RESPONSE OF *MACROBRACHIUM ROSENBERGII* (de Man) TO CALCIUM AND MAGNESIUM CONCENTRATIONS IN FRESHWATER

By

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THESIS

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DEDICATED TO

MY

PARENTS, BROTHERS

AND SISTERS

DECLARATION

I hereby declare that this thesis entitled **"RESPONSE OF *MACROBRACHIUM ROSENBERGII* TO CALCIUM AND MAGNESIUM CONCENTRATIONS IN FRESHWATER"** is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associate ship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"RESPONSE OF *MACROBRACHIUM ROSENBERGII* TO CALCIUM AND MAGNESIUM CONCENTRATIONS IN FRESHWATER"** is a record of research work done independently by Sri. **T.V. SHIVAKUMAR** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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INTRODUCTION

1. INTRODUCTION

Crustaceans form one of the most valuable food resources of the world by virtue of their importance as items of esteemed delicacy and as items of exports. Of late, its capture fishery has failed to keep pace with the ever-increasing demand. Intensive efforts are underway in many parts of the world to work out cost effective technologies to take up the culture.

The increasing demand for prawns, especially the larger varieties, for export has created a situation where the exploitation is extended to all possible areas and times. This tendency towards overexploitation resulted in a fear of depletion of stock. Various types of human interference like indiscriminate reclamation of estuarine areas, construction of regulators, dams, salinity barriers and pollution due to urbanisation and industrialisation have added to the malady. The spread of waterweeds like *Eichhornia* and *Salvinia* also impedes the normal life and reproductive cycle of many inland fishes and prawns. All these developments prompted to turn the attention more and more towards the culture of important species for increasing production. This has helped the development of the culture of freshwater prawns.

Among the freshwater prawns of the genus *Macrobrachium*, the best known and the most popular species is the giant freshwater prawn *Macrobrachium rosenbergii*, not only because of the many pioneering works done on this species, but also because of other factors like its comparatively tame and less cannibalistic behaviour, fast growth rate, shorter larval period and high tolerance to a wide range of temperature and salinity

(Ling and Costello, 1976). Subramanyam (1980, 1984) has described the salient features of farming this species in mono- and polyculture systems under diverse environmental conditions.

Macrobrachium rosenbergii has a wide distribution in the Indo-Pacific region including India, Sri Lanka, Myanmar, Thailand, Malaysia, Singapore, Indonesia, Philippines, Cambodia, Indo-China, Vietnam, Laos, North Australia, North-Eastern Guinea (Holthuis and Rosa, 1965) and it has been transferred repeatedly from its natural location to other parts of the world for purposes of research and culture. In India it has been recorded from Hoogly estuarine system (West Bengal), Mahanadi system (Orissa), Cauvery system (Tamil Nadu), Krishna-Godavari system (Andhra Pradesh), Vembanad lake and adjacent waters (Kerala), Thana creek and others rivers of the west coast of Maharashtra and Narmada and Tapti system in Gujarat. The distribution of this species is limited to about 200 km interior from the coastal belt (Ling, 1969).

Though freshwater prawns were used to be collected from the wild and grown to marketable size in impoundments earlier, true culture systems began to develop in the early 1960^{S}. Shao-wen Ling (1977) in Penang, Malaysia, succeeded in closing the life cycle of the giant long legged prawn, *Macrobrachium rosenbergii*, which stimulated widespread interest in the commercial culture of this and other members of the family palaemonidae.

1.1 Status of culture:

Although it is commercially cultured in the Southeast Asian countries, freshwater prawn culture in India is not an organised one unlike shrimp or carp culture. This is mainly due to the lack of sufficient supply of seed. In some states like Maharashtra, Andhra Pradesh and Gujarat, seed collected from the wild is used for culture. But wild seed is usually mixed with those of other small species of less importance and may be of uneven size and different age groups. With the establishment of hatcheries in the public and private sectors, particularly in Andhra Pradesh, Kerala, Tamil Nadu and West Bengal in the late eighties scientific culture of freshwater prawns started in India. At present the important *Macrobrachium* hatcheries functioning in India include the Bengal Scampi Tech (Digha, West Bengal), Andhra Prawn Hatcheries (Bhimavaram, Andhra Pradesh), Maharaja Hatcheries (Nellore), United Freshwater Prawn Hatchery (Trichi, Tamil Nadu), Aquaplaza Hatcheries P. Ltd. (Cherai, Kerala), Rosen Fisheries (Trissur, Kerala), Maveli Hatchery (Alappuzha, Kerala), and Nature's Way Hydrofauna (Cherthala, Kerala), Fisheries College Hatchery, (Panangad, Kerala) etc.

1.2 Resource potential:

In 1997, aquaculture production of freshwater prawns in India was about 453 mt out of the global production of 60,995 mt. The contribution from Asia alone is about 92%. Even though the resources for freshwater prawn culture in India are rich and varied, the present production of the country is low when compared to the potential, which exists.

The lucrative penaeid shrimp culture industry in India is faced with numerous problems like white spot and other diseases. Further, the supreme court of India has banned shrimp culture in coastal areas within 500 m from the high tide mark. However, there is tremendous opportunity for freshwater prawn culture in the country, with the rich and varied resources.

Production data of the giant freshwater prawn from major countries during 1997 (F.A.O report, 1999) is as follows:

China	- 42,851 Metric tons
Thailand	- 7,800
Taiwan	- 7,554
Ecuador	- 800
Brazil	- 560
India	- 453
Malaysia	- 143
Mexico	- 112
Dominican Rp.	- 90
Other countries	- 632
Total	- 60,995

Calcium is the most important element for growth and development of crustaceans. Calcium plays an important role in water alkalinity, hardness, buffer action and in the formation of skeletal parts of animals. For aquatic organisms, especially crustaceans, molluscs and shellfishes the calcium content in water is very important and necessary for new shell formation following molting. Most of the animals accumulate

calcium in the body from the water surrounding it and calcium can form complex compounds with carbonate and phosphate, which cause precipitation of the calcarious complex in animal tissue thus hardening the skeleton. Animals are supposed to be controlling the precipitation of calcium carbonate in their body by withdrawal of calcium-carbonate-phosphate complex. A drop in calcium absorption affects the body mechanism. Smitherman *et al.* (1967) found that growth and survival of the crayfish (*Procambarus clarkii*) in experimental pools were low when total water hardness was less than 20 ppm.

Addition of lime to pond with acid sulphate soils increases the pH, allowing better release of nutrients, particularly phosphorus. This, in turn, enhances plankton growth and the production of other organisms in the food chain. Boyd (1974) found that liming increased production of benthic organisms. The lime reacts with bottom mud and neutralizes acidity by exchanging basic for acidic ions on cation exchange sites. In instances where fertilization has proved unsuccessful, acid soil conditions should be suspected. Addition of lime, with no fertilization, may increase production simply by allowing release of nutrients locked up with pond mud.

The present study is aimed at finding out growth, survival, food intake and food conversion ratio of *Macrobrachium rosenbergii* in different concentrations of calcium and magnesium in freshwater and the optimum range of calcium and magnesium required for successful scampi culture.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

All forms of aquatic animals require various minerals for their normal life processes. Unlike most terrestrial animals, fish and shellfish have the ability to absorb some inorganic elements from their diets and also from their external environment in both freshwater and seawater (Lall, 1989).

2.1 Functions and Metabolism of calcium

Calcium is one of the most abundant cations in the body of fish and shellfish. Calcium is essential in bone formation and maintenance of skeletal tissues. Calcium ions are widely distributed in soft tissues also (Podoliak, 1974). Other functions of calcium include muscle contraction, blood clot formation, nerve transmission, maintenance of cell membrane integrity and activation of several enzymes. In cell membrane, calcium is closely bound to phospholipid, where it controls the permeability of membrane and thus regulating the uptake of nutrients by the cell (Lall, 1989).

The calcium requirement of fish and shellfish is met generally by their ability to derive these ions directly from the environment. Unlike terrestrial animals, bone is not the major site of calcium regulations in aquatic animals. Gas exchange across gills in fish provides them with continuous access to an unlimited calcium reservoir present in the water. Thus, calcium influx and efflux occurs at the gills, fins, and oral epithelia. All these structures are actively involved in calcium

transport in marine fish, but the gills are the most important sites of calcium regulation in both freshwater and marine organisms. However, calcium ion transport across gills appears to be more efficient in freshwater. The gut is not the major site of calcium absorption in marine fish, which drink it copiously (Bjornsson and Nilsson, 1985). Evidence indicates that some minerals (Mg, Sr, Zn, and Cu) may reduce calcium absorption (Podoliak, 1970). The endocrine control of calcium metabolism in fish is not fully understood (Milhaud *et al*, 1980). Prolactin stimulates calcium uptake in tilapia (Bonga *et al.*, 1984 and Kaneko *et al*, 1993).

The absorbed calcium is deposited in bone and exoskeleton. Generally, the rate of uptake, the deposition pattern and retention by the skeletal tissues appear to be similar in freshwater and marine species and it is independent of the type of bones, i.e., cellular or acellular bone. The chemical composition of the bony layer of scales is similar to that of other skeletal tissues but differs physiologically in calcium metabolism. An extensive study of Berg (1968) on calcium and strontium metabolism showed that the calcium exchange rate of fish scales was three times greater than that in bones. The scales are also the sites of labile calcium storage. In fish with smooth skin, such as eels and catfish, the cellular bones must also play an important role in calcium turnover. Resorption of scales occurs in fasting salmon during spawning migration. Principally gills and kidneys excrete calcium. Faeces also contain endogenous calcium secreted in the gut (Lall, 1989).

2.2 Function and metabolism of magnesium

Magnesium is an essential ion in many fundamental enzymatic reactions in intermediary metabolism. These include those that transfer phosphate groups (phosphokinase), hydrolyze phosphate and pyrophosphate groups (phosphokinase and pyrophosphatase) and acetyl coenzyme A (thiokinase)in fatty acid oxidation, and activate amino acid synthesis (amino acid synthetase). Magnesium is also essential in skeletal tissue metabolism and neuromuscular transmission (Lall, 1989).

Most of the magnesium in crustaceans is located in the exoskeleton. The remainder is found within the cells of soft tissues. Magnesium plays an important role in the respiratory adaptations of aquatic organism (Houston, 1985). Magnesium is necessary in body fluids for osmoregulation and also, to maintain integrity of smooth muscle and exoskeleton. Freshwater organisms derive magnesium ions either by active uptake from the environment or from the dietary sources. Deficiency causes flaccid muscle in aquatic organisms (Lovell, 1998).

There are two mechanisms concerned in the process of ionic regulation of magnesium in crustaceans (Robertson, 1960).

1. Selective excretion of ions from the blood by means of the paired antennal glands.

2. Controlled uptake of ions through the gills.

Antennal glands are part of the mechanism of ionic regulation. They produce a secretion in which certain ions, chiefly Mg^{++} and SO_4^{-}, are selectively excreted, and the remainder, including K^+, conserved.

The urinary secretions produced by the antennal glands are iso-osmotic with the blood, but show striking ionic differences, particularly in having much higher concentrations of Mg^{++} and So_4^{-} and much higher concentrations of K^+ and Ca^{++}. High level of Mg^{++} and So_4^{-} excretion is found in species with low values of these ions in the blood (Robertson, 1960).

Reports on magnesium requirement levels for crustaceans in freshwater, their tolerance limit and ability of a crustacean to regulate shell magnesium levels are not available.

Jussila *et al* (1995) reported that magnesium and manganese were minor components of the branchial exoskeleton. There were no correlation between environmental magnesium and their concentrations in crayfish exoskeletons. Females generally had higher concentrations than males.

Mavrin *et al* (1992) found that increase of calcium concentration against the background of low magnesium content improves the growth of fish and their resistance to infectious diseases and water flow.

Gibbs and Brayan (1972) after analysing the samples from Barbuda, Leeward Islands, reported that the strontium/calcium atom ratio of the exoskeleton of the fiddler crab *Uca bulgersi* Holthuis is proportional to that of the environment, while the atom ratio magnesium/calcium of the exoskeleton is fairly constant regardless of the environmental ratio. Laboratory experiments on *Carcinus maenas* demonstrated that the strontium/calcium atom ratio of the exoskeleton is, to a large extent, determined by the ratio in the environment at the time of the deposition of the new exoskeleton.

2.3 Transepithelial movement of calcium in crustaceans

Calcium is the major inorganic component of the exoskeleton and it is used to calcify the new exoskeleton. Calcium metabolism in crustaceans is complex and is associated with the moulting cycle. In freshwater decapods (*Procambarus clarkii*), calcium from the eroding exoskeleton is transferred to the haemolymph during premoult and then stored in the hepatopancreas (Fiber and Lutz, 1985) for use during the next moult. In marine decapods, replacement of body calcium lost during ecdysis is achieved mainly by calcium absorption from seawater (Parado-Estepa *et al.*, 1989).

