 0.0035
250	0.0304 ± 0.0042	0.0375 ± 0.0027	0.0351^{b} ± 0.0024
Mean	0.0325^{x} ± 0.0030	0.0319^{x}± 0.0024	

c)

Alkalinity	Hardness 80	Hardness 160	Mean
10	0.0449 ± 0.0062	0.0448 ± 0.0060	0.0445^{a} ± 0.0061
100	0.0373 ± 0.0057	0.0360 ± 0.0079	0.0366^{a} ± 0.0050
250	0.0131 ± 0.0119	0.0182 ± 0.0089	0.0165^{b} ± 0.0069
Mean	0.0308^{x} ± 0.0050	0.0308^{x}± 0.0053	

significantly ($P<0.01$) higher at moderate to high (100-250mg l^{-1}) alkalinity than at low (10mg l^{-1}) alkalinity waters but the effect of water hardness was minimal (Two-way ANOVA, Table 5.11b). The statistical analysis indicated significant ($P<0.05$) interaction between alkalinity and hardness but the direction of the interaction was not clear. The effect of water hardness on the length specific calcium content in cast carapaces in experiments 2 and 3 was not significant (Tables 5.4 and 5.5 respectively).

Differences in length specific calcium content between intermoult and cast carapaces

In all 3 experiments the t-test showed that the length specific calcium content was significantly ($P<0.001$) higher by 94-188% in intermoult than in cast carapaces (Table 5.9). In experiment 1, the differences in length specific calcium content between intermoult and cast carapaces decreased ($P<0.05$) with increase in alkalinity (Table 5.11c) but no effect of water hardness was detected. In experiment 2 where alkalinity was moderate (100mg l^{-1}), the differences were significantly higher ($P<0.01$) at the lowest water hardness (20mg l^{-1}) than at higher hardnesses tested (Table 5.4); but the wide range of water hardnesses in experiment 3 did not significantly affect the differences in length specific calcium content between intermoult and cast carapaces where alkalinity was low (25mg l^{-1}) (Table 5.5).

5.3.5.4 Magnesium

Magnesium concentrations in intermoult carapaces

In experiment 1, alkalinity but not hardness had a significant effect ($P<0.05$) on the magnesium concentrations in intermoult carapaces (Two-way ANOVA, Table 5.12a), where magnesium concentrations were high at the two extreme alkalinities. The interaction effect of alkalinity and hardness on intermoult magnesium concentrations was significant ($P<0.05$) but the direction of the interaction was not clear due to there being only two levels of water hardness tested. In experiment 2, one-way ANOVA demonstrated that significantly higher ($P<0.001$) magnesium concentrations in intermoult carapaces occurred in the lowest water hardness (20mg l^{-1}) and then tended to decline with increasing hardness up to 160mg l^{-1}. However it increased again at 320mg l^{-1} hardness (Table 5.4). In experiment 3 where 20, 500 and 1000mg l^{-1} hardnesses were tested, the magnesium concentrations in intermoult carapaces tended to rise with increase in water hardness (Table 5.5) though they did not differ significantly.

Magnesium concentrations in cast carapaces

In experiment 1, magnesium concentrations in cast carapaces did not vary significantly due to either alkalinity or hardness (Table 5.12b). In experiment 2,

Table 5.12 Effect of alkalinity and hardness on the magnesium concentrations (mg.g dry wt^{-1}) in a) intermoult, b) cast and c) differences between cast and intermoult carapaces of postlarval *M. rosenbergii*. Values are means ± SE (n= 6-9). Mean values of the respective columns (n=20-24) and rows (n=12-16) with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 80	Hardness 160	Mean
10	2.89 ± 0.21	4.08 ± 0.36	3.38^{a} ± 0.26
100	2.26 ± 0.13	2.73 ± 0.22	2.54^{b} ± 0.16
250	4.25 ± 0.68	3.20 ± 0.36	3.79^{a} ± 0.35
Mean	3.08^{x} ± 0.27	3.29^{x} ± 0.22	

b)

Alkalinity	Hardness 80	Hardness 160	Mean
10	4.29 ± 0.43	4.17 ± 0.58	4.24^{a} ± 0.33
100	3.47 ± 0.55	4.71 ± 0.44	4.21^{a} ± 0.38
250	4.48 ± 0.65	3.36 ± 0.21	3.73^{a} ± 0.29
Mean	4.12^{x} ± 0.30	3.40^{x} ± 0.25	

c)

Alkalinity	Hardness 80	Hardness 160	Mean
10	1.40 ± 0.41	0.09 ± 0.43	0.86^{a} ± 0.35
100	1.22 ± 0.46	1.98 ± 0.58	1.67^{a} ± 0.39
250	0.23 ± 0.74	0.15 ± 0.44	0.18^{b} ± 0.37
Mean	1.04^{x} ± 0.31	0.71^{x} ± 0.34	

however, water hardness significantly affected ($P<0.01$) the magnesium concentrations in cast carapaces where high magnesium concentrations were recorded again at the two extreme levels of water hardness (Table 5.4). Magnesium concentrations in experiment 3 increased with increase in water hardness without showing any significant differences among the treatment groups (Table 5.5). It was noted that magnesium concentrations in cast carapaces in experiments 2 and 3 followed a similar trend to that of the intermoult magnesium concentrations.

Differences in magnesium concentrations between cast and intermoult carapaces

The t-test indicated that magnesium concentrations were significantly higher by 27-29% in cast than in intermoult carapaces in experiments 1 and 2, and in experiment 3 although the concentrations were higher by 13% in cast carapaces, they did not differ significantly from those in intermoult carapaces (Table 5.9). In experiment 1, the differences were significantly higher in moderate and low alkalinity than in high alkalinity waters, but the differences in magnesium concentrations between cast and intermoult carapaces did not differ significantly in relation to water hardness (Table 5.12c). Water hardnesses in experiments 2 and 3 did not show any significant effect on the differences in magnesium concentrations between cast and intermoult carapaces (Tables 5.4 and 5.5 respectively).

5.4 Results (Juveniles)

The results presented here demonstrate the independent effects of environmental alkalinity and hardness and also the interaction effects between alkalinity and hardness on the incidence of white muscle syndrome, survival, growth (in terms of final weight, growth rate, moulting frequency and carapace length increment per moult) and carapace mineralization in juvenile *M. rosenbergii*.

5.4.1 Water Quality

The water quality parameters of alkalinity, hardness and pH of culture media were found to be the same as in the postlarval study (section 5.3.1). Ammonia and nitrite nitrogen levels however were a little higher than in postlarval tanks. In this study total ammonia increased from 0.01 - 0.25mg $N\text{-}l^{-1}$ and nitrite from 0.01 - 0.30mg $NO_2\text{-}N\ l^{-1}$ over the 48 hour period between water changes. These levels were also judged unlikely to be harmful to juvenile prawns (Wickins 1976).

5.4.2 White Muscle Syndrome

Like postlarval prawns (section 5.3.2), the abdomens of some juvenile prawns in this study also became opaque and white, but in juveniles this syndrome appeared one

moult later than in postlarval prawns. The juveniles suffering from white muscle syndrome also looked severely stressed, showed sluggish movement and had a low appetite for food. Other behavioural characteristics were similar to those of the postlarval prawns. The highest alkalinity (250mg l^{-1}) in combination with all hardness levels tested produced the higher percentages of white muscle syndrome (Fig IIa). In experiment 1 two-way ANOVA indicated that juveniles suffered a significantly higher incidence of white muscle syndrome in high alkalinity (250mg l^{-1}) than in low and moderate alkalinity (10 and 100mg l^{-1}), and hardness levels of 80 and 160mg l^{-1} produced significantly higher incidence than did 20mg l^{-1} (Table 5.13a). No significant interaction between alkalinity and hardness was detected. No significant differences were found in white muscle syndrome among the juvenile prawns held in the wide range of water hardnesses tested in experiments 2 and 3 (Tables 5.14 and 5.15 respectively).

5.4.3 Survival

In experiment 1, high alkalinity (250mg l^{-1}) particularly in conjunction with low hardness (20mg l^{-1}) resulted in the poorest survival (Table 5.13b). Moderate alkalinity (100mg l^{-1}) allowed survival in excess of 67%, and the lowest alkalinity (10mg l^{-1}) in combination with all hardnesses resulted in 100% survival. The interaction of alkalinity and hardness on prawn survival was significant (Two-way ANOVA, $P<0.01$). In experiments 2 and 3 the survival of prawns was 100% in all hardness

Table 5.13 Effect of alkalinity and hardness of water on a) the white muscle syndrome (%), and b) survival (%) in juvenile *M. rosenbergii*. Values are means ± SE (n=9 for white muscle and 3 for survival). Mean values (n=27 for white muscle and 9 for survival) along the respective columns and rows sharing common supercript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	0	33 ± 17	33 ± 19	22^{a} ± 11
100	0	33 ± 17	33 ± 19	22^{a} ± 11
250	78 ± 11	78 ± 22	89 ± 11	81^{b} ± 4
Mean	26^{x} ± 26	48^{y} ± 15	52^{y} ± 19	

b)

Alkalinity	Hardness 20	80	160	Mean
10	100 ± 00	100 ± 00	100 ± 00	100^{a} ± 00
100	67 ± 33	89 ± 11	100 ± 00	85^{a} ± 10
250	22 ± 22	67 ± 19	100 ± 00	63^{b} ± 23
Mean	63^{x} ± 23	85^{y} ± 10	100^{y} ± 00	

Table 5.14 The effects of increasing hardness at constant, moderate alkalinity (100mg l^{-1} as $CaCO_3$) on white muscle syndrome, survival, final weights, growth rate, intermoult period, carapace length increment, length specific dry weights of carapaces, length specific calcium content, calcium and magnesium concentrations in carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=9). Mean values along the rows with common superscript letters did not show significant differences at 95% LSD intervals.

Alkalinity (mg l^{-1} $CaCO_3$)	100	100	100	100
Hardness (mg l^{-1} $CaCO_3$)	20	80	160	320
White muscle syndrome (%)	11[a]	11[a]	22[a]	0[a]
Survival (%)	100	100	100	100
Final weight of prawns (g)	0.97[a] ± 0.04	0.89[a] ± 0.06	0.92[a] ± 0.06	0.88[a] ± 0.06
Growth rate (mg day^{-1})	13.70[a] ± 0.64	12.14[ab] ± 1.06	9.63[bc] ± 1.36	9.72[c] ± 1.12
Intermoult period (days)	7.92[a] ± 0.25	7.70[a] ± 0.30	8.11[a] ± 0.32	8.41[a] ± 0.39
Carapace length increment (% $moult^{-1}$)	7.53[a] ± 0.54	6.85[a] ± 0.47	5.05[b] ± 0.37	4.83[b] ± 0.47
Length specific dry weight of intermoult carapaces	1.50[a] ± 0.11	1.37[a] ± 0.12	1.22[a] ± 0.16	1.31[a] ± 0.08
Length specific dry weight of cast carapaces	0.59[a] ± 0.05	0.56[a] ± 0.06	0.56[a] ± 0.05	0.57[a] ± 0.06
Diff.in length sp. dry wt between intermoult & cast carapaces	0.90[a] ± 0.13	0.83[a] ± 0.13	0.66[a] ± 0.16	0.74[a] ± 0.06
Calcium concentrations in intermoult carapaces (mg.g^{-1})	193.28[a] ± 5.61	199.70[a] ± 5.21	195.29[a] ± 4.41	194.49[a] ± 2.01
Calcium concentrations in cast carapaces (mg.g^{-1})	219.35[a] ± 4.01	228.17[a] ± 2.41	223.76[a] ± 2.41	230.17[a] ± 3.61
Diff.in calcium conc. between cast & intermoult carapaces	14.09[a] ± 8.14	28.30[ab] ± 6.38	28.43[ab] ± 3.63	35.69[b] ± 3.31
Length specific calcium content in intermoult carapaces	0.29[a] ± 0.02	0.28[a] ± 0.02	0.24[a] ± 0.03	0.26[a] ± 0.17
Length specific calcium content in cast carapaces	0.12[a] ± 0.01	0.13[a] ± 0.01	0.13[a] ± 0.01	0.13[a] ± 0.02
Diff.in length sp. calcium content between intermoult & cast carapaces	0.16[a] ± 0.02	0.15[a] ± 0.03	0.11[a] ± 0.03	0.12[a] ± 0.02
Magnesium concentrations in intermoult carapaces (mg.g^{-1})	4.24[a] ± 0.55	2.49[b] ± 0.24	2.11[b] ± 0.70	2.79[b] ± 0.07
Magnesium concentrations in cast carapaces (mg.g^{-1})	4.23[a] ± 0.23	3.23[b] ± 0.31	3.34[b] ± 0.19	3.55[b] ± 0.15
Diff. in magnesium concentrations between cast & intermoult carapace	-0.02[a] ± 0.5	0.74[ab] ± 0.25	1.23[b] ± 0.17	0.72[ab] ± 0.35

Table 5.15 The effects of low and high hardness combined with low alkalinity (25mg l^{-1} as $CaCO_3$) on white muscle syndrome, survival, final weights, growth rate, intermoult period, carapace length increment, length specific dry weights of carapaces, length specific calcium content, calcium and magnesium concentrations in carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=9). Mean values along the rows with common superscript letters did not show significant differences at 95% LSD intervals.

Alkalinity (mg l^{-1} $CaCO_3$)	25	25
Hardness (mg l^{-1} $CaCO_3$)	20	1000
White muscle syndrome(%)	0[a]	11[a]
Survival (%)	100	100
Final weight of prawns (g)	0.74[a] ± 0.05	0.77[a] ± 0.09
Growth rate (mg day^{-1})	9.60[a] ± 1.35	9.78[a] ± 1.82
Intermoult period (days)	8.39[a] ± 0.26	8.28[a] ± 0.30
Carapace length increment (% $moult^{-1}$)	6.30[a] ± 0.78	6.20[a] ± 0.95
Length specific dry weight of intermoult carapaces	1.26[a] ± 0.51	1.28[a] ± 0.52
Length specific dry weight of cast carapaces	0.50[a] ± 0.04	0.48[a] ± 0.04
Diff. in length specific dry wt between intermoult & cast carapaces	0.75[a] ± 0.31	0.80[a] ± 0.33
Calcium concentrations in intermoult carapaces ($mg.g^{-1}$)	198.50[a] ± 4.01	207.71[a] ± 7.22
Calcium concentrations in cast carapaces ($mg.g^{-1}$)	226.16[a] ± 6.02	231.38[a] ± 4.41
Diff.in calcium concentrations between cast & intermoult carapaces	27.67[a] ± 5.61	23.26[a] ± 2.81
Length specific calcium content in intermoult carapaces	0.25[a] ± 0.02	0.26[a] ± 0.03
Length specific calcium content in cast carapaces	0.11[a] ± 0.01	0.11[a] ± 0.01
Diff.in length sp. calcium content between intermoult & cast carapaces	0.13[a] ± 0.02	0.15[a] ± 0.03
Magnesium concentrations in intermoult carapaces ($mg.g^{-1}$)	2.19[a] ± 0.22	2.89[a] ± 0.19
Magnesium concentrations in cast carapaces ($mg.g^{-1}$)	2.53[a] ± 0.15	4.13[a] ± 0.24
Diff. in magnesium concentrations between cast & intermoult carapaces	0.69[a] ± 0.16	1.24[a] ± 0.15

levels tested (20 to 1000mg l^{-1}) (Tables 5.14 and 5.15 respectively).

