DEVELOPMENT OF INNOVATIVE LOW COST LARVICULTURE TECHNOLOGIES OF THE GIANT FRESH WATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)

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DOCTOR OF PHILOSOPHY

By

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DECLARATION

I, **Saritha Thomas**, do hereby declare that the thesis entitled "**DEVELOPMENT OF INNOVATIVE LOW COST LARVICULTURE TECHNOLOGIES OF THE GIANT FRESH WATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)**" is a genuine record of research work done by me under the supervision of **Dr. B. Madhusoodana Kurup**, Professor and Director, School of Industrial Fisheries, Cochin University of Science and Technology, Cochin -16 and has not been previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title of any University or institution.

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Saritha Thomas

Certificate

This is to certify that the thesis entitled "DEVELOPMENT OF INNOVATIVE LOW COST LARVICULTURE TECHNOLOGIES OF THE GIANT FRESH WATER PRAWN, MACROBRACHIUM ROSENBERGII (DE MAN)" to be submitted by ***Mrs. Saritha Thomas,*** *is an authentic record of research work carried out by her under my guidance and supervision in partial fulfillment of the requirement of the degree of* ***Doctor of Philosophy*** *of Cochin University of Science and Technology, under the* ***Faculty of Marine Sciences.***

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Saritha

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LIST OF ABBREVIATIONS

BSN	-	Brine shrimp nauplii
BFT	-	Biofloc Technology
CH	-	Carbohydrate
CMFRI	-	Central Marine Fisheries Research Institute
CUSAT	-	Cochin University of Science and Technology
GWC	-	Green Water Culture
MLS	-	Mean Larval Stage
MSGWS	-	Modified Static Green Water System
PL	-	Post Larvae
SIF	-	School of Industrial Fisheries
S.D	-	Standard Deviation
TAN	-	Total Ammonia Nitrogen
THB	-	Total Heterotrophic Bacteria
TVB	-	Total *Vibrio* Bacteria

Chapter 1

INTRODUCTION

1.1 General Introduction

Fresh water prawns of the genus *Macrobachium* are distributed throughout the tropical and subtropical regions of the world. *Macrobrachium rosenbergi* (De Man 1879) is the largest species of the genus; the males reach a total length of 320mm and for the females it is 250mm (Ismael and New, 2000). *M. rosenbergii* is commonly known as the giant fresh water prawn. It's trade name is 'Scampi' and it is locally known as 'Aattu konchu' or 'Kuttanadan Konchu'. From the aquaculture point of view, the species has drawn much attention due to its larger size, resistance to diseases and high demand in both domestic and export markets.

1.1.1 Aquaculture of Giant fresh water prawn

Modern aquaculture of *M. rosenbergii* originated in 1960's (New 2000) following the pioneering attempts made by Shao Wen Ling and Takuji Fujimara in completing the life cycle of prawn in captivity and undertaking the larviculture on a commercial scale. Therefore, Ling and Fujimura are considered as the 'Fathers' of fresh water prawn farming (New 2000). While Ling discovered the significance of brackish water in successful completion of larval rearing (Ling and Merican, 1961), Fujimura developed mass rearing techniques for commercial scale post larval production (Fujimura and Okamoto, 1972). Following the mass production of fresh water prawn post larvae, the aquaculture production of the species was initiated during 1970's and *M. rosenbergii* had been introduced into many countries where it was not indigenous.

A very rapid global expansion was noticed since 1995 which has been attributed to the huge production from China and rapid take off of farming in India and Bangladesh (New, 2005). The average annual expansion of fresh water prawn farming (excluding China) over the period 1992 to 2001 has been estimated as 11% whereas the expansion rate between 1999 and 2001 was over 20% (New, 2005). The production of *M. rosenbergii* alone (excluding China) was expanding at the rate of 12% per year in the decade 1992 to 2001. However between 1999 and 2001, the production of the species increased at an annual rate of 86 % in India and 19 % in Thailand. The global annual production of *M. rosenbergii* in 2007 was over 2,21,000 tonnes (FAO, 2009). The main contributor was China (1,24,520 tonnes which is 56.3 % of the total production) followed by Thailand (27,650 tonnes – 12.5 % of the total production) and India (27,262 tonnes – 12.3 % of the total production) (FAO, 2009).

Fresh water prawn farming in India is mainly focussed on the giant fresh water prawn *Macrobachium rosenbergii*. In the past, the culture of the species formed a part of the poly culture activity in the fresh water fish farms. Since the prawns fetched high price in the market, monoculture was taken up and the culture activity developed at a slow pace in the beginning. However, due to the collapse of the shrimp aquaculture (*Penaeus monodon*) in India during mid 1990's due to outbreak of diseases, sustainability issues and implementation of coastal zone regulation (CRZ) combined with the good demand in domestic and export markets for fresh water prawns, there was a growing interest in the culture

of fresh water prawns. This ended up in a sudden spurt in the fresh water prawn farming activities from 2000 onwards (Bojan *et al.*, 2006). Sustained production infra structure facilities such as hatcheries, feed units, etc., resistance to the diseases, comparative easiness in procurement of licenses and permissions, and environmental sustainability are the various factors attributed to the accelerated development and growth of fresh water prawn farming sector (Bojan *et al.*, 2006).

A 327% increase of scampi production from scientific farms was registered during the period 1999 - 2000 (7140 mt) to 2002 - 2003 (30,450 mt) (Bojan *et al.*, 2006). The area under culture was also increased by 188% during the same period. This trend of increased production continued until 2005 - 2006 when a production of 42,820 mt was registered and contribution of 9,264 mt to the export (Table 1.1) (MPEDA Annual report 2007-08). Unfortunately a decrease in the production and export has been noticed in the following years. As compared to 30,115 mt of scampi produced from about 30,042 ha during 2006-07, the production further declined to 27,262 mt from an area of 50,206 ha during the year 2007- 08 (Table 1.2). It can be seen that, although a 67% increase in culture area was reported during 2007 – 08 (Table 1.2), a 9.47% decrease in production with a 27.5% decrease in value (Table 1.3) was recorded during the same period. This has been mainly attributed to the reduction in the productivity. Andhrapradesh ranked first among the states with a production of 19,887 mt during the year 2007 - 08, while, Kerala ranked

fourth (539.97mt). It is worth noting that despite of a plummeting in the production observed at the national level, a progressive increase in production from 268 MT during the year 2004 - 05 to 539.97 MT during 2007 - 08 was observed in Kerala. The area under culture also increased from 911 ha to 2171.65 ha during the same period (Table 1.2).

Table 1.1 Year-wise production and exports of scampi from India during 1997-98 to 2007-08

Year	Culture production (MT)	Export quantity (MT)
1997--1998		1787.00
1998--1999	3900.00	1909.00
1999--2000	7140.00	2678.00
2000--2001	16560.00	4756.00
2001--2002	24340.00	9201.00
2002--2003	30460.00	10380.00
2003--2004	35870.00	9040.00
2004--2005	38720.00	9264.00
2005--2006	42820.00	6321.00
2006--2007	30115.00	6129.00
2007--2008	27262.00	4472.00

Source: MPEDA annual report 2007-08

Table 1.2 State wise details of Scampi production from aquaculture during 2004-08

Sl No.	State	Area under Culture (Ha)	Production (MT)	Area under Culture (Ha)	Production (MT)	Area under Culture (Ha)	Production (MT)	Area under Culture (Ha)	Production (MT)
		2004-05	2004-05	2005-06	2005-06	2006-07	2006-07	2007-08	2007-08
1	West Bengal	4395.00	3193.00	4284.00	3751.00	4744.00	4471.00	4744.00	4516.00
2	Orissa	3188.00	470.00	3388.00	680.00	3591.00	856.00	3786.00	915.00
3	Andhra Pradesh	28096.00	34541.00	33556.00	37103.00	17335.00	24056.00	38819.00	19887.00
4	Tamil Nadu	192.00	144.00	536.00	605.00	324.00	449.00	404.35	376.69
5	Kerala	911.00	268.00	1252.00	364.00	1211.00	88.00	2171.65	539.97
6	Karnataka	165.00	44.00	145.00	43.00	90.00	46.00	265.00	180.00
7	Maharashtra*	43.00	60.00	234.00	234.00	2713.00	115.00	15.60	6.95
8	Gujarat**	4.00	3.00	0.00	40.00	34.00	34.00	0.70	0.45
	*Production from reservoirs								808.00
	**Production from Village ponds								32.00
	Total	36990.00	38720.00	43394.00	42820.00	30042.00	30115.00	50206.00	27262.00

Source: MPEDA annual report 2007-08

Table 1.3 Comparison of scampi production from aquaculture during the years 2006-07 and 2007-08

Year	Live weight (MT)	Product weight (MT)	Estimated value (Rs. Crores)
2006 07	30,115	15,058	602.00
2007--08	27,262	13,631	436.00
Increase/decrease	(--) 2853	(--)1427	(--)166.00
Difference %	(--)9.47	(--)9.47	(--)27.57

Source: MPEDA annual report 2007-08

Eventhough more than 25 species of fresh water prawns have been reported from Kerala, *M. rosenbergii* is the only species with aqua culture importance. There exist a vast potential for the development of prawn culture and nearly 65,000 ha comprising of ponds, tanks and check dams are found suitable for fresh water prawn farming following monoculture or polyculture with carps (Sureshkumar and Velayudhan, 2006). The low lying Kari lands of Kuttanadu and Kole lands are also found to be suitable for prawn farming alternating with paddy cultivation. The prawn farming has been considered as an important aquaculture activity in the state due to its domestic as well as export market and the possibility of integration or rotation with paddy cultivation which in turn provides an additional income. The increase in the aquaculture production from the state of Kerala mentioned earlier may be due to the utilisation of many of these suitable areas for culture purposes as seen in the increase of the area brought under the culture.

1.1.2 Landings of *M. rosenbergii* from Vembanad lake

Another important point worth mentioning in this context is the decline of the *M. rosenbergii* fishery in it's home ground, Kuttanad, to a mere 39 tonnes in eighties from 189 - 429 tonnes recorded during 1957- 62 period (Harikrishnan and Kurup, 2006). The authors have recorded a further decline

in the landings of prawns to 28.86 tonnes during 2000 - 01. They have also estimated an economic loss of Rs 30 million in 2000 - 01 as compared to the landings of 112.85 t during 1994 - 95. Scientific ranching has been suggested by the authors as a rehabilitation measure to improve the population of *M. rosenberii* in its natural habitat. On the other hand, Sureshkumar and Velayudhan (2006) reported a catch of 1,224 t of the prawn from the Vembanad lake during the year 2002 which was attributed to the large scale ranching of hatchery reared seed into natural waters. Therefore, the availability good quality seed is also a pre-requisite for undertaking rehabilitation of the species in natural waters.

1.1.3 Seed production of *M. rosenbergii*

Successful aquaculture operations also require the supply of sufficient good quality of the seed at the right time of stocking. Consequently, the technology of seed production is an important part of the aquaculture systems. The successful completion of lifecycle of *M. rosenbergii* in captivity was accomplished by Shao-Wen Ling in early 1960's. Following this the mass culture technology for commercial scale seed production was perfected by Takuji Fujimura in late 1960's.

Two main types of hatchery systems for seed production of *M. rosenbergii* have been described by New (2002). They include the flowthrough system and the dynamic closed system otherwise known as recirculation system. Flow through hatchery systems are based on regular water exchange, in order to reduce toxic substances that accumulate in the larval rearing water. It can be a clear water system or a green water system. Clear water system

envisages the rearing of larvae in clear water devoid of algae. The green water system makes use of waters rich in algae for larval rearing. However, the clear water system is generally favoured due to the easier management and higher efficiency (Correia *et al.*, 2000; New, 2002). The recirculation system is based on continuous circulation of larval water through physical and biological filters to remove solid and nitrogenous wastes. Since this system tends to increase larval stress and mortality, it is not recommended for larviculture of *M. rosenbergii* (New, 2002).

Subsequent to success achieved in seed production of *M. rosenbergii* in Kerala during mid 1990's (Sebastian and Nair, 1995; Kurup *et al.*, 1998), a few fresh water prawn hatcheries came to be established with varying installed capacities. As per recent reports, there are 76 fresh water prawn hatcheries in the country (Murthy, 2006), out of which 14 are in Kerala (Suresh kumar and Velayudhan, 2006). Most of hatcheries are not able to produce at installed capacities due to various reasons (Murthy, 2006) and the average survival in the commercial hatcheries was found to be 30% with a long duration of 25-40 days required for the completion of one rearing cycle. The lack of adequate supply of seed has been reported (Murthy, 1996; Reddy, 1997; Kurup, 2003; Thanuja, 2007) as a major bottle neck in the expansion of fresh water prawn aquaculture as well as implementation of various rehabilitation programmes. Thanuja (2007) has also reported a requirement of 10,000 million seeds in the coming years for the development of 2 lakh ha water area for Scampi culture. However, in Kerala, there are 14 hatcheries with a total annual installed capacity of 140 million post larvae. Sureshkumar

and Velayudhan (2006) have pointed out the lack of demand of seed within the state as a major problem faced by the hatchery operators. The same authors have mentioned the import of cheap poor quality seed by some hatcheries from the neighbouring states and its supply as a major problem faced by the farmers. One of the major reasons for outbreak of white tail (muscle) disease at nursery and juvenile stage has been attributed to the poor quality of prawn seed (Murthy, 2006). Against this background, it can be hypothesised that the supply of good quality seed at a low cost is a major constraint in taking up the aquaculture of the species as well as implementation of various rehabilitation programmes.

1.1.4 Background of the study

The heavy dependence on *Artemia* nauplii is a major constraint in the seed production of *M. rosenbergii*. *Artemia* which is otherwise known as 'Brine shrimp' is a highly preferred live feed in most fish and crustacean hatcheries. Its ready availability as storable 'on demand' live feed has led to wide acceptance across the world. In spite of being a convenient, suitable, and an excellent larval feed, it is very costly.

Artemia cysts account for more than 50% of the variable costs in fresh water prawn hatcheries (New 2002). Several studies have been conducted to substitute *Artemia* with live or inert feeds with varying degrees of success (Lovett and Felder, 1988; Kumlu and Jones, 1995; Alam *et al.*, 1993a, b; Barros and Valenti, 2003a, b; Kovalenko *et al.*, 2002; Murthy *et al.*, 2008). In addition to the very high costs incurred, it becomes sometimes scarce (Lavens *et al.*, 2000) and is found to be nutritionally inadequate (Barros and Valenti,

2003b; Lober and Zeng, 2009). The fluctuations in the nutrient content of *Artemia* nauplii has also been well documented (Leger *et al.*, 1986; Rodgers and Barlow, 1987; Lavens *et al.*, 1989). Moreover, there is possibility for the accumulation of brine shrimp exuvia and shed cyst capsules leading to bacterial degradation which in turn fouls the water resulting in larval mortalities. Hence, the partial or complete replacement of brine shrimp nauplii (BSN) is one of the major researchable issues in the larviculture of the giant prawn, the results are having much practical application not only in resolving issues pertaining to the non availability of *Artemia* nauplii but also is useful in reducing the cost of production.

The use of algae is reported to enhance the post larval production of *M. rosenbergii* in several instances (Cohen *et al.*, 1976; Maddox and Manzi, 1976; Manzi *et al.*, 1977). Kurup (2003) suggested the adoption of modified static green water system of larval rearing as solution to many problems encountered in the larviculture of the species. The rapid growth in the number of hatcheries and production of post larvae since 2000 in Vietnam has been attributed to the adoption of the same system for larval rearing (Phuong *et al.*, 2006). In Vietnam, modified static green water system of larviculture is widely used at backyard level. Kurup (2003) also found the results of preliminary trials conducted using the technology at the prawn hatchery of School of Industrial Fisheries (SIF) highly successful with a survival ranging between 34 - 80% within a period of 28 - 32 days as compared to average survival of 30% recorded for the commercial Scampi hatcheries of the country in a period of 25 - 40 days. Moreover, the quantity of *Artemia* cysts and custard feed

required for production of postlarvae is low when compared to the other systems (Kurup, 2003). The other advantages include its simplicity, eco-friendly nature, low labour requisite, lower requirement of sea water enabling setting up of hatcheries at places far away from the sea, all of which make the system more economically viable and feasible. Unfortunately, no further studies were made on the suitability of adoption of the modified static green water system in the state. Therefore, attempts were also made in this study to standardise and improvise the system with respect to Indian conditions. The organic farming of fresh water prawn is also getting importance due to increased market demand, which in turn requires organically produced seed without using chemicals and antibiotics.

One of the major constraints in adopting the static system in backyard hatcheries is the heavy accumulation of toxic nitrogenous nutrients like total ammonia nitrogen (TAN) and nitrite nitrogen. Removal of excessive nitrogen from the culture system is most commonly carried-out by frequent exchange and replacement of rearing water. However this practice is constrained by the following reasons:

1. Environmental regulations prohibit the release of nutrient rich water into environment;
2. The danger of introducing pathogens into the external water;
3. The high expense incurred in pumping huge volume of water.

Another approach is based on means to encourage and enhance nitrification of ammonium and nitrites to the relatively inert nitrate species. This is often done by employing biofilters, essentially immobile surfaces serving as

substrate to the nitrifying bacteria. A high surface area with immobilized nitrifying biomass enables a high nitrifying capacity in a controlled environment. One problem associated with biofiltration is the high cost involved and the need to treat and digest a large mass of feed residues and the expertise required for maintaining such systems.

An additional strategy that is getting more attention presently is the removal of ammonium from water through its assimilation into microbial proteins by addition of carbonaceous materials to the system (Avnimelech, 1999). If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. A further important aspect of this process is the potential utilization of microbial proteins as a source of feed protein for fish or shrimp. This, however, depends upon the ability of the animal to harvest such bacteria and to digest and utilize the microbial protein. Controlling inorganic nitrogen by manipulating carbon / nitrogen ratio is a potential method for aquaculture systems. This approach offers a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in the rearing water. Applicability of the same approach in earthen stagnant ponds is not trivial and has to be further studied in static hatchery systems. Thus a pioneer attempt was made at the Fresh water prawn hatchery, SIF, CUSAT in controlling the accumulation of toxic nitrogen species in static hatchery system by the application of biofloc technology (BFT) which ultimately results in making the modified static green water system economically feasible by a ensuring a steady and increased post larval production.

Thus, the specific objectives of the present study are:

1. To evaluate the usefullness of a microalga and blood worms in partially or completely substituting *Artemia* nauplii, thereby reducing the cost of production and maximizing the post larval production by adopting clear water system.
2. To improvise the modified static green water system of larviculture by optimizing the stocking density.
3. To evaluate the effectiveness of different algal media in achieving maximum post larval production using the modified static system.
4. To optimize the quantity of *Artemia* nauplii used in the modified static system with the microalga *Isochrysis galbana* as the rearing medium.
5. To optimize quantity of carbohydrate addition to control carbon / nitrogen ratio in the most suitable way to increase postlarval production.
6. To asses the efficiency of various types of carbohydrates in the control of inorganic nitrogen in the modified static green water system.
7. To optimize the stocking density in carbohydrate added systems and to compare the addition of carbohydrate in intensive systems
8. To reduce the water based inorganic nitrogen discharge in to environment thus making larval rearing of *M. rosenbergii* more economically, ecologically and environmentally sustainable.
9. To enable the production of healthy post larvae without the use of antibiotics making the post larval production more organic and economically viable.

1.2. Review of Literature

1.2.1. Importance of live feed in larval rearing

The aquaculture production during 1970s almost entirely depended on the capture of wild seed and subsequent stocking and growing in ponds, tanks or cages. Since the domestication of many marine and brackish water aquaculture species was successfully achieved during 1980s and 90s, the hatchery production of seed has become a routine operation. The rearing of larvae requires specific culture techniques since the developing larvae are usually very small, extremely fragile and generally not physiologically fully developed. Generally they have underdeveloped perception organs and digestive system and this makes their feeding during the early stages very difficult. This is more complicated in animals like fresh water prawns, where in, the larvae have to pass through different larval stages which might be having specific nutritional requirements. Therefore, the larval nutrition, and in particular that of the first-feeding larvae, has become one of the major bottlenecks preventing the full commercialization of many farmed fish and shellfish species (Lavens and Sorgeloos, 1996). Consequently, it becomes essential for the larvae to be supplied with a food source which is easily digestible, contains enzyme systems which allow autolysis and supplies all the essential nutrients required by the larvae.

Generally the formulated feeds are not able to meet these requirements in most of the cultured species. Most of the live feeds, on other hand, seems to be nutritionally adequate, provides much better contrast than artificial feeds and generally have a triggering effect by their continuous movement, allowing

an enhanced perception by the feeding larva (Lavens and Sorgeloos, 1996). The swimming activity of the live feed is also supposed to provide a better distribution in the rearing water column which enables more frequent encounter with the developing larvae which is especially true in the case of *Macrobrachium rosenbergii* larvae. The early stages of *M. rosenbergii* are non-active hunters and they seem to capture food by chance encounter (Moller, 1978). Therefore, the assumed absolute need for live feed is considered to be a limiting factor in commercial culture of larvae of many fish and crustacean species (Kovalenko *et al.*, 2002).