The regulation of calcium in most crustaceans is especially challenging owing to the highly mineralized cuticle that must be recalcified after each moult, a process that often occurs in environments with low concentrations of calcium. The

gill and carapace epithelia separate the major calcium containing compartments of the body and therefore, see large changes in the rate of calcium flux through the moult cycle. Large changes in the ultra structure of these cells do not, however, correlate well with the periods of calcium movement and probably reflect other physiological events. Despite the challenges to regulating calcium levels of various acclimation salinities and moult stages, the calcium concentration in the blood is maintained relatively constant (Neufeld and Cameron, 1993).

Moulting is the most important process dominating the biology of crustaceans. Significant metabolic changes mediated by neuroendocrine control occur throughout the moult cycle (Passano, 1960 and Skinner, 1985). Moult stages have been documented in many groups of crustaceans including penaeids (Robertson *et al.*, 1987). A scheme to describe the moult cycle of natantians was devised by Drach and Techernigovtzeff (1967) and Chu *et al.*, (1988). The five major stages in this scheme are A (early postmoult), B (late postmoult), C (intermoult) D (premoult) and E (ecdysis).

Hashem *et al.*, (1993) reported that both calcium and magnesium contents in the exoskeleton increased during the time between early postmoult to intermoult and decreased at premoult, demonstrating deposition and reabsorption of these minerals during the moult cycle. The low amount of calcium and magnesium in the hepatopancreas suggests a limited role of the organ in the storage of these minerals in premoult.

The calcium ion is not only a regulatory agent in physiological processes but also the primary cation used in biomineralized structures. Maintenance of an extremely low intracellular concentration of calcium is particularly problematic in those cases where there are large calcium fluxes across tissue layers resulting from changes in the overall calcium balance of an organism. Organisms such as crustaceans that have highly calcified structures thus present particular challenges for the maintenance of proper intra- and extracellular concentrations of calcium; relatively large quantities of calcium must be moved across epithelial layers and through the circulatory system. The accretionary growth of crustaceans entails discarding the old cuticle, normally representing the loss of a massive quantity of calcium. In most crustaceans, a large quantity of calcium must subsequently be replaced in a relatively short period to regain the structural and protective functions of the mineralized cuticle. In addition to massive fluxes of calcium into the cuticle after the moult, in some cases this is preceded by a period of calcium transport in the opposite direction as the cuticle is partially demineralized (Sparkes and Greenaway, 1984; Wheatly and Ignaszewski, 1990).

Crustaceans employ a diversity of strategies allowing them to mineralize the cuticle, while occupying habitats with a wide range of calcium availabilities. They inhabit environments ranging from hypersaline, where there is a relative abundance of calcium, to freshwater or terrestrial environments, where acquiring sufficient amounts of calcium is more difficult (Mantel and Farmer, 1983). Even for those species living in freshwater, calcium concentrations vary greatly. Some crayfishes

inhabit water with calcium concentrations of less than 100 μmol/l (Malley, 1980), whereas euryhaline crabs such as *Callinectes sapidus* typically inhabit freshwater with a calcium concentration of around 1mmol/l (Cameron, 1978). Species living in water with low concentrations of calcium often reduce the need to acquire large amounts of calcium from the environment by shifting it to the blood or to organs of the gastrointestinal tract prior to moulting. Marine crustaceans, in contrast, tend to rely more on the availability of calcium in the external milieu by taking up large quantities of calcium via the gill. The prominent role of calcium in the physiology of crustaceans, coupled with the diversity of habitats occupied by these organisms, therefore, presents unique examples of calcium regulation at both the organismic and cellular levels.

While there is little information on the mechanisms of calcium transport in crustaceans, changes in the ultra structure and physiology of calcium transporting tissues have given indications of factors that are important for calcium regulation.

2.4 Ultra structure of epithelia involved in calcium transport

By virtue of their location as barriers between the primary calcium-containing compartments of the body, the gill and carapace epithelia are intimately involved in the regulation of calcium levels in crustaceans and provide the most information with respect to calcium transport. The gills function as the primary interface between the external medium and the blood, while movements of calcium

between the blood and carapace occur across the epithelial layer underlying the carapace. The various epithelia of the gastrointestinal tract are similarly located to see changing fluxes of calcium resulting from dietary or calcium storage functions, but there is little knowledge about these epithelia.

2.4.1 Gill epithelium:

The epithelium lining the gill is the tissue that has been most extensively studied with regard to ionoregulatory processes in aquatic crustaceans. The gill can be partitioned into a thin (<1 μm) epithelium, presumed to function primarily in respiration, and a thick (approximately 10 μm) epithelium, presumed to function mainly in ionoregulation (Copeland and Fitzjarrell, 1968). While the thin epithelium contains few organelles and little cellular elaboration, the thicker epithelium has many of the characteristics of other transporting epithelia: an abundance of mitochondria, a large cell surface area and well defined cell junctions (Berridge and Oschman, 1972). Ultrastructural evidence for the role of the thick epithelium in ionoregulation comes from the large structural changes observed during salinity acclimation (Compere *et al.*1989). Functionally, the localization of Na^{+}/K^{+2} ATPase activity in cells of the thick epithelium suggests that these cells are the site of ionoregulatory processes (Neufeld *et al.* 1980); by extension, these same cells are considered to be the most likely candidate for the site of calcium regulation.

While there are changes in gill ultrastructure with salinity acclimation, there is little indication of which morphological changes might be involved in calcium regulation as opposed to the regulation of other ions. The physiological events at the moult are better suited for a correlation of calcium transport with cell structure, since the period of maximum flux occurs at a well-defined period and can be temporarily separated from other physiological changes. There is little information on what ultrastructural changes occur in the gill through the moult cycle. Unlike Andrews and Dillaman (1993), who report no change in the epithelium of *Procambarus clarkii* through the moult cycle, Neufeld and Cameron (1993) observed significant changes in the epithelial height and mitochondrial abundance in the gill epithelium of *Callinectes sapidus* acclimated to low salinity. The increased cell activity occurred during the premoult period, however, correlated with the period when organic materials are deposited onto the new cuticle rather than the period of calcium deposition. Aside from the presence of multivesicular bodies, the gill epithelium at postmoult had the same appearance as that at intermoult. These results suggest that the metabolic expenditure by the gills for calcium transport is similar to the metabolic expenditure during the intermoult period, even when *Callinectes sapidus* moults in low salinities where calcium uptake must occur by active transport (Newfeld and Cameron, 1992).

2.4.2 Carapace epithelium:

Regardless of whether calcium for mineralization comes from the external medium or from stored reserves, calcium deposited onto the cuticle must move across the epithelial layer that forms a barrier between the cuticular space and the blood. In the various crustaceans studied, substantial changes in ultrastructure are observed through the moult period (Roer and Dilaman, 1984). In *Callinectes sapidus* this layer is quite thin during intermoult, having very few organelles and little cell surface elaboration. As with the gill epithelium, they observed an enlargement of the carapace epithelium occurs far in advance of the moult and this enlargement was concomitant with the appearance of more mitochondria, endoplasmic reticular and vesicles. Hypertrophy of the epithelium at this stage is expected, given that synthesis of the organic portion of the carapace begins early in the premoult stage. It is surprising, however, that the carapace epithelium decreases greatly in both size and complexity shortly after moult when large quantities of calcium are being deposited into the new carapace. The general regression of the carapace requires little cellular effort in comparison with other physiological functions of the epithelium during the moult period, as is the case in the gill epithelia.

2.5 Calcium concentration in the blood

As the main calcium-transporting tissues, the gill and carapace epithelia are the primary epithelia responsible for the maintenance of appropriate calcium

concentration in the blood and cuticle. Changes in the calcium concentration of the blood are, therefore, due either to differences in flux rates across these tissues or to changes in the size of the blood compartment. Many more studies have described the pattern of calcium regulation in the blood in response to the two primary physiological challenges for calcium regulation, salinity acclimation and moulting (Greenaway, 1985), than have described the actual mechanisms responsible for this pattern. Despite the challenges placed on crustaceans by moulting and the diversity of strategies used to deal with the large requirement for calcium, calcium concentration in the blood is generally maintained within a narrower range than that of other ions, no doubt because of its numerous regulatory functions (Neufeld and Cameron, 1993).

The calcium concentration of haemolymph in both spiny lobster and blue crab increases prior to moult and then decreases postmoult (Travis, 1955; Haefner, 1964). Calcium may be withdrawn from haemolymph by epidermis and deposited in integument (Drach, 1939; Travis 1955). Haemolymph calcium concentration does not drop to zero because calcium ions are replaced from surrounding water via gills (Robertson, 1960) as a normal osmoregulatory process. This uptake of environmental calcium may be a rate-limiting factor in later stages of the hardening process of marine crustcea (Cole, 1940). Travis and Friberg (1963) proposed that the amount of calcium accumulated in exo- and endocuticles is dependent on the concentration of calcium ions in the environment and the number of sites, which

would serve as nuclei for calcium crystal formation. However, the calcium accumulation rate is thought to be dependent on the rate of synthesis of these sites.

2.5.1. Effect of salinity on the calcium concentration in the blood:

The concentration of total calcium in the blood is relatively constant not only within a species acclimated to various salinities but also between species which normally inhabit a variety of salinities. The concentration of total calcium in the blood is most commonly between 10 and 20 mmol/l (Mantel and Farmer, 1983). Since calcium movements are thermodynamic processes, however, the concentration of free calcium is actually the more relevant variable. The marine species *Callinectes sapidus* (Neufeld and Cameron, 1992) and *Carcinus maenas* (Greenaway, 1976) have approximately 30% of calcium in the bound form. While the freshwater crustaceans *Gammarus pulex* (Wright, 1979) and *Austropotamobius pallipes* (Greenaway, 1972) has a larger percentage (50-60%) of the total calcium in a bound form, the freshwater *Holthuisana transversa* has only 20% of calcium in the bound form at intermoult (Sparkes and Greenaway, 1984). Within a single species, the concentrations of free and total calcium in the blood of *Callinectes sapidus* are independent of the acclimation salinity, indicating that, for this euryhaline crab, the regulation of calcium relies on compensatory mechanisms rather than on a shift in the concentration of calcium maintained in the blood. There are no other studies indicating whether this is a general feature in euryhaline

crustaceans, but it appears that total and free calcium may be equally independent of acclimation salinity (Neufeld and Cameron, 1993).

2.5.2 Effect of moulting on the calcium concentration in the blood:

While salinity appears to have little effect on the calcium concentration in the blood, the regulatory mechanism for calcium is obviously presented with an added challenge during the moult cycle of crustaceans, when there are large fluxes of calcium. Among various species, the concentration of total calcium consistently shows an increase during the period prior to the moult (Greenaway, 1985). At least in *Callinectes sapidus*, this is most likely to be due to changes in blood protein and concomitant changes in the amount of bound calcium rather than to a change in the concentration of free calcium (Neufeld and Cameron, 1992). The most extreme example of a premoult rise in blood calcium is found in terrestrial/freshwater species of crabs such as *Holthuisiana transversa*, where the total concentration of calcium can increase 150-fold to a concentration of over 2 mol/l by the formation of calcite spherules, accounting for approximately 30% of the blood volume (Sparkes and Greenaway, 1984). The premoult increase in calcium concentration in most species is followed by a decrease at ecdysis, presumably due to dilution of the blood by the large quantity of water taken up as the body swells. Although the decrease in total calcium at moult in the blood of most species is relatively small, blood calcium level drops substantially in some species that take up water containing very low concentrations of calcium (Wright, 1980).

Free calcium in the blood of *Callinectes sapidus* acclimated to low salinity changes little from intermoult to postmoult, a pattern of stasis also found in the freshwater crayfish *Austropotamobius pallipes* (Greenaway, 1974a,b). The greatest change reported is for *Holthuisanan transversa*, where calcium activity decreases by 25% at moulting (Sparkes and Greenaway, 1984). In other cases, the drop in total calcium is great enough that free calcium must drop by a substantial degree as well (Wright, 1980). Although the changes in free calcium are relatively small, a drop in the calcium activity after moulting would serve to create a more favourable electrochemical gradient for calcium uptake after the moult. In the case of *Callinectes sapidus*, where there is a slight drop in both free (Towle and Mangum, 1985) and total calcium (Cameron, 1989), the electrochemical gradient for calcium is directed inwards and net calcium influx may occur by passive mechanisms (Cameron, 1989). Even in crustaceans inhabiting water with lower concentrations of calcium, where the electrochemical gradient is directed outwards and uptake must occur by active transport, a lower calcium concentration in the blood after the moult may serve to reduce passive losses or to lessen the electrochemical gradient against which calcium is transported.