5.4.4 Growth

5.4.4.1 Mean Final Weight

In experiment 1, juveniles exposed to moderate and high (100 and 250mg l^{-1}) alkalinity waters exhibited significantly ($P<0.001$) lower final weights than those in low alkalinity (10mg l^{-1}) waters, and prawns exposed to 80 and 160 mg l^{-1} hardness levels again exhibited lower ($P=0.05$) final weights than those in 20mg l^{-1} water hardness. The interaction between alkalinity and hardness was close to being significant ($P=0.06$) (Two-way ANOVA, Table 5.16a). In experiments 2 and 3, the final weights of juveniles were not affected by the wide range of water hardnesses (20-1000mg l^{-1}) where alkalinity level was 100 and 25mg l^{-1} respectively (Tables 5.14 and 5.15).

5.4.4.2 Growth Rate (mg day^{-1})

In experiment 1, two-way ANOVA revealed that alkalinity and hardness both had a significant effect ($P<0.001$) on growth of juvenile prawns (Table 5.16b) where growth tended to decline progressively with increase in alkalinity (Fig IIIa) but was reduced less by increasing hardness, particularly at low alkalinity levels. The

Table 5.16 Effect of alkalinity and hardness on a) mean final weight (g), and b) growth rate (mg day^{-1}) of juvenile *M. rosenbergii*. Values are means ± SE (n=9). Mean values (n=27) of the respective columns and rows sharing common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	Hardness 80	Hardness 160	Mean
10	0.78 ± 0.04	0.63 ± 0.06	0.58 ± 0.02	0.66^{a} ± 0.03
100	0.55 ± 0.04	0.55 ± 0.06	0.57 ± 0.04	0.56^{b} ± 0.03
250	0.55 ± 0.04	0.48 ± 0.01	0.53 ± 0.03	0.52^{b} ± 0.02
Mean	0.63^{x}± 0.03	0.55^{y} ± 0.02	0.56^{y} ± 0.03	

b)

Alkalinity	Hardness 20	Hardness 80	Hardness 160	Mean
10	9.47 ± 1.08	5.99 ± 0.94	5.43 ± 0.82	6.96^{a} ± 0.63
100	7.04 ± 0.65	5.37 ± 0.95	5.27 ± 0.50	5.89^{a} ± 0.43
250	3.75 ± 0.58	3.06 ± 0.60	3.51 ± 0.39	3.43^{b} ± 0.30
Mean	6.75^{x} ± 0.63	4.80^{y} ± 0.53	4.74^{y} ± 0.37	

interaction effect between alkalinity and hardness on growth was just significant ($P=0.05$). In experiment 2, the growth rate of prawns was significantly inhibited ($P<0.05$) by the increased levels of water hardness (Table 5.14) where alkalinity was 100mg l^{-1}. Like postlarvae, the growth rate of juvenile prawns in experiment 3 did not show any significant differences between low and very high (20 and 1000mg l^{-1}) hardness levels when the alkalinity was low (25mg l^{-1}). (Table 5.15).

5.4.4.3 Intermoult Period

In experiment 1, alkalinity ($P<0.001$) and hardness ($P<0.05$) influenced the intermoult period of juvenile prawns, where intermoult period was significantly longer in low alkalinity (10mg l^{-1}) than in moderate and high alkalinity (100 and 250mg l^{-1}) waters (Fig IIIb), and a longer intermoult period was again recorded at the lowest level of water hardness (Table 5.17a). The interaction effect of alkalinity and hardness on intermoult period was not significant (Two-way ANOVA). In contrast, the intermoult period did not show any significant differences due to water hardness in experiments 2 and 3 (Tables 5.14 and 5.15 respectively).

5.4.4.4 Carapace Length Increment (% $moult^{-1}$)

In experiment 1, carapace length increment per moult was significantly affected

Table 5.17 Effect of alkalinity and hardness on a) intermoult period (days), and b) carapace length increment (% moult^{-1}) of juvenile *M. rosenbergii*. Values are means ± SE (n=9). Mean values (n=27) of the respective columns and rows sharing common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	10.70 ± 0.54	9.15 ± 0.42	8.67 ± 0.30	9.62^{a} ± 0.31
100	9.75 ± 0.47	8.40 ± 0.46	7.92 ± 0.44	8.42^{b} ± 0.43
250	8.61 ± 0.49	8.85 ± 0.54	8.59 ± 0.29	8.74^{b} ± 0.63
Mean	9.49^{x} ± 0.64	8.91^{y} ± 0.53	8.31^{y} ± 0.37	

b)

Alkalinity	Hardness 20	80	160	Mean
10	7.22 ±0.62	4.96 ±0.48	4.25 ±0.55	5.48^{a} ±0.39
100	5.45 ±0.38	4.23 ±0.60	4.73 ±0.54	4.82^{a} ±0.31
250	3.38 ±0.25	3.98 ±0.53	4.01 ±0.44	3.79^{b} ±0.24
Mean	5.34^{x} ±0.42	4.45^{xy} ±0.31	4.33^{y} ±0.29	

by alkalinity ($P<0.001$) and hardness ($P<0.05$) with significant interactions ($P<0.01$) between alkalinity and hardness (Two-way ANOVA, Table 5.17b). The increment per moult was higher in low to moderate alkalinity (10-100mg l^{-1}) than in high alkalinity (250mg l^{-1}) waters (Fig IIIc). Carapace length increment per moult was greatest at the low alkalinity and low hardness combination. In experiment 2 where alkalinity was 100mg l^{-1}, the carapace length increment per moult decreased significantly ($P<0.01$) with increase in water hardness (Table 5.14) but no differences due to water hardness were observed in experiment 3 where alkalinity was 25mg l^{-1} (Table 5.15).

5.4.5 Carapace Mineralization

5.4.5.1 Thickness of Carapace

It was noticed that the inner surface of cast carapaces was always rough while that of dissected intermoult carapaces was smooth. The thickness of carapaces from juvenile prawns was measured only from experiment 1 and the measurements were too few for statistical analysis. However, carapace thicknesses ranged from 52 to 71μm (intermoult) and 40 to 58μm (cast), and the differences in thickness between intermoult and cast carapaces decreased with increase in alkalinity and hardness (Table 5.18 a, b and c).

Table 5.18 Effect of alkalinity and hardness on the thicknesses (μm) of a) intermoult, b) cast and c) differences between intermoult and cast carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=2-3).

a)

Alkalinity	Hardness 20	80	160	Mean
10	70.8	63.4	52.0	62.1 ± 5.5
100		65.0	69.0	67.0 ± 2.0
250	71.0	70.0	52.5	64.5 ± 6.0
Mean	70.9 ± 0.1	66.1 ± 2.0	57.8 ± 5.6	

b)

Alkalinity	Hardness 20	80	160	Mean
10	52.5	48.2	40.0	46.9 ± 3.7
100		50.0	56.7	53.4 ± 3.4
250	57.7	58.0	55.5	57.1 ± 0.8
Mean	55.1 ± 2.6	52.1 ± 3.0	50.7 ± 5.4	

c)

Alkalinity	Hardness 20	80	160	Mean
10	18.3	15.2	12.0	15.2 ± 1.8
100		15.0	12.3	13.7 ± 1.4
250	13.5	12.0	-3.0	7.5 ± 4.3
Mean	15.9 ± 2.4	14.1 ± 1.0	7.1 ± 4.1	

5.4.5.2 Length Specific Dry Weights of Carapaces

Length specific dry weight of intermoult carapaces

In experiment 1, the length specific dry weight of intermoult carapaces of juvenile prawns decreased progressively ($P<0.001$) with increased alkalinity (Fig V) and a significantly higher ($P<0.01$) length specific dry weight was recorded in the lowest (20mg l^{-1}) water hardness than in 80 and 160mg l^{-1} (Table 5.19a). In contrast, in postlarval prawns the length specific dry weights of intermoult carapaces (Table 5.8a) were not affected either by alkalinity or water hardness possibly due to lack of data in treatment combinations of the lowest hardness (20mg l^{-1}) with alkalinities. Experiments 2 and 3 made with juveniles, alkalinity levels of 100 and 25mg l^{-1} respectively, did not significantly influence the length specific dry weight even over the wide range of water hardnesses tested (Tables 5.14 and 5.15 respectively). In contrast the length specific dry weights of carapaces of postlarval prawns in experiment 2 (with alkalinity of 100mg l^{-1}) were significantly higher in the media of the lowest water hardness (20mg l^{-1}).

Length specific dry weight of cast carapaces

In experiment 1 with juvenile prawns, significantly higher ($P<0.05$) length specific dry weight of cast carapaces were recorded in low (20mg l^{-1}) hardness than

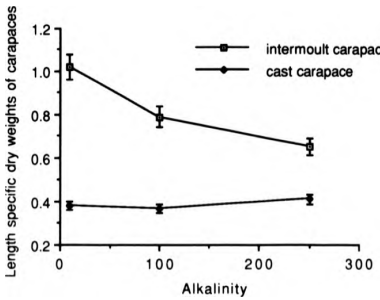


Figure V Effect of alkalinity (mg l^{-1} as $CaCO_3$) on length specific dry weights of intermoult and cast carapaces of juvenile *M. rosenbergii*. Vertical bars are ± SE (n=19-26 both for intermoult and cast carapaces.

Table 5.19 Effect of alkalinity and hardness on length specific dry weights of a) intermoult, b) cast and c) their differences between intermoult and cast carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=2-9). Mean values (n=20-27) along the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	1.30 ± 0.07	0.93 ± 0.11	0.85 ± 0.07	1.02^{a} ± 0.06
100	0.90 ± 0.09	0.76 ± 0.11	0.74 ± 0.05	0.79^{b} ± 0.05
250	0.69 ± 0.12	0.65 ± 0.04	0.64 ± 0.05	0.65^{c} ± 0.04
Mean	1.00^{x} ± 0.08	0.79^{y} ± 0.06	0.75^{y} ± 0.04	

b)

Alkalinity	Hardness 20	80	160	Mean
10	0.48 ± 0.02	0.34 ± 0.03	0.33 ± 0.02	0.38^{a} ± 0.02
100	0.38 ± 0.05	0.35 ± 0.02	0.39 ± 0.04	0.37^{a} ± 0.02
250	0.40 ± 0.03	0.39 ± 0.03	0.44 ± 0.04	0.41^{a} ± 0.02
Mean	0.43^{x} ± 0.02	0.36^{xy} ± 0.02	0.39^{y} ± 0.02	

c)

Alkalinity	Hardness 20	80	160	Mean
10	0.81 ± 0.06	0.59 ± 0.08	0.53 ± 0.06	0.64^{a} ± 0.05
100	0.53 ± 0.07	0.42 ± 0.11	0.35 ± 0.06	0.42^{b} ± 0.05
250	0.29 ± 0.10	0.27 ± 0.04	0.20 ± 0.07	0.25^{c} ± 0.05
Mean	0.57^{x} ± 0.07	0.43^{y} ± 0.06	0.37^{y} ± 0.04	

at higher (80 and 160mg l^{-1}) hardness levels but alkalinity exerted no significant influence (Table 5.19b). The interaction effects of alkalinity and hardness was significant ($P<0.05$) where alkalinity level >100mg l^{-1} tended to increase length specific dry weights at the higher (160mg l^{-1}) hardness tested. In contrast to juveniles, the cast carapaces of postlarval prawns showed a significant increase with increasing alkalinity. At a hardness level of 160mg l^{-1} the increase was progressive (Table 5.8b) and may be the result of a poor ability in postlarval prawns to withdraw material from the carapace prior to casting. Water hardnesses in experiments 2 and 3 did not influence the length specific dry weights of cast carapaces significantly (Tables 5.14 and 5.15). Similar observations were made on postlarval prawns (Tables 5.4 and 5.5)

Differences in length specific dry weight between intermoult and cast carapaces

In all 3 experiments with juveniles, the t-test showed that the length specific dry weights were significantly ($P<0.001$) higher by 73-158% in intermoult than in cast carapaces (Table 5.20), and similar results were observed in postlarval prawns (Table 5.9). However in juveniles the results of experiment 1 showed that the differences decreased significantly ($P<0.001$) with increased alkalinity and significantly higher ($P<0.05$) differences were recorded at the lowest water hardness (20mg l^{-1}) than at the 80 and 160mg l^{-1} hardness levels (Table 5.19c). Again, as in postlarval prawns, the direction of the differences was not clear though the lowest differences were recorded in the highest alkalinity tested (250mg l^{-1}) (Table 5.8c). Water hardness did not exert

Table 5.20 The combined mean values of carapace length specific dry weights, calcium and magnesium concentrations, length specific calcium content and the differences between intermoult and cast carapaces of juvenile *M. rosenbergii* from 3 different experiments.

Expt No	Parameters	Cast carapace	Intermoult carapace	Difference (%)	Sig. level
1	Length specific dry weight	0.3875	0.8301	114	P<0.001
	Calcium (mg.g dry wt^{-1})	204.51	182.46	12	P<0.001
	Length specific calcium	0.0806	0.1458	81	P<0.001
	Magnesium (mg.g dry wt^{-1})	3.11	2.42	29	P<0.001
2	Length specific dry weight	0.7771	1.3461	73	P<0.001
	Calcium (mg.g dry wt^{-1})	222.56	195.69	14	P<0.001
	Length specific calcium	0.1286	0.2633	105	P<0.001
	Magnesium (mg.g dry wt^{-1})	3.59	2.90	24	P<0.001
3	Length specific dry weight	0.4928	1.2693	158	P<0.001
	Calcium (mg.g dry wt^{-1})	228.57	203.71	12	P<0.001
	Length specific calcium	0.1127	0.2566	128	P<0.001
	Magnesium (mg.g dry wt^{-1})	3.33	2.54	31	P<0.001

any significant influence on the differences in length specific dry weights between intermoult and cast carapaces of juveniles in moderate and low alkalinity (experiments 2 and 3, and Tables 5.14 and 5.15 respectively). In postlarval prawns (experiment 2 with alkalinity of 100mg l^{-1}) the differences were higher in the lowest water hardness (Table 5.4).