The important live feed used in larval rearing of various fishes and crustaceans is constituted by various microalgae, infusoria, rotifers, brine shrimp, cladocerans, tubifex worms and blood worms. Although live feed has proven successful for raising the larvae of many species, there are many problems associated with their use. Shortcomings include variable nutrient composition and availability, potential introduction of pathogens into the culture system and high costs of labour and infrastructure required (Kovalenko *et al.*, 2002). Moreover, production of live diets on a commercial scale is complicated, expensive and unreliable in supply and nutritional value (Sorgeloos *et al.*, 1983; Langdon *et al.*, 1985; Jones *et al.*, 1993). Live feed production is further associated with a need for skilled personnel, dedicated equipment and facilities, and need for micro algae culture as food source for the live prey (zooplankton) (Holme *et al.*, 2009). However, in spite of the progress made in understanding the nutritional requirements of larval and post larval stages of cultivable fishes and shell fishes, the commercial culture of

early stages of these animals still require the use of live feed. Among all the live diets, it is the brine shrimp nauplii that constitute the most widely used feed item. The unique property of the small branchiopod crustacean *Artemia* to form dormant embryos otherwise known as the cysts, accounts to a great extend to the designation of a convenient, suitable, or excellent larval food source that it has been credited with (Stappen, 1996).

1.2.1.1. *Artemia* in the larval rearing of giant fresh water prawn

One of the main factors affecting larviculture of fresh water prawns is feeding strategies, which must be adjusted to behavior and nutritional needs of the larvae (Loya-Javellana, 1989). Brine shrimp nauplii (BSN) are an indispensable component in the larval rearing of the giant fresh water prawn. The use of *Artemia* nauplii for feeding prawn larvae has advantages such as easy management, adequate size and high content of essential nutrients available for the predator (Lavens *et al.*, 2000). *M. rosenbergii* hatchery operations are heavily dependent on the use of live BSN, either solely or in combination with prepared diets (New, 1990). The early stages of prawn are supposed to prefer live feeds. Moreover the mineral and micronutrient needs of the larvae are not yet fully understood to be incorporated in the formulated feeds (Reddy, 1997).

Ling (1969 b) first successfully reared *M. rosenbergii* through all larval stages using live zooplankton (rotifer, cyclops, copepods and insect larvae), chopped fish, shell fish and steamed yolk *ad libitum*. However, the attempts made by several workers to substitute BSN with other live feeds have only been partially successful. Aniello and Singh (1982) and Ang and Cheah

(1986) found that dead *Moina* were not a suitable substitute for BSN in the larval rearing of *M. rosenbergii*. However, live *Moina micrura* were found to be a suitable feed for the prawn larvae during later larval stages (Alam *et al.*, 1993a). The larvae fed a 50:50 combination of the cladoceran and BSN, were in turn found by authors to perform in a superior way than that fed *Artemia* alone. The weaning study conducted following the success in using *Moina micrura* (Alam *et al.*, 1993b) confirmed the usefulness of the cladoceran, as the larvae fed the weaning diets were found to develop faster than those fed BSN alone. Lovett and Felder (1988) have reported that the rotifer, *Brachionus plicatilis* was neither a suitable substitute for BSN nor a useful supplement in the larviculture of *M rosenbergii*. However, Thomaz *et al.* (2004) and Murthy and Yogesh babu (2006) achieved some degree of success and were able to substitute BSN with *B. plicatilis* up to a particular level without affecting the post larval production. The effect of replacement of BSN with the nematode *Panagrellus redivivus* on the growth and survival of fresh water prawn was studied by Silva and Rodrigues (1997). The authors have suggested 34 % and 66 % replacement after V^{th} and $VIII^{th}$ larval stages respectively. However, a total replacement of BSN with the worm resulted in complete mortality of the larvae by 6^{th} day. The comparison of the performance of three types of larval diets, led to the conclusion that chopped *Tubifex* worms or *Lamellidens* meat alone were not a good substitute for *Artemia* nauplii, whereas, prepared feed combined with BSN given as one overnight feed can be used instead of using BSN alone throughout the larval rearing cycle to reduce the cost of seed production (Mohanta and Rao, 2000).

Nair *et al.* (2007) reported the use of Cyclop-eeze (a commercial product prepared from a marine copepod organism) to economically replace BSN at 50 % level with a significantly improved survival and carotenoid composition of *M. rosenbergii* larvae.

Despite considerable efforts to develop supplemental diets (Sick and Beaty, 1975; Sandifer *et al.*, 1977; Murai and Andrews, 1978; Corbin *et al.*, 1983; Cheah *et al.*, 1987), the rearing of *M. rosenbergii* larvae relies almost completely on the use of live *Artemia* nauplii. In view of the high cost and occational scarcity, the dependence on *Artemia* nauplii is therefore a major concern in the expansion of *M. rosenbergii* hatcheries, especially in the Asian region (Hagwood and Willis, 1976; Hanson and Goodwin, 1977; Ong *et al.*, 1977; Aniello and Singh, 1982). Moreover, the cost of feeding accounts for as much as 60 % of total production costs in *M. rosenbergii* hatcheries (Hagwood and Willis, 1976). However, most of the fresh water prawn hatcheries now a days are using a combination of BSN and prepared feed for producing post larvae. Nevertheless, some researchers have achieved success in replacing BSN with formulated inert diets, particularly towards the advanced zoeal stages. Barros and Valenti (2003 a) suggest the use of BSN only until stage VI. According to them from stage VII to VIII, the diet should be supplemented with wet food and from stage IX onwards, supplemental diet could be either wet or dry food depending on cost and availability. Microencpsulated diet was found to sustain *M. rosenbergii* larvae from Z5-6 to PL with 28 % survival (Kumlu and Jones, 1995). The authors have attributed the inability of early larvae of *M. rosenbergii* to survive on artificial diets, to the under developed

gut of the larvae and limited enzymatic capabilities. Stage Z5-6 in fresh water prawn coincides with a rapid increase in the volume of hepatopancreas and the formation of filter apparatus and these morphological changes in the gut structure appears to enable the larvae to utilize artificial diets after Z5-6 stage. Kovalenko *et al.* (2002) found that survival of larvae fed a high moisture content semi-purified microbound diet (from 5^{th} stage) did not differ significantly from that of BSN fed larvae. The authors have also found a weaning protocol unnecessary since the larvae show no preference when a mixture of live and inert diets are provided.

The early larval stages of carideans like *M. rosenbergii* have been reported as predominantly carnivorous, have low concentrations of amylase and trysin and lack flexibility with respect to their feeding habits, but at stage VI, the production of digestive enzymes increases abruptly (Kamarudin *et al.*, 1994; Kumlu and Jones, 1995; Jones *et al.*, 1997; Barros and Valenti, 1997). The early zoeal stages of the giant fresh water prawn in spite of having good visual power are non-active hunters (Daniels *et al.*, 1992) and capture food by chance encounter (Moller, 1978). Therefore, in addition to all other advantages, the chances of colliding with a moving prey makes BSN more acceptable. The larvae also have to depend on a highly digestible prey which may provide exogenous prey enzymes (Jones *et al.*, 1993). Although exogenous digestive enzymes are not essential for digestion of formulated diets by fifth stage and beyond of the larvae of carnivorous *M. rosenbergii* (Kovalenko *et al.*, 2002), the size of BSN also favours (Lavens *et al.*, 2000) its use in the larval rearing of the giant fresh water prawn. The other factor that

makes the BSN a much accepted live food in the larviculture of *M. rosenbergii* is the availability as a storable on demand live feed which makes them more convenient and least labour intensive (Lavens *et al.*, 2000).

Nevertheless, the dependence on BSN has its drawbacks as well. The most important among them has been listed as the high cost, occasional scarcity and wide fluctuation in the nutrient content and hatchability depending on the geographic location and season of harvest and fouling of water and larval entrapment by bacterial degraded brine shrimp exuvia (Leger *et al.*, 1986; Lovett and Felder, 1988; Sorgeloos and Van Stappen, 1995; Kumlu, 1999; Kovalenko *et al.*, 2002; Araujo and Valenti, 2007; Murthy *et al.*, 2008; Holme *et al.*, 2009). The supply of higher quantities of BSN than required also leads to problems related to water quality (Barros and Valenti, 2003b). Moreover, according Daniels *et al.* (1992) and Barros and Valenti (2003a), *Artemia* nauplii do not provide all the nutrients needed in the final stages of *M. rosenbergii* larvae. Thus, feeding managements using alternative diets to replace or compliment *Artemia* nauplii feeding, should be more suitable and may decrease production costs (Araujo and Valenti, 2007).

1.2.2. Algae in aquaculture systems

Algae are the major living resources in the aquatic habitat. The role of micro-algae in aquaculture systems can be in stabilizing the water quality, providing nutrition to the organisms and microbial control. All the more, micro-algae are indispensable in the commercial rearing of majority of the economically important fishes and shell fishes. However suitable algal species have to be selected based on their mass-culture potential, cell size,

digestibility and overall food value for the feeding animal. The advantages of controlled green-water larval culture technologies for fishes and shell fishes have been reviewed extensively by Palmer *et al.* (2007). The better survival and growth in these controlled green water systems has been attributed by the authors due to the following reasons

- Better direct and indirect nutrition of the larvae
- Lower stress level
- Enhanced environmental conditions for feeding from increased turbidity, light scattering and attenuation, and visual contrast enhancement
- Improved water quality due to stripping of nitrogenous substances and increasing oxygenation rates
- Chemical and digestive stimulus
- Antibacterial properties of algae.

Significant advantages of adding phytoplankton to larval fish rearing systems has been documented by several authors (Howell, 1973; Scott and Baynes, 1979; Barahona-Fernandes, 1982; Van der Meeren, 1991; Naas *et al.*, 1992; Hernandez-Cruz *et al.*, 1994; Gulbrandsen *et al.*, 1996; Liao *et al.*, 2001; Papandroulakis *et al.*, 2001, 2002; Faulk and Holt, 2005). Advantages of using green water cultures in the larval rearing of several species of fishes and prawns in Australia has been described in detail by Palmer *et al.* (2007). According to the authors, closed mesocosm was built around bacteria and microorganisms associated with exponential growth phase of outdoor mass cultures of the green microalgae, *Nannochloropsis occulata*. Even with high

densities of fish larvae, water exchange was generally not found to be necessary until the final stages, when significant levels of BSN were fed daily (Palmer *et al.*, 2007). Further, the low water exchange approach was found to enrich the growth media, created savings in rotifer requirements, eliminated tank cleaning, reduced accidental losses and physical damage of healthy larvae, and greatly simplified hatchery procedures whilst increasing production capacities. Higher levels of total ammonia nitrogen was sometimes encountered in these systems. Even though the toxic unionized form of ammonia was found to be in the detrimental range towards the completion of the rearing period, no ill effects for *Lates calcarifer* larvae were observed (Palmer *et al.*, 2007). No mortality was noticed as a result of high levels of unionized ammonia and the harvested fry were found to be robust without exhibiting any stress-induced fainting typical of fish fry reared under intensive conditions. The authors attributed the better overall performance of the larvae to their feeding on the wide variety of diatoms, protozoans and bacteria developed in these highly intensive low water exchange green water cultures. The opportunist organisms thus developed may be responsible in keeping the waste products in check via accelerated aerobic decomposition of organic wastes.

Low water exchange green water cultures have been used in shrimp larval rearing in various ways (Cook and Murphy, 1969; Liao *et al.*, 1983). Chiang and Liao (1985) have reported the use of green water cultures for rearing *Penaeus monodon* larvae in large tanks of 10 to 50 tonnes along with increased stocking densities and artificial feeds. Although excessive levels of

ammonia were detected in larval culture water of *Mugil cephalus* using green water constituted by *N. oculata*, due to application of ammonium sulphate and urea as fertilizers, significantly high growth and survival were observed when compared with the cultures without phytoplankton (Tamura *et al.*, 1994). The contrast enhancement provided by the alga *Tetraselmis suecica* in larval rearing of greenback flounder, *Rhombosolea tapirina* provided higher rotifer consumption rates than clear water, particularly at low prey densities and resulted in increased survival (Shaw *et al.*, 2006). Enriching rotifers and *Artemia* with live *I. galbana* in conjunction with green water culture systems improves the growth and survival of Cobia larvae (*Rachycentron canadum*) in recirculating aquaculture systems (Faulk and Holt, 2005).

Microalgae like *Tetraselmis* sp. has been shown to inhibit growth of pathogens (*Vibrio* sp.), at the same time promoting the growth of other bacteria which better supported larval survival (Regunathan and Wesley, 2004). Several other studies have shown that active compounds in fresh and processed microalgae like *Phaeodactylum tricornutum* and *Skeletonema costatum*, *Chaetoceros* sp., *T. suecica* and *Dunaniella tertiolecta* (Cooper *et al.*, 1983; Viso *et al.*, 1987; Austin and Day, 1990; Marques *et al.*, 2006) reduce the potential for viral infection (Wang, 2003). Synergistic actions between micro algae and their bacterial flora has also been suggested by several authors (Avendano and Requelme, 1999; Vine *et al.*, 2006). Hence Palmer *et al.* (2007) have strongly supported striving for naturally fit and healthy microflora derived from well managed live feed cultures allowing

natural selection to draw the process other than introduction of specific bacteria through the addition of probiotics.

The use of cyanobacterium *Spirulina platensis* was found to effectively control all nitrogenous compounds in *P. monodon* culture tanks (Chuntapa *et al.*, 2003). One of the techniques that has been reported to work against luminous bacteria is the green water (Corre *et al.*, 2000). The use of green water with microalga *Chlorella* has also been suggested as a potential alternative method in the control of luminous bacterial populations in shrimp ponds (Tendencia and Pena, 2003). The authors have also described the system as low cost, easy to manage and environment friendly reducing the use of antibiotics and other chemicals which causes environmental deterioration. Higher growth for Pacific white shrimp (*Litopenaeus vannamei*) has been achieved in static green water systems than in intensive clear water systems (Tacon *et al.*, 2002). The authors have attributed the ability of Pacific white shrimp to obtain additional nutrients from food organisms endogenously produced within the green water system as one of the reasons for better growth and feed performance in outdoor green water. The nutritional contribution of floc to shrimp in mesocosm culture has shown to reduce or eliminate the need for a dietary source of fish oil in *L. vannamei* (Izquierdo *et al.*, 2006). In shrimp culture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon, 1982; Diab and Shilo, 1986). Phytoplankton are considered as the principal regulators of ammonia levels in fish ponds (Tucker, 1996; Hargreaves, 1997). Phytoplankton is therefore playing an important role in uptake, which prevents

the accumulation of toxic levels of ammonium and urea (Buford and Gilbert, 1999). Their study have also shown that although the phytoplankton community can adapt to higher dissolved nitrogen concentrations by increasing uptake, there is a threshold beyond which regeneration will exceed uptake, and nitrogen will accumulate. Dissolved nitrogen is thought to stimulate phytoplankton growth and this inturn benefits the shrimp by shading them, preventing the growth of benthic algae, maintaining oxygen levels, reducing ammonia to non-toxic levels and providing an additional food source for zooplankton and shrimp (Buford, 1997).

1.2.2.1. Algae in larval rearing of *M rosenbergii.*

As in the larviculture of finfishes and shell fishes reviewed in the previous section (1.2.2), the use of algae is found to enhance the growth and survival of *M. rosenbergii* larvae. According to Maddox and Manzi (1976), algal supplements increased survival of the larvae and production of post larvae and decreased length of time to metamorphosis. *Phaeodactylum tricornutum*, *Isochrysis galbana* and *Pseudoisochrysis paradoxa* were reported as the most valuable supplements. The use of algae was found to increase the survival by 30 % than control treatments without algae. Unialgal supplements especially of those of Chrysophyta significantly increased survival of larvae and production of postlarvae in both static and recirculating larval rearing systems of *M. rosenbergii* (Manzi *et al.*, 1977).

However, still confusion exists regarding the role of the larvae in the larviculture of *M. rosenbergii*. Joseph (1977) by conducting fatty acid analysis of the larvae reared in different algal medium concluded that algae are not

used as supplemental feed by the larvae, as the fatty acids of the larvae from different treatments were similar without any of the unusual algal fatty acids. The improved post larval production in a shorter period was attributed to the possible uptake of algae by the prawn larvae (Thresiamma *et al.*,2006; Devika *et al.*, 2006; Lober and Zeng, 2009). The ingestion of algal cells by the prawn larvae was also reported by Manzi *et al.* (1977). The possible ingestion of algae in the rearing medium has also been reported by Sebastin (2006). According to Cohen *et al.* (1976), the primary role of the algae is maintenance of water quality. The same authors observed no direct nutrition provided by the algae to the *M. rosenbergii* larvae. However, algal supplementation, as a part of formulated diet was found to enhance the growth of the larvae and post larval production in the larval rearing of *M. rosenbergii* (Khatoon *et al.*, 2009). Lober and Zeng (2009) studied the effect of algal concentration on the growth of Australian strain of *M. rosenbergii* and suggested a density of *Nannochloropsis* sp. above 12.5 x 10^5 cells/ml. However, the possibility of nutritional contribution of the algae to the prawn larvae have not been ruled out by the authors. The probable enrichment of the BSN by the algae, which in turn forms the food of prawn larvae has also been reported by some authors (Thresiamma *et al.*, 2006; Lober and Zeng, 2009).

In spite of most of the hatchery operators favouring the adoption of clear water systems (New, 1995), the green water systems are still in use and they are reported (Phuong *et al.*, 2006) to have contributed much to the establishment of large number of hatcheries and enhanced post larval production of *M. rosenbergii* in Vietnam. The study conducted using the green

water systems which are defined as self cleaning systems due to the presence of beneficial bacteria has also been found to increase the postlarval production considerably in the Indian subcontinent (Kurup, 2003). According to the author, the whole system works together as a biological filter, which plays the key role in maintaining the water quality and keeping the green water static, for a prolonged period without causing any stress conditions to the larvae. The rearing of *M. rosenbergii* larvae in open earthen ponds has been reported in Thailand (Tunsutapanich *et al.*, 1996, 1997). In these zero water exchange green water systems, naturally occurring plankton comprised the sole food item upto 7 days of culture followed by BSN and egg custard and the final survival rates were estimated to be around 68 %. The authors reported an increased supply of post larvae by adopting this novel technology. The other advantages has been projected as decreased volume of *Artemia* cysts required, lower water requirement, short larval cycle and less labour.

1.2.3. The significance of stocking density

Larval culture density is an important factor in determining the efficiency of hatchery production (Doroudi and Southgate, 2000). Larval survival and growth was inversely related to stocking density in the culture of spiny lobster (*Jasus edwardsii*) larvae. Development time increased as a function of stocking density, average survival decreased concomitantly and growth decreased linearly with increasing stocking density in the larvae of *Penaeus indicus* (Emmerson and Andrews, 1981). Palmer *et al.* (2007) have reported an inverse relation between stocking density and survival in Barramundi larvae. Although larval growth was found to be similar during the

early days of larviculture, lower density tanks had the longer fry at harvest. However, these growth differences were considered by the authors to be small when compared with the potential for improved productivity at higher densities.

Higher initial stocking densities were reported (Suharto *et al.*, 1982; Correia *et al.*, 1988; Hsieh *et al.*, 1989; Valenti, 1993; 1995; Kurup *et al.*, 1998) in the multistage larval rearing systems of *M. rosenbergii*. Not only growth, but also the survival of the prawns reared under intensive conditions in closed systems were better at low than at high stocking densities (Forster and Beard, 1974; Sandifer and Smith, 1976). Although survival was found to be more at lower stocking densities, due to the higher biomass at harvest, higher stocking densities were found to be optimal in the nursery phase culture of *M. rosenbergii* in cages (Marques *et al.*, 2000).

Even if survival was not affected, growth of *P. monodon* juveniles was inversely related to stocking density during intensive production (Arnold *et al.*, 2006). Reduced growth and survival at higher densities are attributed to a number of factors like, a decrease in the availability of space and natural food sources (Maguire and Leedow, 1983; Peterson and Griffith, 1999); an increase in adverse shrimp behavior such as cannibalism (Abdussamad and Thampy, 1994); the degradation of water quality (Nga *et al.*, 2005); and accumulation of undesirable sediment (Arnold *et al.*, 2005, 2006).

However, increasing the stocking density did not have a negative effect on the survival and growth in grow out of *M. rosenbergii* (Asaduzzaman *et al.*, 2009) and nursery rearing of *P. monodon* (Arnold *et al.*, 2009) with the

addition of substrate as well as carbohydrate, therefore, greater production outputs were achieved at higher density. It was also found that mean TAN and nitrite concentrations were significantly higher at higher density with no added substrate (Arnold *et al.*, 2009).