2.6 Flux rates of calcium

It was estimated that about one-fifth of the calcium content in the exoskeleton of prawn is removed at premoult. Although the possibility of rapid calcium reabsorption just before ecdysis cannot be excluded, this finding supports

the view that much calcium is lost with the exuvia. Reabsorbed calcium is believed to be taken up into the haemolymph and then eliminated or stored (Passano 1960). Elevation of calcium concentration in haemolymph at premoult has been demonstrated in *Penaeus duorarum* (Bursey and Lane, 1971) and *Penaeus chinensis* (Chu *et al.*, 1988). It has been suggested that the hepatopancreas is responsible for calcium mobilization in the moult cycle, particularly for the storage of calcium during premoult (Glynn, 1968).

2.6.1 Intermoult:

There are few reports of the rate of unidirectional influx for crustaceans during the intermoult period. Those rates reported suggest that unidirectional influx is low at intermoult, at least in comparison with the massive fluxes at postmoult. The freshwater crayfish *Austropotamobius pallipes, Gammarus pulex* and *Orconectes virilis* have influx rates of 0.014 (Greenaway, 1972), 0.287 (Wright, 1979) and 0.77 mmol/kg/h (Malley, 1980), respectively. The only influx rate measured for a marine crustacean, *Carcinus maenas*, is 0.5 mmol/kg/h (Greenaway, 1976). While there is some exchange of calcium between the blood and carapace at intermoult (Dall, 1965; Greenaway, 1976; Roer, 1980; Henry and Kormanik, 1985), exchange across this layer is certainly relatively low in comparison with postmoult fluxes.

2.6.2 Premoult:

During intermoult, there is either no net flux (Greenaway, 1976; Wheatly and Ignaszewski, 1990; Neufeld and Cameron, 1992) or only a small loss (Greenaway, 1972; Wright, 1979) of calcium, but some species lose a substantial amount of calcium immediately prior to the moult as the old cuticle to be greater than the flux in the opposite direction. When detectable, the magnitude of the calcium flux from the cuticle at premoult is generally not comparable to the large postmoult influx but it can represent a significant change from intermoult fluxes. In still other cases, movement of calcium onto the new cuticle begins prior to the moult (Henry and Kormanik, 1985; Wheatly and Ignaszewski, 1990).

2.6.3 Postmoult:

There are more reports of flux rates for the postmoult period when the net flux onto the new carapace is very high. The measurement of net fluxes across the gill is facilitated by the good correlation between apparent hydrogen excretion and net calcium uptake (Cameron, 1985; Wheatly and Ignaszewski, 1990). Calcification of the cuticle occurs by the reaction $Ca^{2+} + HCO_3^- = CaCO_3 + H^+$, in which bicarbonate is provided either directly from the external solution or by hydration of metabolic CO_2, which results in the production of an extra hydrogen ion for excretion. A reduction in net influx of calcium might be expected in calcium-limited environments. The evidence suggests that freshwater species are able to accumulate calcium as rapidly as their marine counterparts.

The rate of net flux gives a lower boundary for the rate of unidirectional influx occurring across the gill, since the body is permeable to calcium. Crustaceans living in environments where the electrochemical gradient is directed outwards at the time of calcium uptake are especially apt to lose a proportion of the calcium taken up, since there is a general increase in permeability to water and ions after the moult (Lockwood and Andrews, 1969).

2.7 Active transport of calcium

The studies mentioned previously suggest a role for permeability and electrochemical forces in the regulation of calcium. As an alternative mechanism of acclimation, calcium regulation may rely on compensatory mechanisms. In the case of crustaceans that inhabit low salinities or freshwater, assessment of the electrochemical gradient for calcium implicates active transport across the gill epithelium. In *Callinictes sapidus* acclimated to low salinity, they found the equilibrium potential for calcium to be more negative than the transepithelial potential at all moult stages, indicating that active transport is responsible for the calcium movements (Neufeld and Cameron, 1992). Active transport is similarly required for calcium uptake in *Austropotamobius pallipes* (Greenaway, 1974b) and *Gammarus pulex* (Wright, 1979) when in water with low concentrations of calcium. The electrochemical gradients for calcium in *Austropotamobius pallipes* and *Callinectes sapidus* are similar at intermoult and postmoult, since there is little change in the chemical or electrical gradients for calcium (Greenaway, 1974b;

Neufeld and Cameron, 1992). The large increase in net uptake of calcium is therefore due to an increase in the influx component.

In mammalian models, where there is much more information on the mechanism of calcium transport, there is still disagreement about the details of calcium movement at the cellular level (Bawden, 1989). The most plausible mechanism involves apical entry into the cell down the steep electrochemical gradient created by a low internal calcium concentration and a negative potential. Calcium may cross the cell *via* intracellular shuttles that prevent drastic elevations in intracellular calcium concentration that would perturb cell metabolism. The energy-utilizing component of this model consists of the calcium transporters, which are located on the basolateral membrane, extruding calcium against a large electrochemical gradient. On the basis of this model, investigations of calcium transport in crustacean tissues have attempted to identify the presence of a calcium transporter whose activity correlates with periods of calcium fluxes.

2.7.1 Ca^{2+}-ATPase:

While the Ca^{2+}-ATPase is considered to be the transport mechanism in many other tissues, attempts to correlate its activity with periods of Ca^{2+} flux in crustacean tissues have been unsuccessful. In *Callinectes sapidus* acclimated to seawater Ca^{2+}-ATPase activity in the gills was equal at all stages of the moult cycle (Cameron. 1989). Epithelial Ca^{2+}- stimulated ATPase activity increased after the moult, although the increase was not proportional to the increase in fluxes

(Cameron, 1989). Morris and Greenway (1992) found a Ca^{2+} stimulated ATPase with high affinity (K_m 6-35 μ mol/l) in *Leptograpsus variegatus* that did not increase at postmoult and was therefore considered to be primarily for intracellular regulation. A Ca^{2+} -stimulated ATPase with a similar K_m (4-9 μmol/l) is found in the gills of the land crab *Birgus latro*, where it probably functions as a calcium transporter during urine reprocessing (Morris *et al.* 1991). Unfortunately, the method of measuring Ca^{2+}-ATPase is beset by difficulties, making it difficult to distinguish calcium-translocating activity from the activity of other ATPases that are stimulated by calcium (Walters, 1990). Alkaline phosphatase activity is particularly dependent on calcium and it is possible that much of the Ca^{2+}-stimulated ATPase activity with a high K_m and pH maximum, such as that found in sea water-acclimated *Callinectes sapidus*, may represent alkaline phosphatase.

2.7.2 Na^+/Ca^{2+} exchange:

A second transporter that could be involved in transepithelial movement of calcium movements is the Na^+/Ca^{2+} exchanger that operates in transepithelial movement of calcium in tissues of some organisms (Flik *et al.* 1990). Roer (1980) used various transport inhibitors to demonstrate the dependence of calcium transport on substances that affect sodium transport, suggesting a role for Na^+/Ca^{2+} exchange in addition to Ca^{2+} -ATPase in the movement of calcium from the blood to the carapace. Similar conclusions were reported by Towle and Mangum (1985), who found an increase in Na^+/Ca^{2+} -ATPase activity in the carapace epithelium of

blue crabs after the moult. Na^+/Ca^{2+} exchange has yet to be conclusively demonstrated in crustacean tissues, however, and its role in transepithelial transport of calcium in crustaceans remains speculative. Measurements of the electrochemical gradient across the carapace epithelium have not been made and it is not clear whether movements are by passive or active mechanisms. Precipitation of calcium into the more alkaline carapace compartment (Cameron and Wood, 1985) may lower the calcium activity in this compartment and create an electrochemical gradient favouring passive movement from the blood to the carapace.

2.7.3 Ca^{2+}/H^+ exchange:

Ahearn and Franco, (1990) recently demonstrated that calcium can be transported by the Na^+/H^+ antiporter in crustacean antennal glands, suggesting a role for this transporter in the overall calcium regulation of crustaceans. This transporter is also located in the gills of crustaceans (Shetlar and Towle, 1989), where calcium uptake correlates well with apparent hydrogen excretion.

The gill and carapace epithelia of crustaceans obviously have fluxes of calcium that vary considerably in magnitude and direction according to moult stage. The similarity of calcium concentrations in the blood and the high rates of calcium uptake after the moult in various species suggest that the reliance on calcium is equally high for crustacean species that live in environments with a broad range of calcium availabilities. The mechanisms responsible for calcium regulation probably

vary according to species and habitat. But data suggest that changes in the electrochemical gradient depend on the particular conditions and can therefore change rates of passive fluxes. Permeability to calcium appears to vary with habitat, as it does for other ions. Finally changes in active transport must also be responsible for some of the changes in flux rates, particularly after the moult. Despite the importance of calcium in the life cycle of crustaceans, there is a notable lack of information on the cellular mechanism of active transport.

2.8 Growth response of *M. rosenbergii* (de Man) to different levels of hardness

One of the most important factors in determining the success of an aquaculture operation is water quality. On the list of important parameters that should be measured are alkalinity and hardness.

Although levels of alkalinity and hardness are often similar in a body of water, they are independent of each other and can be quite different. Alkalinity is a measure of the water's capacity to neutralize acids. Bicarbonates (HCO_3^-) and carbonates (CO_3-) represent the major forms of alkalinity. Alkalinity is expressed as equivalent concentration of calcium carbonate. Hardness represents the concentration of divalent cations in water; the principal cations are calcium and magnesium, but manganese and ferrous iron can also contribute to hardness. Stickney (1979) stated that most freshwater fish have been shown to grow well at total hardness values of >= 20mg/l, when ample food is available. Piper *et al.*

(1982) indicated that fish grows well over a wide range of water hardness, and list a range of 120-400 mg/l as optimum. Neither the minimum nor the maximum acceptable water hardness concentrations necessary to permit optimum fish growth have been established, but total hardness may not be of major importance.

Unlike fish, crustaceans appear to be more sensitive to levels of water hardness. Smitherman *et al.* (1967), while conducting experiments to determine the effects of supplemental feeding and fertilization on production of red swamp crayfish, found that treatment effects were masked by the total hardness of the water. Additional studies on red swamp crayfish by de la Bretonne *et al.* (1969) found very poor growth and survival rates in water with levels of hardness less than 50 mg/l. They found that the minimum concentration for best growth was somewhere between 50 and 100 mg/l, with no further enhancement of growth at 150 and 200 mg/l. The study did not investigate hardness levels above 200 mg/l.

Despite wide spread commercial interests in the *Macrobrachium spp.* little information is available on their tolerance to water hardness and on their ability to regulate shell calcium levels. Juveniles and adults moult frequently, between every 5 to 40 days, respectively, and require exogenous sources of cations, particularly calcium for successful shell mineralization. Records of satisfactory hardness levels for culture range widely; Wickins (1982) suggested from 65 to 200 mg/l $CaCO_3$. But New and Singholka (1985) recommended levels above 40 and below 150 (Preferably less than 100 mg/l $CaCO_3$), while Vasquez *et al.* (1989) showed that

while growth was better at 112 than at 20 mg/l $CaCO_3$, growth was reduced at 225 and 450 mg/l $CaCO_3$. Bartlett and Enkerlin(1983) and Howlader and Turjoman (1984) reported on growth of *M. rosenbergii* in high hardness waters from mainly groundwater sources. While the latter study found that the growth was adversely affected by the high hardness levels (688-987 mg/l $CaCO_3$). Bartlett and Enkerlin (1983) found that hardness levels between 940 and 1060 mg/l did not adversely affect growth, but their water had relatively low alkalinity (58-86 mg/l when expressed as $CaCO_3$). Cripps and Nakamura (1979) reported decreasing growth rate of *M. rosenbergii* with increasing total hardness over the range of 65-500 mg/l. Fujimura (1975) pointed out retarded growth rates, high incidence of encrustations of epistylis and precipitation of calcium carbonate on the prawn exoskeleton at hardness levels greater than 300 mg/l. Bartlett and Enkerlin (1983) studied the growth of *M. rosenbergii* in asbestos asphalt ponds in hard water and on a low protein diet. The total hardness of the water was 1000 ppm and the diet with a 14% protein content was used. They found that water high in calcium but low in carbonates may not inhibit growth in *M. rosenbergii.*

Soft waters, with extremely low alkalinity and are found to be not as productive as hard waters (Boyd 1974). Boyd recommends increasing alkalinity with the application of limestone ($CaCO_3$) if total alkalinity or hardness is less than 20 mg/l as $CaCO_3$. The net results are increased phytoplankton and benthic productivity and thereby an increased production of the cultured aquatic organism.