5.4.5.3 Calcium

Calcium concentrations in intermoult carapaces

In experiment 1, alkalinity had a significant effect, ($P<0.01$) but water hardness had no effect, on the calcium concentrations in intermoult carapaces (Two-way ANOVA, Table 5.21a). Here, calcium concentrations were significantly higher in the intermoult carapaces from prawns exposed to moderate and high (100 and 250 mg l^{-1}) alkalinity than in those from low (10mg l^{-1}) alkalinity waters (Fig IVa). No significant interaction between alkalinity and hardness was detected. The calcium concentrations of intermoult carapaces in experiments 2 and 3 did not differ in response to the wide range of water hardnesses tested (Tables 5.14 and 5.15 respectively). No differences in the trend of calcium concentrations were noticed between postlarval and juvenile prawns. The contrast was that the calcium concentrations appeared to be higher in postlarvae than in juveniles, probably due to the higher proportion of organic matter in carapaces of juveniles. Unfortunately no

Table 5.21 Effect of alkalinity and hardness on calcium concentrations (mg.g dry wt^{-1}) in a) intermoult, b) cast and c) their differences between cast and intermoult carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=2-9). Mean values (n=20-27) along the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	175.24 ± 4.81	175.64 ± 3.61	175.24 ± 1.60	175.24[a] ± 2.01
100	180.85 ± 2.41	184.86 ± 3.61	192.88 ± 4.01	185.66[b] ± 2.41
250	188.47 ± 4.41	185.26 ± 5.21	186.06 ± 4.81	186.47[b] ± 2.81
Mean	181.65[x] ± 2.41	181.65[x] ± 2.41	184.46[x] ± 2.81	

b)

Alkalinity	Hardness 20	80	160	Mean
10	202.10 ± 6.42	192.48 ± 4.41	196.49 ± 6.42	196.89[a] ± 3.21
100	197.69 ± 3.21	208.12 ± 5.21	212.93 ± 6.02	206.11[b] ± 3.21
250	208.52 ± 3.61	212.53 ± 6.82	211.73 ± 5.61	210.93[b] ± 3.21
Mean	202.91[x] ± 2.81	204.11[x] ± 3.61	206.92[x] ± 3.61	

c)

Alkalinity	Hardness 20	80	160	Mean
10	27.27 ±2.41	16.84 ±3.61	21.25 ±7.22	21.65[a] ±2.81
100	16.84 ±1.20	23.26 ±4.01	20.05 ±2.81	20.05[a] ±1.60
250	20.05 ±2.41	27.27 ±2.81	25.66 ±8.82	24.46[a] ±4.01
Mean	21.65[x] ±2.41	22.06[x] ±2.81	22.46[x] ±1.60	

references are available on the differences in organic matter content between postlarval and juvenile prawns.

Calcium concentrations in cast carapaces

The calcium concentrations in cast carapaces from juvenile prawns were significantly greater ($P<0.05$) in moderate to high (100-250mg l^{-1}) alkalinity than in low (10mg l^{-1}) alkalinity waters while hardness again exerted no significant influence. (Experiment 1, Two-way ANOVA, Table 5.21b, Fig 1Vb). No interaction effect between alkalinity and hardness was detected. Also in experiments 2 and 3, the calcium concentrations in cast carapaces were not affected by the wide range of water hardnesses tested (Tables 5.14 and 5.15 respectively). Like intermoult carapaces, calcium concentrations in cast carapaces of both postlarvae and juveniles were similarly influenced by the alkalinity and hardness. Again it was noticed in cast carapaces that the calcium concentrations were higher in postlarvae than in juveniles.

Differences in calcium concentrations between cast and intermoult carapaces

In all three experiments with juvenile prawns, the t-test showed that calcium concentrations were significantly ($P<0.001$) higher by 12-14% in cast than in intermoult carapaces (Table 5.20) while in postlarvae the differences were 10-11%

(Table 5.9). In experiment 1 with juveniles no significant differences due to treatments were noted (Table 5.21c). Similarly, the wide range of water hardnesses in experiments 2 and 3 did not influence the differences in calcium concentration between cast and intermoult carapaces significantly (Tables 5.14 and 5.15 respectively). The influence of the external environment on the trend of the differences in calcium concentrations (cast - intermoult) between postlarvae and juveniles was similar.

5.4.5.4 Length specific calcium content of carapaces

Length specific calcium content of intermoult carapaces

In experiment 1, a significantly higher ($P<0.001$) length specific calcium content was recorded in intermoult carapaces from prawns exposed to low alkalinity waters and the values decreased significantly with increase in water alkalinity (Table 5.22a). In postlarval prawns the decrease was not as progressive as in juveniles although significantly lower values were found in the highest alkalinity tested (Table 5.11a). In juveniles the effect of water hardness was not significant ($P=0.6$) but multiple range analysis indicated that intermoult carapaces from juveniles exposed to 20mg l^{-1} water hardness had a significantly higher length specific calcium content than those in 80 and 160mg l^{-1} water hardnesses. The interactions between alkalinity and hardness were significant ($P<0.05$) and seemed to be due to the treatment combination

Table 5.22 Effect of alkalinity and hardness on the length specific calcium content of a) intermoult, b) cast and c) differences between intermoult and cast carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=2-9). Mean values (n=20-27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	0.2223 ±0.0173	0.1436 ±0.0178	0.1689 ±0.0238	0.1830[a] ±0.0145
100	0.1516 ±0.0180	0.1450 ±0.0224	0.1464 ±0.0110	0.1517[b] ±0.0122
250	0.0971 ±0.0145	0.1160 ±0.0051	0.1127 ±0.0128	0.1094[c] ±0.0063
Mean	0.1711[x] ±0.0224	0.1343[y] ±0.0096	0.1388[y] ±0.0100	

b)

Alkalinity	Hardness 20	80	160	Mean
10	0.0961 ±0.0068	0.0645 ±0.0078	0.0639 ±0.0036	0.0729[a] ±0.0055
100	0.0952 ±0.0065	0.0865 ±0.0101	0.0639 ±0.0036	0.0861[a] ±0.0098
250	0.0742 ±0.0043	0.0862 ±0.0087	0.0846 ±0.0087	0.0822[a] ±0.0045
Mean	0.0850[x] ±0.0051	0.0801[x] ±0.0073	0.0785[x] ±0.0063	

c)

Alkalinity	Hardness 20	80	160	
10	0.1439 ±0.0184	0.0791 ±0.0162	0.1084 ±0.0202	0.1073[a] ±0.0128
100	0.1162 ±0.0125	0.0586 ±0.0107	0.0624 ±0.0176	0.0656[b] ±0.0101
250	0.0234 ±0.0159	0.0298 ±0.0070	0.0281 ±0.0161	0.0274[c] ±0.0078
Mean	0.0802[x] ±0.0237	0.0542[x] ±0.0081	0.0599[x] ±0.0133	

of low hardness and high alkalinity which gave the lowest value. Water hardnesses with moderate and low alkalinity in experiments 2 and 3 respectively did not exert any significant influence on the length specific calcium content in intermoult carapaces (Tables 5.14 and 5.15 respectively). The major difference in the length specific calcium content between postlarvae and juveniles was that the content was about 100% higher in juveniles than in postlarvae indicating that the length specific calcium content in carapaces increases with size of prawns.

Length specific calcium content of cast carapaces

The length specific calcium content of cast carapaces was not affected either by hardness or alkalinity in any of the 3 experiments (Tables 5.22b, 5.14 and 5.15 for experiments 1, 2 and 3 respectively). By contrast, in experiment 1 with postlarval prawns, length specific calcium content in cast carapaces of postlarvae was higher in high alkalinities (Table 5.11b) which might be a reflection of the higher concentrations of calcium in the intermoult carapaces of postlarvae compared to juveniles (section 5.4.5.3).

Differences in length specific calcium content between intermoult and cast carapaces

In all three experiments with juvenile prawns, the t-test showed that the length specific calcium content was significantly ($P<0.001$) higher by 81-128% in intermoult than in cast carapaces (Table 5.20). Similarly postlarval prawns also had a higher length specific calcium content in intermoult than in cast carapaces. In experiment 1 with juveniles the differences in length specific calcium content between intermoult and cast carapaces decreased significantly ($P<0.001$) with increased environmental alkalinity (Table 5.22c). In the case of postlarvae the trend was apparently progressive (Table 5.11c) although statistically, no difference existed between alkalinity levels of 10 and 100mg l^{-1}. In none of the three experiments was the differences in length specific calcium contents between intermoult and cast carapaces influenced by water hardness (Tables 5.22c, 5.14 and 5.15 for experiments 1, 2 and 3 respectively).

5.4.5.5 Magnesium

Magnesium concentrations in intermoult carapaces

In experiment 1 with juveniles, alkalinity and hardness both had a significant effect ($P<0.001$) on the magnesium concentrations in intermoult carapaces (Two-way

ANOVA, Table 5.23), where significantly higher magnesium concentrations were recorded in intermoult carapaces from juvenile prawns exposed to 10mg l^{-1} alkalinity than those from 100 and 250mg l^{-1} alkalinity waters. Additionally, magnesium concentrations were higher in carapaces from prawns of low water hardness (20mg l^{-1}) than those in 80 and 160mg l^{-1} hardness levels. It was interesting that increased alkalinity significantly enhanced deposition of calcium in intermoult carapaces of both postlarvae and juveniles (Tables 5.10a and 5.21a) but magnesium deposition was not enhanced by high external alkalinities (5.12a and 5.23a). In experiment 1 with juveniles, the interaction effect between alkalinity and hardness on magnesium concentrations was also significant ($P<0.001$). Higher magnesium concentrations were recorded in high alkalinity x low hardness and again in low alkalinity x high hardness combinations. In experiment 2 (Table 5.14) significantly ($P<0.001$) higher magnesium concentrations in intermoult carapaces were recorded from prawns exposed to the lowest level of water hardness (20mg l^{-1}) and the concentrations tended to decline with increasing hardness up to 160mg l^{-1}, except under the very low alkalinity treatment (10mg l^{-1}) of experiment 1, and again tended to a rise at hardness level of 320mg l^{-1}. Similar results occurred in postlarval prawns. In experiment 3 where the effect of two (20 and 1000mg l^{-1}) water hardnesses were compared at 25mg l^{-1} alkalinity, significantly higher ($P<0.001$) magnesium concentrations in intermoult carapaces were recorded from prawns exposed to the higher water hardness (Table 5.15). Like calcium, the magnesium concentrations were apparently higher in postlarval prawns (Table 5.12) than in juveniles (5.23) which perhaps relates to the lower content of organic matter in carapaces of postlarval prawns.

Table 5.23 Effect of alkalinity and hardness on magnesium concentrations (mg.g dry wt^{-1}) in a) intermoult, b) cast and c) differences between cast and intermoult carapaces of juvenile *M. rosenbergii*. Values are means ± SE (n=2-9). Mean values (n=20-27) of the respective columns and rows with common superscript letters did not show significant differences at 95% LSD intervals.

a)

Alkalinity	Hardness 20	80	160	Mean
10	2.79 ± 0.18	2.38 ± 0.13	3.20 ± 0.13	2.79^{a}±0.12
100	2.49 ± 0.15	2.20 ± 0.23	1.58 ± 0.23	2.09^{b}±0.12
250	3.30 ± 0.22	2.07 ± 0.27	1.76 ± 0.25	2.38^{b}±0.22
Mean	3.30^{x}±0.13	2.22^{y}±0.12	2.18^{y}±0.22	

b)

Alkalinity	Hardness 20	80	160	Mean
10	2.91 ± 0.15	3.56 ± 0.48	4.14 ± 0.27	3.54^{a}±0.24
100	3.04 ± 0.36	2.54 ± 0.22	2.82 ± 0.27	2.80^{b}±0.17
250	3.06 ± 0.45	2.69 ± 0.24	3.23 ± 0.60	3.00^{ab}±0.25
Mean	3.10^{x}±0.19	2.93^{x}±0.22	3.40^{x}±0.28	

c)

Alkalinity	Hardness 20	80	160	Mean
10	0.12 ± 0.07	1.18 ± 0.49	0.94 ± 0.31	0.75*±0.22
100	0.55 ± 0.22	0.34 ± 0.29	1.24 ± 0.17	0.71*±0.16
250	-0.23 ±0.54	0.62 ± 0.39	1.48 ± 0.39	0.62*±0.30
Mean	0.15^{x} ±0.17	0.71^{xy}±0.25	1.22^{y} ± 0.16	

Magnesium concentrations in cast carapaces

In experiment 1 with juveniles, the alkalinity effect (P=0.058) on magnesium concentrations in cast carapaces was found to be significant by multiple range analysis which indicated that magnesium concentrations in carapaces cast from prawns held in low alkalinity (10mg l^{-1}) were significantly higher than those in 100mg l^{-1} alkalinity waters (Table 5.23b) while no differences were noticed with postlarvae (Table 5.12b). Water hardness in experiment 1 did not show any significant influence on magnesium concentration in cast carapaces. However in experiments 2 and 3 magnesium concentrations in cast carapaces followed a similar trend to that of the intermoult carapaces, and once again, highest magnesium concentrations (>4mg.g dry wt^{-1}) were found at the two extremes of water hardnesses 20 and 1000mg l^{-1} (Tables 5.14 and 5.15 respectively). Similar observations were made in experiment 2 with postlarvae (Table 5.4) where magnesium concentrations were higher at the two extreme hardness levels (20 and 320mg l^{-1}). In experiment 3, however, postlarvae showed a gradual, but statistically insignificant in the hardness treatments of 20, 500 and 1000mg l^{-1} (Table 5.5).