1.2.4. Role of carbohydrate addition in aquaculture systems

Deterioration in the water quality particularly in the static larval rearing systems has been one of the major constraints in the hatchery production of *M. rosenbergii* (Murthy, 1996). Dissolved nitrogen compounds are the most important water quality parameters in closed systems (Valenti and Daniels, 2000). Since the *M. rosenbergii* larviculture is usually conducted in intensive systems maintaining high stocking densities, larvae might be exposed to high nitrite and ammonia concentration (Tomasso, 1994). According to Chen and Lei (1990), nitrogenous compounds such as ammonia and nitrite resulting from excess feed and excretory products, often cause deterioration of water quality in hatchery system. Ammonia and nitrites can cause severe mortalities in *M. rosenbergii larvae* (Valenti and Daniels, 2000). The most common method to over come this problem is the frequent water exchange. However, this method is laborious and often expensive (Thomson *et al.*, 2002) and may increase the risk of disease causing agents or may cause eutrophication in rivers and coastal waters (Ziemann *et al.*, 1992). Recirculatory hatchery systems have also been used to maintain low ammonia and nitrite levels by means of nitrification (Valenti and Daniels, 2000). However, this is rather expensive and during an imbalance in the process, nitrite levels may rise in water (Russo and

Thurston, 1991; Valenti and Daniels, 2000; Jensen, 2003). The use of external bio-filters which has been practiced successfully for years in hatcheries and nurseries, on the other hand, are quite costly, both in investment and in operation (Avnimelech, 2006). At the same time, good water quality is essential for ensuring survival and adequate growth rate (Boyd, 1990; Burford, 1997). Another hatchery system which can be adopted and the application of which is gaining importance recently, in intensive culture systems with high aeration and zero water exchange, is the Biofloc technology (BFT) otherwise known as the activated suspension technique (AST).

The organic residues serve as growth substrates for bacteria, leading to transition of the system to more and more heterotrophic dominance (Avnimelech, 2006). A proper manipulation of the microbial biomass enables to control water quality, mostly through the conversion of potentially toxic inorganic nitrogen species to microbial protein. Therefore BFT utilizes the co-culture of heterotrophic bacteria and algae under controlled conditions within the culture system (Kurup, 2009). Colt and Armstrong (1981) reported that accumulation of toxic inorganic nitrogen species such as NH_4^+ and NO_2^- in water is one of the major problems affecting the sustainability of shrimp farming. Ammonia-N is a highly toxic compound because it can easily cross most biological membranes and cause pH alterations which may reduce survival rates and impair various physiological mechanisms (Schmidt-Nielson, 1983; Campbell, 1991).

The low toxic inorganic nitrogen levels in the BFT ponds (Wahab *et al.*, 2003) and utilization of microbial cells as feed act as favorable factors for the augmented shrimp production (Avnimelech, 1999; Burford *et al.*, 2003, 2004). Extensive conditions and carbohydrate addition to the water column also resulted in a significant increase in the total heterotrophic bacteria (THB) count, together with observed lower total ammonia nitrogen (TAN) concentrations in water and sediment (Bronk, 2002; Hari *et al.*, 2004). Carbohydrate addition also resulted in a significant reduction in NO_2^--N concentration in the water column, which can be attributed to low availability of TAN as substrate for nitrification (Avnimelech, 1999; Hari *et al.*, 2004). The reduction in TAN and NO_2^--N levels observed in carbohydrate added treatments could only be attributed to the increased THB population, which immobilized TAN for the synthesis of new bacterial cells (Hari *et al.*, 2004).

The benefits of activated suspension technique has been described extensively for shrimp culture (Buford *et al.*, 2003, 2004; Hari *et al.*, 2004; Wasielsky *et al.*, 2006) and for finfish culture (Avnimelech, 1999; Milstein *et al.*, 2001; Serfling, 2006; Avnimelech, 2007). Where as there are only few works on its applicability and advantages of the technology to *M. rosenbergii* growouts (Asaduzzaman *et al.*, 2008; 2009) and apparently no work in fresh water prawn larviculture. The technique has also proved to be beneficial in the nursery rearing of *P monodon*, where in the use of carbon source and artificial substrates was found to positively influence growth and production of shrimp juveniles in addition to providing more favourable

water quality conditions irrespective of elevated stocking densities (Arnold *et al.*, 2009).

In aquaculture systems the most frequent limiting factor for heterotrophic bacteria is carbon whereas nitrogen and phosphate are seldom limiting (Leonard *et al.*, 2002). Avnimelech (1999) pointed out the use of the C/N ratio as a control element of toxic nitrogenous metabolites in aquaculture systems. Schneider *et al.* (2005) have observed that if carbon and nitrogen are well balanced in the solution, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass. Several carbohydrate sources are used in aquaculture systems to manipulate C/N ratio in the most beneficial way. Fontenot *et al.* (2007) have reported that the C:N ratio of 10:1 gave the best results in terms of maximum inorganic nitrogen removal from shrimp aquaculture waste water, by adding molasses or ammonium salt. On the other hand, at higher C/N ratios, the heterotrophic bacteria out-compete nitrifiers for available space and resources (Crab *et al.*, 2007). Immobilization of ammonium by heterotrophic bacteria is much rapid because the growth rate and microbial biomass yield per unit substrate of heterotrophs are a factor 10 higher than that of nitrifying bacteria (Hargreaves, 2006).

According to Gomez-Gil *et al.* (2000), it is important to provide larvae with a healthy environment that includes a beneficial microbial community. Moreover, eliminating bacteria from rearing tanks employing antimicrobials to control bacterial populations can lead to serious problems. The indiscriminate use of antimicrobial compounds in hatcheries and farms of

fresh water prawns and marine shrimp in India to control bacterial population (Karunasagar *et al.*, 1994; Abraham *et al.*, 1997; Sahul Hameed and Balasubramanian, 2000; Sahul Hameed *et al.*, 2003), can lead to the development of antibiotic-resistant microorganisms, multiple antibiotic resistance, resistance transfer to pathogenic bacteria and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens (Frappaolo and Guest, 1986). The microbial community inside the gut of some animals confers some degree of resistance to or protection against disease (Fox, 1988) and in natural populations of aquatic animals, the microflora of gut might reflect that of the aquatic environment (Gomez-Gil *et al.*, 2000). The administration of a mixture of bacterial strains positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *Vibrio harveyi* and white spot syndrome virus (Balcazar, 2003). Competitive exclusion of potential pathogenic bacteria effectively reduces or eliminates the need for antibiotic prophylaxis in intensive larviculture systems (Garriques and Arevalo, 1995). Bacterial antagonism is a common phenomenon in nature; therefore the microbial interactions play a major role in equilibrium between competing beneficial and potentially pathogenic microorganisms (Balcazar *et al.*, 2006). According to Al-Harbi and Uddin (2004), high bacterial abundance is not a disadvantage in *M. rosenbergii* larviculture systems. In their opinion, if the bacteria are not pathogenic, high bacterial abundance may indicate a potential for organic matter recycling, self cleaning potential and re-mineralization.

However, the utilization of microbial protein depends on the ability of the target animal to harvest the bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). Burford *et al.* (2004) suggested that 'flocculated particles' rich in bacteria and phytoplankton could contribute substantially to the nutrition of the *Litopenaeus vannamei* in intensive shrimp ponds. The contribution of biofloc to the nutrition of cultured shrimp, *P. monodon* (Hari *et al.*, 2004; Panjaitan, 2004) and tilapia (Avnimelech, 1999, 2007) are well documented. Although not nutritionally significant, unattached bacteria were found to be ingested by planktivorous carps (Rahmatullah and Beveridge, 1993) and the numbers ingested increased with time and bacterial concentration in the media. Moreover, the ingestion process, according to the authors, was probably by passive means.

The nutritional value of bioflocs, as well as their morphological characteristics, according to Schryver *et al.* (2008) is dependent on a large set of operational parameters in BFT aquaculture systems. Among them mixing intensity, dissolved oxygen, organic carbon source, organic loading rate, temperature and pH are the most important and are interrelated. The various advantages of the application of BFT to shrimp culture systems in the state of Kerala has been summarized by Kurup (2009) as

- It is the best means for the control of toxic inorganic nitrogen in water and for accumulating production of microbial protein by adjusting C/N ratio.

- It can convert uneaten nitrogen for being utilized to produce microbial protein rather than generating toxic component
- Microbial protein, the end product which is suspended in the system as microbial flocs can be utilized as feed by shrimps
- The level of protein utilization is doubled in microbial reuse system
- The dense heterotrophic microbial biomass decreases the outbreak of microbial diseases and finally
- The technology enables high yield under environmentally and economically sustainable system.

Chapter 2

SUBSTITUTION OF BRINE SHRIMP NAUPLII WITH *TETRASELMIS CHUII* IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII*

2.1 Introduction

Microalgae are an essential food source in commercial rearing of various animals including larval stages of many crustacean species. They are an indispensable component in the feeding of penaeid larvae. In addition, microalgae are used in the mass culture of several zooplankters which in turn serve as food for larval and early juvenile stages of crustaceans and fish.

Tetraselmis sp is a motile Parsinophyte, moving very actively in the rearing medium. They are widely used for feeding penaeid larvae, bivalve mollusc larvae and post larvae, *Artemia* and marine rotifers. *Tetraselmis chuii* is an alga extensively used in the feeding of penaeid larvae. It is having a protein content of 31 % on a dry weight basis (Lavens and Sorgeloos, 1996). *Macrobrachium rosenbergii* larvae do not actively search for food (New, 2002) and capture food by accidental collision (Moller, 1978). Gulbrandsen *et al.* (1996) suggested the use of larger micro algae like *Tetraselmis* sp, which is ingested by larval fish, may provide critical micronutrients. However, the use of *T. chuii* as a feed in the larviculture of giant fresh water prawn has never been evaluated. Against these backgrounds, a preliminary study was conducted to assess the effectiveness of the alga *T. chuii* (which is motile, nutritious and widely used in the larviculture of many crustacean and fish species) in replacing the highly priced and sometimes scarce *Artemia* nauplii which is considered as an indispensable component in the larval rearing of the giant fresh water prawn.

The objectives of the present experiment were to

1. Examine the usefullness of alga *Tetraselmis chuii* in fully or partially replacing *Artemia* nauplii in the larviculture of the giant fresh water prawn.
2. Reduce the cost and increase economic viability of post larval production by reducing the quantity of *brine* shrimp nauplii.

2.2 Materials and Methods

The experiment was carried out following completely randomised design with five overnight feeding regimes (including the control) with three replicates each (Table 2.1).

2.2.1 Brood stock management

Berried prawns with greyish black eggs were procured from the wild and transported to the hatchery in wide mouthed black plastic cans of 50 l capacity. The brooders were disinfected (New and Singholka, 1985) and kept in well aerated brackish water having a salinity of 5 ppt in 150 l FRP tanks. Feeding was not done as the berried prawns with black eggs invariably hatched by the next day morning.

2.2.2 Algal culture and hatching of *Artemia* Cysts

The alga *Tetraselmis chuii* was grown using the Modified Walne's medium (Lavens and Sorgeloos, 1996), the composition of which is given in Table 2.2. The stock culture was grown at a salinity range of 20 – 25 ppt in 3 liter Hoffkins flask. Before feeding the prawn larvae, the salinity was gradually reduced to the range of 12 – 14 ppt in mass culture media maintained outside.

For *Artemia* nauplii (also mentioned as BSN) production, OSI brand *Artemia* cysts were used. The required quantity of cysts were weighed, hydrated, decapsulated using commercial grade sodium hypochlorite and kept in 25 ppt saline water with vigorous aeration for hatching (approximately I g cyst/ I sea water). Artificial light was also provided for hatching of the cysts. The nauplii hatched in 18 – 24 hours. They were harvested and washed thoroughly before feeding.

2.2.3 Larval rearing

The experiment was carried out using clear water method of larval rearing in 100 I FRP tanks with 50 I rearing water. The water used for the experiment was treated following New (2002). Larvae were stocked at the rate of 100 numbers / I. They were fed with *Artemia* nauplii or algae (depending on the treatment) on the second day of hatching in the morning and evening. From the third day onwards feeding with live feed was done only in the evening (5 PM). The inert feed used in the experiment was egg custard, the composition of which is given in Table 2.3 (Kurup, 2003). The ingredients were mixed well in an electric mixer and steam cooked for about 20 minutes. After cooling it was stored in the refrigerator and used whenever required. Egg custard was fed *ad libitum* to the larvae by dispersing it in the rearing water evenly using dropper, starting on the third day when it was done only once in the morning. On the fourth day the feeding frequency was increased to two times and there after to four times daily at 8 30 AM, 10 30 AM, 12 30 PM and 3 PM after passing through the sieves to get the required particle size (Kurup, 2003, Table 2.4). Before dispersing the custard feed, the aeration was turned

off in order to enable surfacing of the larvae. *Artemia* was given at the rate of 4 numbers/ ml (100 %) in the control and the alga *T. chuii* was given at the rate of 1 lakh cells / ml in the treatment with 100 % alga (T4). The quantity of live feed supplied in the other treatments were adjusted accordingly. Siphoning of the waste and water exchange (20 - 50 %) was done daily before the live feed was given in the evening.

2.2.4 Water quality and evaluation of various treatments

The daily water quality parameters measured included salinity and temperature which was measured during the morning hours using Atago refractometer and mercury thermometer respectively. pH and dissolved oxygen were estimated twice weekly using pH meter (Eutech Cyberscan model 510) and Winklers method (APHA, 1995) respectively.

The final evaluation of experimental diets was done based on the post larval production, time required for the larvae to metamorphose to subsequent stages and their relative survival. Mean larval stage was used to find out the development of larvae. Mean larval stage was calculated using the formula, MLS = $\sum$(S x Ps) where MLS is the mean larval stage, S is the larval stage number and Ps is the proportion of larvae at stage S (Lovett and Felder, 1988).The larval stages were identified following Uno and Kwon (1969). The larval progression and relative survival of larvae in each tank was estimated every fifth day. The estimation of percentage composition of the different larval stages and survival continued till 25th day when considerable number of post larvae started appearing in all the tanks and it was difficult to draw uniform samples (Alam *et al.*, 1993a). In the mean time, daily observations were made

in the tanks to assess the settlement of post larvae from 20th day of the experiment and the appearance of first post larva in each tank was noted down. On completion of rearing cycle (when more than 95 % of larvae metamorphosed to PL), total length (from the tip of the rostrum to the end of the telson) and wet weight of 50 post larvae which were randomly taken from each tank was also measured as an additional parameter for assessing the efficiency of different feeding regimes.

2.2.5 Statistical analysis

Data analysis was done by one-way ANOVA using SPSS 16. Significant differences between the treatments were determined using Duncan's multiple range test (DMRT) ($P < 0.05$). Data expressed in percentages were normalised by arcsine transformation (Zar, 1984). However non transformed data is given in the table (Table 2.7).

2.3 Results

The water quality parameters observed in all the experimental tanks (Table 2.5) were well within the acceptable range (New and Singholka, 1985; New, 2002) for the larval rearing of *M. rosenbergii*. Temperature was found be to fluctuating between 27 to 30^{0}C in the present experiment. The optimum temperature for larval rearing of *M. rosenbergii* is in the range of 28 to 31^{0}C (New, 2002). pH values ranged between 7.64 and 8.16. Salinity fluctuated between 12 and 14 ppt while dissolved oxygen varied between 6.49 to 7.39 ppm during the larval rearing period. Slightly alkaline waters (pH 7 to 8.5) is suggested by Valenti and Daniels (2000)) and salinity around 12 ppt and dissolved oxygen near saturation level have been suggested as optimal by

New (2002) for the larval rearing of *M. rosenbergii*. As recommended by the above authors, the physico chemical parameters of the rearing water was maintained stable without fluctuation through out the rearing period.

The highest mean survival of 24.4 ± 0.87 % was recorded in the control followed by the treatment T1 wherein the mean survival was found to be 22.6 ±1.5 %. No significant difference was observed among the control and T1 with respect to mean survival.

The treatment T4 in which there was a complete substitution of Brine shrimp nauplii (BSN) by the algae, no larvae were found to progress beyond 3rd larval stage and most of them were found dead by 3rd and 4th days with a complete mortality on the 5th day of the experiment. The larvae were found to be generally weak by the third larval rearing day. Hence, for convenience of interpretation, data pertaining to this treatment was omitted from the graphs and final production tables.

Although the duration for settlement of 95 % of post larva was observed as 31 days in both control and T1 and the MLS showed significant variation ($P<0.05$) among them only on the 5th day of the experiment, the appearance of the first post larva was delayed in T1 (average of 26 days) and it took significantly more number of days than in the control where it was 24 days. The final mean length and wet weight of the larvae were also significantly lower ($P<0.05$) in T1 than in the control. The MLS recorded in the different treatments during the experimental period is presented in Table 2.6 and the final percentage survival, duration of the larval rearing period, mean length and the wet weight of the larvae are depicted in Table 2.7.

The treatments T2 and T3 with 50 % and 75 % replacement of BSN respectively recorded average survival of 17.96 % and 15 % which was significantly lower ($P<0.05$) than T1 and the control. The duration of the experiment (settlement of more than 95 % post larvae) was longer and took an average of 32 and 34 days in treatments T2 and T3 respectively. The appearance of the post larva was also delayed (28 days in T2 and 29 days in T3) and was significantly different from that of the control and T1. The total length of the post larvae in T2 and T3 was also significantly lower than in the control, but was not different from that in T1. The wet weight of the post larvae recorded in T2 was comparable with in T1 and significantly lower than in the control. Where as, the wet weight of post larvae in T3 was significantly lower than T1 and the control ($P<0.05$).

2.4 Discussion

As seen from Tables 2.6 and 2.7 and Fig. 2.1 and Fig. 2.2, the control supplied with 100 % brine shrimp nauplii (BSN) recorded significantly faster growth and better survival than all the other treatments. This is indicative of the poor performance of all other treatments due to the reduction in the BSN supplied in various treatments. The survival and MLS of larvae in T1 was not significantly different from the control, however it was definitely lower in T1 when compared to the control.

Microalgae has been widely used as a feed in the larval rearing of fishes crustaceans and molluscs. *Tetraselmis* sp. is used as a feed for penaeid larvae, bivalve mollusc larvae and post larvae, abalone larvae and zooplankton like BSN and marine rotifers (Lavens and Soegeloos, 1996).

However, generally the larvae of *M. rosenbergii* larvae is judged primarily as carnivorous as indicated by the higher levels of enzymes trypsin and esterase in the early larval stages (Kamaruddin *et al.*, 1994). Jones *et al.* (1993) also reported the first feeding prawn larvae as carnivorous rather than herbivorous based on enzyme studies. Cohen *et al.* (1976) concluded that *M. rosenbergii* larvae do not feed directly on algae (*Tetraselmis* sp) and that the role of algae in larviculture is primarily to purify the medium by assimilating ammonia. However, the authors conducted the experiment by introducing prawn of particular age into ^{14}C labeled algal cultures. Cook and deBaissac (1994) also reported the inability of the prawn larvae to derive nutrition from the algae by investigating the fatty acid composition of larvae reared in green and clear water. No significant difference in the fatty acid profiles was detected in the larvae reared in the different rearing media and it was concluded by the authors that phytoplankton contributed little to larval energy metabolism. Joseph (1977) was also not able to perceive any fatty acid assimilation by the larvae from algal populations in *M. rosenbergii* rearing tanks. Contrary to this, Thresiamma *et al.* (2006) has stated that the micro alga *Isochrysis galbana* was probably acting as a convenient feed to both prawn larvae and BSN which resulted in a higher survival in the treatments which used the micro alga when compared to the control in which no alga was used.

However, based on the results of the present experiment (all the larvae perished in the treatment T4), it can be concluded that the prawn larvae were not able to derive considerable nutrition from the micro algae especially during the early stages. The prawn larvae might have ingested the *T. chuii* cells, but

they were not able to assimilate considerable nutrition from the algae and as a result, all of them died in spite of feeding the larvae with egg custard from the third day of larval rearing. The surviving larvae in the treatment without BSN (T4) were found to be generally weak by the third day followed by considerable mortality leading to 100 % mortality on the fifth day. This result of the present study is concurring with that of Sick and Beaty (1975) who could not demonstrate a nutritional effect of phytoplankton added to the fresh water prawn larval rearing systems.