Huner and Brown (1985) have indicated mass mortality of *M. rosenbergii* above 180 mg/l, total alkalinity. It was also suggested by these authors that water with hardness levels of 300 mg/l or greater and alkalinity levels above 180 mg/l should be avoided.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The aim of the study was to find out the growth response of *Macrobrachium rosenbergii* post larvae to various concentrations of calcium and magnesium in freshwater and to find out the optimum range of calcium and magnesium required in freshwater for successful scampi culture.

The experiment was designed in two stages. The first stage was a bioassay for 3 days and the second a culture experiment for a period of 21 days. The experiment was carried out during the period from December1999 to February 2000.

3.1 Experimental animals

The post larvae of *M. rosenbergii* required for the experiment were procured from the College of Fisheries, Panangad. All the experimental post-larvae were selected from a single brood and acclimated to freshwater and laboratory conditions prior to use.

Before the start of the experiment, the animals were starved for a day and weighed. The mean initial weight of post larvae was 4.0 mg and the mean length was 1.15 cm. They were acclimatised to petridish feeding.

3.2 Design and procedure

The experiment was conducted in two stages. The first stage of the experiment was a bioassay for three days to understand the maximum and minimum tolerance levels of *M. rosenbergii* to calcium and magnesium in freshwater was carried out in 600ml capacity Borosil transparent glass beakers.

A range of 0 to 5000 mg/l and 0 to 10,000 mg/l of calcium and magnesium respectively were selected. In the case of calcium up to 1000 mg/l, an interval of 50 mg/l (i.e. 0, 50, 100, 150, 200, 250, ----------1000 mg/l) and from 1000 to 5000 mg/l an interval of 500 mg/l (i.e. 1500, 2000, 2500, 3000, ---------5000 mg/l) were taken.. Whereas in the case of magnesium the intervals were 100 mg/l for 0 to 1000 mg/l and 1000 mg/l for 1000 to 10,000 mg/l. 500 ml of water was used in a 600 ml glass beaker for the experiments.

Based on the results from the first experiment the second experiment was carried out for calcium alone. The levels used were 5, 10, 20, 50, 100, 200, 300, 400, 500, 600, 700 and 800 mg/l. For each treatment two tanks were used. For the second set of experiment circular flat bottom fiber glass tanks with capacity of 83 litres (diameter 55 cm, height 35 cm) were used. In each tank twenty prawns were assigned randomly. The second set of rearing experiment was for a period of 21 days.

The different levels of calcium in water for the experiment were obtained by adding calculated quantities of $CaCl_2$ stock solution in water. The stock solution was prepared by dissolving dihydrous calcium chloride crystals in water. After preparing the solution the calcium level in it was again estimated using standard titration methods and the differences in the concentration of calcium between calculated value and the titration value was only 5 %.

The feeding was done *ad libitum*. A one time feeding, at 6 Hrs was resorted. To make the collection of left out feed easy the feeding was done in a petridish kept immersed at the bottom of the tank. Before the next feeding left over feed was

collected by carefully taking out the petridish. The collected feed was dried in an air oven and transferred to desiccator prior to weighing. The petridishes were cleaned properly before every next feeding.

During morning hours before feeding, bottom of the tank was siphoned to remove faeces and settled particles. Complete water exchange was done in all the tanks once in a week to maintain water quality. The bottom and sides of the tanks were scrubbed and the suspended particles were allowed to settle. The settled particles were siphoned before water exchange.

3.3 Rearing facilities

The experiment was carried out in a closed shed, roofed with transparent fiberglass reinforced plastic sheets, which permit moderate light conditions. Adequate ventilation was also available. The shed was provided with good drainage and proper fresh and brackish water supply.

Clear filtered freshwater drawn from a well was used for the experiment. The experimental tanks were filled to a level of 21.1 cms so as to get effective volume of 50 litres. Before filling the tanks the water was passed through 100 micron meshed nylon bolting cloth.

3.4 Preparation of feed

3.4.1 Feed ingredients and processing:

A feed proposed by Sherief (1989) using clam meat, groundnut oil cake, rice bran and tapioca powder was used for feeding the animals. Fresh clam meat

(*Villorita cyprinoides)* was purchased from local market, washed thoroughly and steamed in an autoclave at ambient pressure for 15 minutes and then dried in an electric dryer for 12 hours at 60^0 C. Fresh and mould free groundnut oil cake was also dried in an electric dryer for 6 hours at 60^0 C. The dried ingredients were powdered separately in a pulverizer and passed through a sieve of 250 microns. The sieved ingredients were packed separately in airtight plastic containers till they were used for feed preparation. Rice bran and tapioca powder were also sieved through a 250 microns sieve.

3.4.2 Formulation of the diet:

The proportion of the ingredients used for the preparation of pelleted feed was given below.

1. Clam meat : 40 %
2. Ground nut oil cake : 25 %
3. Rice bran : 25 %
4. Tapioca floor : 10 %

The experimental feed was prepared by mixing required quantity of ingradients. The respective ingradients were weighed accurately and all the ingradients were mixed well in a dry mortar. The dry mixture was made into dough by adding sufficient volume of distilled water (1: 1.25 w/v) and mixed well in a mortar. The dough was transferred to a bowl and steam cooked for 30 minutes in an autoclave at ambient pressure. The cooked dough was palletized using hand operated extruder with a die of 2mm diameter to a clean tray as a single layer and dried at 60^0 C for 12 hours in an electric dryer. The dried pellets with a diameter of 2mm were

crumbled into small pieces and packed in airtight plastic bottles and stored at 4^0 C in a refrigerator until use.

3.4.3 Proximate composition of prepared feed:

Proximate composition of the experimental diet was analysed to evaluate the nutrient status.

To estimate moisture levels Boyd's (1979) method was used. The sample was heated to 105^0 C for 30 minutes and then dried at 65^0 C till a constant weight was obtained.

The crude protein content was estimated by Microkjeldahl's method (AOAC 1990). The nitrogen content was multiplied by a factor of 6.25 to arrive at crude protein content.

Crude fat was extracted using petroleum ether (B.P. 40-60^0 C) in a soxhlet extraction apparatus for 6 hours.

The ash content was estimated by burning the sample at 550 $\pm 10^0$ C for 6 hours in a muffle furnace.

The carbohydrate content was found out by Hastings (1976) difference method as nitrogen free extract (NFE).

$$NFE = 100 - (\% \text{ Crude protein} + \% \text{ Crude fat} + \% \text{ ash})$$

3.5 Monitoring of water quality parameters

Water quality and physicochemical parameters like temperature and pH was measured at three-day interval and dissolved oxygen content, total alkalinity and

total hardness was measured weekly. For measuring the water quality parameters the following methods were followed.

1. Temperature - with mercury bulb thermometer with on accuracy of 0.1^0 C.

2. pH - Using universal pH indicator solution manufactured by Glaxo (India) Ltd.

3. Dissolved oxygen - Standard Winkler method (Strickland and Parson 1972).

4.Total alkalinity - by acidimetric titration method (APHA *et al.*, 1981).

5.Total hardness- by chemical method (APHA *et al.*, 1981).

3.6 Evaluation criteria

The parameters like net weight gain, specific growth rate (SGR), percentage survival and food conversion ratio (FCR) were determined at various concentrations of calcium and magnesium in freshwater.

3.6.1 Net weight gain:

Net weight gain gives the increase in weight of prawns during the experimental period when reared in different levels of calcium in water. It was calculated by using the formula:

Mean wet weight gain = Final weight -Initial weight

3.6.2 Percentage growth:

The percentage growth of the animal was calculated using the following formula.

$$\% \text{ Wet weight gain} = \frac{\text{Mean final body weight} - \text{Mean initial body weight}}{\text{Mean initial body weight}} \times 100$$

3.6.3 Specific growth rate (SGR):

Growth performance was measured in terms of specific growth rate (SGR). Since it is a more refined and improved growth index than absolute weight gain of percentage growth rate (Hepher, 1988). In the present study SGR was calculated by using the following formula.

$$SGR = \frac{(\ln W_f - \ln W_i)}{T2-T1}$$

Where,

W_i = Weight at day T1

W_f = Weight at day T2

T2 - T1 = Duration of experiment in days.

The calculated values give average percentage increase in body weight per day over 21 days.

3.6.4 Survival rate:

The survival rates of the prawns are expressed in terms of percentage. This was calculated as follows.

$$\text{Survival rate} = \frac{\text{(Initial number - Number of animal died)}}{\text{Initial number}} \times 100$$

3.6.5 Food conversion ratio (FCR):

This refers to the ability with which an animal can convert the feed consumed into edible and muscle tissue (Devendra, 1989). Food conversion ratio gives an idea about the amount of feed required to produce unit increase in weight of prawn. It is the most commonly used index to measure efficiency of diet used in the experiment trial.

$$FCR = \frac{\text{Average weight of food consumed/ prawn (dry wt.)}}{\text{Average live wet weight gain/prawn}}$$

3.7 Statistical analysis

A Completely Randomized Design **(CRD)** was used for the Experimental study.

All indices, such as growth, specific growth rate, survival rate, food conversion ratio and protein efficiency ratio were subjected to comparison using analysis of variance (ANOVA) and treatment difference studied at 5 % level of significance. Pairwise comparison of treatment values was done using critical difference based on students 't' distribution at 5 % level significance (Snedecor and Cochran, 1968).

RESULTS

4. RESULTS

4.1 Lethal concentration

The results of the probit analysis for *Macrobrachium rosenbergii* post larvae during 48 hrs exposure to various concentrations of calcium and magnesium is given in the Table 1 & 2.

4.2 Growth

The data regarding the gain in weight of *M. rosenbergii* post larvae in different concentrations of calcium in freshwater are presented in Table 3.

The average live weight gain of *M. rosenbergii* post larvae in treatments T_1 (5 mg/l), T_2 (10 mg/l), T_3 (20 mg/l), T_4 (50 mg/l), T_5 (100 mg/l), T_6 (200 mg/l), T_7 (300 mg/l), T_8 (400 mg/l), T_9 (500 mg/l), T_{10} (600 mg/l), T_{11} (700 mg/l) and T_{12} (800 mg/l) was found to be 29.69, 34.45, 34.45, 34.34, 34.7, 36.55, 35.39, 45.75, 40.02, 35.62, 34.06 and 32.99 mg respectively. The highest average live weight gain of 45.75 mg was obtained in treatments T_8 (400 mg/l) followed by 40.02 mg in T_9 (500mg/l). The lowest average live weight gain of 26.69 mg was obtained in treatment T_1 (5mg/l). The treatment of T_8 (400 mg/l) and T_9 (500 mg/l) showed no significant difference. Similarly there is no significant difference between treatments T_1 (5 mg/l), T_4 (100 mg/l), T_{11} (700 mg/l), T_2 (10 mg/l), T_3 (50 mg/l) and T_5 (200 mg/l). But between these two groups there is a significant difference.

Table 1. Results of bioassay analysis for the tolerance limit of *M. rosenbergii* post larvae during 48 hr exposure to various high concentration of calcium in freshwater.

Exposure period (hrs)	LC 50 (ppm)	95% Fiducial limits* (ppm)		Slope function (b)	Intercept (a)
		Lower	Upper		
48 hrs	1562.85	1000	3000	3.98	-7.7189
Regression Equation = -7.7189 + 3.982 X					

Table 2. Results of bioassay analysis for the tolerance limit of magnesium in freshwater for *M. rosenbergii* post larvae during 48 hrs exposure.