Differences in magnesium concentrations between cast and intermoult carapaces

In all three experiments with juveniles, magnesium concentrations were significantly (P<0.001) higher by 24-31% in cast than in intermoult carapaces (Table

5.20) whereas in postlarval prawns cast carapaces had 13-29% higher concentrations than in intermoult carapaces (Table 5.9). Statistical analyses of the data from juveniles in experiment 1 indicated that alkalinity had no influence on the differences in magnesium concentrations between cast and intermoult carapaces (Table 5.23c), but all three experiments with juveniles showed that increasing water hardness significantly increased the differences in magnesium concentrations between cast and intermoult carapaces (except hardness 320mg l^{-1} of experiment 2) (Tables 5.23c, 5.14 and 5.15 for experiments 1, 2 and 3 respectively). The differences also increased in postlarval prawns but statistically were not significant.

5.5 Discussion (Postlarvae and Juveniles)

5.5.1 Water Quality

It was noticed that alkalinity and hardness of treatment waters increased slightly with time, possibly due to evaporative loss of water. The small changes were judged to be insignificant. The pH of water was quite stable and did not show any major fluctuations. Inevitably, the high alkalinity waters had higher pH values than the low alkalinity waters. In this study the lowest pH (6.8) was recorded from the lowest alkalinity waters (10mg l^{-1} as $CaCO_3$) and the highest pH (8.6) was recorded from the highest alkalinity waters (250mg l^{-1}) (Table 5.1). Both pH levels are well within the tolerable range for the species.

5.5.2 White Muscle Syndrome

The stress condition, termed "the white muscle syndrome", affected postlarval prawns more than it affected juveniles. It also occurred earlier in postlarvae than juveniles. Environmental stress due to high alkalinity and high hardness in experiment 1 seemed to be responsible for the higher incidence of white muscle syndrome both in postlarval and juvenile prawns. Later, experiment 3, which had higher hardness levels (20-1000mg l^{-1}) but lower alkalinity (25mg l^{-1}) than in experiment 1 (hardness 20-160mg l^{-1} and alkalinity 10-250mg l^{-1}), demonstrated that high hardness did not influence the occurrence of white muscle syndrome either in postlarvae or juveniles, and the results suggested that the effects of water hardness in experiment 1 and 2 was due to its interaction with the higher alkalinity (>100mg l^{-1}).

The occurrence of white muscle syndrome following exposure to water of different hardness levels has not been reported in earlier studies (Heinen 1977, Cripps and Nakamura 1979, Bartlett and Enkerlin 1983, Howlader and Turjoman 1984, Vasquez *et al.* 1989 and Brown *et al.* 1991). In this study two factors which might possibly have had some influence on the occurrence of stress were ammonia and pH of water. However both ammonia and pH levels in all 3 experiments were similar and were maintained within safe levels. Again it is of interest that white muscle syndrome was not reported for *M. rosenbergii* during studies of elevated pH and ammonia levels (Wickins 1976, Sarver *et al.* 1979, and Straus *et al.* 1991) although a similar condition, idiopathic muscle necrosis (whitish coloration and muscle opacity)

in penaeids (*Penaeus aztecus*) has been associated with environmental stressors such as salinity and temperature fluctuations (Lakshmi *et al.* 1978), hypoxia, osmoregulatory problems, overcrowding and hyperactivity associated with handling (Venkataramiah 1971a and b) and by narcotizing with quinaldine (Johnson 1974). Histopathological studies of the necrotic muscles showed no pathogens (Rigdon and Baxter 1970, Lakshmi *et al.* 1978). *M. rosenbergii* is also reported to be affected by muscle necrosis (Fujimura and Okamoto 1970, Nash *et al.* 1987, Sinderman 1977 and Akiyama *et al.* 1982, Sarver *et al.* 1982). However in this study high alkalinity seemed to produce the higher incidence of white muscle syndrome while high hardness levels did not exacerbate the condition. Juveniles were more resistant while postlarval prawns were more vulnerable to white muscle syndrome especially at high environmental alkalinities.

5.5.3 Survival

This study revealed a trend in the survival of prawns where the high alkalinity in conjunction with low hardness produced poor survival of both postlarval and juvenile prawns. No postlarval prawns survived at the treatment combination of the highest alkalinity (250mg l^{-1}) and the lowest hardness (20mg l^{-1}), and at this level only 22% juveniles survived (Tables 5.3 and 5.13). These prawns died during the late premoult or while moulting. Juvenile prawns did not show any sign of physical weakness or white muscle syndrome before death, but some postlarvae especially at the highest

alkalinity exhibited the syndrome before death. In one case study a 40% prawn mortality was reported to be associated with the occurrence of a similar white muscle syndrome (Akiyama *et al.* 1982). An idiopathic muscle necrosis has been described in postlarval *M. rosenbergii* in which a "milky"-white opacity of the body is followed rapidly by death (Nash *et al.* 1987). This was considered to be due to stress in the hatchery conditions, and conditions had also been observed in postlarval prawns in ponds. In this study it seemed that the severe ionic imbalance in treatment combinations of high alkalinity and low hardness and/or the stressful effect due to high alkalinity alone were the most likely causes of high mortality especially during the moulting period. In experiments 2 and 3 where a wide range of water hardnesses (20-1000mg l^{-1}) were tested at moderate (100mg l^{-1}) and low (25mg l^{-1}) alkalinity levels respectively, survival of postlarvae and juveniles was 100% (Tables 5.4 and 5.5 for postlarvae, and 5.14 and 5.15 for juveniles). Heinen (1977) also did not find any influence of water hardness (10-310mg l^{-1} as $CaCO_3$) on the survival of postlarval *M. rosenbergii* where total alkalinity of water ranged from 84-130mg l^{-1} as $CaCO_3$.

The possible effects of pH and ammonia on prawn mortality may also be discussed. Straus *et al.* (1991) reported that high pH levels can cause mortality of *M. rosenbergii* and they suggested: a) postlarvae should not be exposed to pH >9.0 nor NH_3-N >1.0mg l^{-1} in the pH range of 8.5-9.0, and b) juveniles should not be exposed to pH >9.5 nor NH_3-N > 2.0mg l^{-1} at pH 8.5. In this study the highest pH (8.6) was recorded in the highest alkalinity level (250mg l^{-1} as $CaCO_3$). Although the pH level in this study was within the range suggested by Straus *et al.* (1991), the alkalinity

level in experiment 1 (which produced the highest occurrence of white muscle syndrome and poorest survival) was about 3 times higher than in the study of Straus *et al* (1991). This high alkalinity in the present study appears to be a major threat to prawn survival. Sandifer and Smith (1985) reported that alkalinity levels above 180mg l^{-1} occasionally resulted in mass mortality of prawns in South Carolina.

While in experiment 2 prawn survival was 100% at moderate alkalinity (100mg l^{-1}) and low hardness (20mg l^{-1}), the reason for lower survival of both postlarvae and juveniles with moderate alkalinity in experiment 1 is unclear. Any or all of the following factors might, have possibly been responsible for the observed differences in survival:

a) The prawns in experiment 1 were from different parental stock and possibly had inherited less resistance than those used in experiment 2.

b) During the larval stage all prawns were fed *Artemia* nauplii. The *Artemia* used in the Institute were originally from two sources (Salt Lake and San Francisco Bay). It is thus possible that the animals of experiment 1 had have been fed *Artemia* of poorer quality as larvae and had produced weaker postlarvae and juveniles as a result.

c) The time of the experiment was conducted could be another factor that might have affected the survival, but even so the experiments were conducted in the same

controlled environment.

d) Undetected chemical impurities in the stock solutions might also have produced discrepancies.

The results of this study reveal that;

a) both postlarval and juvenile *M. rosenbergii* survived a wide range of water hardness provided the alkalinity of water was low,

b) high environmental alkalinity and its interaction with low water hardness resulted in the poorest survival of both postlarvae and juvenile prawns, and it might be suggested that the ionic imbalance in high alkalinity and low hardness waters was the likely cause of prawn mortality especially during late premoult and while moulting, and

c) juveniles exhibited better resistance than postlarval prawns to waters high in alkalinity and low in hardness.

5.5.4 Growth

5.5.4.1 Mean Final Weight

Due to significant interactions ($P<0.05$) between alkalinity and hardness the mean final size of postlarval prawns in experiment 1 were reduced by increased alkalinity and hardness (Table 5.6 a). In a similar experiment the final weights of juveniles were also reduced by increased alkalinity and hardness (Table 5.16 a). Finally, the results of experiment 3 (Tables 5.5 for postlarvae and 5.15 for juveniles) where alkalinity was 25mg l^{-1} suggested that higher water hardness was not a factor to blame for the production of smaller sized prawns. Heinen (1977) also did not find any effect of water hardness (10-310mg l^{-1} as $CaCO_3$) on the mean final weight of postlarval *M. rosenbergii*, but interestingly he recorded that well water of hardness levels similar to his artificial treatment waters but with less bicarbonate alkalinity produced significantly larger prawns. Brown *et al.* (1991) reported smaller prawns from water of increased hardness, but the results of the present study suggest that perhaps the interference of high alkalinity reduced the size of prawns in their study where alkalinity levels also increased with the increase of water hardness due to the way the media were made up.

5.5.4.2 Growth Rate

The growth rate (mg day^{-1}) of both postlarval and juvenile prawns was found to be sensitive to higher alkalinities. Experiment 1 demonstrated that the interaction effects ($P<0.05$) between alkalinity and hardness resulted in significant growth inhibition (in both size groups) at higher alkalinity and hardness levels (Tables 5.6b and 5.16b). Further, the results of experiment 2 revealed that alkalinity level of 100mg l^{-1} and above can inhibit the growth of postlarval and juvenile prawns as water hardness increases (Tables 5.4 and 5.14 respectively). Finally, the results of experiment 3 with an alkalinity level of 25mg l^{-1} ruled out any significant role of water hardness in growth inhibition and it appeared that both postlarval and juvenile *M. rosenbergii* were capable of growing in a wide range of water hardnesses provided the alkalinity of water was low.

Alkalinity and its interaction with water hardness thus plays a very significant role in determining the growth and survival of postlarval and juvenile *M. rosenbergii*. Alkalinity levels >100mg l^{-1} can inhibit growth rate with increased hardness and, again similar alkalinity levels with low water hardness might cause high mortality in both size groups of prawns.

Vasquez *et al.* (1989) observed growth inhibition of *M. rosenbergii* with increased water hardness when alkalinity was low (10mg l^{-1} as $CaCO_3$). Similarly, in experiment 1, the lowest alkalinity (10mg l^{-1} as $CaCO_3$) resulted in slower growth

rate of postlarvae and juveniles with increasing water hardness. It would appear that while alkalinity level of 10mg l^{-1} was too low to be compatible with water hardness over 80mg l^{-1}, alkalinity of 25mg l^{-1} was satisfactory.

Although sufficient separate studies on postlarval and juvenile prawns have not been published, previous studies on *M. rosenbergii* reported both inhibition of growth with increased level of water hardness (Cripps and Nakamura 1979, Howlader and Turjoman 1984, Vasquez *et al.* 1989, Brown *et al.* 1991) and no inhibition even at very high levels of water hardness (Heinen 1977, Bartlett and Enkerlin 1983). The results of the present study suggests that differences in the alkalinity of the waters and their interactions with water hardness were responsible for the conflicting results in the previous studies.

5.5.4.3 Intermoult Period

One of the most interesting results of this study was that both postlarval and juvenile prawns in high alkalinity waters moulted more frequently (i.e. intermoult period was short) but, paradoxically, grew at a slower rate (mg day^{-1}) than those in low alkalinity waters. Although experiments 1 and 2 for postlarvae, and experiment 1 for juveniles demonstrated that higher water hardness also enhanced the moulting frequency in prawns, comparison with the results of experiment 3, with low alkalinity (25mg l^{-1}), suggested that high environmental alkalinity (100mg l^{-1} and above) was

responsible for the higher moulting frequency. Brown *et al.* (1991) reported high moulting frequency but slow growth among the prawns exposed to higher levels of water hardness (above 53mg l^{-1} as $CaCO_3$) but, the present study suggests that this was probably a result of the water alkalinity increasing with the increase of water hardness in their study. The alkalinities of water in Brown *et al.* (1991) were similar to those described in Chapter 3 (Table 3.2).

5.5.4.4 Carapace Length Increment (% moult^{-1})

Like growth rate (section 5.5.4.2), carapace length increment per moult in postlarval and juvenile prawns also declined with increased alkalinity. Initially the results of experiments 1 and 2 revealed that increased hardness with higher alkalinity levels (>100mg l^{-1}) reduced the carapace length increment per moult both in postlarvae and juveniles. But later experiment 3 (with 25mg l^{-1} alkalinity) did not detect any significant influence of the wide range of water hardnesses (20-1000mg l^{-1}) on carapace length increment. Although high environmental alkalinity increased the moulting frequency both in postlarval and juvenile prawns, it seems likely that growth, in respect of final weight, daily weight increase, and carapace length increment per moult, was reduced due to high alkalinity.

The combination of high moulting frequency with slow growth provides further evidence that the moulting process is not necessarily always associated with a positive

size increase. Prawns may moult without achieving any growth or even when growth is negative i.e. size is decreasing. *M. rosenbergii* has been observed to moult in aluminium treatment waters even though they were losing weight at the rate of 0.6% day^{-1} (Baker 1989). Some prawns in this study particularly at the highest alkalinity tested, were moulting while the carapace lengths were declining.

It thus appears that the frequent moulting in high alkalinity waters was thus occurring at the expense of growth rate although this may have provided some advantage in allowing excretion of excess $CaCO_3$ which might otherwise have accumulated to harmful levels in the exoskeleton. Some evidence of how the high alkalinity might have stimulated the moulting frequency in prawns is discussed below.

5.5.5 Carapace Mineralization

5.5.5.1 Carapace Thickness (Table 5.18)

The few measurements made of carapace thickness were taken only from juvenile prawns. In general, intermoult carapaces were 20-35% thicker than cast carapaces, with the exception that at the highest alkalinity and high hardness treatment, cast carapaces were slightly thicker than the equivalent intermoult carapaces. Huner and Lindqvist (1985) also observed cast exoskeletons to be less dense than intact exoskeletons in *Astacus astacus*, but they did not collect intermoult and cast

exoskeleton from the same crayfish. However it was interesting to note that the inner surface of cast *M. rosenbergii* carapaces was always rough while that of the dissected carapaces was smooth. The roughness of the inner surface of cast carapaces could relate to the dissolution and reabsorption of the inner layer(s) during the premoult stage. This supports the findings of Dall (1965a) who studied the changes in the integument during the moult cycle of *Metapenaeus* sp. and noticed that during premoult (stage D) there was some decrease in the overall thickness of the old cuticle due to reabsorption.