While conducting a study on the larval rearing of green back flounder, *Rhombosolea tapirina* using turbid green water constituted by *Tetraselmis suecica*, Shaw *et al.* (2006) reported higher rotifer consumption rates than clear water particularly at low prey densities enabling reduction in the feeding ration. Barahona- Fernandes (1982) reported a two fold increase in the survival of larvae of marine fish, *Dicentrarchus labrax* when the same micro alga (*T. suecica*) was added to the larval rearing medium. This was observed in spite of daily water exchange which considerably reduced the algal densities. According to the author, *T. suecica* has the added advantage of avoiding algal settlement in green water culture systems during times of low aeration and reduced algal densities due to its natural motility. While studying the relationship between algae and larval nutrition, Maddox and Manzi (1976) reported that all the seven algal species used for the larviculture of *M. rosenbergii* were useful in enhancing the post larval production, among them, the diatom *Phaeodactylum tricornutum* was found to be the most effective one.

On the contrary, no such beneficial effect of the algae could be demonstrated in the present experiment. Since the larval rearing of *M. rosenbergii* was carried out in the present experiment using the clear water system with regular siphoning of waste and water exchange, the algae were not found grow to make the water green. The concentration of the algae used in the present study in treatment T4 was comparable to that in penaeid larval culture tanks where a density of 80,000 to 100000 cells of *Tetraselmis* sp. /ml was maintained (FAO, 2007). In another feeding regime for penaeid larvae using a combination of *Chaetoceros neogracile* and *T. chuii* (Lavens and Sorgeloos, 1996), although, the concentration of the latter used as feed for mysis stages (M1 to M3) varied from 20,000 to 30,000 cells/ml, *Chaetoceros neogracile* was used at a higher density of 50,000 to 1 lakh cells / ml, increasing the total number of algae supplied to the penaid mysis above I lakh cells/ml. Cohen *et al.* (1976) used a concentration of 10^6 cells/ ml for ^{14}C labelled algal medium, where as the algal density was maintained between 1.1 to 1.4 x 10^5 cells /ml by Thresiamma *et al.* (2006) in the larviculture of *M. rosenbergii.* Compared to this the density of algal cells fed to the prawn larvae in the present study was on the lower side (25,000, 50,000 and 75,000 cells /ml of rearing medium in T1, T2 and T3 respectively). In addition some of the algal cells might have been lost while conducting cleaning and water exchange, still lowering the available number of algae.

Moreover, the results of the present study fails to establish various advantages of algae in the larval rearing of fishes and crustaceans like enhanced survival and growth, resistance to diseases, enrichment of live feed

as claimed by many authors (Barahona-Fernandes, 1982; Naas *et al.*, 1992; Austin *et al.*, 1992; Reghunathan and Wesley, 2004; Thresiamma *et al.*, 2006; Palmer *et al.*, 2007). Most of all, the controlled production of micro-algae is a complex and expensive procedure (Lavens and Sorgeloos, 1996).

It would thus appear that *M. rosenbergii* is unable to utilize algae as food especially during the early larval stages and the lower density at which the algae were supplied to the prawn larvae. It is worth reporting that even the comparatively larger size and the natural motility of the alga *T. chuii* was not utilized by the larvae of *M. rosenbergii* in the present experiment due to the reasons discussed above.

2.5 Conclusion

The results of the present study showed that the *M. rosenbergii* larvae were not able to derive considerable nutrition from the alga *T. chuii*, especially during the early larval stages and when it is fed at lower densities. Though not significantly different from the control, a lower post larval production was recorded in TI (with 75 % *Artemia* nauplii) without considerable reduction in the cost of production since *T chuii* is also a live feed and additional facilities are required for maintaining its culture. Hence the use of the alga *T. chuii* as feed for the *M. rosenbergii* larvae is not recommended.

Table 2.1 Feeding regimes in different treatments in the larviculture of *M. rosenbergii*

Treatments	live feed
T1	75 % A + 25 % T
T2	50 % A + 50 % T
T3	25 % A + 75 % T
T4	*Tetraselmis chuii* only
Control	*Artemia* nauplii only

Table 2.2 Composition and preparation of Walne's medium

Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric chloride ($FeCl_3$)	0.8 g[a]
Manganous chloride ($MnCl_2$, $4H_2O$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA[b], di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate (NaH_2PO_4, $2H_2O$)	20.0 g
Sodium nitrate ($NaNO_3$)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water[c]	Heat to dissolve
Solution B	
Zinc chloride ($ZnCl_2$)	2.1 g
Cobaltous chloride ($CoCl_2$,6 H_2O)	2.0 g
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$, $4H_2O$)	0.9 g
Cupric sulphate ($CuSO_4$, $5H_2O$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml fresh water[c]	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B_1	0.2 g
Solution E	25.0 ml
Make up to 200 ml with fresh water[c]	
Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate (Na_2SiO_3, $5H_2O$)	40.0 g
Make up to 1 litre with fresh water[c]	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1 g
Make up to 250 ml with fresh water[c]	
Solution F (for culture of *Chroomonas salina* - used in addition to solutions A and C, at 1 ml per liter of culture)	
Sodium nitrate ($NaNO_3$)	200.0 g
Make up to 1 litre with fresh water[c]	
(a) Use 2.0 g for culture of *Chaetoceros calcitrans* in filtered sea water;	
(b) Ethylene diamine tetra acetic acid;	
(c) Use distilled water if possible.	

Table 2.3 Composition of egg custard fed to larvae of *M rosenbergii*

Ingredients	Quantity
Milk powder	15 g
Egg yolk	1
Lecithin	1%
Squid oil + cod liver oil	1.5%
Vitamin C	100 mg/kg
Red colour	1 pinch
Distilled water	3-4 ml

Table 2.4 The particle size of custard feed given to the *M. rosenbergii* larvae

Mesh size of the sieve (µ)	Larval stage
200-300	II - IV
300 - 400	IV – V
400 – 500	VI – VIII
500 – 600	IX – X
650 – 1000	XI

Table 2.5 Average ±s.d of various water quality parameters recorded during the experimental period in the larviculture of *M rosenbergii*.

Parameters	Treatments			
	T1	T2	T3	C
Temperature (^{0}C)	28.01 ± 1	28.06 ± 0.96	28 ± 0.98	28.04 ± 0.94
pH	7.99 ± 0.13	8 ± 0.11	7.87 ± 0.09	7.89 ± 0.12
Salinity (ppt)	13.41 ± 0.55	13.54 ± 0.56	13.57 ± 0.57	13.34 ± 0.54
Dissolved oxygen (mg/l)	7.03 ± 0.18	7.06 ± 0.16	6.99 ± 0.16	7.01 ± 0.21

Table 2.6 Mean Larval Stages in different treatments in the larviculture of *M. rosenbergii*

Treatments	Days				
	5	10	15	20	25
T1	3.13 ± 0.06^{b}	4.93 ± 0.06^{a}	6.57 ± 0.06^{a}	7.87 ± 0.06^{a}	10.07 ± 0.06^{ab}
T2	2.8 ± 0.1^{c}	4.66 ± 0.15^{b}	6.37 ± 0.15^{ab}	7.77 ± 0.06^{ab}	9.93 ± 0.06^{b}
T3	2.63 ± 0.06^{d}	$4.6 \pm .1^{b}$	6.17 ± 0.25^{b}	7.63 ± 0.15^{b}	9.7 ± 0.1^{c}
Control	3.33 ± 0.06^{a}	5.1 ± 0.1^{a}	6.63 ± 0.12^{a}	7.9 ± 0.1^{a}	10.1 ± 0.1^{a}
Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)					

Table 2.7 Production details of *M rosenbergii* post larvae in various treatments in the larviculture carried out to evaluate the substitution of BSN with alga *T chuii*

Treatments	Stocking density(larvae/litre)	Mean % survival	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
T1	100	22.6 ± 1.5^{a}	26 ± 1^{b}	31^{a}	8.82 ± 0.38^{b}	9.11 ± 0.4^{bc}
T2	100	17.96 ± 1.57^{b}	28^{c}	32^{b}	8.76 ± 0.44^{b}	9.07 ± 0.39^{cd}
T3	100	15 ± 1.4^{c}	29^{d}	34^{c}	8.64 ± 0.43^{b}	8.87 ± 0.42^{d}
Control	100	24.4 ± 0.87^{a}	24^{a}	31^{a}	9.12 ± 0.55^{a}	9.36 ± 0.56^{a}
Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)						

Fig.2.1 Mean Larval Stages in different treatments in the larviculture of *M. rosenbergii* carried out for substituting BSN with the alga *T. chuii.*

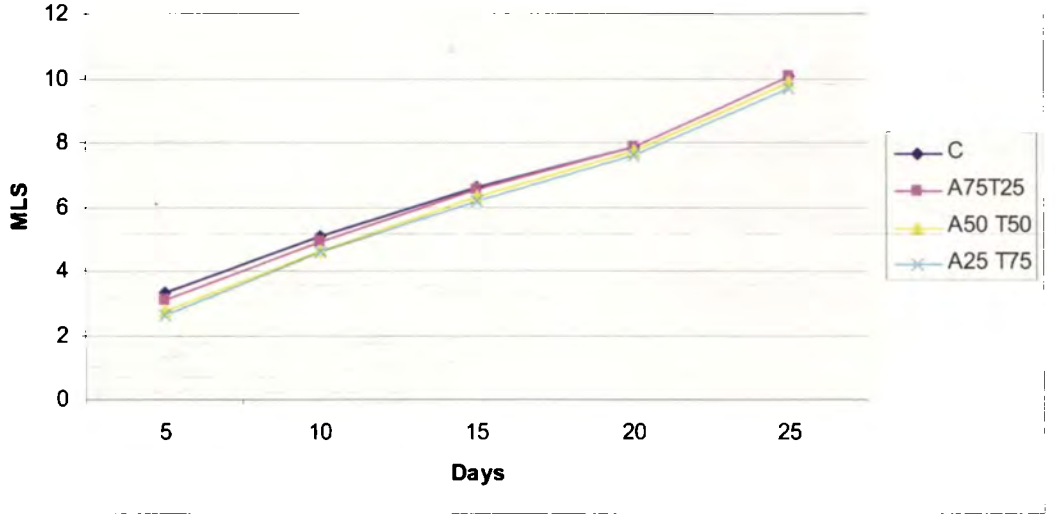
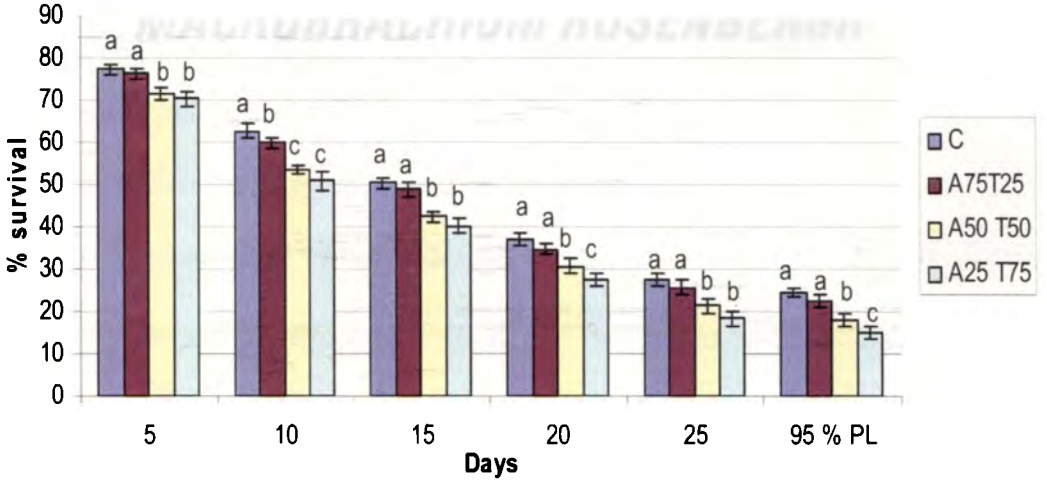


Fig 2.2 Mean percentage survival in different treatments in the larviculture of *M. rosenbergii* carried out for substituting BSN with the alga *T. chuii.*



Vertical lines represent s.d. Means in each day with identical letters are not significantly different.

Chapter 3

SUBSTITUTION OF BRINE SHRIMP NAUPLII WITH CHIRONOMID LARVAE IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII*

3.1 Introduction

Macrobrachium rosenbergii, the giant fresh water prawn, with the trade name 'Scampi' and locally (in Kerala) known as 'Aattu konchu' or 'Kuttanadan konchu', is the most preferred prawn for fresh water aquaculture. There has been a growing interest in the farming of this species in India due to its faster growth rate and good market demand. The seed production of *Macrobrachium rosenbergii* was successfully carried out in India during late 1990's (Sebastin and Nair, 1995; Kurup *et al.*, 1998). But the heavy dependence on *Artemia* for the larval rearing of Scampi continues to be a problem in its larviculture due to the high cost of *Artemia* cyst and scarcity often observed in its availability. Too much dependence on *Artemia* has been identified as a major constraint in the expansion of *M. rosenbergii* hatcheries (New, 1990). Cost of *Artemia* cyst has been reported (New, 2002) to account for more than 50 % of the variable costs of the commercial fresh water prawn hatcheries. Thus finding out a suitable substitute to replace *Artemia* partially or completely will substantially reduce the operational cost of fresh water prawn hatcheries functioning in the country. The other disadvantage in the use of the brine shrimp nauplii is the variation in its nutritional value especially when strains from different geographical sources are used for shrimp and prawn species (Leger *et al.*, 1986). Very significant differences in performance of *Artemia* were reckoned with different batches from the same geographic *Artemia* sources, containing different amounts of EPA (Eicosapentaenoicacid) (Leger *et al.*, 1985). The yields obtained in terms of growth and survival of mysid shrimp, *Mysidopsis*

bahia were proportional to the amount of EPA. Moreover, the bacterial degradation of the brine shrimp exuvia and shed cyst capsules accumulate in culture vessels, which results in fouling of water and entangling of larvae, thereby resulting in increased larval mortalities (Lovett and Felder, 1988).

In this context an attempt was made in the present experiment to substitute *Artemia* with an insect larva – the *Chironomous* which is commonly known as the 'blood worm'. *Chironomous* is an insect belonging to the Order Diptera of Class Insecta of Phylum Arthropoda. The larva of this insect is a staple food item of young ones of many carnivorous fishes besides serving as an important food item for many fishes and cultured invertebrates (Yusoff *et al.*, 1996; Fernando, 1994; Tidewell *et al.*, 1997; Wais *et al.*, 1999). The chironomid larva is also reported to have a protein content of 52.1 % on a dry weight basis (Tidwell *et al.*, 1997). According to De La Noue and Choubert (1985) chironomid larvae are excellent source of protein whereas Mclarney *et al.* (1974) stated that they are rich in lipids, vitamins and minerals. More over, the use of the larva has been reported in the nursery rearing of *M. rosenbergii* (Prasad, 1996). Besides this, blood worm, have the advantage of culturing it using a very simple and inexpensive method.

Thus the objectives of the present study were

1. To evaluate the effectiveness of the *Chironomous* larva in substituting *Artemia* nauplii fully or partially in the larviculture of *M. rosenbergii* and
2. To reduce the cost of post larval production.

3.2 Materials and Methods

3.2.1 Brood stock management

Berried prawns with greyish black eggs were collected from the wild and transported to the hatchery in wide mouthed black plastic cans. They were disinfected (New and Singholka, 1985) and kept in 150 l FRP tanks with aerated brakish water having a salinity of 5 ppt. The berried prawns with black eggs invariably hatched by the next day morning.

3.2.2 Live feed culture

The *Chironomous* larvae were reared using chopped cabbage leaves immersed in trays filled with water. The *Chironomous* flies were attracted to the decaying vegetable leaves to lay eggs and the larvae were ready in a week's time. For feeding the prawn larvae, the blood worms were collected, washed and transferred to clear water in beakers. Before feeding, the worms were taken out of water, sliced using a sharp knife and passed through sieves to get the required particle size. Large number of trays was kept to attract the flies so as to ensure a steady supply of worms through out the period of the experiment.

For *Artemia* nauplii production, OSI brand *Artemia* cysts were used. The required quantity of cysts were weighed, hydrated, decapsulated using commercial grade sodium hypochlorite and kept in 25 ppt saline water with vigorous aeration for hatching (approximately l g cyst/ 1 l sea water). Artificial light was also provided for hatching of the cysts. The cyst hatched in 18 – 24 hours. They were harvested and washed thoroughly before feeding.

3.2.3 Larval rearing

The larval rearing was carried out using clear water method in 100 l FRP tanks with 50 l rearing water. The water used for the experiment was treated following New and Singholka (1985). Larvae were stocked at the rate of 100 numbers / l. The experiment was carried out following completely randomised design with five overnight feeding regimes with three replicates for each treatment (Table 3.1). The larvae were fed daily with *Artemia* or blood worm particles of particular size in the morning (8.30 AM) and evening (5 PM) from the second day onwards. From the third day onwards the live feed was given only in the evening. Egg custard (Kurup, 2003) was fed only once in the morning on the third day, followed by two times on the fourth day and four times daily at 8 30 AM, 10 30 AM, 12 30 PM and 3 PM after passing through the sieves to get the required particle size (Kurup, 2003) from the fifth day of the experiment. The blood worm particles were passed through 400 µm and 600 µm mesh size sieves before feeding to stages I – IV and for V to post larva respectively (Alam *et al.*, 1993a). *Artemia* was given at the rate of 4 numbers/ ml in the control and the percentage replacement was done on wet weight basis. Siphoning of the waste and water exchange (20 - 50 %) was done daily before the live feed was given in the evening.

Salinity and temperature was measured daily during the morning hours using Atago refractometer and mercury thermometer respectively. pH, dissolved oxygen and ammonia were estimated twice weekly using pH meter (Eutech Cyberscan model 510), Winklers method (APHA, 1995) and alkaline phenol method (Grasshoff *et al.*, 1983) respectively.

The larval stages were identified following Uno and Kwon (1969). The larval progression and relative survival of larvae in each tank was estimated every third day. Thus the final evaluation of experimental diets was done based on the post larval production, time required for the larvae to reach each stage and their relative survival. Mean larval stage was used to find out the development of larvae. Mean larval stage was calculated using the formula, MLS = $\sum$(S x Ps) where MLS is the mean larval stage, S is the larval stage number and Ps is the proportion of larvae at stage S (Lovett and Felder, 1988). The estimation of percentage composition of the different larval stages and survival continued till 27th day when considerable number of post larvae started appearing in all the tanks and it was difficult to draw uniform samples (Alam *et al.*, 1993a). Mean while all the tanks were observed daily for the settlement of post larvae after 20 days and the days on which the first post larva appeared in each tank was noted down. The experiment was terminated when more than 95 % larvae metamorphosed to post larvae in the larval rearing tanks. The percentage mortality compared to the initial number of larvae was calculated from the percentage survival values and was used as an additional criterion for the assessment of the various treatments. On completion of the experiment, 50 post larvae were randomly taken from each tank to measure the total length (from the tip of the rostrum to the end of the telson) and wet weight.

3.2.4 Statistical analysis

Data analysis was done by ANOVA using SPSS 16. Significant differences between the treatments were determined using Duncan's multiple

range test (DMRT) ($P<0.05$). Data expressed in percentages were normalised by arcsine transformation (Zar, 1984). However, non transformed data is given in the tables (Tables 3.4; 3.9; 3.10).

3.3 Results

The values of various water quality parameters recorded in the different treatments throughout the rearing period is given in Table 3.2. All the parameters were found to be within the recommended range for larval rearing of the giant fresh water prawn (New and Singholka, 1985). The lowest and highest temperatures recorded during the experiment were 26^0C and 29^0C respectively. Salinity in all the treatments was found to vary between 11 and 14 ppt where as pH ranged between 7.61 and 8.03. Dissolved oxygen fluctuated between 6.79 and 7.33 mg/ l and ammonia between 2.1 and 2.42 µg/ l.

The final production details of the experiment are presented in Table 3.4. The highest mean survival of 32.69 ± 0.97 % was recorded in the control and it was significantly higher than that observed in all other treatments (Figure 3.1, Table 3.4) whereas the lowest of 10.6 ± 1.36 % was observed in T4 with no *Artemia* nauplii given throughout the experiment. The final production of post larvae in the different tanks was found to be directly proportional to the quantity of *Artemia* supplied. In other words, as the percentage of *Artemia* nauplii supplied increased from 25 to 100, the survival increased from 10.6 ± 1.36 % to 29.94 ± 1.98 %. The larval progression estimated in terms of mean larval stage (Table 3.3) also showed that the larvae in the control and the treatment T1 (A75C25) were metamorphosing at

a faster rate (higher mean larval stage) and did not differ significantly at any stage of the experiment.

Appearance of the first post larva was noted on the 25th day in T1 and the control, whereas it took 28 days for settlement of first post larva in T4. The average time taken for completion of the experiment was 31 days in the control and T1 when more than 95 % of the larvae settled as post larvae. However it was 32.3, 33.3 and 35.33 days respectively for T2, T3 and T4. The highest mean length and wet weight were also recorded in T1, closely followed by the control with no significant difference between them.