Exposure period (hrs)	LC 50 (ppm)	95% Fiducial limits* (ppm)		Slope function (b)	Intercept (a)
		Lower	Upper		
48 hrs	7823.48	5000	10,000	7.684	-24.916
Regression Equation = -24.916 + 7.684 X					

Table 3. Growth of *M. rosenbergii* (PL) on different levels of calcium concentration in freshwater

Treat-ment	Repli-cation	Av. initial weight (mg)	Av. final weight (mg)	Net. gain in weight (mg)	Av. live weight gain (mg)	% weight gain	Mean ± S.E.
T_1	1	4	32.18	28.18	29.69	704.5	742.25
5 mg	2	4	35.20	31.20		780.0	±65.232
T_2	1	4	39.60	35.60	34.45	890.0	861.25
10 mg	2	4	31.20	33.30		832.5	±65.232
T_3	1	4	40.20	36.20	34.45	905.0	861.25
20 mg	2	4	36.70	32.70		817.5	±65.232
T_4	1	4	35.00	31.00	34.34	775.0	821.00
50 mg	2	4	41.68	37.68		867.0	±65.232
T_5	1	4	38.10	34.10	34.70	852.5	867.50
100 mg	2	4	39.30	35.30		882.5	±65.232
T_6	1	4	42.60	38.60	36.55	965.0	913.75
200 mg	2	4	38.50	34.50		862.5	±65.232
T_7	1	4	40.30	36.30	35.385	905.0	884.625
300 mg	2	4	38.47	34.47		861.75	±65.232
T_8	1	4	51.94	47.94	45.75	1198.5	1143.75
400 mg	2	4	47.56	43.56		1089.0	±65.232
T_9	1	4	43.00	39.00	40.015	975.0	1000.38
500 mg	2	4	45.03	41.03		1025.75	±65.232
T_{10}	1	4	37.33	34.33	35.615	858.25	890.375
600 mg	2	4	40.90	36.90		922.5	±65.232
T_{11}	1	4	40.70	36.70	34.06	917.5	815.50
700 mg	2	4	35.42	31.42		785.5	±65.232
T12	1	4	40.30	36.30	32.99	907.5	824.75
800 mg	2	4	33.68	29.68		742.0	±65.232

Table 4. Results of analysis of variance of the data on the growth of *M. rosenbergii* (PL) to different levels of calcium in freshwater

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Treatment	11	223260	20296.36	4.76963*
Error	12	51064	4255.334	
Total	23	274324		

*** Significant at 5% level**

Critical difference = 142.1425

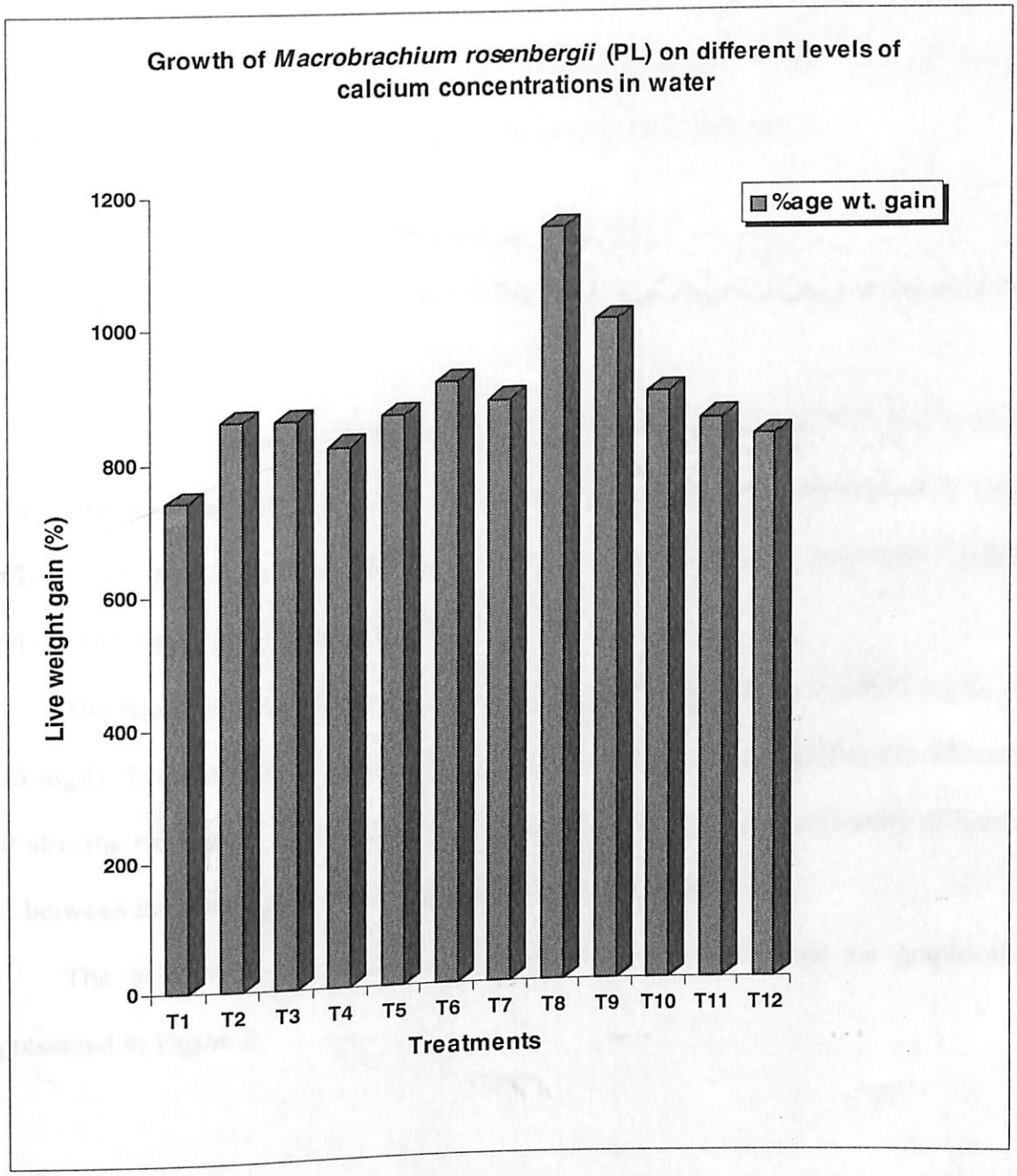
Table 5. Comparison of treatment means based on critical difference

Treatments Mean	T_1 742.25	T_4 821.0	T_{12} 824.75	T_{11} 851.5	T_2 861.25	T_3 861.25
Treatments Mean	T_5 867.5	T_7 884.63	T_{10} 890.38	T_6 913.75	T_9 1000.38	T_8 1143.75

T_1 T_4 T_{12} T_{11} T_2 T_3 T_5 T_7 T_{10} T_6 T_9 T_8

Under scored treatment means are not significantly different.

Figure: 1.


Growth of *Macrobrachium rosenbergii* (PL) on different levels of calcium concentrations in water
%age wt. gain
Live weight gain (%)
1200
1000
800
600
400
200
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
Treatments

Analysis of variance of the data on percentage shows significant difference ($P<0.05$) among various treatments (Table 4). Graphical representation of percentage live weight gain values for different treatments was shown in Figure 1.

4.3 Specific growth rate

The data on specific growth rate of prawns under various treatments are given in Table 6.

Analysis of variance of the data shows significant difference ($P<0.05$) in SGR values between treatments (Table 7). The prawns in treatments T_8 (400 mg/l) & T_9 (500 mg/l) gave the highest SGR (0.1199 & 0.1142), followed by those in treatments T_6 (200 mg/l - 0.1102) and T_7 (300 mg/l- 0.1089).

The treatments T_9 (500 mg/l), T_6 (200 mg/l), T_7 (300 mg/l), T_{10} (600 mg/l), T_5 (100 mg/l), T_2 (10 mg/l), T_3 (20 mg/l), and T_4 (50 mg/l) are not significantly different and also the treatments T_8 (400 mg/l) and T_9 (500 mg/l), are not significantly different. But between these two groups there is a significant difference.

The SGR values of prawns obtained in various treatments are graphically represented in Figure 2.

Table 6. Specific growth rate of *M. rosenbergii* (PL) to different levels of calcium in fresh water

Treat-ment	Repli-cation	Av. initial weight (mg)	Av. final weight (mg)	Av. live wt. gain (mg)	Specific growth rate	Mean ± S.E.
T_1	1	4	32.18	28.18	0.0993	0.10145
5mg	2	4	35.20	31.20	0.1036	±0.0035
T_2	1	4	39.60	35.60	0.1092	0.10775
10 mg	2	4	31.20	33.30	0.1063	±0.0035
T_3	1	4	40.20	36.20	0.1099	0.10770
20 mg	2	4	36.70	32.70	0.1055	±0.0035
T_4	1	4	35.00	31.00	0.1033	0.10745
50 mg	2	4	41.68	37.68	0.1116	±0.0035
T_5	1	4	38.10	34.10	0.1073	0.10805
100 mg	2	4	39.30	35.30	0.1088	±0.0035
T_6	1	4	42.60	38.60	0.1126	0.11020
200 mg	2	4	38.50	34.50	0.1078	±0.0035
T_7	1	4	40.30	36.30	0.1100	0.10890
300 mg	2	4	38.47	34.47	0.1078	±0.315
T_8	1	4	51.94	47.94	0.1220	0.11990
400 mg	2	4	47.56	43.56	0.1178	±0.315
T_9	1	4	43.00	39.00	0.1131	0.11420
500 mg	2	4	45.03	41.03	0.1153	±0.315
T_{10}	1	4	37.33	34.33	0.1064	0.10855
600 mg	2	4	40.90	36.90	0.1107	±0.315
T_{11}	1	4	40.70	36.70	0.1105	0.10715
700 mg	2	4	35.42	31.42	0.1038	±0.315
T12	1	4	40.30	36.30	0.1100	0.10575
800 mg	2	4	33.68	29.68	0.1015	±0.315

Table 7. Results of analysis of variance of the data on the specific growth rate of *M. rosenbergii* (PL) to different levels of calcium in freshwater

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Treatment	11	4.499555E-04	4.090504E-05	3.235864*
Error	12	1.516938E-04	1.264115E-05	
Total	23	6.016493E-04		

*** Significant at 5% level**

Critical difference = 7.747E-03 (0.0077)

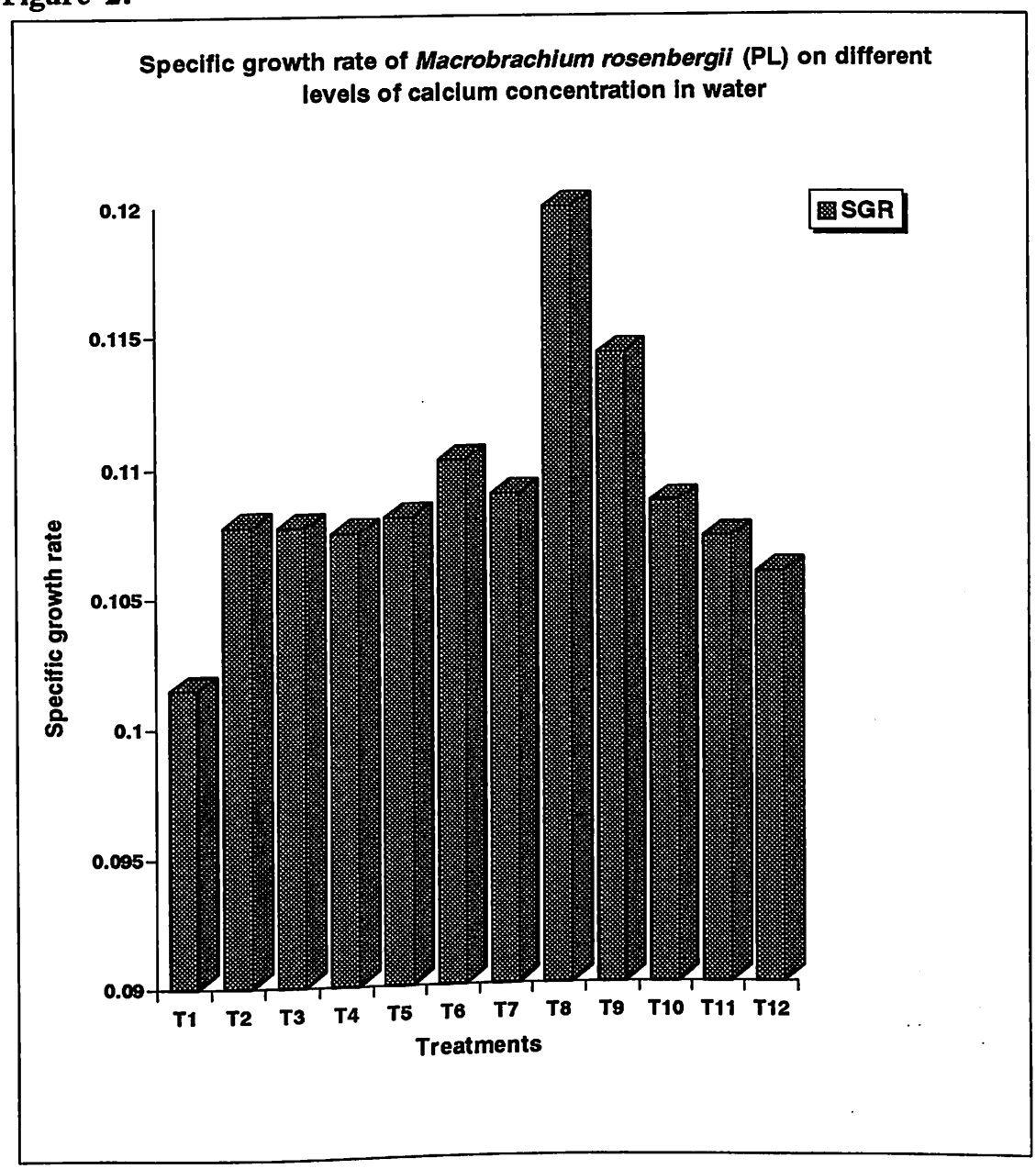
Table 8. Comparison of treatment means based on critical difference

Treatments						
Mean	T_1 0.10145	T_{12} 0.10575	T_{11} 0.1 0715	T_4 0.10745	T_3 0.1077	T_2 0.10775
Treatments Mean	T_5 0.10805	T_{10} 0.10855	T_7 0.1089	T_6 0.1102	T_9 0.1142	T_8 0.1199

T_1 T_{12} T_{11} T_4 T_3 T_2 T_5 T_{10} T_7 T_6 T_9 T_8

Under scored treatment means are not significantly different.