Because of insufficient data, the influence of environmental alkalinity and hardness on carapace thickness was not clear. However, it appeared that the thickness of both intermoult and cast carapaces tended to decline with increased water hardness. On the other hand no trend of intermoult carapace thickness emerged that could be attributed to alkalinity although the thickness of cast carapaces did increase with increased alkalinity. It was noted that as the environmental alkalinity and hardness increased, the differences in thickness between intermoult and cast carapaces declined indicating that less mineral/organic matter was being lost or withdrawn from the old carapace. It is also quite important to mention here that at the highest alkalinity and hardness combination tested, cast carapaces were slightly thicker than the equivalent intermoult carapaces (Table 5.18c) suggesting that the prawns in such a stressful environment may not have been able to withdraw organic matter from their exoskeleton or that there was less withdrawable organic matter present.

5.5.5.2 Length Specific Dry Weights of Carapaces

It was noticed that the length specific dry weight of intermoult carapaces followed the same trend as growth rate (sections 5.3.4.2 and 5.4.4.2). Both postlarvae and juveniles that had higher growth rates produced intermoult carapaces of higher length specific dry weight indicating the presence of a high quantity of organic matter and/or minerals in the carapaces. In other words intermoult carapaces of larger prawns had higher length specific dry weights than those of smaller prawns. Similarly, Wickins (1984b) and Brown *et al.* (1991) recorded increased length specific dry weight of intermoult carapaces with size of prawns.

The effects of environmental alkalinity and hardness on the length specific dry weights of postlarval carapaces were not pronounced. This might have been due to: a) the smaller sizes of carapace and b) lack of data in one of the treatment combinations where all postlarvae died before carapace collection. However in juvenile prawns, increased alkalinity progressively decreased the length specific dry weights of intermoult carapaces (Fig V). Since the growth rate of prawns decreased with alkalinity (section 5.5.4.2), it is possible that intermoult carapaces in high alkalinity contained less organic matter than those in low alkalinity waters. Unfortunately the organic matter in carapaces was not measured in this study. Again the results of experiments 2 and 3 (Tables 5.14 and 5.15) indicated that the significant effect of water hardness observed in experiment 1 on length specific dry weights of intermoult carapaces was not an independent effect of water hardness but resulted

from its interaction with increased alkalinity.

The cast carapaces were thinner (section 5.4.5.1) and had lower length specific dry weights than intermoult carapaces. Irrespective of treatments, all 3 experiments demonstrated that postlarval prawns had 109-187% and juveniles had 73-158% higher length specific dry weight in intermoult than in their cast carapaces which suggests that the withdrawal and/or loss of organic matter/minerals from the old exoskeleton took place during premoult or while moulting and resulted in lower thicknesses and lower length specific dry weights of cast carapaces compared to intermoult carapaces.

High environmental alkalinity tended to produce cast carapaces of slightly higher length specific dry weights (Tables 5.8b and 5.19b) suggesting less withdrawal from the old exuviae in such environments. Again the results of experiments 2 and 3 (Tables 5.4 and 5.5 for postlarvae and 5.14 and 5.15 for juveniles) demonstrated that water hardness did not influence the length specific dry weights of cast carapaces.

The differences in length specific dry weights between intermoult and cast carapaces were higher in low alkalinity and low hardness levels (Tables 5.9 for postlarvae and 5.20 for juveniles) suggesting that reabsorption from the old exoskeletons prior to casting was more pronounced in that environment. However, the measurements of length specific dry weights of carapaces were not enough to clarify whether only organic matter or minerals or both were reabsorbed from old exoskeleton prior to casting.

5.5.5.3 Calcium Concentrations in Carapaces

Postlarval and juvenile prawns in this study exposed to a wide range of water hardnesses (20-1000mg l^{-1} as $CaCO_3$) demonstrated their ability to maintain and regulate the calcium concentrations in their intermoult carapaces (Tables 5.4, 5.5, 5.10 a for postlarvae and 5.14, 5.15 and 5.21a for juveniles). It is presumed that because of this splendid ability to regulate the environmental calcium, the survival and growth of postlarval and juvenile *M. rosenbergii* were not greatly affected by water hardness. Stern *et al.* (1987) also indicated that *M. rosenbergii* has the ability to regulate its internal ionic composition (Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}) in media of widely different concentrations.

Although both the groups of prawns were capable of regulating carapace calcium concentrations over a wide range of water hardness, high environmental alkalinity increased the deposition of Ca^{2+} ions in the intermoult carapaces (Tables 5.10a and 5.21a). This increased Ca^{2+} concentration in carapaces at high environmental alkalinity might be associated in some way with the more frequent moulting of prawns held in high alkalinity waters (sections 5.5.4.3). With regard to calcium deposition, *M. rosenbergii* in this study perhaps behaved in a similar way to the freshwater crayfish observed by Greenaway (1974b) who found that addition of bicarbonate to the medium enhanced net uptake of calcium by postmoult crayfish while uptake was reduced in the absence of external bicarbonate. This also indicated that excessive calcium mineralization could occur when environmental alkalinity was

high.

The calcium concentrations in cast carapaces followed the same pattern as in intermoult carapaces. However, the postlarval prawns had 4-11% and juveniles had 12-14% higher calcium concentrations in cast than in intermoult carapaces (Tables 5.9 and 5.20), which suggests that juveniles were more capable than postlarval prawns in withdrawing organic matter from their old exuviae prior to casting and that this resulted in a higher concentration of calcium in cast carapaces. Although the average calcium concentrations were higher in cast carapaces, some individual postlarvae in this study, especially those in higher water hardnesses, showed higher calcium concentrations in intermoult than in cast carapaces. The reason for this is unclear. It could be the result of poor withdrawal ability by some of the postlarval prawns who left more organic matter in their old exoskeleton and which resulted in a lower concentration of calcium in cast exuviae. Brown *et al.* (1991) also reported higher calcium concentrations in intermoult than in cast carapaces of smaller size prawns. Reports on exoskeletal calcium concentrations in postlarval prawns are still quite insufficient for comparison with this data. However, Wickins (1984b), Dall (1965a) and, Huner and Lindqvist (1985) observed higher calcium concentrations in cast than in intermoult carapaces of juvenile *Penaeus monodon*, *Metapenaeus* sp. and crayfish respectively. The higher concentrations of calcium in cast carapaces might be the result of withdrawal of organic matter from the carapaces prior to ecdysis. It is worth mentioning here that Fieber and Lutz (1982) recorded calcium concentrations higher in premoult than in intermoult carapaces, thus supporting the idea that withdrawal of

organic matter takes place at the premoult stage.

The present study also demonstrated that cast carapaces were thinner (section 5.5.5.1) and had a significantly ($P<0.001$) lower length specific dry weight than intermoult carapaces (Tables 5.9 and 5.20). It is likely that during ecdysis the inner endocuticle, which is composed of non-calcified chitin-protein (Richards 1951, Cited in Dall 1965a), was perhaps absorbed during the premoult stage, leaving behind the calcified exocuticle thus resulting in higher calcium concentrations in cast carapaces. Dall (1965a) reported that no inorganic materials were reabsorbed from the cuticle prior to moulting but there was a reduction of 39% chitin protein in cast carapaces of *Metapenaeus* sp. From this study it is concluded that the withdrawal of organic matter prior to moulting was the most likely cause of higher calcium concentrations in cast than in intermoult carapaces.

5.5.5.4 Length Specific Calcium Content of Carapaces

While the length specific calcium content of intermoult carapaces ranged from 0.06 to 0.09 in postlarval prawns, it ranged from 0.15 to 0.26 in juveniles indicating that the length specific calcium content increases with carapace thickness and size of prawns. Although the calcium concentrations (mg.g dry wt^{-1}) in intermoult carapaces were lower than in the cast carapaces of both postlarval and juvenile prawns (Tables 5.9 and 5.20 respectively), it is interesting that postlarval prawns had 94-188% and

juveniles had 81-128% higher length specific calcium content in intermoult than in cast carapaces. The lower length specific calcium content of cast carapaces suggests that calcium was lost from the exuviae either by reabsorption by the animal during premoult stage or by loss to the environment while moulting. In *Carcinus*, only 5% of cuticular calcium was reabsorbed at ecdysis (Lafon 1948, Cited in Dall 1965b); and immediately after moult the total calcium of *Austropotamobius pallipes* was only 10% of the intermoult level and most of the calcium removed from the exoskeleton during the premoult period was lost to the medium in soluble form (Chasemartin 1967, Cited in Greenaway 1974a). Greenaway (1974a) also found that newly moulted crayfish contained only 17% of its calcium content at the previous intermoult stage. These low percentages of body calcium in postmoult crayfish, and the low length specific calcium content of cast carapaces compared to the intermoult carapaces observed in the present study supports the idea that calcium is lost to the environment during ecdysis and less is reabsorbed along with the organic matter during premoult stage. McWhinnie *et al* (1969) reported a rise in blood calcium during premoult but the real increase was occurring in a protein-bound fraction.

The influence of water hardness on the length specific calcium content of carapaces was not pronounced either in postlarvae or juveniles. Although higher length specific calcium contents were recorded in intermoult carapaces at the lowest level of water hardness (Table 5.4 for postlarvae and Table 5.22a for juveniles), this was possibly due to its significant interaction with alkalinity (section 5.3.5.3 for postlarvae and section 5.4.5.4 for juveniles). Finally experiment 3 revealed no

significant influence of water hardness on the length specific calcium content of carapaces of either postlarvae or juveniles. However, the effect of environmental alkalinity on the length specific calcium content of carapaces of postlarval and juvenile prawns was significant; higher length specific calcium content occurring in prawns exposed to lower levels of alkalinity (Tables 5.11a and 5.22a). In contrast, the calcium concentrations (mg.g dry wt^{-1}) in carapaces were higher in high alkalinity waters (Tables 5.10a and 5.21a) which might be due to the fact that the increased deposition of calcium and frequent moulting reduced the ability of prawns to synthesize more organic matter when they were exposed to high alkalinity waters. This also might be one of the reasons for slow growth of prawns in high alkalinity waters.

The differences in length specific calcium content between intermoult and cast carapaces decreased with increased alkalinity (Tables 5.11c and 5.22c) indicating that less material was being lost or withdrawn from the old carapaces in high alkalinity waters, or it might be that the carapaces in high alkalinity waters contained less withdrawable organic matter.

5.5.5.5 Magnesium Concentrations in Carapaces

In postlarval and juvenile *M. rosenbergii*, concentrations of magnesium in carapaces were much less than those of calcium (Tables 5.9 and 5.20). Although

calcium deposition in intermoult carapaces was enhanced by high environmental alkalinity (section 5.5.5.3), higher magnesium concentrations in intermoult carapaces of juvenile prawns were observed in the lowest alkalinity waters (Table 5.23a). Magnesium regulation by postlarval prawns in experiment 1 was not clear due to lack of data in one of the treatment combinations. Similarly, in experiments 2 and 3 no clear pattern of magnesium mineralization in relation to water hardness emerged (Tables 5.4 and 5.5 for postlarvae, and 5.14 and 5.15 for juveniles). Unexpectedly, the magnesium concentrations in intermoult carapaces of both postlarvae and juveniles in experiment 2 were highest at the two extreme levels of water hardness (20 and 320mg l^{-1} as $CaCO_3$). The higher magnesium in carapaces from the lowest water hardness might be because the magnesium ion is essential for exoskeletal calcification and the prawns tended to conserve more magnesium when its concentration was too low in the environment. At the other extreme, magnesium concentrations in intermoult carapaces increased in treatments where total water hardness levels were >320mg l^{-1} as $CaCO_3$ (>1.6mmol Mg^{2+} l^{-1}), indicating that the prawns were possibly less capable of regulating the magnesium when its environmental concentration was high. Although at least an order of magnitude different, it would be of interest to mention here the reports of Zanders (1980) who studied the magnesium regulation in *Carcinus maenas*. He reported that over the range of external concentrations between 10 and 150-175mmol Mg^{2+} l^{-1}, the magnesium concentrations in hemolymph increased steadily but at a slower rate than the rise in the medium, and as the external concentrations was increased above about 175mmol Mg^{2+} l^{-1}, magnesium regulation appeared to break down and the blood

magnesium concentrations increased in parallel to the external rise. It is possible that the magnesium regulation in freshwater prawn *M. rosenbergii* might similarly break down at extreme environmental levels. Stern *et al.* (1987) also recorded an elevated concentrations of magnesium in the hemolymph of *M. rosenbergii* exposed to waters high in magnesium. The experiment in Chapter 4 (section 4.3.5, Table 4.9) also demonstrated the presence of higher magnesium in carapaces from prawns exposed to waters of Ca:Mg ratio of 1:4 than 4:1 and 1:1 ratios, and Hagerman (1980) suggested that magnesium might easily enter the body due to a favourable concentration gradient.

In this study, postlarval prawns had 13-29% and juveniles had 24-31% higher magnesium concentrations in cast than in intermoult carapaces which further supports the hypothesis that withdrawal of organic matter from the old carapaces took place prior to moulting. The greater differences in juveniles than postlarvae again indicating that the juveniles were more efficient than postlarvae in withdrawing organic matter from their old exoskeleton prior to ecdysis.

Dall (1965a), Wickins (1984b) and Fieber and Lutz (1985) also found higher magnesium concentrations in cast than intermoult carapaces. In this study with juvenile prawns the differences in magnesium concentrations between cast and intermoult carapaces (cast - intermoult) increased with the increase in water hardness (Tables 5.23c, 5.14 and 5.15) and this loss of excess magnesium in the cast exoskeleton may also be an advantage in high hardness waters particularly if

regulating ability is poor.

5.6 Conclusion

This study demonstrated, possibly for the first time that both postlarvae and juveniles were adversely affected by high environmental alkalinity not water hardness. Postlarvae were found to be more sensitive to elevated alkalinity than juveniles.

Environmental stress due to high alkalinity >100mg l^{-1} resulted in the occurrence of white muscle syndrome both in postlarvae and juveniles where the incidence were more prevalent and occurred earlier in postlarvae than in juveniles.

The high alkalinity (250mg l^{-1}) and low hardness (20mg l^{-1}) combination resulted in the poorest survival of prawns. Mortality occurred mainly during late premoult and while moulting suggesting that the ionic imbalance perhaps exerted a fundamental, adverse influence on the moulting physiology. Postlarval prawns again suffered higher mortality than juveniles in such an environment.