The percentage mortality in various treatments is depicted in Fig. 3.2. The lowest mean mortality among all the treatments and the control was observed after the period of 9-12 days in T1 with 25 % replacement of *Artemia* nauplii. It is also clearly visible that the mortality rates in all the treatments were comparable with the control after the critical period of 9-12 days (Fig. 3.2).

3.4 Discussion

The direct relation between survival and the quantity of *Artemia* nauplii in concert with the rate of larval metamorphosis observed in terms of mean larval stage (MLS) is a strong evidence which shows that *Artemia* nauplii are an indispensable part of the larval rearing of *Macrobrachium rosenbergii* and a substitution of the quantity of *Artemia* nauplii even by 25 % has brought down the survival rate by more than 2 %. According to Lavens *et al.* (2000), newly hatched *Artemia* nauplii is the most successful starter diet for the *M. rosenbergii* larvae. Lovett and Felder (1988) failed to establish the rotifer

Brachionus plicatilis as a suitable substitute or dietary supplement for *Artemia* nauplii in the larviculture of *M. rosenbergii*. Among the three diets experimented, namely *Tubifex* worm, *Lamellidens* meat and a combination of BSN and prepared feed, Mohanta and Rao (2000) found that a higher production could be attained by using a combination feed of brine shrimp nauplii and prepared feed.

In spite of the highest survival being achieved in the control the percentage mortality was the lowest in T1 after 12 days of hatching (Fig. 3.2). Further more, the percentage mortality in the entire blood worm fed treatments were considerably low and did not differ significantly with the control after the 12^{th} day (Table 3.9). Agard (1999) reported that the effective feeding of *M. rosenbergii* larvae start at stage VI when the yolk reserves disappear and the digestive tract is completely developed. Moreover, the prawn larvae are reported (Deru, 1990) to have a poorly developed larval gut until stage V to VI, they are having a small hepatopancreas and lack anterior midgut diverticulae due to which digestive enzyme production in early larval stages may be low and they have to rely on exogenous prey enzymes which may be supplied by the live food source (Jones *et al.*, 1993). Additionally, during the early stages, the prawn larvae are more or less carnivorous and their food preferences change to more of an omnivorous nature from stage VII onwards (Barros and Valenti, 1997). The heavy mortality recorded in the *Chironomous* fed treatments before the 12^{th} day of the experiment can therefore be attributed to the inefficient utilisation of the blood worm particles by the larvae during the early stages. Most larvae attained a MLS higher than V by the 12^{th} day in the

present experiment (except T4 with a MLS of 4.83). Consequently, after the period of 9 to 12 days the larvae were capable of utilising the blood worm particles in a more effective way when most of the larvae metamorphosed to stage V or higher. Kamarudin *et al.* (1994) and Kumlu and Jones (1995) have reported an increased acceptability of inert diets from stage VI onwards and after due to development of digestive tract and increase in enzyme activity. It may therefore be inferred that the *Chironomous* larvae cut into small pieces behaved more or less like inert diets and were found to be acceptable to the prawn larvae after V^{th} to VI^{th} larval stages. Nair *et al.* (2007), experimenting with the use of Cyclop-eeze as a substitute for *Artemia* nauplii in the larval rearing of *M. rosenbergii* observed that the prawn larvae were feeding on the former which was very similar to feeding on a wet particle feed.

The supply of exogenous enzymes and the motility are considered as two important qualities of *Artemia* nauplii that has made it a highly preferred food item of *M. rosenbergii* larvae especially during the early larval stages. The *M. rosenbergii* larvae are non active hunters during the early stages (Daniels *et al.*, 1992) and capture food by chance encounter (Moller, 1978). So they prefer a live zooplankton with good concentration in the rearing water during the early stages. Besides in *M. rosenbergii* mechanoreception is reported (Barros and Valenti, 1997) as the only mechanism to detect food during the early larval stages (II to VI) whereas stage VII larvae and onwards are more capable of exploring food resources from the medium and also present increased nutritional demand (Barros and Valenti, 2003a). The blood worm particles were neither moving on their own to collide more frequently

with the larvae as *Artemia* nauplii nor could they supply the needed exogenous enzymes. This could be the an additional explanation for the poor performance of blood worm particles during the early stages, that is up to stages V to VI or less in the present investigation.

Alam *et al.*(1993a), working on substitution of *Artemia* nauplii with *Moina micrura*, found out that the ingestion of *Moina* was more efficient after VI to VII larval stages. Also a 50:50 combination of *Artemia* and *Moina* was found useful for a faster metamorphosis of the larvae in a later period than larvae fed *Artemia* alone. This was attributed to the inefficiency of the prawn larvae in capturing larger sized *Moina* during the early stages due to which *Artemia* performed well while during the later stages the 50:50 combination of *Moina* and *Artemia* conferred the benefits of both size classes as well as the nutritional benefits of mixed diets. However, in the present study, the larvae in the control supplied with *Artemia* nauplii through out the experiment performed well and resulted in the highest post larval production than the four treatments. Nevertheless, it could be observed (Table 3.3) that the larvae in T1 were progressing in a comparable way with the control, as there is no significant difference observed between T1 and the control with respect to the mean larval stages. More over, it is worth reporting that the mean larval stages of the larvae in T1 were slightly higher than in the control towards the completion of the experiment. The appearance of the first post larvae and the settlement of 95 % of post larvae occurred on the same day (25^{th} and 31^{st} day respectively, Table 3.4) both in the control as well as T1. Besides, the mean total length and wet weight of the larvae fed 75 % *Artemia* nauplii and 25 %

Chironomous larvae were found to be slightly superior than in the control. This implies that there is a positive effect of combined feeds as the larvae grow bigger. The larvae in the treatment T1 had the supplementary advantage of having blood worm particles in addition to considerable number (3/ml) of *Artemia* nauplii and egg custard. Many researchers (Valenti *et al.*, 1998; Pitipornchai, 1998; Daniels *et al.* 1992) have reported that *Artemia* nauplii do not provide all the nutritional requirements of *M. rosenbergii* for its late larval stages and it has to be supplemented with an inert diet. Further more, several workers (Gruffyd and Beaumont, 1972; Lewis, 1975; Helm, 1977; Stone, 1988) have reported a beneficial effect of mixed diet on marine invertebrate larvae. In the present study the decreased rate of mortality towards the later stages, in the larvae supplied with a mixed diet may be due to the increased nutritional demands of the later stages (Barros and Valenti, 2003a) which could not have been provided by *Artemia* nauplii alone.

The larvae in treatments T2 and T3, though had the benefit of having a combination of all the three types of feeds, could not perform well in any aspect. It appears that they were not able to get sufficient nutrition during the early larval stages due to comparatively lower quantities of *Artemia* nauplii fed (2 and 1/ ml respectively) to them than T1 or control which might have resulted in an overall poor performance in the survival and larval metamorphosis. The larvae in the treatment T4 fed only with *Chironomous* larvae and egg custard showed significant mortality during the early stages (up to 12^{th} day which corresponds to a mean larval stage of 4.83± 0.06). Despite the fact that the percentage mortality in T4 and control were not

significantly different there after, the larvae were never able to regain growth and the lowest mean length, wet weight, MLS and survival were registered for T4 larvae. In similar substitution studies, the larvae given either *Moina micrura* throughout the larval cycle (Alam *et al.*, 1993a) or the copepod Cyclop-eeze only, after the V^{th} larval stage (Nair *et al.*, 2007),the larvae did not develop well and recorded the lowest survival among all the treatments and the control.

Though chironomids are reported (Sugden, 1973) to have a protein content of 56 % and are highly digestible (De La Noue and Choubert, 1985) they did not form a satisfactory food item for the early larval stages of *M. rosenbergii* as the larvae given more and more quantities of this feed were showing progressively poor development and survival. However, the lower percentage mortality observed in the treatments fed with *Chironomous* larvae after stage V and above suggests a possible replacement of *Artemia* nauplii with *Chironomous* larvae in the larval stages VI and above. The death of the *Chironomous* larvae (resulted in reduced motility) and the possible leaching of the nutrients on slicing to smaller particle sizes must also have resulted in reduction of the nutritional value of the chironomids.

When stocked at the rate of 100 larvae/litre and fed with sliced Tubifex worms (3-5 times daily, *ad libitum*), Mohanta and Rao (2000) achieved a survival ranging between 9 and 13 %. A lower survival of 7 to 9% was attained by using *Lamellidens* meat where as it varied between 20 and 25 % on using prepared feed and brine shrimp nauplii in the same experiment and the larval rearing cycle lasted for 40 to 45 days. 50 % replacement of *Artemia* nauplii

with a marine copepod Cyclop-eeze (Nair *et al.*, 2007) undertaken from stage V onwards and above resulted in a final survival of 55.87 % which was higher than the control in which the percentage survival was 48.44 %. However it was reduced to 27.58 % when *Artemia* nauplii was completely replaced by the copepod after stage V. They maintained an initial stocking density of 75 larvae / litre. Murthy *et al.* (2008) recorded a survival rate of 38.9 % and 31.1 % on feeding the *M. rosenbergii* larvae with two different larval diets in combination with *Artemia* nauplii. The fresh water prawn larvae in the above mentioned experiment were stocked at a much lower rate of 30/litre. Murthy and Yogeshbabu (2006) found that a 30 % substitution of brine shrimp nauplii with rotifer *Brachionus plicatilis* yielded a post larval survival of 42.22 % which was not significantly different from the control with a survival of 43.33 %. Here also initial stocking of the prawn larvae was done at a density of 30/litre. In a two phase larval rearing adopted by Kurup *et al.*(1998), the survival percentage varied between 19.06 and 47.41 % with an initial stocking density of 27 to 48 larvae / litre in the second phase. The time taken for settlement of post larvae varied from 33 to 36 days.

The final production obtained in the present experiment in the control with *Artemia* nauplii and egg custard are comparable with the above results achieved in India in the larviculture of the giant fresh water prawn, *M. rosenbergii* following the clear water system. Even the survival of 29.94 ± 1.98 % achieved in T1 having the supply of only 75 % BSN seems to be comparable with the average survival of 30 % reported from commercial fresh water prawn hatcheries in India (Murthy, 2006). The survival obtained (10.6 ±

1.36) by feeding the prawn larvae with *Chironomous* larvae and egg custard is also very much similar to that achieved by *ad libitum* feeding with *Tubifex* worms (Mohanta and Rao, 2000). The average duration of the experiment (35.33 ± 0.58 days) for this treatment as well as the other treatments and the control (31 to 34 days) was also comparatively much shorter when compared to those recorded by Mohanta and Rao (2000), however, it was similar to Kurup *et al.* (1998), who had reported a period of 33 to 36 days. On the contrary, the survival rates recorded in the present investigation were lower when compared to Nair *et al.* (2007). Valenti and Daniels (2000) reported the final production of 50 and 60-80 post larvae / litre following open and closed clear water systems respectively, and this is slightly higher when compared to the present finding.

3.5 Conclusion

Based on the results of the present study, it can be concluded that it is not possible to substitute *Artemia* nauplii completely with *Chironomous* larvae in the seed production of *Macrobrachium rosenbergii*. However, a 25 % substitution of *Artemia* nauplii can be possible especially in the rearing of advanced larval stages of V and above. Nevertheless more concerted studies on a larger scale have to be done before transferring this technology to the commercial hatcheries of the giant fresh water prawn. In order to draw a better conclusion, it is recommended that further studies can be carried out by weaning the prawn larvae from *Chironomous* larvae to *Artemia* nauplii at different larval stages and by feeding the *Chironomous* larvae as a whole without slicing them into smaller particles so as to reduce the nutrient leaching.

Table 3.1 Treatments with different percentage of live feed in substituting BSN with blood worms in the larviculture of *M rosenbergii*

Treatments	Feeding regimes
T1	75 % *Artemia* + 25 % *Chironomous*
T2	50 % *Artemia* + 50 % *Chironomous*
T3	25 % *Artemia* + 75 % *Chironomous*
T4	*Chironomous* larva only
Control	*Artemia* nauplii only

Table 3.2 Average ± s.d of various water quality parameters recorded during the experimental period in substituting BSN with blood worms in the larviculture of *M. rosenbergii*

Parameters	Treatments				
	Control	T1	T2	T3	T4
Temperature (^{0}C)	27.73 ± 0.81	27.67 ± 0.83	27.72 ± 0.77	27.69 ± 0.76	27.7 ± 0.82
pH	7.87 ± 0.11	7.85 ± 0.10	7.86 ± 0.08	7.84 ± 0.09	7.88 ± 0.10
Salinity (ppt)	11.98 ± 0.56	11.98 ± 0.81	12.23 ± 0.75	12.3 ± 0.74	12.23 ± 0.68
Dissolved oxygen (mg/l)	7.13 ± 0.10	7.06 ± 0.14	7.11 ± 0.12	7.12 ± 0.10	7.10 ± 0.12
Ammonia(µg/l)	2.23 ± 0.07	2.24 ± 0.09	2.22 ± 0.08	2.24 ± 0.08	2.25 ± 0.09

Table 3.3 Effect of substitution of BSN with blood worms on the mean larval stages of *M rosenbergii* larvae under various feeding regimes.

Treatments	Days								
	3	6	9	12	15	18	21	24	27
A75 C25	2.1 ± 0.1^{ab}	3.4 ± 0.06^{ab}	4.5 ± 0.1^{a}	5.53 ± 0.06^{a}	6.67 ± 0.06^{ab}	7.33 ± 0.06^{a}	8.4^{a}	9.5^{a}	10.47 ± 0.06^{a}
A50 C50	2.13 ± 0.06^{ab}	3.37 ± 0.06^{ab}	4.3 ± 0.1^{b}	5.37 ± 0.06^{b}	6.53 ± 0.06^{bc}	7.2 ± 0.1^{b}	8.2 ± 0.1^{b}	9.33 ± 0.06^{b}	10.27 ± 0.06^{b}
A25 C75	2.13 ± 0.06^{ab}	3.3 ± 0.1^{b}	4.33 ±0.12^{b}	5.23 ± 0.06^{c}	6.47 ± 0.06^{c}	7.17 ± 0.06^{b}	8.1^{b}	9.2 ± 0.1^{c}	10.13 ± 0.06^{c}
C100	2 ± 0.1^{b}	2.83 ±0.15^{c}	3.77 ± 0.06^{c}	4.83 ± 0.06^{d}	6.2 ± 0.1^{d}	6.93 ± 0.06^{c}	7.8 ± 0.1^{c}	8.83 ± 0.06^{d}	9.67 ± 0.06^{d}
CONTROL	2.17 ± 0.06^{a}	3.53 ± 0.06^{a}	4.5 ± 0.06^{a}	5.6 ± 0.1^{a}	6.7 ± 0.1^{a}	7.33 ± 0.06^{a}	8.4 ± 0.1^{a}	9.47 ± 0.06^{a}	10.4 ± 0.1^{a}
Mean ± s.d of three replicate groups; Means in each column sharing same superscript letter doesnot differ significantly (P>0.05)									

Table 3.4 Effect of substitution of BSN with blood worms on the survival, appearance of post larvae, duration of rearing period, total length and wet weight of post larvae of *M rosenbergii* under various feeding regimes.

Treatments	Stocking density(larvae/litre)	% survival	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
T1	100	29.94 ± 1.98^{b}	25^{a}	31^{a}	9.48 ± 0.04^{a}	9.79 ± 0.04^{a}
T2	100	22.48 ± 1.42^{c}	26.67 ± .58^{b}	32.3 ±0.58^{b}	9.24 ± 0.07^{b}	9.51 ± 0.07^{b}
T3	100	19.12 ± 0.8^{d}	27^{b}	33.3 ± 0.58^{c}	8.95 ± 0.13^{c}	9.30 ± 0.16^{bc}
T4	100	10.6 ± 1.36^{e}	28^{c}	35.33 ± 0.58^{d}	8.68 ± 0.192^{d}	9.08 ± 0.2^{c}
Control	100	32.69 ± 0.97^{a}	25^{a}	31^{a}	9.47 ± 0.05^{a}	9.78 ± 0.54^{a}
Mean ± s.d of three replicate groups; Means in each column sharing same superscript letter doesnot differ significantly (P>0.05).						

Table 3.5 Mean percentage survival of *M rosenbergii* larvae and post larvae in different treatments in the substitution of BSN with *Chironomous* larvae

Treatments	Days									
	3	6	9	12	15	18	21	24	27	95 % PL
A75C25	89.87 ± 1.21^{b}	80.07 ± 1^{b}	68.13 ± 0.8^{b}	55.87 ± 0.9^{b}	45.07 ± 0.9^{b}	39.07 ± 0.9^{b}	35.07 ± 0.9^{b}	32.07 ± 1.1^{b}	30.73 ± 1.7^{b}	29.94 ± 1.98^{b}
A50C50	88.73 ± 0.64^{bc}	78.8 ± 0.72^{b}	66.07 ± 0.31^{c}	53.73 ±0.6^{c}	42.87 ± 0.81^{c}	35.8 ± 0.72^{c}	30.8 ± 0.72^{c}	26.8 ± 0.72^{c}	24.53 ± 0.5^{c}	22.48 ± 1.42^{c}
A25C75	87.07 ± 0.5^{cd}	76.4 ±0.72^{c}	64.47 ± 0.5^{d}	52.6 ± 0.7^{c}	41.87 ± 0.81^{c}	33.8 ± 0.72^{d}	28.13 ± 1.21^{d}	23.73 ± 0.64^{d}	21.27 ± 0.7^{d}	19.12 ± 0.8^{d}
C	85.73 ± 0.3^{d}	73.73 ±0.64^{d}	57.73 ± 0.64^{e}	44.07 ± 0.5^{d}	33.07 ± 1^{d}	25.93 ±1.1^{e}	19.93 ± 0.7^{e}	15.07 ± 1^{e}	12.67 ± 1.14^{e}	10.6 ± 1.36^{e}
A (control)	92.07 ± 1.1^{a}	84 ± 1^{a}	74.07 ± 1.1^{a}	63.07 ± 1.1^{a}	52.13 ± 1.21^{a}	45.07 ± 0.9^{a}	40.13 ± 1.21^{a}	36.27 ± 0.46^{a}	34.07 ± 0.31^{a}	32.69 ± 0.97^{a}

Mean ± s.d ; Means in each column sharing same superscript does not differ significantly (P>0.05).

Table 3.6 Mean percentage mortality of the *M rosenbergii* larvae between particular days in the substitution of BSN with *Chironomous* larvae

Treatments	Days									
	0 to 3	3 to 6	6 to 9	9 to 12	12 to 15	15 to 18	18 to 21	21 to 24	24 to 27	27 to PL
A75C25	10.13 ± 1.21^{b}	9.8 ± 0.2^{b}	11.93 ± 0.31^{b}	12.27 ± 0.7^{ab}	10.8 ± 0.8^{a}	6^{a}	4^{a}	3 ± 0.2^{a}	1.33 ± 0.61^{a}	0.79 ± 0.34^{a}
A50C50	11.27 ± 0.64^{b}	9.93 ± 0.11^{bc}	12.73 ± 0.46^{b}	12.33 ± 0.42^{ab}	10.87 ± 0.42^{a}	7.07 ± 0.12^{b}	5^{ab}	4^{a}	2.27 ± 0.23^{b}	2.05 ± 0.94^{b}
A25C75	12.93 ± 0.5^{c}	10.67 ± 0.31^{c}	11.93 ± 0.7^{c}	11.87 ± 1.21^{a}	10.73 ± 0.83^{a}	8.07 ± 0.12^{c}	5.67 ± 0.58^{ab}	4.4 ± 0 87^{a}	2.47 ± 0.31^{b}	2.17 ± 0.12^{b}
C	14.27 ± 0.31^{c}	12 ± 0.87^{d}	16 ± 0.2^{d}	13.67 ±1.14^{b}	11 ± 1.44^{a}	7.13 ± 0.12^{b}	6 ± 1.8^{b}	4.87 ± 1.7^{a}	2.4 ± 0.2^{b}	2.07 ± 0.63^{b}
A (control)	7.93 ± 1.1^{a}	8.07 ± 0.12^{a}	9.93 ±0.12^{a}	11^{a}	10.93 ± 0.12^{a}	7.07 ± 0.31^{b}	4.93 ± 0.31^{ab}	3.87 ± 1.6^{a}	2.2 ± 0.2^{b}	1.38 ± 0.71^{ab}

Mean ± s.d ; Means in each column sharing same superscript does not differ significantly (P>0.05).