Figure 2.


Specific growth rate of *Macrobrachium rosenbergii* (PL) on different levels of calcium concentration in water
SGR
0.12
0.115
0.11
0.105
0.1
0.095
0.09
Specific growth rate
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
Treatments

4.4 Survival rate

The data on survival rate of *M. rosenbergii* PL reared on different levels of calcium in freshwater are given in Table 9 & Figure 3.

The highest average survival percentage of 92.5 was obtained in the treatment T6 (200 mg/l), followed by a survival rates of 85% in T2 (10 mg/l), T5 (100 mg/l) & T8 (400 mg/l) and 80 % in T1 (5 mg/l) & T3 (20 mg/l). The lower survival percentage of 65% was observed for the treatments T9 (500 mg/l). But the analysis of variance (Table 10) of the data on survival percentage showed no significant difference among the treatments.

4.5 Food conversion ratio

FCR values of *M. rosenbergii* post larvae reared on different levels of calcium concentrations in freshwater are given in Table 11 & Figure 4. The mean FCR values ranged from 2.728 to 4.0745 in various treatments.

The lowest FCR of 2.728 was obtained in treatment T8 (400 mg/l) and highest FCR of 4.0745 in the treatment T_1 (5 mg/l).

Analysis of variance of FCR values showed no significant difference among the treatments (Table 12). Graphical representation of FCR values of post larvae prawns in various treatments is given in Figure 4.

4.6 Proximate composition of the diet

Proximate composition of the formulated pelleted feed is given in Table 13.

Table 9. Survival rate of *M. rosenbergii* (PL) on different levels of calcium in freshwater

Treat-ment	Repli-cation	Initial Stocking No.	Final Survival	Survival rate %age	Mean Survival rate
T_1 5 mg	1	20	17	85	80
	2	20	15	75	
T_2 10 mg	1	20	15	75	85
	2	20	19	95	
T_3 20 mg	1	20	18	90	80
	2	20	14	70	
T_4 50 mg	1	20	14	70	82.5
	2	20	19	95	
T_5 100 mg	1	20	18	90	85
	2	20	16	80	
T_6 200 mg	1	20	17	85	92.5
	2	20	20	100	
T_7 300 mg	1	20	11	55	70
	2	20	17	85	
T_8 400 mg	1	20	18	90	85
	2	20	16	80	
T_9 500 mg	1	20	11	55	65
	2	20	15	75	
T_{10} 600 mg	1	20	15	75	77.5
	2	20	16	80	
T_{11} 700 mg	1	20	17	85	72.5
	2	20	12	60	
T12 800 mg	1	20	15	75	72.5
	2	20	14	70	

Table 10. Results of analysis of variance of the data on the survival rate of *M. rosenbergii* (PL) to different levels of calcium in freshwater.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Treatment	11	1336.453	121.4957	0.7429038*
Error	12	1962.5	163.5417	
Total	23	3298.953		

*** Not significant at 5% level**

Figure 3.

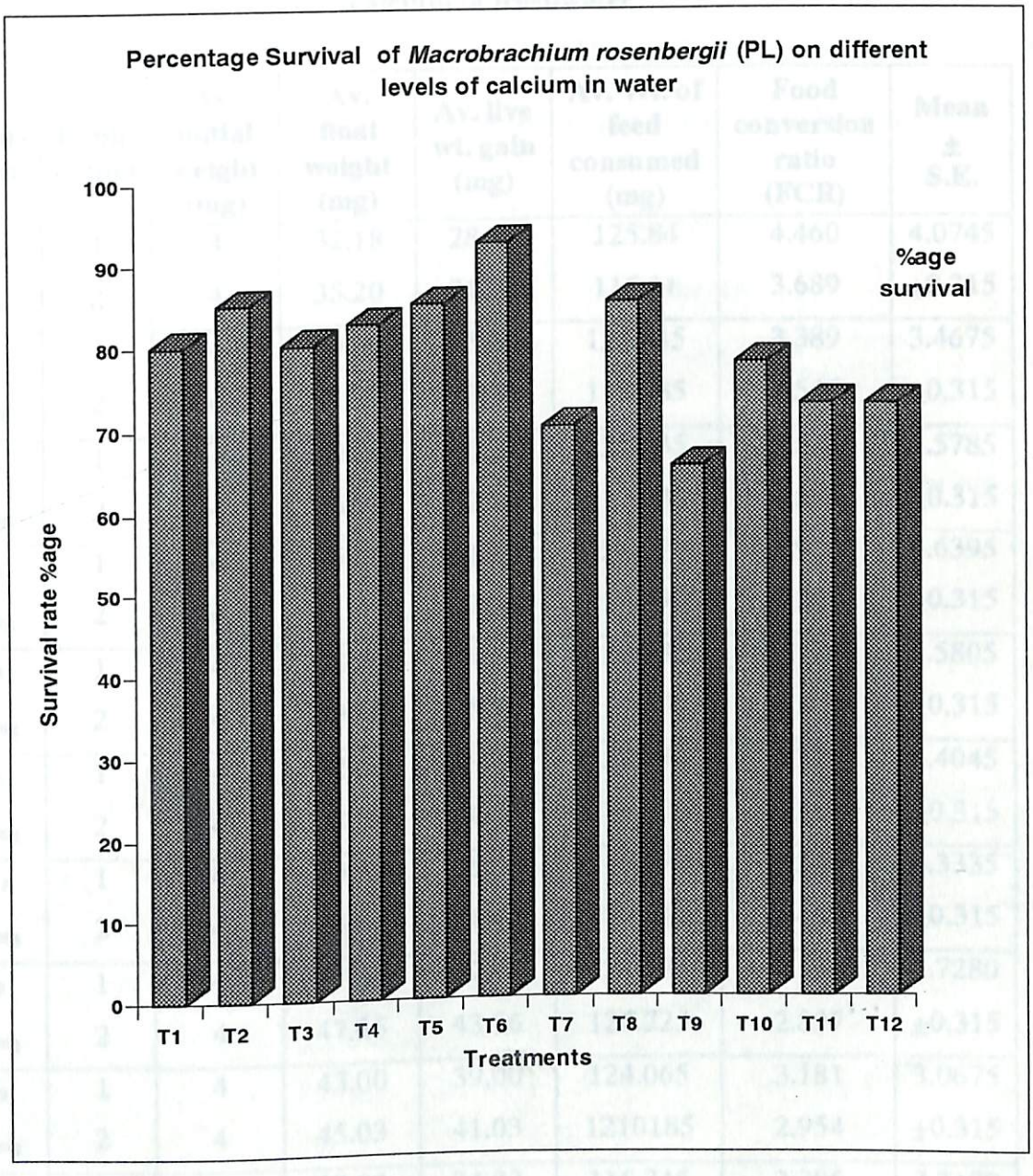


Table 11. Food conversion ratio of *M. rosenbergii* (PL) to different levels of calcium in freshwater

Treat-ment	Repli-cation	Av. initial weight (mg)	Av. final weight (mg)	Av. live wt. gain (mg)	Av. Wt. of feed consumed (mg)	Food conversion ratio (FCR)	Mean ± S.E.
T_1	1	4	32.18	28.18	125.84	4.460	4.0745
5mg	2	4	35.20	31.20	115.11	3.689	±0.315
T_2	1	4	39.60	35.60	120.645	3.389	3.4675
10 mg	2	4	31.20	33.30	118.085	3.546	±0.315
T_3	1	4	40.20	36.20	129.535	3.578	3.5785
20 mg	2	4	36.70	32.70	117.035	3.579	±0.315
T_4	1	4	35.00	31.00	121.770	3.928	3.6395
50 mg	2	4	41.68	37.68	119.490	3.351	±0.315
T_5	1	4	38.10	34.10	128.755	3.776	3.5805
100 mg	2	4	39.30	35.30	119.490	3.385	±0.315
T_6	1	4	42.60	38.60	127.670	3.776	3.4045
200 mg	2	4	38.50	34.50	119.490	3.385	±0.315
T_7	1	4	40.30	36.30	118.170	3.255	3.3335
300 mg	2	4	38.47	34.47	117.615	3.412	±0.315
T_8	1	4	51.94	47.94	122.585	2.559	2.7280
400 mg	2	4	47.56	43.56	126.225	2.897	±0.315
T_9	1	4	43.00	39.00	124.065	3.181	3.0675
500 mg	2	4	45.03	41.03	1210185	2.954	±0.315
T_{10}	1	4	37.33	34.33	116.245	3.386	3.3170
600 mg	2	4	40.90	36.90	119.870	3.248	±0.315
T_{11}	1	4	40.70	36.70	120.210	3.275	3.3830
700 mg	2	4	35.42	31.42	109.705	3.491	±0.315
T12	1	4	40.30	36.30	114.640	3.158	3.6540
800 mg	2	4	33.68	29.68	123.170	4.150	±0.315

Table 12. Results of analysis of variance of the data on the food conversion ratio of *M. rosenbergii* (PL) to different levels of calcium in freshwater

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Treatment	11	2.40863	0.2189664	2.205345*
Error	12	1.191467	9.928894E-02	
Total	23	3.600098		

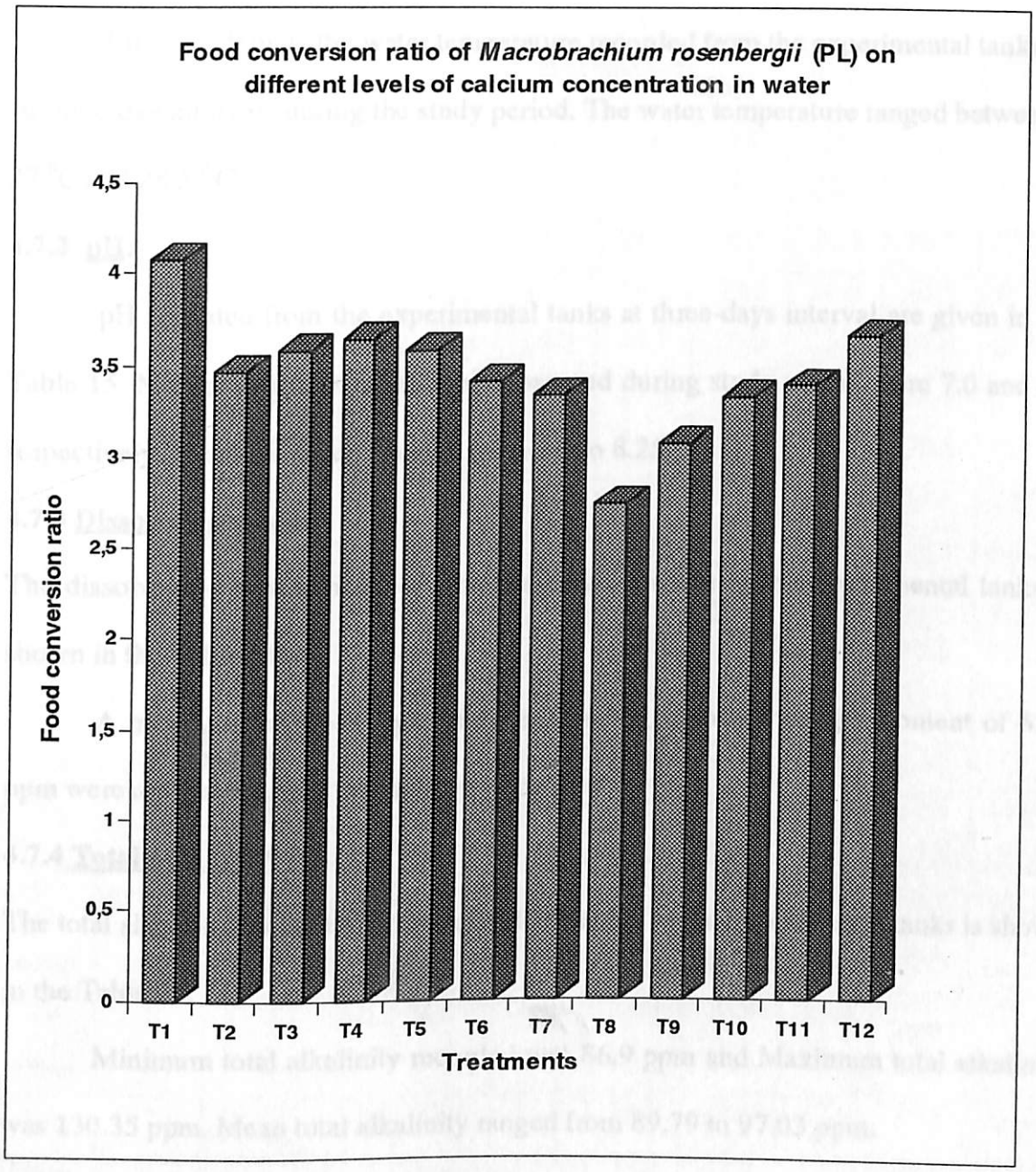
* Not significant at 5% level

Table 13. Proximate composition of the formulated pelleted feed

Proximate composition	%
Moisture	4.44
Protein	39.25
Fat	6.76
Ash	6.25
Carbohydrate	43.30

***Dry weight basis**

Figure 4.