Both postlarval and juvenile *M. rosenbergii* demonstrated their ability to survive and grow satisfactorily over a wide range of water hardnesses provided the alkalinity of water was low (in this study 25mg l^{-1} as $CaCO_3$).

High environmental alkalinity enhanced the moulting frequency both in postlarvae and juveniles, and this happened at the expense of the growth rate of prawns. The experiments provided further evidence for the independence of the mechanisms governing moulting, and size increment achieved at moult, in crustacea. This is the first time this independence has been shown to be affected by alkalinity.

The prawns of both size groups were able to regulate calcium concentrations in their intermoult carapaces over a wide range of water hardnesses (in this study 20-1000mg l^{-1}), but increased alkalinity resulted in a higher deposition of calcium in the intermoult carapaces. Cripps and Nakamura (1979) observed hemolymph calcium concentrations in intermoult *M. rosenbergii* exposed to different water hardness (where alkalinity levels also increased with water hardness), and reported that the hemolymph calcium concentrations did not differ due to environment. This suggests that any excess calcium in the hemolymph will either be excreted or deposited in exoskeleton. The frequent moulting in high alkalinity waters thus might have some advantage if it enabled prawns to get rid of excess exoskeletal $CaCO_3$ before it reached harmful levels.

CHAPTER 6

EFFECTS OF ENVIRONMENTAL ALKALINITY ON CALCIUM-STIMULATED DEPHOSPHORYLATING ENZYME ACTIVITY IN THE GILLS OF POSTMOULT AND INTERMOULT GIANT FRESHWATER PRAWNS *M. ROSENBERGII*.

6.1 Introduction

The adult giant freshwater prawn *M. rosenbergii* moults every 20-40 days during peak growth periods (Ling and Merican 1961), and each moult causes a substantial calcium deficit (Fieber and Lutz 1982) which must be replaced from the environment. Calcium may be limited in many freshwater environments, where uptake of calcium from water into the hemolymph occurs against an electrochemical gradient and is governed by the availability of bicarbonate ions in the water (Greenaway 1985).

In fish, a calcium-dependent enzyme Ca^{2+}-ATPase is associated with active uptake of calcium from water into the blood across the gill membrane (Ma *et al.* 1974, Burdick *et al.* 1976, Fenwick 1976, Shephard and Simkiss 1978, Watson and Beamish 1980, Bansal *et al.* 1985). A high affinity, calmodulin-dependent, Ca^{2+}-ATPase is involved in the active transport of calcium ions across the fish gill membrane (Flik *et al.* 1983, 1984, 1985). A calcium-stimulated enzyme (Ca^{2+}-

ATPase) is also present in the hepatopancreas of the blue crab, *Callinectes sapidus* (Fox and Rao 1978), and in nerve of the lobster, *Homarus americanus* (Ghiasuddin and Matsumura 1979); but has not been looked for in crustacean gills (Greenaway 1985) until recently (Cameron 1989, Morris and Greenaway 1992). This is surprising since the gills are thought to be the major channel of calcium uptake from the environment (Dall 1965b). Although this has only been demonstrated in crayfish the translocation process is believed to occur across the gill epithelium (Chaisemartin 1965). Horiuchi (1977) and Stern *et al.* (1984) demonstrated the presence of Na-K-ATPase in the gills of the freshwater crayfish, *Procambarus clarkii* and the giant freshwater prawn, *M. rosenbergii* respectively. There has been no report of the presence and activity of calcium transport enzyme systems in the gills of the giant freshwater prawn *M. rosenbergii*. A high affinity Ca^{2+}-ATPase activity has now been identified in crab gills (*Leptograpsus variegatus*) but its specific activity did not change during the moult cycle and it was judged not to be contributing significantly to the increased calcium influx from the water during postmoult (Morris and Greenaway 1992).

It has been observed that juvenile *M. rosenbergii* exposed to water of high alkalinity (250mg l^{-1} as $CaCO_3$) had a significantly higher ($P<0.01$) moulting frequency than those in low alkalinity water (25mg l^{-1} as $CaCO_3$) (8.5 as opposed to 10.7 days) (Chapter 5, Table 5.17 a). It might be expected that, under these conditions, rapidly moulting juvenile prawns would have a higher rate of calcium metabolism and that this would be reflected by increased activity of ion transport

enzyme systems particularly in the gills.

A study of calcium-stimulated branchial enzyme activity in *M. rosenbergii* exposed to low and high alkalinity water was therefore conducted in order to investigate:

a) whether a calcium-stimulated dephosphorylating enzyme was present in prawn gills, that could be associated with calcium transport across the gills;

b) the effect of environmental alkalinity on the branchial enzyme activity;

c) the differences in enzyme activity between immediate postmoult and intermoult prawns.

6.2 Materials and Methods

6.2.1 Physical Facility, Number of Prawns, Size, Husbandry and Duration of Exposure in the Media

The experiment location, environment, vessels, water system, aeration and source of animals were as described in Chapter 2. Eighteen sub-adult freshwater prawns *M. rosenbergii* of mean weight 5.53g (± SE = 0.37) were selected for this study. The

prawns were housed individually in plastic tanks (section 2.4) each containing 9 litres of water. Stock solutions were as described in Table 2.1 and culture media were prepared as described in section 2.3.3 by adding appropriate quantities of stock solutions. The concentrations of chemical salts in the media are shown in Table 6.1. Each tank was provided with an airstone (section 2.5.3) and two plastic biological filters. The tanks were covered with black polythene sheet in order to: a) protect the prawns from excessive illumination; b) prevent escapes and c) reduce evaporation. The treatment water was completely renewed every 48 hours with minimum disturbance to the prawns (section 2.5.2). Prawns were fed as described in section 2.5.1. The exposure period of the test animals in the test media ranged from 7 to 36 days.

6.2.2 Design of Experiment

In this study eighteen prawns were exposed to two alkalinity treatments, 25mgl^{-1} and 250mgl^{-1} as $CaCO_3$. Total water hardness in both the treatments was same (20mgl^{-1} as $CaCO_3$). The experimental tanks were randomly distributed into two fibre glass troughs as described in section 2.4.

Table 6.1 Chemical composition of culture media. All chemicals used were of AnalaR grade (BDH, Poole, Dorset).

No.	Chemical	Inclusion $mg.l^{-1}$ in the culture media	
		Alkalinity ($mg.l^{-1}$ as $CaCO_3$)	
		25	250
1	$CaCl_2.6H_2O$	21.9	21.9
2	$MgCl_2.6H_2O$	20.3	20.3
3	KCl	7.1	7.1
4	Na_2SO_4	178.0	178.0
5	$NaHCO_3$	5.3[a]	400.0[b]

[a] and [b] produced water of total alkalinity 25 and 250 $mg.l^{-1}$ as $CaCO_3$

6.2.3 Moult Checking

Moulting of prawns was checked each morning and evening. The prawns were allowed to moult just once in their respective treatment waters. All the prawns moulted at night except two who moulted during day time. Gills of six prawns from each treatment were taken at the following mid intermoult stage and those of three prawns from each treatment were taken within one-day postmoult for determination of the enzyme activity. The intermoult stage was determined as described in Peebles (1977).

6.2.4 Preparation of Gill Homogenate

Approximately 40-45mg of gill tissue were dissected out and quickly rinsed in ice cold homogenizing medium, blotted dry, and weighed. The homogenizing medium contained 0.25M sucrose (Sigma), 20mM imidazole (pH 7.2) and 5mM Na_2EDTA (Sigma). The following procedures were carried out at 0°C. The weighed gills were quickly transferred into polyethylene vials containing half of the final volume of the homogenizing medium. The gills were then homogenized in a tissue homogenizer (Ultrataurrax) for 40s and diluted to the final tissue concentration of $5mg.ml^{-1}$ of the homogenate and homogenized again for another 40s. For the optimum enzyme activity, the tissue concentration of $5mg.ml^{-1}$ was determined by preliminary trials. The homogenates were filtered through a double layered sterile

gauze, and taken for enzyme assay.

6.2.5 Incubation and Enzyme Assay

The homogenates were incubated in two different incubation media to hydrolyse the ATP. The ATPase activity was determined from the amount of inorganic phosphate (P_i) liberated from the ATP during incubation with the gill homogenate (enzyme suspension). The calcium-stimulated enzyme activity was calculated as the difference between the rates of inorganic phosphate liberation from ATP in the presence and absence of calcium in the incubation media. Self hydrolysis of ATP was also determined under the same experimental conditions in the absence of the enzyme.

At the beginning, 100µl of homogenate or enzyme suspension (43-53µg protein) were added to 1.8ml of incubation medium A (3mM $CaCl_2$ and 30mM imidazole buffer at pH 7.8) and also to 1.8ml of incubation medium B (only 30mM imidazole at pH 7.8). The optimum pH of the incubation medium for higher enzyme activity was pH 7.8 (Bansal *et al.* 1985). Both the media were pre-warmed at 37°C for 10 minutes before adding the homogenate. To initiate the reaction, 100µl Na_2ATP (Sigma) was added to each reaction tube to give a final concentration of 3mM ATP in the reaction medium (Bansal *et al.* 1985; Mollah 1991). The total volume of the reaction medium in each tube was 2.0ml. The incubation was carried out for 20

minutes in a thermostatically controlled water bath at 37°C (Watson and Beamish, 1980) with occasional shaking. Under these conditions, a linear relationship was obtained between ATP hydrolysis and protein concentration of the enzyme (Horiuchi, 1977; Mollah, 1991). After incubation, the reaction was stopped by adding 2ml ice-cold 10% trichloroacetic acid, while immersing the tubes in crushed ice. After 10 min the tubes were centrifuged (MSE High Speed) at 12,000g for 10 min at 4°C. A 1ml sample of supernatant was taken from each tube for the determination of P_i liberated during incubation. Incubations were carried out in duplicate for each sample and the mean was used to calculate the ATPase activity.

6.2.6 Determination of Inorganic Phosphate (P_i)

Inorganic phosphate liberated from the hydrolysis of ATP was determined colorimetrically by the method of LeBel *et al.* (1978). This is the modification of the method of Fiske and Subbarow (1925) and claims to be superior with respect to sensitivity and colour stability. This method is also free from interferences of ATP with EDTA, sucrose and other commonly used chemicals in enzyme assay.

In preliminary trials, the supernatant fraction showed a higher rate of enzyme activity than the precipitates of the centrifuged gill homogenate, and hence the supernatant of the homogenate was taken for the ATPase activity measurement in this study.

Briefly, 1ml of supernatant, 3ml reagent A (copper acetate : 0.25% $CuSO_4.5H_2O$ and 4.6% sodium acetate trihydrate in 2N acetic acid) and 0.5ml reagent B (5% ammonium molybdate) were mixed in a vortex mixer. Then 0.5ml reagent C (2% Elon in 5% sodium sulphite) was added and mixed. After 15 min, the absorbence was measured at 870 nm using a Kontron spectrophotometer. Standard phosphate solution (1mg P_i/ml) was prepared by dissolving anhydrous KH_2PO_4 (0.4387g/100ml) in distilled water (LeBel *et al.* 1978). The specific activity of enzymes was calculated after the estimation of protein content in the enzyme suspension.

6.2.7 Estimation of Protein

The protein content of the homogenate was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as a standard. Each estimation was carried out on a 100µl sample.

The specific activity was expressed as micromoles of inorganic phosphate (P_i) liberated per mg of protein per hour.

6.2.8 Statistical Analysis

The t-test was used to calculate the level of any significant differences in branchial enzyme activity between the treatment means and also between the two moult stages.

6.3 Results

Calcium-stimulated dephosphorylating enzyme activity was found in the gills of intermoult and immediate postmoult freshwater prawns *M. rosenbergii* (Table 6.2). Intermoult prawns exposed to the higher alkalinity showed significantly higher ($P<0.05$) branchial enzyme activity (1.30 μmol P_i.mg protein^{-1} hr^{-1}) than those exposed to the lower alkalinity (0.58 μmol P_i.mg protein^{-1} hr^{-1}). However, at the immediate postmoult stage, no significant difference in the enzyme activity was detected between the prawns held at low and high alkalinity (2.11 and 1.29 μmoles P_i.mg protein^{-1} hr^{-1}, $P>0.05$).

A comparison of branchial enzyme activity between postmoult and intermoult prawns showed that at low alkalinity, the immediate postmoult prawns had significantly higher ($P<0.01$) calcium-stimulated enzyme activity (2.11 μmoles P_i.mg protein^{-1} hr^{-1}) than did those at the intermoult stage (0.58 μmol.P_i.mg protein^{-1} hr^{-1}) (Table 6.2). At high alkalinity, however, no significant differences were detected in branchial enzyme activity between the postmoult and intermoult stages (1.29 and 1.30 μmol P_i.mg protein^{-1} hr^{-1}) ($P>0.05$, Table 6.2).

Table 6.2 Comparison of branchial calcium-stimulated dephosphorylating enzyme activity in postmoult and intermoult *M. rosenbergii* exposed to different water alkalinities. Values are means ± SE (n=6 and 3 for intermoult and postmoult prawns respectively).

Alkalinity ($mg.l^{-1}$ as $CaCO_3$)	Enzyme activity ($\mu mol\ P_i.mg\ protein^{-1}.hr^{-1}$)		P value
	Postmoult prawns	Intermoult prawns	
25	2.11 ± 0.33	0.58 ± 0.22	P<0.01
250	1.29 ± 0.04	1.30 ± 0.19	P>0.05
P value	P>0.05	P<0.05	

6.4 Discussion

6.4.1 Enzyme Activity and Calcium Uptake

In freshwater Crustacea calcium uptake occurs against an electrochemical gradient and is generally believed to take place at the gills (Chaisemartin 1965, Dall 1965b). A rapid increase in uptake occurs immediately postmoult while subsequent shell mineralization is believed to continue throughout most of the intermoult period. This study demonstrates, the presence of a calcium-stimulated, dephosphorylating enzyme system in the gills of the giant freshwater prawn *M. rosenbergii* and shows, possibly for the first time that the enzyme activity was affected by external alkalinity and the moult stage.