Fig 3.1 Effect of substitution of BSN with blood worms on the percentage survival of the larvae and post larvae in treatments with different feeding regimes in the larviculture of of *M. rosenbergii*

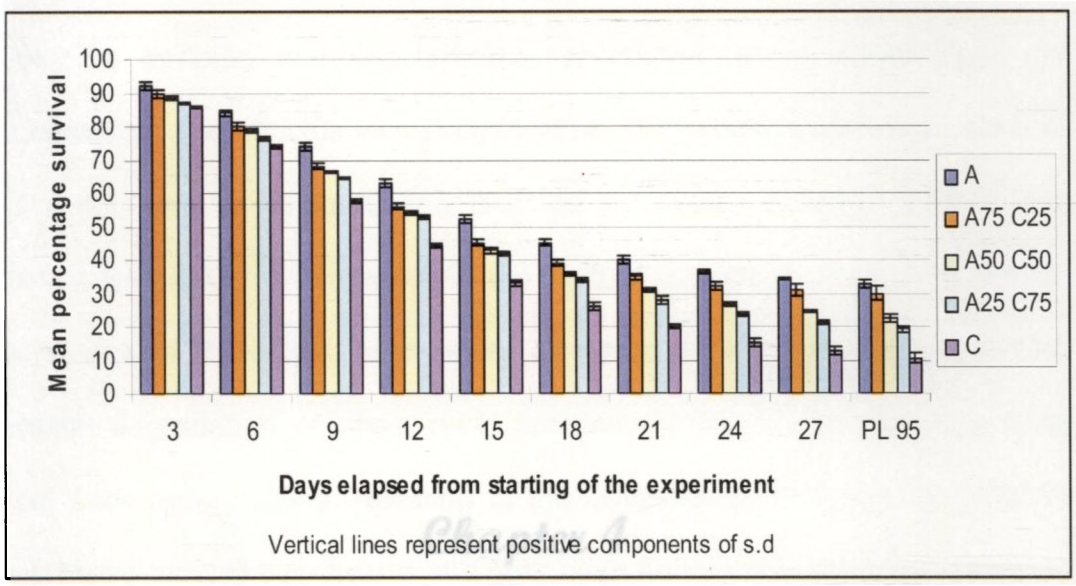
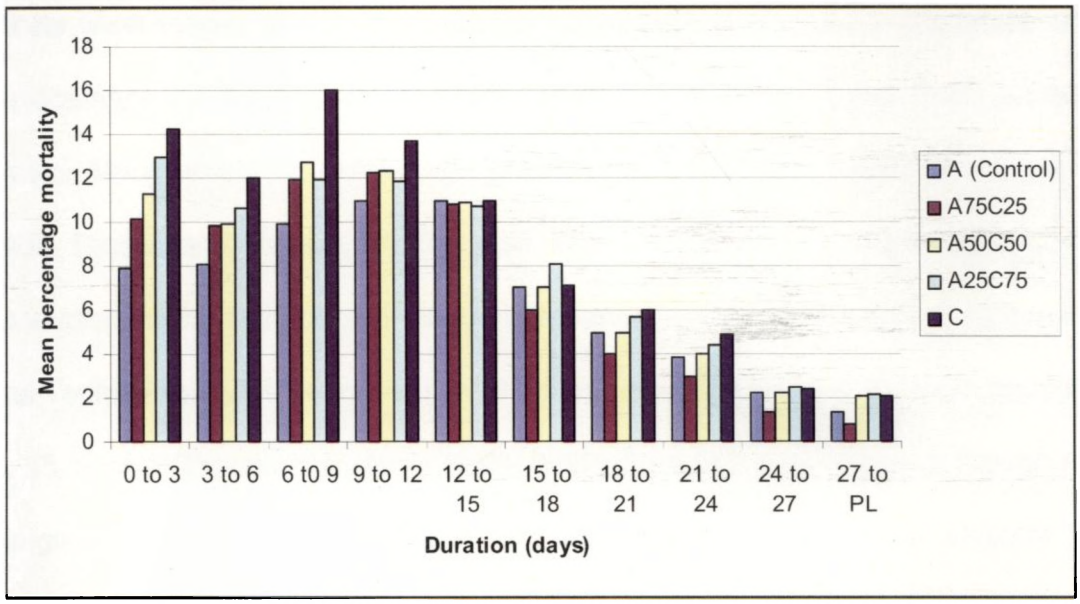


Fig 3.2 Mortality of the larvae of *M. rosenbergii* in different feeding regimes in the substitution of BSN with blood worms



Chapter 4

WEANING OF *MACROBRACHIUM ROSENBERGII* LARVAE FROM *ARTEMIA* NAUPLII TO CHIRONOMID LARVAE

4.1 Introduction

Artemia nauplii have been used as an essential live feed in the fresh water prawn hatcheries. The availability of brine shrimp nauplii cysts are subject to periodic and unpredictable shortages, as a result there are unpredictable price fluctuations (Martin *et al.*, 2006) which ultimately result in high cost of post larval production. Besides, the seed production is beset with other problems such as variability in the nutrient content depending on season and source of BSN cyst, entrapment of larvae in brine shrimp exuvia and bacterial degradation of shed cyst capsules. These factors together bring about poor water quality, resulting in the outbreak of diseases. In order to avoid these casualties, several attempts have been made to replace *Artemia* nauplii with other locally available live feed or inert diets (Meyers and Hagwood, 1984; Jones *et al.*, 1997; Lovett and Felder, 1988; Kumarly *et al.*, 1989; Samocha *et al.*, 1989; Silva and Rodriguez, 1997). Though concerted efforts were made, only a few became successful to find out a substitute or replacement for *Artemia* for different larval stages of the giant fresh water prawn, *Macrobrachium rosenbergii* (Alam *et al.*, 1993a, b; Kovalenko *et al.*, 2002; Barros and Valenti, 2003a; Nair *et al.*, 2007). In this regard, a study conducted at the School of Industrial Fisheries, Cochin University of Science and Technology, was successful in providing a replacement of *Artemia* nauplii by 25 % with *Chironomous* larva in the larviculture of the giant prawn though a marginal reduction in the post larval production was observed (refer chapter 3 for details). Nevertheless, the prawn larvae were found to accept blood worm particles well after the vth larval stage and the larval progression and the

survival rates were found to be superior than the control in which *Artemia* nauplii alone was used as the live feed (refer chapter 3). The post larvae of the fresh water prawn were also found to aggressively feed on the *Chironomous* larvae as soon as they were available in the rearing medium. The blood worm particles were also reported to be consumed as a natural food by the juvenile and adult *M. rosenbergii* (Tidewell *et al.*, 1997). Against these backdrops, a study was carried out by gradually weaning the prawn larvae from *Artemia* nauplii to *Chironomous* larvae by reducing the quantity of BSN given at different larval stages and substituting it with *Chironomous* larval particles.

Thus the objectives of the present study were

1. To evaluate the effectiveness of gradual weaning of the prawn larvae from *Artemia* nauplii to *Chironomous* larvae.
2. Reducing the dependence on *Artemia* nauplii thereby reducing the cost of post larval production.

4.2 Materials and Methods

The experiment was carried out following completely randomised design with four overnight feeding regimes with three replicates for each treatment (Table 4.1). Feeding with *Artemia* nauplii alone as live feed through out the experimental period was taken as the control which was also maintained in triplicate. In treatment T1(AC4C), only *Artemia* nauplii was given in the second larval stage, the quantity of *Artemia* nauplii was reduced to half in the third stage, and from fourth larval stage onwards, only chironomid larva was given as the overnight live feed. In T2 (AC6C), BSN was reduced to 75 %

in third larval stage, 50 % in IVth, 25 % in Vth and 100 % replacement was effected in the VIth larval stage. Similarly, in the treatment T3, the quantity of *Artemia* nauplii given was reduced to 75 % in the IIIrd stage, 50 % in the Vth stage, 25 % in the VIIth stage and fully replaced by chironomid larvae in the eighth larval stage where as in T4, BSN was reduced to 75 % in the IIIrd stage, 50 % in the Vth stage, 25 % in the VIIIth stage and complete replacement by chironomid larvae was effected in the tenth larval stage .

4.2.1 Brood stock management

Berried prawns with greyish black eggs were collected from the wild and transported to the hatchery in wide mouthed black plastic cans. They were disinfected (New and Singholka, 1985) and kept in 150 l FRP tanks, one in each tank with aerated brakish water having a salinity of 5 ppt. No feed was provided as the berried prawns with black eggs invariably hatched by the next day morning.

4.2.2 Live feed culture

The *Chironomous* larvae were reared using chopped cabbage leaves immersed in trays filled with water. The *Chironomous* flies were attracted to the decaying vegetable leaves to lay eggs and the larvae were ready in a week's time. For feeding the prawn larvae, the blood worms were collected, washed and transferred to clear water in beakers. Before feeding, the worms were taken out of water, sliced using a sharp knife and passed through sieves to get the required particle size. Large number of trays was kept to attract the flies so as to ensure a steady supply of worms through out the period of the experiment.

For *Artemia* nauplii production, OSI brand *Artemia* cysts were used. The required quantity of cysts were weighed, hydrated, decapsulated using commercial grade sodium hypochlorite and kept in 25 ppt saline water with vigorous aeration for hatching (approximately I g cyst/ I sea water). Artificial light was also provided for hatching of the cysts. The cyst hatched in 18 – 24 hours. They were harvested and washed thoroughly before feeding.

4.2.3 Larval rearing

The larval rearing was carried out using clear water method in 100 I FRP tanks with 50 I rearing water. The water used for the experiment was treated following New and Singholka (1985). Larvae were stocked at the rate of 100 numbers / I. The experiment was carried out following completely randomised design with five overnight feeding regimes with three replicates for each treatment (Table 4.1). The larvae were fed daily with *Artemia* nauplii in the morning (8 30 AM) and evening (5 PM) from the second day onwards. From the third day onwards, the live feed (BSN or blood worms depending on the treatment) was given only in the evening. Egg custard (Kurup, 2003) was fed only once in the morning on the third day, followed by two times on the fourth day and four times daily at 8 30 AM, 10 30 AM, 12 30 PM and 3 PM after passing through the sieves to get the required particle size (Kurup, 2003) from the fifth day of the experiment. The blood worm particles were passed through 400 µm and 600 µm mesh size sieves before feeding to stages I – IV and for V to post larva respectively (Alam *et al.*, 1993a). *Artemia* was given at the rate of 4 numbers/ ml in the control and the percentage replacement was

done on wet weight basis. Siphoning of the waste and water exchange (20 - 50 %) was done daily before the live feed was given in the evening.

Salinity and temperature was measured daily during the morning hours using Atago refractometer and mercury thermometer respectively. pH, dissolved oxygen and ammonia were estimated twice weekly using pH meter (Eutech Cyberscan model 510), Winklers method (APHA, 1995) and alkaline phenol method (Grasshoff *et al.*,1983) respectively.

The larval progression and relative survival of larvae in each tank was estimated every third day. Thus the final evaluation of experimental diets was done based on the post larval production, time required for the larvae to reach each stage and their relative survival. Mean larval stage was used to find out the development of larvae. Mean larval stage was calculated using the formula, MLS = $\sum$(S x Ps) where MLS is the mean larval stage, S is the larval stage number and Ps is the proportion of larvae at stage S (Lovett and Felder, 1988). The estimation of percentage composition of the different larval stages and survival continued till 27th day when considerable number of post larvae started appearing in all the tanks and it was difficult to draw uniform samples (Alam *et al.*, 1993a). Mean while all the tanks were observed daily for the settlement of post larvae after 20 days and the days on which the first post larva appeared in each tank was noted down. On completion of the experiment (when more than 95 % of larvae metamorphosed to PL), 50 post larvae were randomly taken from each tank to measure the total length (from the tip of the rostrum to the end of the telson) and wet weight.

4.2.4 Statistical analysis

Data analysis was done by ANOVA using SPSS 16. Significant differences between the treatments were determined using Duncan's multiple range test (DMRT) ($P<0.05$). Data expressed in percentages were normalised by arcsine transformation (Zar, 1984). However, non transformed data is given in the tables (Table 4.4).

4.3 Results

The water quality parameters are shown in Table 4.2 which was found to be within the optimal range required for larval rearing of the giant fresh water prawn (New and Singholka, 1985; Correia *et al.*, 2000). The temperature recorded during the experiment ranged between 26^0C and 28^0C. Salinity in all the experimental tanks was in the range of 12 to 13 ppt while pH fluctuated between 7.58 and 8.02. Where as dissolved oxygen values fluctuated between 6. 91 to 7.53 mg/ l. Total ammonia nitrogen varied between 2.11 and 2.39 µg/ l.

The highest production was recorded in the control (Mean survival – 32.3 ± 1.25 %) followed by T4 (31.7 ± 0.66 %) and the lowest was recorded in T1 (11.29 ± 0.67 %). The mean survival in treatments T2 and T3 were 19.11± 0.94 % and 27.3 ± 0.75 % respectively. The appearance of the post larva was first noted in the control (mean number of days – 24 ± 0.6), however, it did not differ significantly with T4 where it appeared on the 25^{th} day. This was followed by T3, T2 and T1 in which the mean number of days on which the first post larva appeared was on 26, 26.3 ± 0.6 and 26.7 ± 0.6 days respectively. The mean larval stage (indicator of larval progression) also

followed a similar trend with the highest values recorded in the control followed by T4, T3, T2 and T1.

Although the highest post larval production of 32.3 ± 1.25 % was encountered in the control in which only *Artemia* nauplii was given as the live feed (Table 4.4), the treatment T4 (AC 10 C) in which there was complete replacement of *Artemia* nauplii after tenth larval stage did not differ significantly with the control in terms of post larval production or larval progression (Table 4.3, 4.4; Fig 4.1 and Fig 4.2). Moreover the total length and wet weight of the post larvae in T4 recorded slightly higher values when compared to the control. The highest mean total length of 9.56 ± 0.39 mm of the post larvae was recorded in T4 followed by the control (9.55 ± 0.28 mm), T3 (9.31± 0.36), T2 (9.07 ± 0.43) while it was lowest was in T1 (8.96 ± 0.34). The average wet weight of the larvae were also found to be higher in T4 (9.88 ± 0.37mg) when compared to the control (9.81 ± 0.31), however the difference was not significant. The experiment was terminated on an average duration of 30.7 days in the control followed by 31 days in T4. The time taken for settlement of 95 % of post larva was the longest in T1 where an average of 34.7 days was taken for the completion of the experiment.

4.4 Discussion

Co-feeding is a common practise in weaning of larvae from live feed to inert diets. In respect of marine fish larvae co feeding of live prey with formulated diets for a short period was found to be better for successful weaning before feeding with formulated diets alone (Person Le Ruyet *et al.*,1993; Hart and Purser, 1996; Kolkovski *et al.*, 1997a, b; Rosenlund *et al.*,

1997). In the present trial conducted by gradual weaning to chironomid larve, the treatment AC10C did not show significant difference with the control in the post larval production or larval progression. In addition, the final length and wet weight though not significantly different, were found to be higher when compared to the control. This can be attributed to the advantages of a mixed diet as observed by Alam *et al.* (1993b), while weaning *Macrobrachium rosenbergii* from *Artemia* nauplii to *Moina micrura*. Better survival and growth were reported in sea bass fry (Fermin, 1991) when fed with a mixture of *Artemia* and *Moina* in the ratio 1:1. Many other researchers (Lewis, 1975; Helm, 1977; Stone, 1988) have reported the beneficial effects of feeding mixed diet to the larvae of marine invertebrates. Moreover, the use of supplemental diets is suggested along with *Artemia* nauplii by several authors (Daniels *et al.*, 1992; New, 1995; Valenti and Daniels, 2000) during the later larval stages of *M. rosenbergii* as the brine shrimp nauplii does not fulfil the nutritional requirements of advanced zoea. *Artemia* nauplii were also found to be inadequate for the halibut larvae when they had reached a certain size (Maeland *et al.*, 1999). Besides, the incorporation of *Chironomous* larvae in the feed was found to improve growth and survival in *M. rosenbergii* under laboratory conditions (chapter 3). The better growth observed in the present experiment in the treatment AC10C can therefore be attributed to the beneficial effect of mixed diet containing *Artemia* nauplii, *Chironomous* larva and egg custard for a prolonged period.

While carrying out a weaning experiment, Alam *et al.* (1993b), have observed a higher survival and growth when *M. rosenbergii* larvae were fed a

combination of *Moina micrura* and *Artemia* nauplii than to the larvae fed *Artemia* nauplii only. Conversely, performance of the control with *Artemia* nauplii only given as the live feed through out the experimental period was superior to all the other treatments but for the total length and wet weight of the post larvae which showed highest values in the treatment AC10C. It is worth reporting that till the complete replacement of *Artemia* nauplii by *Chironomous* larva in the X^{th} larval stage in the treatment AC10C, the percentage survival of the larvae (in the stages I to IX) was higher than that in the control.

In AC4C, though same initial quantity of *Artemia* nauplii was supplied in the second larval stage as in the control, the quantity was reduced to 50 % by the third stage and no more of it was given once the larvae reached the IV^{th} zoeal stage. Thereafter blood worms were the only overnight feed provided to the prawn larvae. Therefore the performance of the larvae in treatment AC4C (survival of 11.29 ± 0.67 %) was more or less similar to that in the treatment (C) in the previous experiment in which only *Chirnonomous* larval particles were given as live feed to the prawn larvae(survival of 10.6 ± 1.36 %) from the second larval stage (refer Chapter 3). Thus the results of the present study fully concurs with the results of previous study conducted with percentage substitution of BSN with *Chironomous* larvae in the larval rearing of giant fresh water prawn which once again confirms the fact that the early stages of the prawn larvae are unable to derive sufficient nutrition from inert diets. The treatment AC6C in which the brine shrimp nauplii was fully substituted by chironomid larvae by sixth stage, also did not give satisfactory results.

According to Jones *et al.* (1997), the prawn larvae exhibit mandibular characteristics of a carnivore till third stage after which they show more of omnivorous characteristics. Barros and Valenti (2003a) recommended to feed the *M. rosenbergii* larvae with wet diets after the VIIth to VIIIth larval stages and with wet or formulated diets after IXth stage. The lower survival and growth recorded both the treatments T1 and T2 can be explained as the inability of the larvae in utilising the blood worm particles in the early larval stages. A similar observation was made in the previous experiment where maximum mortality occurred in the different treatments until 12th day after which the mortality in the tanks fed a combination of *Artemia* nauplii and chironomid larvae were found to be lower or comparable to that in the control (refer chapter 3).

In the present study, the larvae started dying at a faster rate (Fig. 4.1) by day 9 in AC4C, once there was total replacement of *Artemia* nauplii by chironomid larvae. In AC6C wherein the quantity of brine shrimp nauplii was reduced to half by the fourth larval stage, there was high mortality in the immediate days following the reduction (9th day in Fig, 4.1). Where as in treatments AC8C and AC10C in which the reduction of BSN to 50 % was effected only from Vth larval stage, the larvae were not found affected due to the reduction of quantity and their steady progression was observed without any problem (Table 4.3, Fig. 4.2). A decrease in mortality was encountered in all the treatments in which a combination of BSN and chironomid larvae were supplied after the 12th day of the experiment (refer chapter 3). The result of the present study is concurring with the above and therefore it can be

concluded that *Artemia* nauplii are required in sufficient quantities (not less than 3 numbers/ml) in the rearing of early larval stages especially till it attains stage V. Kovalenko *et al.* (2002) and Nair *et al.* (2007) have experimented with the substitution of BSN commencing from the V^{th} larval stage. Agard (1999) reported the beginning of effective feeding at stage V when the yolk reserves are supposed to be disappeared and the digestive tract becomes completely developed. Jones *et al.* (1997) explains the lack of tryptic enzyme response to artificial diet seen in early stages of caridean larvae as the lack of flexibility with respect to feeding behaviour during these stages. More over, changes in the perception ability and selectivity of food after capture has been reported in the advanced larval stages of *M. rosenbergii* (Barros and Valenti, 2003a). The results of the present study is in accordance with the results of afore mentioned researchers.

It is also remarkable to note that the complete substitution of BSN, even in the advanced larval stages, stage VIII and X respectively in treatments T3 and T4 adversely affected the percentage survival. This indicates that the presence of BSN although in small quantities is desirable in *M. rosenbergii* larval rearing. On the contrary, this finding disagrees with the observations of Kovalenko *et al.* (2002) who reported that a complete substitution of BSN with inert diets did not affect the feeding or the survival of the *M. rosenbergii* larvae after stage V.

The reasons that can be attributed for the inability of prawn larvae in capturing and utilising the chironomid larval particles during early larval stages thus includes poorly developed larval gut until stage V to VI (Deru, 1990),

dependence on exogenous prey enzymes which may be supplied by the live food source (Jones *et al.*, 1993) and the carnivorous nature of the larvae before stage VII (Barros and Valenti, 1997). Kamarudin *et al.* (1994) and Kumlu and Jones (1995) have reported an increased acceptability of inert diets from stage VI onwards and after, which is attributed to the development of digestive tract and increase in enzyme activity. Moreover, the *M. rosenbergii* larvae are non active hunters during the early stages (Daniels *et al.*, 1992) and capture food by chance encounter (Moller, 1978). So they prefer a live zooplankton with good concentration in the rearing water during the early stages. The above mentioned reasons might have led to the inferior performance of the prawn larvae which were supplied with a higher proportion of blood worm particles during the early larval stages. Whereas, in AC10C the weaning was gradual and the quantity of BSN was reduced to 75 %, only in the VIIIth larval stage. By then the larvae were more capable of exploring and utilising inert diets, and hence the larvae in this treatment performed in a superior way compared to the other treatments. In addition to having sufficient quantity of BSN, the larvae were also supplied with another supplementary diet, the blood worms. The advantages of a mixed diet and the inadequacy of BSN alone as food for prawn larvae have been discussed earlier in this chapter.