Food conversion ratio of *Macrobrachium rosenbergii* (PL) on different levels of calcium concentration in water
Food conversion ratio
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
Treatments

4.7 Physicochemical parameters

4.7.1 Water temperature:

Table 14 depicts the water temperature recorded from the experimental tanks at three-day intervals during the study period. The water temperature ranged between 27 ^{0}C and 28.5 ^{0}C.

4.7.2 pH:

pH recorded from the experimental tanks at three-days interval are given in the Table 15. Minimum and maximum pH observed during study period were 7.0 and 8.5 respectively. Mean pH values ranged from 7.0 to 8.25.

4.7.3 Dissolved oxygen:

The dissolved oxygen content of water recorded weekly in the experimental tanks is shown in the Table 16.

A minimum of 4.681 ppm and a maximum dissolved oxygen content of 6.24 ppm were obtained during the study period.

4.7.4 Total alkalinity:

The total alkalinity content of water recorded weekly in the experimental tanks is shown in the Table 17.

Minimum total alkalinity recorded was 86.9 ppm and Maximum total alkalinity was 130.35 ppm. Mean total alkalinity ranged from 89.79 to 97.03 ppm.

Table 14. Water temperature (^{0}C) in the experimental tanks during study period

(Range shown in parenthesis)

Treat-ment	Repli-cation	3 days interval								Mean ± S.D. (Range)
		0	3	6	9	12	15	18	21	
T_1	1	27.0	27.5	27.5	28.0	28.1	26.5	26.5	26.5	27.21± 0.62
5 mg	2	27.0	27.6	27.5	28.0	28.1	26.5	26.6	26.5	(27.0 – 28.1)
T_2	1	27.2	27.2	27.5	28.3	28.2	26.5	26.6	26.5	27.10 ± 0.53
10 mg	2	27.0	27.3	27.4	28.3	28.1	26.5	26.5	26.5	(27.0 – 28.3)
T_3	1	27.1	27.2	27.5	28.5	27.8	27.3	26.9	26.8	27.40 ± 0.51
20 mg	2	27.0	27.5	27.5	28.5	27.7	27.4	26.9	26.8	(27.0 – 28.5)
T_4	1	27.2	27.2	27.4	28.0	27.9	27.4	27.5	27.5	27.53 ± 0.24
50 mg	2	27.3	27.5	27.5	28.0	27.8	27.5	27.5	27.4	(27.2– 28.0)
T_5	1	27.0	27.1	27.5	28.5	28.0	28.0	27.5	27.5	27.63 ± 0.40
100 mg	2	27.0	27.5	27.5	28.0	28.0	28.0	27.5	27.6	(27.0 – 28.5)
T_6	1	27.0	27.3	27.5	28.0	28.0	28.0	27.6	27.5	27.61 ± 0.33
200 mg	2	27.0	27.4	27.5	28.0	28.0	28.0	27.5	27.6	(27.0 – 28.0)
T_7	1	27.0	27.2	27.5	28.0	28.0	28.0	27.6	27.5	27.62 ± 0.35
300 mg	2	27.2	27.3	27.5	28.0	28.2	28.0	27.5	27.5	(27.0 – 28.2)
T_8	1	27.0	27.3	27.5	28.0	28.1	28.3	27.6	27.5	27.61 ± 0.36
400 mg	2	27.0	27.3	27.5	28.0	28.0	28.5	27.5	27.5	(27.0 – 28.1)
T_9	1	27.0	27.5	27.5	28.0	28.0	28.3	27.8	27.7	27.76 ± 0.42
500 mg	2	27.0	27.5	27.5	28.5	28.2	28.3	27.8	27.7	(27.0 – 28.5)
T_{10}	1	27.0	27.4	27.4	28.0	28.0	28.0	27.6	27.6	27.63 ± 0.33
600 mg	2	27.0	27.5	27.5	28.0	28.0	28.0	27.5	27.6	(27.0 – 28.0)
T_{11}	1	27.0	27.2	27.5	28.5	28.0	28.0	27.6	27.5	27.68 ± 0.44
700 mg	2	27.0	27.4	27.5	28.5	28.0	28.0	27.6	27.6	(27.0 – 28.5)
T_{12}	1	27.0	27.2	27.5	28.0	28.2	28.0	27.6	27.5	27.50 ± 0.33
800 mg	2	27.3	27.2	27.5	28.0	28.0	28.0	27.6	27.5	(27.0 – 28.0)

Table 15. Fluctuation of pH in the experimental tanks during study period

(Range shown in parenthesis)

Treat-ment	Repli-cation	3 days interval								Mean ±S.D. (Range)
		0	3	6	9	12	15	18	21	
T_1 5 mg	1	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0 ± 0.0
	2	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	(7.0 – 7.0)
T_2 10 mg	1	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0 ± 0.0
	2	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	(7.0 – 7.0)
T_3 20 mg	1	7.0	7.0	7.5	7.0	7.5	7.0	7.0	7.0	7.218 ± 0.24
	2	7.5	7.0	7.5	7.5	7.0	7.5	7.5	7.0	(7.0 – 7.5)
T_4 50 mg	1	7.0	7.5	7.5	7.5	7.0	7.5	7.5	7.5	7.375 ± 0.21
	2	7.0	7.5	7.5	7.5	7.0	7.5	7.5	7.5	(7.0 – 7.5)
T_5 100 mg	1	7.5	8.0	7.5	8.0	7.5	7.5	8.0	8.0	7.75 ± 0.25
	2	7.5	8.0	7.5	8.0	8.0	7.5	8.0	7.5	(7.5– 8.0)
T_6 200 mg	1	8.0	8.5	8.0	8.0	8.0	8.0	8.5	8.5	8.156 ± 0.23
	2	8.0	8.0	8.0	8.0	8.0	8.0	8.5	8.5	(8.0 – 8.5)
T_7 300 mg	1	8.0	8.0	8.0	7.5	8.0	8.0	7.5	8.0	7.968 ± 0.27
	2	8.5	8.0	8.0	7.5	8.0	8.5	8.0	8.0	(7.5 – 8.5)
T_8 400 mg	1	8.0	8.0	8.5	8.0	8.0	8.0	8.5	8.0	8.125 ± 0.21
	2	8.0	8.0	8.5	8.5	8.0	8.0	8.0	8.0	(8.0 – 8.5)
T_9 500 mg	1	8.5	8.5	8.0	8.0	8.5	8.5	8.5	8.5	8.343 ± 0.23
	2	8.5	8.5	8.0	8.0	8.5	8.5	8.0	8.5	(8.0 – 8.5)
T_{10} 600 mg	1	8.0	8.0	8.5	8.0	8.5	8.0	8.0	8.5	8.218 ± 0.24
	2	8.0	8.5	8.5	8.0	8.5	8.0	8.0	8.5	(8.0 – 8.5)
T_{11} 700 mg	1	8.0	8.0	8.0	8.5	8.0	8.5	8.5	8.5	8.25 ± 0.25
	2	8.0	8.0	8.5	8.5	8.0	8.0	8.5	8.5	(8.0 – 8.5)
T_{12} 800 mg	1	8.5	8.0	8.5	8.0	8.5	8.0	8.0	8.0	8.218 ± 0.24
	2	8.0	8.5	8.5	8.0	8.5	8.5	8.0	8.0	(8.0 – 8.5)

Table 16. Dissolved oxygen (ppm) in the experimental tanks during study period

(Range shown in parenthesis)

Treatment	Replication	Weeks			Mean ± S.D. (Range)
		1	2	3	
T_1 5 mg	1	5.46	6.24	6.24	5.98+0.36
	2	5.46	6.24	6.24	(5.46 – 6.24)
T_2 10 mg	1	5.46	6.24	6.24	5.85+0.39
	2	5.46	5.46	6.24	(5.46 – 6.24)
T_3 20 mg	1	6.24	6.24	5.46	5.85+0.39
	2	5.46	6.24	5.46	(5.46 – 6.24)
T_4 50 mg	1	4.68	5.46	6.24	5.72+0.58
	2	6.24	5.46	6.24	(4.68 – 6.24)
T_5 100 mg	1	5.46	5.46	5.46	5.85±0.39
	2	6.24	6.24	6.24	(5.46 – 6.24)
T_6 200 mg	1	5.46	6.24	5.46	5.85±0.39
	2	5.46	6.24	6.24	(5.46 – 6.24)
T_7 300 mg	1	6.24	6.24	5.46	5.85±0.39
	2	5.46	6.24	5.46	(5.46 - 6.24)
T_8 400 mg	1	5.46	6.24	5.46	5.98±0.36
	2	6.24	6.24	6.24	(5.46 - 6.24)
T_9 500 mg	1	6.24	6.24	6.24	5.85±0.39
	2	5.46	5.46	5.46	(5.46 - 6.24)
T_{10} 600 mg	1	6.24	5.46	5.46	5.59±0.53
	2	6.24	5.46	4.681	(4.681- 6.24)
T_{11} 700 mg	1	5.46	5.46	5.46	5.46±0.45
	2	5.46	6.24	4.681	(4.681- 6.24)
T_{12} 800 mg	1	5.46	5.46	4.681	5.33±0.29
	2	5.46	5.46	5.46	(4.681- 5.46)

Table 17. Total alkalinity (ppm) in the experimental tanks during study period

(Range shown in parenthesis)

	Replication	Weeks			Mean ± S.D. (Range)
		1	2	3	
T_1	1	86.9	95.59	26.07	69.52 + 30.92
	2	86.9	95.59	26.07	(26.07-95.59)
T_2	1	86.9	95.59	86.9	89.79 + 4.09
	2	86.9	95.59	86.9	(86.9 - 95.59)
T_3	1	86.9	95.59	86.9	91.24 ± 6.63
	2	86.9	104.28	86.9	(86.9–104.28)
T_4	1	130.35	95.59	86.9	97.03 ± 15.39
	2	86.9	95.59	86.9	(86.9–130.35)
T_5	1	86.9	104.28	86.9	91.24 ± 6.63
	2	86.9	95.59	86.9	(86.9–104.28)
T_6	1	86.9	95.59	86.9	98.48 ± 15.59
	2	130.35	104.28	86.9	(86.9–130.35)
T_7	1	86.9	95.59	86.9	89.79 ± 4.09
	2	86.9	95.59	86.9	(86.9–95.59)
T_8	1	86.9	95.59	86.9	89.79 ± 4.09
	2	86.9	95.59	86.9	(86.9–95.59)
T_9	1	86.9	95.59	86.9	89.79 ± 4.09
	2	86.9	95.59	86.9	(86.9–95.59)
T_{10}	1	86.9	95.59	86.9	97.03 ± 15.39
	2	130.35	95.59	86.9	(86.9–130.35)
T_{11}	1	86.9	95.59	86.9	89.79 ± 4.09
	2	86.9	95.59	86.9	(86.9–95.59)
T_{12}	1	130.35	95.59	86.9	97.03 ± 15.31
	2	86.9	95.59	86.9	(86.9–130.35)

DISCUSSION

5. DISCUSSION

5.1 Growth:

Growth and survival of *Macrobrachium rosenbergii* post larvae were investigated under conditions of different calcium concentrations varying from 5 to 800 mg/l. Maximum growth was obtained at 400 mg/l and it did not change significantly in 500 mg/l calcium concentration also. While, it declined at lower and higher levels of calcium in water.