In this study, the low environmental alkalinity used was 25mgl^{-1} as $CaCO_3$ which is the average total alkalinity of many Malaysian freshwaters (Ang Kok Jee, Personal communication), a natural habitat of *M. rosenbergii*. The prawns exposed to this environment showed significantly higher ($P<0.01$) calcium-stimulated enzyme activity at the postmoult than during the intermoult stage (Table 6.2). The increase was about 264%. It is well established that the prawns lose calcium during each moult, and therefore, it is quite reasonable to suppose that in the days immediately after moulting they will quickly take up calcium from the environment for rapid calcification of their exoskeleton. The significantly higher branchial calcium-stimulated enzyme activity among the postmoult prawns compared to the intermoult prawns at low alkalinity

suggests that the enzyme may be associated with calcium uptake from the environment.

Doneen (1981) found two kinetic forms of Ca^{2+}-ATPase enzymes in *Gillichthys mirabilis* gill membranes, one having a low affinity and the other a high affinity for calcium. He also suggested that Ca^{2+} transport in fish gills may involve both the low and high affinity Ca^{2+}-ATPases operating at different sites or by different mechanisms. Parker *et al.* (1985) reported that low affinity Ca^{2+}-ATPase activity is assayed well above the intracellular Ca^{2+} concentration (3mM Ca^{2+}) (which may also interact with Mg^{2+}-ATPase) while high affinity Ca^{2+}-ATPase is within the μM range (100μM) of intracellular Ca^{2+} concentration. Later, Flik *et al.* (1983) re-evaluated the characteristics of Ca^{2+}-ATPase activity and suggested that calcium-activated ATP hydrolysis in the plasma membranes of eel gills partially results from nonspecific alkaline phosphatase activity. They also suggested that more than one type of enzyme is involved in transepithelial Ca^{2+}-transport and that a high affinity Ca^{2+}-ATPase activity may play a key role. At least two types of calcium-stimulated dephosphorylating enzymes (nonspecific alkaline phosphatase, also called low affinity Ca^{2+}-dependent ATPase in some literature; and a high affinity Ca^{2+} ATPase) are known from crustacean gill tissue viz. *Birgus latro* (Morris *et al.* 1991) and *Leptograpsus variegatus* (Morris and Greenaway 1992). These enzyme may be distinguished by their kinetics and under the conditions reported here (3 mM $CaCl_2$), it is likely that the activity observed in *M. rosenbergii* was that of a low affinity, Ca^{2+}-stimulated ATPase, probably alkaline phosphatase.

In a recent study on marine crab (*L. variegatus*), Morris and Greenaway (1992) did not find any increase in enzyme activity (high affinity Ca^{2+}-ATPase) at the postmoult stage, and therefore supported the idea that an alternative mechanism, such as a Na^{+}/Ca^{2+} exchanger was also involved in calcium transport. This exchanger responds to higher intracellular levels of calcium but has a much greater translocating capacity than Ca^{2+}-ATPase (Philipson 1985). Roer (1980) suggested that a combination of a Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchange mechanism was responsible for active transport of Ca^{2+} across the hypodermis of *Carcinus*. The passive entry of calcium by diffusion in marine crab *Callinectes sapidus* proposed by Cameron (1989), was however considered unlikely by Morris and Greenaway (1992) because of the very small difference in calcium levels between seawater and haemolymph (Greenaway 1983, Towle and Mangum 1985). In the freshwater environment, however, because of the lower concentration of calcium in the water, passive entry of calcium into the haemolymph would be impossible, and therefore, an active transport mechanism must be present in freshwater crustaceans (Morris and Greenaway 1992).

6.4.2 Effects of Environmental Alkalinity and Moult Stage on Enzyme Activity

In the present study the high environmental alkalinity stimulated the enzyme activity in the freshwater prawn gills. Immediately after moulting, the prawns of the two environmental groups did not show any significant differences in their branchial

calcium-stimulated enzyme activity (Table 6.2); this suggests that calcium uptake mechanisms were equally active, and possibly saturated, in the animals of both groups during immediate postmoult. At the intermoult stage, however, the prawns exposed to the alkalinity of 250 mgl^{-1} $CaCO_3$ showed a significantly greater ($P<0.05$) enzyme activity as compared to those held at 25mgl^{-1} $CaCO_3$ (Table 6.2). The increase was 124% and since this level of activity did not differ significantly from postmoult levels, it is suspected that the activity of the enzyme system at high alkalinity was perhaps always saturated. While it is quite logical to assume that the Ca^{2+} uptake at the postmoult stage would be high due to the need to rapidly recalcify the exoskeleton; intermoult prawns would not necessarily be expected to show such a high enzyme activity if such activity were solely associated with calcium uptake. The data of enzyme activity in this study, do not show whether calcium is going into or coming out of the prawns. It is possible that the observed higher activity in intermoult prawns at high alkalinity might be due increased deposition of calcium in carapaces which was observed in intermoult carapaces and/or also might be associated with the excretion of calcium through gills. In brackish water shrimp for example, Dall (1965 b) reported that while some 90% of ^{45}Ca uptake probably occurs via the gills, and about 70% lost through the gills. The need to excrete calcium would arise if high alkalinity caused increased influx of bicarbonate and with it, excess calcium.

Cameron (1985) studied the effect of external pH, HCO_3^- and Ca^{2+} to determine the nature of rapid net Ca^{2+} influx and apparent net H^+ efflux in the postmoult blue crab, *Callinectes sapidus*. He found both fluxes were strongly inhibited by reductions

in external Ca^{2+}, HCO_3^- or pH. However, Greenaway (1974b) found that addition of bicarbonate to the medium enhanced net calcium uptake by postmoult crayfish while the mechanism was reduced in the absence of calcium. In this experiment, the HCO_3^- level at high alkalinity was 4.76 mmol.l^{-1}. That this level of HCO_3^- enhanced the calcium-stimulated enzyme activity in both the postmoult and intermoult prawns, indicates that the prawns were actively engaged in some metabolic processes probably involving calcium, for example uptake or excretion. It is tempting to speculate that the increased dephosphorylation activity observed in intermoult *M. rosenbergii* exposed to high alkalinity was in some way providing energy for an increased rate of calcium transport, inwards or outwards, perhaps through increased activity of the Na^+/Ca^{2+} exchanger mechanism proposed by Roer (1980).

Kerstetter and Kirschner (1974) observed the presence of a bicarbonate-dependent ATPase in both freshwater and seawater adapted rainbow trout gills, the enzyme activity being higher in sea than in fresh water. If elevated levels of environmental alkalinity increased the inward movement of bicarbonate ions, perhaps through stimulation of such an enzyme system, this would probably be accompanied by an inward transport of cations to maintain electrical neutrality (most probably Ca^{2+}), and this might perhaps have resulted in an increased uptake of calcium from the environment by the intermoult prawns exposed to high alkalinity in this study. It was observed that juvenile *M. rosenbergii* exposed to high alkalinity had significantly higher carapace calcium concentrations than those in low alkalinity water (Chapter 5, Table 5.21). In other words, high alkalinity enhanced calcium deposition in

intermoult carapaces. If, due to high environmental alkalinity, an enhanced rate of calcium uptake continued it seems reasonable to suppose that the prawns would have to get rid of the excess calcium carbonate from their body either by excretion or, if this was not fast enough to prevent excessive calcium deposition in the shell, by frequent moulting. It has indeed been observed that prawns held in high alkalinity water moulted more frequently without showing a corresponding increase in growth (Chapter 5, Tables 5.16 and 5.17). Under these conditions, it is conceivable that the increased effort required to maintain a satisfactory calcium balance by such mechanisms considerably reduced the scope for growth.

6.4.3 Recommendation for Further Study

Since the enzyme activity in the prawn gills did not show whether calcium was being taken up or excreted by intermoult prawns exposed to high alkalinity waters; further investigation on calcium movement using ^{45}Ca in low and high alkalinity waters and also at different moult stages is suggested. Such a study using ^{45}Ca will hopefully elucidate;

a) whether calcium is being taken up and/or excreted by intermoult prawns exposed to high alkalinity waters,

b) the peak period of calcium uptake by the postmoult prawns for rapid exoskeletal

calcification.

CHAPTER 7

FINAL CONCLUSIONS

The studies in this thesis were initiated to investigate the separate effects of environmental alkalinity and hardness levels on postlarvae and juveniles of the giant freshwater prawn *M. rosenbergii*. During the early part of this work, it became necessary to undertake an additional investigation (Chapter 4) into the calcium to magnesium ion ratio in water in order to confirm that variations in these factors were not the cause of the mortality that occurred in the preliminary experiment. This additional investigation also provided some information relevant to the fact that the Ca:Mg ratios in natural waters generally vary from place to place depending on the geology of the area and could also vary in culture ponds due to human intervention, for example during pond management practices involving addition of lime.

During the experiments on alkalinity and hardness the interesting changes that were observed in moulting frequency and carapace mineralization suggested that measurable differences in ion transport mechanisms might be occurring under some treatments. The opportunity to determine phosphorylase activity in gills was therefore taken and yielded new information on the physiological responses of prawns to alkalinity change. In this Chapter conclusions are drawn from each of the investigations in order to integrate the most important findings in the light of the

biological and culture requirements of *M. rosenbergii* and to highlight important areas for further research.

Effects of External Cation Ratio (Ca:Mg) on Prawns

Of the three calcium to magnesium ratios studied (4:1, 1:1 and 1:4) at an alkalinity level of 110mg l^{-1}, the best growth rate (mg day^{-1}) was obtained from the media containing the 1:1 ratio. The prawns in the media where the ratio was 4:1 appeared stressed at high hardness levels, exhibited white muscle syndrome, moulted more frequently but had the slowest growth rate. It seemed likely that prolonged exposure to this medium could eventually lead to increased mortality or disease. On the other hand excess magnesium in the media (i.e. 1:4 ratio) prolonged the intermoult period and resulted in a slower growth rate than did the 1:1 ratio. Indeed, the results of mineralization in intermoult carapaces indicated that magnesium regulation in the prawns exposed to a ratio of 1:4 appeared to be poor. The Ca/Mg atom ratios in carapaces declined as the magnesium levels in the rearing media increased. In the present study however no abnormal behaviour of prawns was noticed when exposed to media of the ratio 1:4. Stern *et al.* (1987) suggested high levels of magnesium in crustaceans hemolymph could affect nerve and muscle activity and in some cases cause animals to become inactive. The experiment, though preliminary in nature, did indicate that extremes of Ca:Mg ratios could have a significant impact on prawn physiology. When at 1:1 ratio however, these seemed to be few problems and this value was therefore chosen for subsequent experiments.

Effects of Alkalinity and Hardness on Postlarval and Juvenile Prawns

The experiments with both postlarvae and juveniles indicated that high environmental alkalinity was responsible for the occurrence of the white muscle syndrome in the prawns, and high hardness did not exacerbate the condition when alkalinity was low (25mg l^{-1}). The prawns suffering from white muscle syndrome appeared to severely stressed, showed sluggish movements and had a low appetite for food. Histopathological studies of a similar condition "necrotic muscle" observed in penaeids have revealed no causative pathogens (Rigdon and Baxter 1970, Lakshmi *et al.* 1978) and the condition is generally regarded as typical of a stress response in Crustaceans. With regard to the incidence of the condition, juvenile *M. rosenbergii* were more resistant to high environmental alkalinities than postlarvae. It is suggested that alkalinity levels of >100mg l^{-1} in conjunction with increasing water hardness >80mg l^{-1} are likely to increase the occurrence of white muscle syndrome in *M. rosenbergii* and should therefore be avoided during culture.

High environmental alkalinity was also implicated as the primary agent responsible for prawn mortality in these experiments. The highest alkalinity tested (250mg l^{-1}) in conjunction with the lowest low hardness (20mg l^{-1}) resulted in the highest mortality of prawns, postlarvae again being more vulnerable. In such media all postlarval prawns died while 22% of the juveniles survived. Most of the prawns died during late premoult or while moulting, thus suggesting that the ionic imbalance in high alkalinity and low hardness combinations perhaps exerted a fundamental,

adverse influence on moulting physiology possibly associated with the translocation of cations and anions within and between the animal and its environment. The results also showed that both postlarval and juvenile *M. rosenbergii* could survive a wide range of water hardness (in this study 20-1000mg l^{-1}) provided the alkalinity of the water was low.

High environmental alkalinity >100mg l^{-1} significantly increased the moulting frequency of both postlarval and juvenile prawns but paradoxically, this more frequent moulting did not give rise to an increased growth rate. It appeared that the frequent moulting in high alkalinity water took place at the expense of the increment achieved at each moult, a result that is not incompatible with the hypothesis that energy, or other resources, were being diverted from growth in order to combat the adverse effects of the excess bicarbonate ions in the water.

Importantly, these experiments demonstrated that high water hardness *per se* was not necessarily a constraint to the growth of *M. rosenbergii*. In fact it was the high environmental alkalinity and its interaction with water hardness that was responsible for the poor survival and inhibition of growth. Previous studies have reported both inhibition of growth in *M. rosenbergii* with increased water hardness (Cripps and Nakamura 1979, Howlader and Turjoman 1984, Vasquez *et al.* 1989, Brown *et al.* 1991) and no inhibition even at very high levels of water hardness (Heinen 1977, Smith *et al.* 1982, Bartlett and Enkerlin 1983). The results of the present study thus provides probably the first experimental evidence that differences in the alkalinity of

the waters were probably responsible for the conflicting results. To reveal the optimum range of alkalinity for optimum growth and survival of postlarval and juvenile *M. rosenbergii* further trials are required. However, the results of this study suggest that the optimum range of alkalinity for both postlarvae and juveniles exists somewhere between 20 and 100mg l^{-1} as $CaCO_3$.

Physical measurements of carapace thickness of juvenile prawns revealed that, in general, intermoult carapaces were 20-30% thicker than cast carapaces. It was also noticed that the inner surface of cast carapaces was always rough and that of dissected intermoult carapaces was smooth. The lesser thickness of cast carapaces and the roughness of their inner surfaces suggested that the inner layer (s) might have been dissolved and reabsorbed by the animals during premoult stage (sections 5.5.5.1 and 5.5.5.3). The thickness of both intermoult and cast carapaces tended to decline with increased water hardness while increasing alkalinity tended to produce thicker cast carapaces, though effects on intermoult carapaces was not discernable (section 5.5.5.1, Table 5.18). The differences in carapace thicknesses between intermoult and cast carapaces thus declined as the environmental alkalinity was increased and indicated that either less mineral or organic matter was being withdrawn from or that more was being deposited in the old exuviae before casting.