Kovalenko *et al.* (2002) have reported that weaning is an unnecessary step in shifting from BSN to micro bound diet in the larviculture of the giant prawn. In total contrast to this, in the present study, the gradual reduction of the BSN over a longer period as in the treatment AC10C could bring about a

reduction in the total quatity of *Artemia* cysts by more than 50 % without affecting the growth of the larvae and the efficiency of post larval production. However, due to slicing of the blood worms, leaching of nutrients might have occurred reducing its nutritional value. Hence, further studies can be conducted by presenting the blood worms as a whole after assessing its acceptability by the prawn larvae.

4.5 Conclusion

A gradual weaning of *M. rosenbergii* larvae from *Artemia* nauplii to *Chironomous* larvae and the complete substitution at stage X was not found to affect the survival and post larval production and therefore it can be recommended for the larviculture of the prawn at a reduced cost of production. However, the behaviour of the larvae in large rearing tanks have to be experimented before applying it on a commercial scale. Also the production of *Chironomous* larvae has to be taken up and its cost of production need to be estimated. The results of the present study further reiterates that *Artemia* nauplii forms an indispensable live feed in the larviculture of the giant fresh water prawn and a partial or complete substitution with other live feeds like *Chironomous* larva will have its adverse effect in the survival and post larval production, irrespective of the stage of substitution. Another salient finding of the study is that the combination of prepared feed, *Chironomous* larval particles and *Artemia* nauplii were giving a better survival until BSN was completely replaced. It can therefore be concluded that in the treatment where in a gradual weaning from BSN to *Chironomous* larva until the quantity of BSN reaches 25 % by the X^{th} larval stage and no further substitution is effected

there after till the complete settlement of post larvae will be far better than the control in which BSN alone was used in combination with egg custard. So a gradual weaning protocol from BSN to *Chironomous* larva till the quantity of BSN reaches 25 % by the X^{th} larval stage and no further substitution is done till the complete settlement of post larvae is recommended for the larviculture of *M. rosenbergii* for maximising the post larval production.

Table 4.1 Different treatments in the weaning of *M rosenbergii* larvae from *Artemia* to chironomid larvae

Feeding regime	% composition of live food									
	Larval stages									
	II	III	IV	V	VI	VII	VIII	IX	X	XI - PL
AC4C (T1)	A 100	A 50 C 50	C 100	C 100	C 100	C 100	C 100	C 100	C 100	C 100
AC6C (T2)	A 100	A 75 C 25	A 50 C 50	A 25 C 75	C 100	C 100	C 100	C 100	C 100	C 100
AC8C (T3)	A 100	A 75 C 25	A 75 C 25	A 50 C 50	A 50 C 50	A 25 C 75	C 100	C 100	C 100	C 100
AC10C (T4)	A 100	A 75 C 25	A 75 C 25	A 50 C 50	A 50 C 50	A 50 C 50	A 25 C 75	A 25 C 75	C 100	C 100
A (Control)	A 100	A 100	A 100	A 100	A 100	A 100	A 100	A 100	A 100	A 100

Table 4.2 Average ± s.d of various water quality parameters recorded during the experimental period in the weaning of prawn larvae from BSN to blood worms

Parameters	Treatments				
	C	T1	T2	T3	T4
Temperature (^{0}C)	26.74 ± 0.72	26.77± 0.69	26.72 ± 0.65	26.77 ± 0.71	26.83 ±0.66
pH	7.85 ± 0.11	7.87 ± 0.06	7.84 ± 0.08	7.91 ± 0.08	7.84 ± 0.11
Salinity (ppt)	12.21 ± 0.41	12.31 ± 0.47	12.37 ± 0.48	12.29 ± 0.46	12.12 ± 0.32
Dissolved oxygen (mg/l)	7.28 ± 0.12	7.26 ± 0.12	7.29 ± 0.08	7.32 ± 0.12	7.31 ± 0.09
Ammonia(µg/l)	2.23 ± 0.08	2.28 ± 0.05	2.23 ± 0.08	2.23 ± 0.08	2.24 ± 0.07

Table 4.3 Effect of weaning of prawn larvae from BSN to blood worms on mean larval stage in different treatments in the larviculture of *M rosenbergii*

Treatments	Days								
	3	6	9	12	15	18	21	24	27
AC4C	2.1 ± .1^{a}	3.5^{b}	4.3^{c}	5.27 ± .06^{b}	6.37 ± .06^{b}	7.13 ± .06^{c}	7.87 ± .06^{d}	9 ± .1^{c}	9.9 ± .1^{c}
AC6C	2.13 ± .06^{a}	3.47 ± .06^{ab}	4.4^{b}	5.4^{b}	6.4 ± .1^{b}	7.2^{bc}	8 ± .1^{c}	9.03 ± .06^{c}	10 ± .1^{c}
AC8C	2.13 ± .06^{a}	3.43 ± .06^{ab}	4.5^{a}	5.6 ± .1^{a}	6.6 ± .1^{a}	7.23 ± .06^{b}	8.17 ± .06^{b}	9.2 ± .1^{b}	10.23 ± .06^{b}
AC10C	2.03 ± .06^{a}	3.53 ± .06^{ab}	4.57 ± .06^{a}	5.67 ± .06^{a}	6.73 ± .06^{a}	7.4^{a}	8.4^{a}	9.43 ± .06^{a}	10.4^{a}
A	2.13 ± .06^{a}	3.5^{a}	4.53 ± .06^{a}	5.6 ± .1^{a}	6.7 ± .1^{a}	7.3 ± .06^{a}	8.4 ± .1^{a}	9.43 ± .06^{a}	10.43 ± .0.06^{a}
Mean ± s.d of three replicate groups; Means in each column sharing same superscript does not differ significantly (P>0.05).									

Table 4.4 Effect of weaning of prawn larvae from BSN to blood worms on the survival, appearance of post larvae, duration of experiment, total length and wet weight of post larvae in the larviculture of *M rosenbergii*

Treatments	Stocking density(larvae/litre)	% survival	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
T1	100	11.29 ± 0.67^{c}	26.7 ± .6^{b}	34.7 ± .6^{d}	8.96 ± 0.34^{d}	9.25 ± 0.33^{c}
T2	100	19.11 ± .94^{c}	26.3 ± .6^{b}	33.3 ± .6^{c}	9.07 ± 0.43^{c}	9.37 ± 0.38^{c}
T3	100	27.3 ± 0.75^{b}	26^{b}	32.3 ± .6^{b}	9.31 ± 0.36^{b}	9.63 ± 0.33^{b}
T4	100	31.7 ± 0.66^{a}	25^{a}	31^{a}	9.56 ± 0.39^{a}	9.88 ± 0.37^{a}
Control	100	32.3 ± 1.25^{a}	24 ± .6^{a}	30.7 ± .6^{a}	9.55 ± 0.28^{a}	9.81 ± 0.31^{a}
Mean ± s.d of three replicate groups; Means in each column sharing same superscript does not differ significantly (P>0.05).						

Fig 4.1 Survival of the larvae and post larvae of *M. rosenbergii* in treatments with different feeding regimes in the weaning of prawn larvae from BSN to blood worms

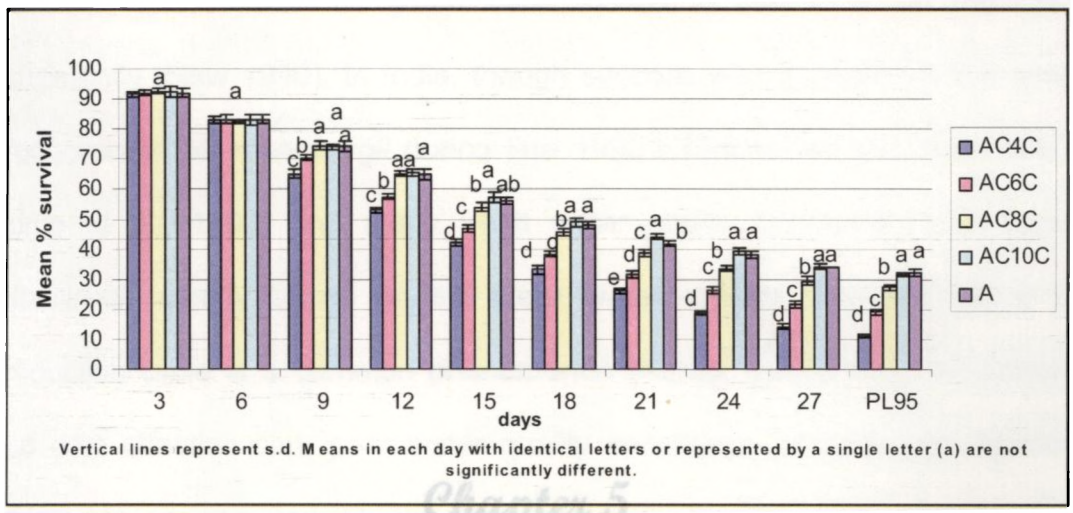
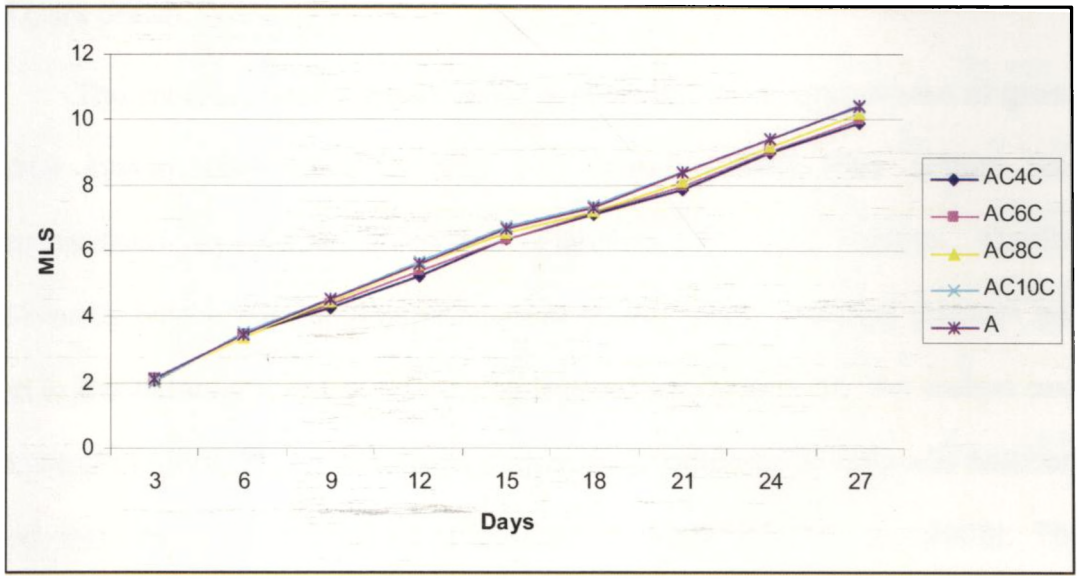


Fig 4.2 Mean Larval Stage in different treatments in the weaning of prawn larvae from BSN to blood worms.



Chapter 5

STANDARDISATION OF STOCKING DENSITY IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII* USING THE MODIFIED STATIC GREEN WATER SYSTEM

5.1 Introduction

The larviculture of *M. rosenbergii* is most widely been carried out using the clear water system as green water system is difficult to be managed consistently (New 1990). In India, though success was achieved in the seed production of *M. rosenbergii* during late 1990's (Sebastian and Nair, 1995; Kurup *et al.*, 1998) and many fresh water prawn hatcheries have been established mostly based on the clear water system, frequent failure of production cycle is a common phenomenon (Kurup, 2003). Lack of efficient and cost effective diet, poor water quality conditions, parasite and disease effects etc. are reported (Murthy, 2006) to be the reasons for the low survival in fresh water prawn hatcheries of the country. Kurup (2003) highlighted the advantages of the modified static green water system (MSGWS) of larviculture as a solution to many of these inherent problems faced in the seed production of giant prawn.

The modified static green water system is the refined version of green water system developed by Ang and Cheah (1986). The system was standardised by Aquaculture and Fisheries Science Institute, Cantho University by the experiments conducted during 1998 - 2002 in Vietnam and led to the establishment of 91 hatcheries and production of 76.5 million post larvae (PL) in the Mekong Delta of Vietnam as compared to only one hatchery and less than1 million of PL produced in 1999 (Hai *et al.*, 2003). The technology which was found to be successful for adoption at backyard levels in Vietnam has been reported (Kurup, 2003) to be very successful in the pioneer rearing trials conducted in Kerala. Unfortunately further studies were

not conducted to standardise the system in Indian conditions and popularise the rather simple technology having higher production efficiency, lower investment cost and suitable for its popularisation at backyard levels. Against this backdrop 6 experiments were conducted at the prawn hatchery complex of SIF, CUSAT to standardise and further improvise the system. The first trial in this regard was done by conducting an experiment to standardise the stocking density in the system.

Generally the green water system envisages low density (20 larvae /l) larval rearing and the average production is usually low (Murthy 1996). Thressiamma *et al.* (2006) stocked the larvae at a density of 50/l and they used a rearing medium of *Isochrysis galbana* with daily exchange of water. Different researchers have used different stocking densities for carrying out studies using M S G W S. Alam *et al.* (1993 a, b) reared *M. rosenbergii* larva using the M S G W S at a density of 30 larvae /l while conducting studies on substitution of *Artemia* naupli. While studying the effectiveness of Vitamin C on the larvae of fresh water prawn, *M. rosenbergii*, Hein *et al.* (2000) stocked the larvae at the rate of 50/l. The same stocking density was adopted by Oanh *et al.* (2000) in his experiment on the use of probiotics in *M. rosenbergii* larviculture. Thus the present experiment was carried with objective to

- Optimise the larval stocking density in the modified static green water system in Indian conditions.

5.2 Materials and methods

The experiment was conducted following the Modified static green water system of larviculture adopted by Kurup (2003) with five stocking

densities (Table 5.1) and a control in which the clear water rearing was followed, by maintaining triplicates for each treatment.

5.2.1 Brood stock management

Berried prawns with black coloured eggs were collected from grow outs and transported in black coloured plastic cans to the hatchery at the School of Industrial Fisheries (SIF). They were disinfected (New, 2002) and transferred to 150 l FRP tanks, one berry / tank and maintained in 5 ppt saline water with continuous aeration. The tanks were covered with nets to prevent escapement of prawns and were kept undisturbed at night. The eggs hatched by next day morning.

5.2.2 Algal culture

Algal inoculum (*Chlorella* sp.) was purchased from Central Marine Fisheries Research Institute, Cochin which was maintained and sub cultured at the Aquaculture Laboratory, School of Industrial Fisheries using modified Walne's medium (Lavens and Sorgeloos, 1996), the composition of which is given in Chapter 2 (Table 2.2). Large scale out door culture was carried out using this inoculum. The inoculum was maintained at a higher salinity (20-25ppt) and before adding to the rearing tanks, the salinity was gradually reduced to 15 ppt. An algal density of 5-10 x 10^5 cells /ml was maintained in the rearing medium. This differs from the methodology of Kurup (2003) wherein natural development of *Chlorella* sp was promoted using Tilapia (*Oreochromis mossambicus*).

5.2.3 Larval rearing

100 l white coloured FRP tanks were used for larviculture experiments. Brackish water with 12 ppt salinity was prepared by mixing sand filtered sea water (25-30 ppt) and chlorinated tap water, treated with 30 ppm active chlorine and was kept with vigorous aeration for 6-7 days to remove chlorine. In addition to this, the water was sterilised by heating using immersion heaters before adding the algae. After cooling, the algal inoculum was added along with the required quantity of nutrient medium (Walne's medium) one day prior to stocking of larvae.

The larvae were stocked at an initial density of 25, 50, 75, 100 and 125 numbers/ l. There was neither water exchange nor cleaning of tanks undertaken for green water system during the rearing cycle. The algal density was maintained from $5x10^5$ to $10x10^5$ cells / ml. A control was maintained which was stocked at the rate of 100 larvae / litre and the clear water system with regular water exchange, tank cleaning and siphoning of wastes was adopted in the triplicate tanks. One air stone was placed vertically 2 cm above from the bottom of each tank so as to provide moderate to strong aeration.

5.2.4 Feeding

Newly hatched *Artemia* nauplii (OSI brand) were fed at density of 4 numbers/ ml divided into two equal rations in the morning and evening (9 AM and 5 PM) till V^{th} larval stage. From V^{th} stage onwards egg custard, which was prepared following Kurup (2003) , was fed four times daily at 9 AM, 11 AM, 2 PM and 4PM while newly hatched *Artemia* nauplii was given only at 5 PM as over night feed. The aeration in the tanks was stopped before feeding with

g custard so as to enable the surfacing of the larvae. The custard feed of rticular size (Kurup, 2003) was fed to the larvae judiciously using a pipette a circular manner with out over feeding.

6.2.5 Evaluation of results

The results of different treatments were assessed based on the time required for larvae to reach each stage and their relative survival. Thirty larvae from each tank were randomly sampled every fifth day and the larval stages were identified following the descriptions of Uno and Kwon (1969). Mean larval stage (MLS) was used for determining the development of larvae and calculated using the formula of Lovett and Felder (1988). MLS= $\sum$(S x Ps) where S is the larval stage number and Ps is the proportion of larvae at stage S. Survival of the larvae were estimated by taking ten 1 litre samples from the rearing medium under strong aeration. The estimation of percentage composition of the different larval stages and survival continued till 20th and 25th day respectively. Since it was not possible to draw uniform samples, due to the benthic habit of post larvae neither staging nor estimation of survival was continued after appearance of considerable number of post larvae (Alam *et al.*, 1993a). However, all the tanks were observed for the appearance of post larvae after 15 days from the start of the experiment and the same was terminated on the day on which more than 95 % of larvae metamorphosed into post larvae.

Temperature, pH and salinity were measured daily using mercury thermometer, pH meter (Eutech Cyberscan model 510), and ATAGO refractometer respectively. Dissolved oxygen was estimated twice a week by

Winkler's method (APHA, 1995). The water samples were filtered through GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrite-N and total ammonia nitrogen (TAN) (Phenol hypochlorite method) (Grasshoff *et al.*, 1983). Total heterotrophic bacteria (THB) were estimated following standard methods and the results are expressed as colony forming units (cfu) / ml (APHA, 1995).

On completion of the experiment, 50 post larvae from each treatment were randomly selected for measuring the total length from tip of rostrum to end of the telson and the wet weight of the body. Data were analysed by Analysis of variance using SPSS 16 statistical software. Duncan's multiple range test (DMRT) was used to analyse the significant difference among means at 5 % probability level. Percentage data were normalised by arcsine transformations. Nonetheless, non transformed data is presented in the Table 5.3.

5.3 Results

The water quality parameters like temperature, pH, salinity and dissolved oxygen in all the rearing tanks were found to be well within the accepted range for larval rearing of *M. rosenbergii* (New, 2002). Where as the TAN and NO_2^- N concentration were found to increase proportionately with increase in stocking density. The control having water exchange throughout the rearing period did not record elevated levels of these inorganic nitrogen species (TAN ranged between 1.93 to 2.27 µg/l with an average of 2.12 ± 0.1 µg/l and Nitrite ranged between 0 to 1.9 µg/l with an average of 0.8 ± 0.58 µg/l) and were found to be within the limits for the normal growth and survival

of *M. rosenbergii* larvae (Correia *et al.*, 2000). The average values recorded in respect of various physio chemical parameters are given in Table 5.2. The variation in the levels of TAN and NO_2^- N are depicted in Fig 5.1 and Fig 5.2 respectively. As there was a wide variation in values of these parameters between the treatment and control, in order to facilitate a better comparison, the values of the control are omitted from the graphs. From the first day onwards all the treatments recorded elevated levels of nitrogenous nutrients compared to controls. The elevation in the values of TAN and Nitrite-N in the various treatments on the first day might be due to the addition of the Walne's medium to the rearing medium which contains nitrogenous compounds to enhance the growth of the algae.

The total heterotrophic bacteria in the control ($1.6 \pm 0.17 \times 10^3$ to $5.8 \pm 0.3 \times 10^3$ cfu /ml) were also found to be significantly lower than the algae added static treatments ($1.03 \pm 0.15 \times 10^5$ to $86 \pm 4.58 \times 10^5$ cfu/ml). Comparing the bacterial counts in the treatments, it appears that the increase in the number of bacteria was directly related to the stocking density. The highest THB in all samples collected were recorded in the treatment with a stocking density of 125 larvae /l. The pattern of variation in THB recorded during the experimental period is given in Fig. 5.3. The THB recorded in the control was significantly low when compared to the treatments. Hence to facilitate better comparison between the treatments with different stocking densities, it is not included in the graph.