Even though the Prawn survived in lower calcium levels (5 to 50 mg/l), the growth was reduced. It was same for the higher calcium levels (600, 700 and 800 mg/l) also. No significant difference in growth rate (29.69 mg to 34.70 mg average live weight gain) was observed at lower calcium levels varying from 5 to 100 mg/l and also for higher calcium levels, like 700 and 800 mg/l (34.89 mg and 32.99 mg average live weight gain respectively). The maximum growth for 400 mg/l (T_8) was 45.75 mg and for 500 mg/l (T_4) it was 45.015 mg.

Reports on required calcium concentration levels in culture water for rearing of *M. rosenbergii* are not available. While reports on hardness requirement for successful scampi culture are available. But these reported levels of hardness for *M. rosenbergii* culture are found to vary very much. Wickins (1982) suggested hardness 65 to 200 mg/l $CaCO_3$, New and Singholka (1985) 40 to 150 (preferably less than 100 mg/l $CaCO_3$). Cripps and Nakamura (1979) reported inhibition of growth at hardness levels above 65 mg/l $CaCO_3$, while Vasquez *et al.* (1989) found that the growth was better at 112 than at

20 mg/l $CaCO_3$, and the growth was reduced at 225 and 450 mg/l $CaCO_3$. In the present study, the requirement of calcium obtained is much higher levels than the above reported hardness levels. The mortality and reduced growth in higher levels of hardness may not be due to the high levels of calcium concentration, but it may be due to the high concentration of carbonate-bicarbonate (CO_3-HCO_3) associated with calcium.

Barlett and Enkerlin (1983) and Howlader and Turjoman (1984) reported growth of *M. rosenbergii* in high hardness waters mainly from groundwater sources. While the latter study found that the growth was adversely affected by the high hardness levels (688-987 mg/l $CaCO_3$), the former study showed that hardness levels between 940 and 1060 mg/l $CaCO_3$ did not adversely affect growth. But their water had relatively low alkalinity (58-86 mg/l when expressed as $CaCO_3$).

Sherif *et al.* (1995) studied the effect of using agricultural drainage water with total hardness 900 to 1500 ppm and total alkalinity 115 to 130 ppm on the growth rate of *M. rosenbergii* in earthen ponds. Different stocking levels (1 and 2 prawns/m^2) were used in six earthen ponds and the daily individual weight increment of prawns was determined after 155 days. There was no significant difference in growth ($p< 0.05$) among the prawns stocked at both densities. The prawns reached an average weight of 52.8 $\pm$ 0.54 gm and 49.4 $\pm$ 0.7 gm at the two densities respectively, with individual increments of 317 $\pm$ 4.6 and 319 $\pm$ 4.5 mg/day. [Prawns stocked at a density of 1 prawn/m^2 (66.3 $\pm$ 3.7 %)]. The average growth rate of daily biomass at a stocking rate

of 2 prawns/m^2 reached 4.2 $\pm$ 0.2 kg/ha/day in comparison with 2.4 $\pm$ 0.1 at a stocking rate of 1 prawn/m^2. This indicates that *M. rosenbergii* can be cultured in higher concentration of hardness, if total alkalinity is within the range.

Patric *et al.* (1997) in their studies on calcium regulation in the freshwater adapted mummichog, report that the chronic low Ca^{2+} exposure (50 μ Eq/l) stimulated Ca^{2+} uptake almost three-fold above control values, whereas chronic high Ca^{2+} exposure (20,000 μ Eq/l) had no effect.

The reduction in growth under conditions of high calcium carbonate hardness (305-638 ppm) reported by Cripps and Nakamura (1979) would appear not to be due to high calcium concentration alone, as the level of calcium in this study was higher. It is possible that high carbonate, or high carbonate associated with high calcium, was responsible for the reduced growth in their study. The results of this study suggest it may not be necessary to avoid sites with hard water if the carbonate-bicarbonate level (normally measured by alkalinity) is also not high.

However, calcium concentration, alkalinity, water hardness and prawn growth relationships need further study.

5.2 Survival

As seen in Table 9, the percentage of survival varies very much between the treatments, but the variation showed no significance on statistical analysis (Table 10). But it can be seen that the survival remained above 80% up to 400 mg/l (T_8) and later on it got reduced as the concentration of calcium increased. The very low survival of 70% obtained with T_7 (300 mg/l) and 55 % obtained with T_9 (500 mg/l), were due to excessive algal growth occurred in the rearing tanks during the course of the experiment. Thus it is seen that as the case of growth when the concentration of calcium in water exceeds above 400 mg/l the survival rate gets proportionately reduced.

5.3 Water quality parameter

Different water quality parameters such as temperature, pH and dissolved oxygen are maintained within the optimum range required for growth of *M. rosenbergii* during the experimental period.

Management of water quality is one of the most important aspects in aquafarming. The level of intensification in aquaculture is now increasing through vertical expansion of scarce land resources by maintenance of high stocking density, use of artificially balanced supplementary feed, intermediate application of fertilizers and manure, providing vigorous aeration and water exchange. Therefore, there is every chance of deterioration of water quality in the culture system if the inputs mentioned earlier are not used optimally. For the judicious management of freshwater prawn

culture, it is imperative to know the different extremes and optimum levels of water quality parameters like temperature, pH, dissolved oxygen, nitrogenous compounds, hardness and alkalinity.

5.3.1 Temperature:

The water temperature influences the growth and survival of any organism and it is one of the important parameters in the hatchery and the grow-out phase of *M. rosenbergii*. It determines to a large degree, the amount of dissolved oxygen in water and life processes such as feeding, reproduction and locomotion are influenced by temperature. *M. rosenbergii* can survive in a wide range of temperature (18 - 33 ^{0}C) without any deleterious effect, provided temperature fluctuations are not severe, sudden and of long duration (Farmanfarmaian and Moore, 1980). New (1990) also reported that *M. rosenbergii* adults can tolerate a wide temperature range of 18 - 34 ^{0}C, while for the larvae the optimum range is 26-31 ^{0}C. Temperature below 14 ^{0}C or above 35 ^{0}C is reported to be lethal for post-larvae, the optimum being 29 - 31 ^{0}C. Dugan *et al.* (1976) achieved the year round spawning of prawns by maintaining a constant temperature and photoperiod of 27.5 ^{0}C and 14 hours light respectively. The weekly range of temperature observed during the present experimental period was 27.4 ^{0}C to 29.5 ^{0}C. The values recorded are within the optimum range suggested for the growth of *M. rosenbergii*.

5.3.2 pH:

pH of water is another important factor which influences the growth and survival of the prawns. In the present study, pH of water was almost uniform in all experimental

tanks and varied between 7.0 and 8.5. New and Singholka (1982) and Sandifer and Smith (1985) reported a pH range of 7.5 to 8.5 as the optimum for culture of *Macrobrachium spp*. Optimum pH for *M. rosenbergii* is reported as 7.0 - 8.5 by Hsich *et al.* (1989). Mohantha and Subramanian (2000) reported that pH slightly greater than 7.0 is considered good for the giant freshwater prawn because certain salts like bicarbonates are to be present essentially for good growth, reproduction and other physiological activities.

5.3.3 Dissolved oxygen:

Dissolved oxygen is one of the most critical water parameter for the hatchery and grow-out operation in the freshwater prawn. Dissolved oxygen helps both in respiration of prawn as well to maintain favourable chemical and hygienic environment. The minimum tolerable concentration of dissolved oxygen in fish and prawn is a function of exposure time, species of the organism, their size, physiological conditions and other factors. Chronic dissolved oxygen concentration results in low food consumption and reduced conversion efficiency.

Dissolved oxygen content of warm water crustaceans habitat should not be less than 4.5 ppm during at least 16 hrs of the day, but no time less than 3 ppm (Mohantha and Subramanian, 2000).

Temperature of water and metabolic rate of prawn influence the physiological need for oxygen. The concentration at which the oxygen becoming limiting is 2.1 ppm at 23 ^{0}C, 2.9 ppm at 28 ^{0}C and 4.7 ppm at 33 ^{0}C. Freshwater prawn *M. rosenbergii*, at

33 ^{0}C becomes oxygen depended at 4.66 mg/l, at 23 ^{0}C and 28 ^{0}C oxygen dependency is 2.08 and 2.90 mg/l respectively.

New and Singholka (1982) reported that an oxygen concentration of 75% saturation was optimum for the growth of *Macrobrachium* Species. Avault (1987) found that dissolved oxygen preferably by 70 % saturation, but levels as low as 3 ppm are tolerable. Vasaquez *et al.* (1989) found that the optimum level of dissolved oxygen in pond conditions for *Macrobrachium* culture is 6-8 ppm. During the present study, weekly dissolved oxygen values in the experimental tanks ranged from 5.43 to 6.24 ppm. No aeration was provided, but weekly water exchange was carried out.

2.3.4 Alkalinity:

The alkalinity measures the buffering ability of water to resist a fall in pH. Alkaline water contains carbonates, bicarbonates and hydroxides.

Shivananda Murthy (1998) reported that alkalinity above 50 ppm have been found suitable for freshwater prawn farming. As seen from the Table in the present study, the total alkalinity in the experimental tanks ranged from 86.9 to 130.35 ppm, but lower alkalinity of 26.07 ppm was recorded in the last week after water exchange, since rain water got mixed into it in treatment T_1 (5 mg/l).

SUMMARY

6. SUMMARY

The present study was aimed at finding out the effect of various concentrations of calcium in freshwater on the growth, survival, food intake and food conversion ratio of *Macrobrachium rosenbergii* post larvae and to determine the optimum range of calcium required in freshwater for successful culture.

1. The experiments were carried out in two stages. The first stage was bioassay analysis for the tolerance limit of calcium and magnesium in freshwater for *M. rosenbergii* post larvae.
2. Based on the results from the first stage bioassay analysis, the second stage *viz.* the rearing experiments were carried out for calcium alone for 21 days. The levels of calcium concentration used were 5, 10, 20, 50, 100, 200, 300, 400, 500, 600, 700 and 800 mg/l. For each treatment two sets of experiments were conducted using two tanks each.
3. The LC 50 of the bioassay analysis for *M. rosenbergii* post larvae during 48 hr exposure in various concentrations of calcium and magnesium were found to be 1562.85 and 7823.48 mg/l respectively.
4. In the rearing experiments of 21 days, the maximum growth of *M. rosenbergii* post larvae was obtained at 400 mg /l (T8) calcium and it declined at lower and higher levels of calcium in water.
5. The survival of *M. rosenbergii* remained above 80% up to 400 mg/l and later on it got reduced as the concentration of calcium increased. As in the case of growth

when the concentration of calcium in water exceeded above 400 mg/l the survival rate got proportionately reduced.

6. The sites that are high in alkalinity are not suitable for the culture of *M. rosenbergii*. It is possible that high carbonate-bicarbonate associated with high calcium, may be responsible for reduced growth and survival of *M. rosenbergii*.
7. The results of this study shows that it may not be necessary to avoid sites with higher calcium concentrations; if the CO_3-HCO_3 (Carbonate-bicarbonate) level (normally measured by alkalinity) is also not high.
8. The experiment also showed that calcium and magnesium are essential elements in the environment for the survival of the organism.
9. The regulation of calcium in crustaceans is important owing to the highly mineralized cuticle that must be recalcified after each moult.
10. Further clarification of the extent to which prawns are affected by the total hardness, water alkalinity (carbonate and bicarbonate) levels, instead of calcium concentrations in water is also needed if reliable recommendations are to be made concerning the suitability of a particular water source for *Macrobrachium* farming.

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7. REFERENCES

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ABSTRACT

RESPONSE OF *MACROBRACHIUM ROSENBERGII* (de Man) TO CALCIUM AND MAGNESIUM CONCENTRATIONS IN FRESHWATER

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ABSTRACT OF THE THESIS

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ABSTRACT

The effect of various concentrations of calcium in freshwater on growth, survival, food intake and food conversion ratio of *M. rosenbergii* post larvae were investigated in order to arrive at the optimum range of calcium required in freshwater for successful culture.

In two stage-designed experiment, the first experiment was bioassay for three days, in order to understand maximum and minimum tolerance level of calcium and magnesium. The LC-50 of calcium and magnesium in freshwater was found to be 1562.85 and 7823.48 mg/l, respectively. Based on the results of bioassay, the rearing experiment was carried out in circular fiberglass tanks for calcium alone. The levels used were 5, 10, 20, 50, 100, 200, 300, 400, 500, 600, 700 and 800 mg/l, i.e. 12 treatments and for each treatment two replication were used. Growth was maximum at 400 mg/l of calcium in freshwater. Better food conversion ratio and survival were also obtained at this level.

The growth declined in lower and higher concentrations of calcium, but the survival remained above 80% up to 400 mg/l and later on it got reduced as the concentration of calcium increased. The food conversion ratio increased at lower and higher levels of calcium.