The physical measurements of carapace thickness could not reliably be made on the small carapaces of postlarvae. Instead the two sizes of animals were compared by means of carapace length specific dry weights. The postlarval and juvenile prawns

that had highest growth rates produced intermoult carapaces of highest length specific dry weights. The high alkalinity not only decreased the growth rate of prawns but also reduced the length specific dry weights of intermoult carapaces. Cast carapaces were thinner (section 5.4.5.1) and also had lower length specific dry weights than intermoult carapaces thus further supporting that reabsorption of material took place from the old exuviae prior to casting. Cast carapaces from high environmental alkalinity tended to have slightly higher length specific dry weights than those from low alkalinity treatment indicating less withdrawal of material from the old exuviae. The reasons for less material being withdrawn while in high alkalinity waters might be due to; a) a reduced amount of withdrawable organic matter being present, and b) failure of the prawns to withdraw much organic matter possibly as a result of heavy deposition of $CaCO_3$ in carapaces during intermoult (section 5.5.5.3).

Further understanding of the separate impacts of alkalinity and hardness on shell mineralization was gained from the measurements of mineral ion concentrations in intermoult and cast carapaces. Both postlarval and juvenile *M. rosenbergii* demonstrated their ability to regulate calcium concentrations in their intermoult carapaces over a wide range of water hardnesses (20-1000mg l^{-1}). It is possible that because of this particularly well developed ability, the survival and growth of both postlarval and juvenile *M. rosenbergii* were not affected by environmental water hardness *per se*. Interestingly it was noticed that increased alkalinity >100mg l^{-1} significantly enhanced calcium deposition in intermoult carapaces and probably the higher moulting frequency in high alkalinity waters was an alternative strategy to

control the accumulation of excess $CaCO_3$ in their exoskeleton. The calcium concentrations in cast carapaces followed the same pattern as in intermoult carapaces. The concentrations of calcium were higher in cast than in intermoult carapaces, providing further evidence for the withdrawal of organic matter from the old exuviae prior to moulting. The results of the differences in calcium concentrations between cast and intermoult carapaces (Tables 5.9 and 5.20) indicated that juveniles were more efficient than postlarvae in withdrawing organic matter from the old exuviae prior to casting.

The total amount of calcium present in carapaces standardised in terms of the lengths of the carapaces, (the length specific calcium content) increased as expected in intermoult carapaces, with increase in carapace thickness and size of prawn. In contrast to calcium concentrations which were higher in cast carapaces due to withdrawal of organic matter prior to ecdysis, the length specific calcium content in intermoult carapaces were higher (94-188% in postlarvae and 81-128% in juveniles) than in cast carapaces. The lower length specific calcium content in cast carapaces suggests that some calcium was lost from the cast exuviae either by reabsorption by the animal during premoult or lost to the environment while moulting. Since, newly moulted crustaceans contained only about 17% of the calcium content of the previous intermoult stage (Greenaway 1974a), the results of the present study support the hypothesis that calcium is lost to the environment during ecdysis and less is reabsorbed along with the organic matter. While the calcium concentrations in intermoult carapaces were higher when environmental alkalinity was high than when

it was low, the length specific dry weights and length specific calcium content of intermoult carapaces were, in contrast, lower in high alkalinity than in low alkalinity waters, suggesting that the intermoult carapaces from high alkalinity waters perhaps contained lower quantities of organic matter. The frequent moulting in high alkalinity waters (probably to get rid of excess calcium and/or carbonate before they reached harmful levels) took place at the expense of energy and growth which perhaps reduced time available and possibly the ability of prawns to synthesize or deposit the normal amount of organic matter.

The magnesium levels in intermoult carapaces were not as well regulated as calcium. Magnesium concentrations in intermoult carapaces generally tended to follow the levels in the water being higher when environmental concentrations were high. This was particularly evident in the preliminary trial in which the Ca:Mg ratio was varied (section 4.3.5).

Effects of Environmental Alkalinity on Calcium-Stimulated Enzyme Activity in Gills

This study found the evidence for the presence of a calcium-stimulated dephosphorylating enzyme in the gills of the giant freshwater prawn, *M. rosenbergii*. The activity of the enzyme was influenced by external alkalinity levels and moult stage. The calcium-stimulated enzyme activity did not show whether calcium was

being taken into or pumped out of the body. At low alkalinity (25mg l^{-1}) the branchial enzyme activity was significantly higher (264%) in the postmoult than in the intermoult stage suggesting that the increased enzyme activity at postmoult stage was due to the uptake of calcium from the environment for exoskeletal calcification. Prawns exposed to high alkalinity (250mg l^{-1}), moulted more frequently without corresponding increase in growth. In these animals the calcium-stimulated dephosphorylating enzyme activity in postmoult and intermoult prawns was virtually same and may have been saturated since the activity did not differ significantly from that in the postmoult prawns. Taken together these findings indicate that the higher enzyme activity might reflect either an increased uptake or excretion of calcium through the gills. The experiments in Chapter 5 indicated however that calcium was well regulated by prawns and it is of course possible that the enzymic activity would vary under different environmental conditions (particularly, since high alkalinity waters enhance calcium uptake, Greenaway 1974b) in order to control calcium and bicarbonate ions. It is also possible that excretion of calcium through the gills may be occurring, but this might not be fast enough to prevent excessive calcium deposition in the shell (Tables 5.10a and 5.21a), in which case the observed increase in moulting frequency could be an alternative mechanism attempting to compensate for the increased uptake of minerals. Under these conditions, the animals had to spend more time and energy in order to maintain a satisfactory calcium balance and thus possibly reduced the scope for growth.

Potential Application of the Results of the Study for Freshwater Prawn Farming

The results of this research have a wide potential application for wherever *Macrobarchium rosenbergii* is cultured. One country with particularly promising conditions for culture is Bangladesh, with it vast freshwater resources. *M. rosenbergii* is an indigenous species in Bangladesh and distributed in a wide variety of freshwater habitats like ponds, lake, rivers, canals and even in inundated paddy fields. BAFRU (1990) conducted a survey of the water quality of 46 Government and non-Government fish farms in Bangladesh (Appendix I), including determination of total hardness and alkalinity. The survey indicates that 11% fish farms (e.g. Shetabgonj, Tajhat, Chagolnaya, Kaherol and Nilphamari fish farms) have waters of total alkalinity levels below 20mg l^{-1} as $CaCO_3$, 72% farms have waters of alkalinity levels 20-110mg l^{-1} and 13% have alkalinity of 120-218mg l^{-1}.

The results of the laboratory trials in the present investigation indicated that alkalinity levels greater than 100mg l^{-1} were responsible for the reduced growth rate of prawns. Although the laboratory results might not exactly be the same in earthen ponds or natural habitats, the results of the present investigation suggest that about 72% of the existing fish farms in Bangladesh are within the suitable range of water quality for freshwater prawn farming. The 11% of sites which have alkalinity level <20mg l^{-1} could also be brought under suitable condition by adding lime to the farm ponds. Only 13% farms which have alkalinity levels >120mg l^{-1} will possibly produce poor performance for *M. rosenbergii* culture. The high alkalinity in these

farms might be either due to human manipulation (e.g. over liming) or due to the geological condition of the area. Human manipulated high alkalinity could be brought down by management practice but the sites of high alkalinity due to geology of the area should be discouraged for the siting of freshwater prawn farms.

It is interesting to note that some of the ground waters from deep tube-wells and hand pump tube-wells have alkalinity levels ranging from 287-401mg l^{-1} as $CaCO_3$ (e.g. Kotchanpur hatchery, Jhenaidah; DoF farm, Jhikorgacha; Grameen Bank farm, Manikgonj and DoF farm, Jessore) (Appendix I). The direct application of these ground waters to prawn farms would undoubtedly damage the prospects for freshwater prawn farming and could not be used without pretreatment.

The BAFRU (1990) survey (Appendix I) report and the results of the study in this thesis suggest that addition of lime in some farms in Bangladesh seems to be essential for prawn farming. But the dosage of lime should be determined by the alkalinity levels of the waters.

The present investigation also indicated that water of high alkalinity and low hardness was one of the suspected cause of high mortality of prawns during premoult and while moulting. It is encouraging that such unbalanced ionic composition of water in Bangladeshi farms were not found in BAFRU (1990) survey.

This study has shown that determining water alkalinity and hardness levels is of vital importance for successful prawn farming.

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Appendix I. Total hardness and alkalinity (both expressed as mg l^{-1} as $CaCO_3$) of waters in some fish farms of Bangladesh. (Source, BAFRU, 1990).

Parameter	Pond water	Shallow tube-well	Hand pump tube-well	Deep tube-well	Location
Hardness	45	-	-	118	BRAC hatchery,
Alkalinity	60	-	-	164	Rajendrapur.
Hardness	146	220	-	-	CARITAS farm,
Alkalinity	180	250	-	-	Bariachar, Gopalgonj.
Hardness	158	-	355	-	CARITAS farm,
Alkalinity	120	-	167	-	Malipara, Natore.
Hardness	50	-	68	-	CARITAS farm,
Alkalinity	53	-	158	-	Dinajpur.
Hardness	25	-	63	-	DoF farm, Burichang,
Alkalinity	32	-	85	-	Comilla.
Hardness	7	-	-	-	DoF farm, Chagolnaya,
Alkalinity	10	-	-	-	Feni.
Hardness	29	-	-	-	DoF farm, Chowmahoni,
Alkalinity	35	-	-	-	Noakhali.
Hardness	197	-	-	>1000	DoF farm, Chuadanga.
Alkalinity	218	-	-	362	
Hardness	31	170	-	-	DoF farm, Debidwar,
Alkalinity	38	95	-	-	Comilla.
Hardness	52	170	-	-	DoF farm, Feni,
Alkalinity	38	120	-	-	Noakhali.
Hardness	46	80	-	-	DoF farm, Jamalpur.
Alkalinity	47	108	-	-	
Hardness	181	-	-	390	DoF farm, Jessore.
Alkalinity	207	-	-	401	
Hardness	89	-	-	>800	DoF farm, Jikorgacha,
Alkalinity	97	-	-	310	Jessore.
Hardness	65	-	-	-	DoF farm, Katchua,
Alkalinity	67	-	-	-	Chandpur.

Appendix I (continued)

Parameter	Pond water	Shallow tube-well	Hand pump tube-well	Deep tube-well	Location
Hardness	144	-	-	-	DoF farm, Khulna.
Alkalinity	119	-	-	-	
Hardness	83	-	-	-	DoF farm, Kishorganj.
Alkalinity	73	-	-	-	
Hardness	169	-	-	320	DoF farm, Kotchanpur,
Alkalinity	179	-	-	287	Jhenaidah.
Hardness	121	-	-	-	DoF farm, Kushtia.
Alkalinity	142	-	-	-	
Hardness	55	167	-	-	DoF farm, Malotipur,
Alkalinity	56	128	-	-	Bogra.
Hardness	55	123	-	-	DoF farm, Mashkanda,
Alkalinity	47	147	-	-	Mymensingh.
Hardness	140	-	-	130	DoF farm, Naogoan.
Alkalinity	133	-	-	83	
Hardness	95	-	-	117	DoF farm, Narshingdi.
Alkalinity	107	-	-	137	
Hardness	131	-	-	434	DoF farm, Natore.
Alkalinity	110	-	-	69	
Hardness	70	-	127	-	DoF farm, Santahar,
Alkalinity	70	-	66	-	Bogra.
Hardness	50	-	-	162	DoF farm, Tongi.
Alkalinity	70	-	-	133	
Hardness	64	-	-	45	DoF farm, Jangalia,
Alkalinity	34	-	-	64	Comilla.
Hardness	32	-	-	-	Grameen Bank farm,
Alkalinity	44	-	-	-	Barguna.
Hardness	100	-	-	-	Grameen Bank farm,
Alkalinity	103	-	-	-	Chatmohar, Pubna.
Hardness	40	174	-	-	Grameen Bank farm,
Alkalinity	37	240	-	-	Gaibandha.

Appendix I (continued)

Parameter	Pond water	Shallow tube-well	Hand pump tube-well	Deep tube-well	Location
Hardness	42	-	-	-	Grameen Bank farm,
Alkalinity	43	-	-	-	Gobindaganj,Gaibandha
Hardness	111	-	-	-	Grameen Bank farm,
Alkalinity	94	-	-	-	Gournandi, Barisal.
Hardness	80	-	-	97	Grameen Bank farm,
Alkalinity	92	-	-	126	Joyshagor, Shirajganj.
Hardness	8	41	-	-	Grameen Bank farm,
Alkalinity	19	43	-	-	Kaherol, Dinajpur.
Hardness	20	166	-	-	Grameen Bank farm,
Alkalinity	26	150	-	-	Lalmonirhat.
Hardness	84	-	365	-	Grameen Bank farm,
Alkalinity	83	-	322	-	Manikganj.
Hardness	94	-	-	-	Grameen Bank farm,
Alkalinity	60	-	-	-	Mehendiganj, Barisal.
Hardness	24	-	78	-	Grameen Bank farm,
Alkalinity	35	-	35	-	Melanda, Jamalpur.
Hardness	56	-	-	-	Grameen Bank farm,
Alkalinity	62	-	-	-	Munshiganj.
Hardness	6	-	20	-	Grameen Bank farm,
Alkalinity	10	-	22	-	Nilphamari.
Hardness	129	-	-	-	Grameen Bank farm,
Alkalinity	101	-	-	-	Noorpur, Pubna.
Hardness	40	-	-	-	Grameen Bank farm,
Alkalinity	40	-	-	-	Patuakhali.
Hardness	24	-	-	36	Grameen Bank farm,
Alkalinity	39	-	-	60	Pulhat, Dinajpur.
Hardness	23	-	-	-	Grameen Bank farm,
Alkalinity	25	-	-	-	Saidpur, Nilphamari.
Hardness	13	-	-	-	Grameen Bank farm,
Alkalinity	11	-	-	-	Setabganj, Dinajpur.

Appendix I (continued)

Parameter	Pond water	Shallow tube-well	Hand pump tube-well	Deep tube-well	Location
Hardness	13	-	70	-	Grameen Bank farm,
Alkalinity	19	-	58	-	Tajhat, Rangpur.
Hardness	84	-	-	-	Grameen Bank farm,
Alkalinity	82	-	-	-	Tangail.

Total number of farms surveyed = 46.
Approximately 11% farms have waters of total alkalinity levels below 20mg l^{-1} as $CaCO_3$, 72% farms have waters of alkalinity levels 20-110mg l^{-1} and 13% have alkalinity of 120-218mg l^{-1}.