The highest survival rate of 63.93% was encountered at the lowest stocking density of 30 larvae/l, while it was lowest at the highest initial stocking

density of 125 larvae/l (survival of 30.56%). The survival rate recorded in the control was 31.46% (Table 5.3). But the highest production (40 ± 1.2 post larvae per litre) was recorded at a stocking density of 100 larvae /litre in the MSGWS. Similarly, the performance of the larvae in terms of mean larval stages (Fig 5.4), appearance of first post larvae, duration of the experiment, length and weight of the post larvae were found to be very much superior at the lower stocking densities. The control took the longest time of 33 days for settlement of more than 95 % of post larvae, which was significantly longer when compared to the time taken in all the other treatments. The total length and wet weight of the post larvae were also lowest in the control which followed the clear water system of larval rearing.

5.4 Discussion

In the larviculture undertaken with lower stocking density, the chemical parameters of the water (lower TAN and NO_2^- N) may be more congenial for the healthy growth of the larvae. In addition the same quantity of *Artemia* nauplii (4 nos /ml) was also fed in all the treatments irrespective of the initial stocking density. The better environmental conditions together with proper nutrition might have led to the better performance of larvae at lower densities. With the increased stocking density, there is the possibility of high accumulation of inorganic nitrogenous compounds in the rearing medium resulting in higher levels of TAN and NO_2^-N. The source of accumulation of inorganic nitrogen species in the rearing medium may be from the excretory products of the larvae together with the unconsumed part of custard feed given to the larvae (Egg custard is fed *ad libitum* to the larvae). The dead and

decaying larvae also might have contributed to the ammonia increase in the rearing media. The increase in TAN and NO_2^- N in the present study is found to be directly proportional to the stocking density of the larvae.

Phuong *et al.* (2000) also reported similar finding in the study conducted to assess the effect of different stocking densities using the MSGWS and recirculating system in the larviculture of *M rosenbergii.* The ammonia nitrogen recorded in the static system was found to be high which ranged between 0.923 to 1.778mg/l and the values were showing an increasing trend with increase in stocking densities. The authors reported a mean percentage survival of 46.4 and 42.1% for stocking density of 90 and 120 larvae /litre and recommended a stocking density ranging from 90 to 120 larvae /litre as the stocking densities to ensure maximum post larval production for recirculating as well as static green water systems. TAN reached levels as high as 2 mg/l (Kurup, 2003) in MSGWS although no harmful effects such as stress or mass mortality were observed. Ang (1983) also made a similar observation and reported values ranging between 1.1 and 5.5 mg/l for TAN with no negative effects on larvae. According to Cohen *et al* (1976) the presence of algae in green water can effectively remove ammonia and other toxicants. The continuous aeration provided in the tanks is also reported to be another reason for the reduction of the amount of toxicants (Kurup, 2003; Phuong *et al.*, 2000).

The results of the present study revealed that, with an increase in the stocking densities the mortality rate was also increased. Nevertheless, even the treatment T5 having an initial stocking density of 125 larvae/l with elevated

levels of toxic nitrogenous compounds, registered a higher mean production (38.2 PL/l) when compared to the control (31.46 PL/l), the latter having all the water quality parameters well within acceptable limits recommended for the larviculture of *M. rosenbergii* (New, 2002; Correia *et al.*, 2000).

A higher initial stocking density resulted in a low survival in closed recirculatory system used in the larval rearing of *M. rosenbergii* (Menasveta and Piyatiratitivokul, 1980). Although a lower growth and survival were reported, biomass increase was found to be significantly higher at elevated initial stocking densities in the nursery rearing of *M. rosenbergii* PL for 20 days in cages (Marques *et al.*, 2000). Where as the same authors did not observe any effect of stocking density on the survival when the advanced prawns (harvested from the previous trial mentioned above) were cultured in cages for an extended period of 60 days. On the contrary, greater survival has been reported by Ang *et al.* (1992) in grow out phase of prawns in cages at a lower stocking density. According to Lanari *et al.* (1989), stocking density has no direct effect on survival rate in the grown out of *Penaeus japonicus*. But Wayban *et al.* (1987) stated that there was a negative correlation between the stocking density and growth of *Penaeus vannamei* adult prawn reared in an earthen pond. However, not only growth, but also survival of prawns reared under intensive culture in closed systems were better at a low stocking densities when compared to high stocking densities (Forster and Beard, 1974; Sandifer and Smith, 1976). The percentage survival was not affected by the initial stocking density where as the mean length was found to have a negative correlation in the larval rearing of summer flounder, *Paraliehthys*

dentatus (King *et al.*, 2000). However in the present study, a reduced survival and growth was observed at higher stocking densities.

Stocking density of the larvae is an important factor in determining the efficiency of hatchery production. Although greater initial stocking density may increase the PL yield, it may have a negative effects on water quality through increased production of metabolic wastes. This may result in reduced growth and survival and /or increased running costs on account of maintaining water quality parameters. On the contrary, suboptimal larval densities are likely to result in under utilisation of the installed capacity of the hatcheries and reciprocately increasing the running costs.

A final survival of 50 – 70 % was achieved in Hawaii hatcheries following green water culture with an initial stocking density of 30-50 larvae/l in a period of 25-35 days (Fast and Leung, 2003). Adopting an initial stocking density of 100 larvae/l, using the same MSGWS a survival in the range of 34 - 80% has been reported by Kurup (2003). The PL appearance was observed as early as 16-20 days and the rearing cycle could be completed in 28-32 days. Phuong *et al.* (2000) observed the post larvae on the 18th day followed by the completion of the larval rearing on the 30th day irrespective of the initial stocking density maintained. However, in the present study, the number of days for appearance of 1st post larva prolonged from 18 to 21 days commensurating with the increase of initial stocking density from 25 to125 larvae/l. So also the duration of larval rearing cycle took 27 and 30.3 days respectively for stocking densities of 25 and 125 larvae /l. The mean survival ranged from 62.93 to 30.56 % and this is comparable to the production

reported by other workers using the green water system (Phuong *et al.*, 2000; Kurup, 2003; Fast and Leung, 2003).

Another highlight of this study is that, the post larval production, duration of rearing cycle and size of post larvae was found to be far inferior in the control, in which the clear water system was adopted when compared to all treatments in which MSGWS was adopted without water exchange. The mean percentage survival achieved in the larviculture of *M. rosenbergii* in the trials conducted at SIF previously with the same clear water technology as in the control of the present study (feeding *Artemia* nauplii at 4 numbers /ml, custard feeding from 3rd day onwards, water exchange and siphoning of waste), ranged between 24.4 and 32.3 % (average 28.35 %) with a mean post larval production ranging between 24.4 and 32.3 PL /l respectively (refer production details of chapters 2, 3 and 4). Where as almost a 41% increase in survival compared with average of 28.35 %, with a production up to 40 PL /l has been registered using the same initial stocking density by the adoption of MSGWS. MSGWS is accepted for its consistent production when compared to the clear water technology which is conventionally being followed for the larviculture of *M. rosenbergii* in India. Several advantages of MSGWS in the larviculture of *M. rosenbergii* has been listed by Kurup (2003) such as technological simplicity which can be adopted at backyard level, lower investment and production costs, limited use of water which enables establishment of hatcheries even in inland areas far away from coast and labour input requirement are very little since there is no need for change of water, removal of wastes etc. from the rearing medium.

The advantages of using algae in the larval rearing (Cohen *et al.*,1976; Hudinaga, 1942; Body and Murai, 1986) and grow out of crustaceans (Chuntapa *et al.*, 2003; Izquierdo *et al.*, 2006) as well as fry and fingerlings of fishes (Faulk and Holt, 2005; Palmer *et al.*, 2007) have been invariably reported by various researchers. Low water exchange by simultaneous culturing of algae have been used for larviculture of shrimp in various ways (Liao *et al.*, 1983). The benefits of regularly adding micro algae to clear water rearing systems has been worked out by Howell (1973). Thressiamma *et al.* (2006) and Devika *et al.* (2006) reported improved survival rates in shorter duration for *M. rosenbergii* larvae reared in *Isochrysis galbana* medium with daily water exchange. Unialgal supplements particularly those of Chrysophyta were found to significantly increase survival of larvae and production of post larvae of *M. rosenbergii* in both static and recirculating water system when compared to control cultures (Manzi *et al.*, 1977). Use of *Nannochloropsis oculata* at higher densities resulted in improved survival and reduced duration of rearing period in larvicultures of Australian strain of giant fresh water prawn, *M. rosenbergii* (Lober and Zeng, 2009). The better environment provided by the algal consortium together with lesser stress due to the lower disturbance in the rearing tanks might have contributed to a higher survival and production in the present study also, when compared to clear water systems.

The THB recorded in the static system was also found to be significantly higher than in the clear water system. It is not clear whether the heterotrophic bacteria form a direct food for the prawn larvae. But the other benefits like competitive exclusion of pathogen and the faster decomposition

of nitrogenous wastes also might have added on to the overall advantage of the static green water system. The dominant benifical bacterial flora present in the static system is reported (Kurup, 2003) to reduce the proliferation of pathogens in the rearing medium which in turn reduced the chance of outbreak of diseases and there by evading the use of antibiotics. The author also finds it unnecessary to use probiotics in such static systems which supports sufficient bacterial flora.

The results of the study using the MSGWS, showed a negative correlation between the initial stocking density and survival rates. Though the total length was lowest at highest initial stocking density of 125 larvae /litre, it did not differ significantly among any of the treatments. Similarly the wet weight in the treatment T5 was found to be significantly lower ($P<0.05$) than in all other treatments. But the mean length of the PL in T4 (100 larvae / l) was not significantly different with that in the treatments with lower initial stocking density. The highest production rate (40 PL /l) was also achieved in the treatment having an initial stocking density of 100 larvae /l followed by treatment with 125 larvae / l. The date of post larval settlement was delayed only by 2 and 3 days for the treatments T4 and T5 respectively when compared to the mean duration of 27.7 days recorded for the treatment with an initial stocking density of 25 larvae /litre. Almost the same expenditure was incurred in all the treatments (BSN was fed at the same rate and custard which was administered at a slightly higher level in the tanks with higher number of larvae was not very expensive. Besides, the brooders could be procured at the cost of Rs. 50 / brooder), whatever be the initial stocking

density that was adopted. Based on the results of the present study, it can be recommended that the initial stocking density of 100 larvae / litre will be optimum in MSGWS to maximise the post larval production and utilisation of available facilities. Moreover, there will not be considerable change in the recurring cost of production irrespective of change in the initial stocking density.

5.5 Conclusion

The optimal initial stocking density in the larviculture of *M. rosenbergii* following modified static green water system is recommended as 100 larvae / litre.

Table 5.1 Stocking densities adopted in different treatments in the larviculture of *M rosenbergii* using MSGWS

Treatments	Stocking density
T1	25
T2	50
T3	75
T4	100
T5	125
Control	100 (Clear water system)

Table 5.2 Average ± s.d of various water quality parameters recorded during the experimental period at various stocking densities in the larviculture of *M rosenbergii* using MSGWS

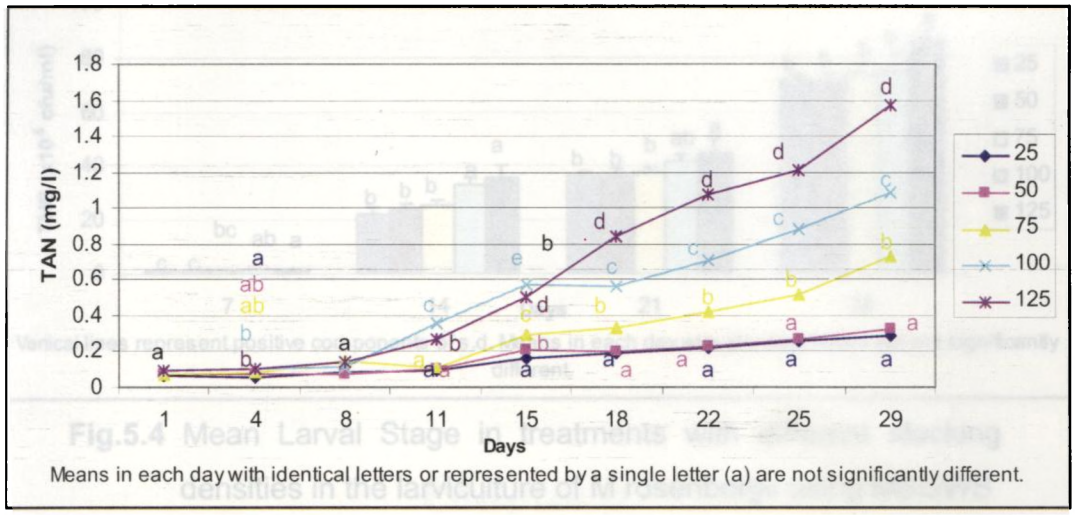
Parameters	Treatments					
	T1	T2	T3	T4	T5	C
Temperature (°C)	27.6 ± 0.72	27.77 ± 0.78	27.69 ± 0.77	27.7 ± 0.75	27.73 ± 0.72	27.71 ± 0.71
pH	8.04 ± 0.14	7.98 ± 0.1	7.97 ± 0.13	8.08 ± 0.15	7.99 ± 0.18	7.95 ± 0.12
Salinity (ppt)	13.18 ± 0.69	13.2 ± 0.83	13.26 ± 0.85	13.28 ± 0.85	13.09 ± 0.67	12.84 ± 0.58
Dissolved oxygen (mg/l)	7.01± 0.18	7.07 ± 0.15	7.02 ± 0.16	6.98 ± 0.19	7.05 ± 0.22	7.12 ± 0.15

Table 5.3 Effect of stocking densities on the survival, appearance of post larvae, duration of rearing period, total length and wet weight of the post larvae in the larviculture of *M rosenbergii* using MSGWS

Treatments	Stocking density(larvae/litre)	% survival	Post larvae Per liter	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
T1	25	62.93 ± 2.57^{a}	15.73 ± 0.64	18^{a}	27^{a}	9.86 ± 0.48^{a}	10.16 ± 0.39^{a}
T2	50	48.53 ± 2.01^{b}	24.26 ± 1	18.3 ± 0.6^{a}	27.7 ± 0.6^{ab}	9.87 ± 0.3^{a}	10.17 ± 0.28^{a}
T3	75	41.87 ± 1.6^{c}	31.4 ± 1.2	19.3 ± 0.6^{b}	28.7 ± 0.6^{bc}	9.77 ± 0.45^{a}	10.05 ± 0.47^{a}
T4	100	40 ± 1.2^{c}	40 ± 1.2	20^{c}	29.7 ± 0.6^{cd}	9.78 ± 0.34^{a}	10.05 ± 0.36^{a}
T5	125	30.56 ± 1.09^{d}	38.2 ± 1.35	21^{d}	30.3 ± 0.6^{d}	9.52 ± 0.5^{a}	9.84 ± 0.43^{b}
Control	100	31.46 ± 1.49^{d}	31.46 ± 1.49	25^{d}	33^{e}	9.51 ± 0.4^{a}	9.74 ± 0.32^{b}

Mean ± s.d of three replicate groups. Means in each column sharing the same superscript letter are not significantly different (P>0.05)

Fig 5.1 Changes in TAN in different treatments with different stocking densities in the larviculture of *M.rosenbergii* using MSGWS



Letters with different colours correspond to the treatments represented by each colour in all the graphs

Fig 5.2 Effect of different stocking densities on nitrite nitrogen in the larviculture of *M.rosenbergii* using MSGWS

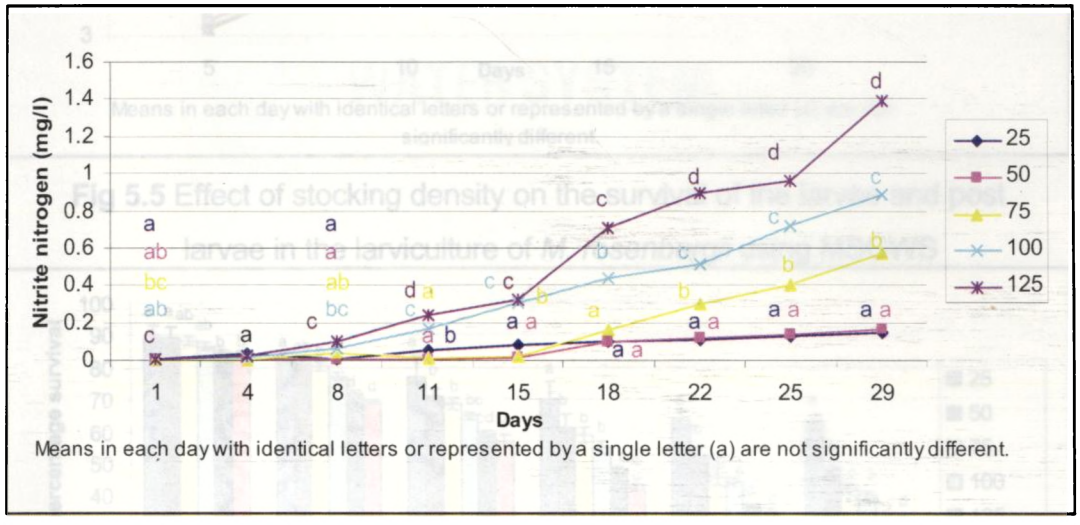


Fig 5.3 THB in treatments with different stocking densities in the larviculture of *M.rosenbergii* using MSGWS

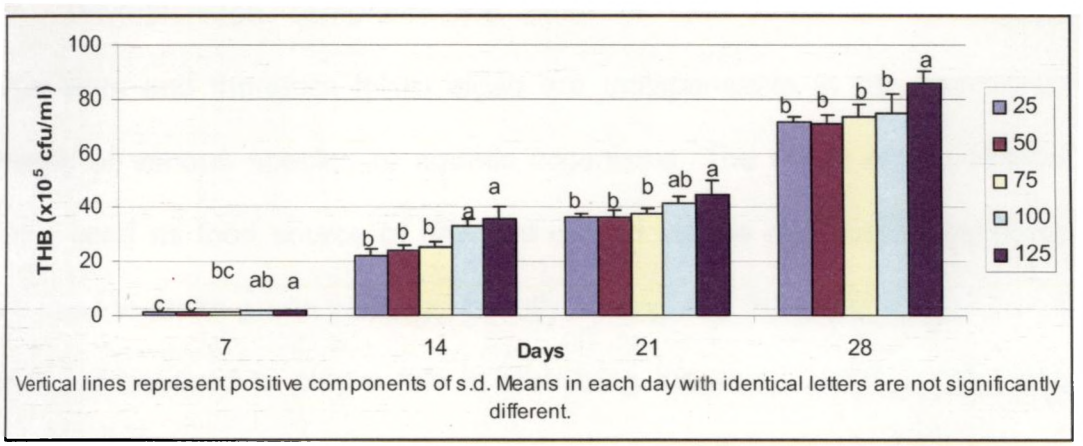


Fig.5.4 Mean Larval Stage in treatments with different stocking densities in the larviculture of M rosenbergii using MSGWS

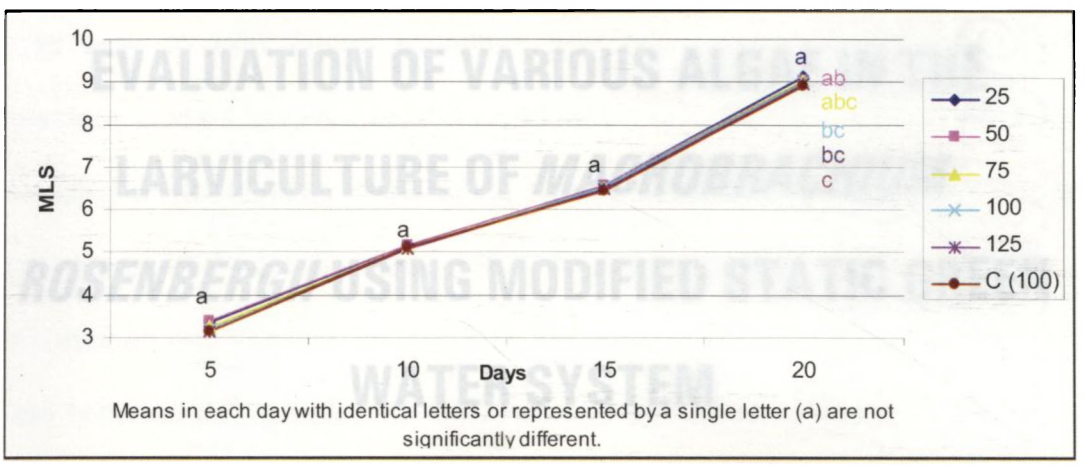
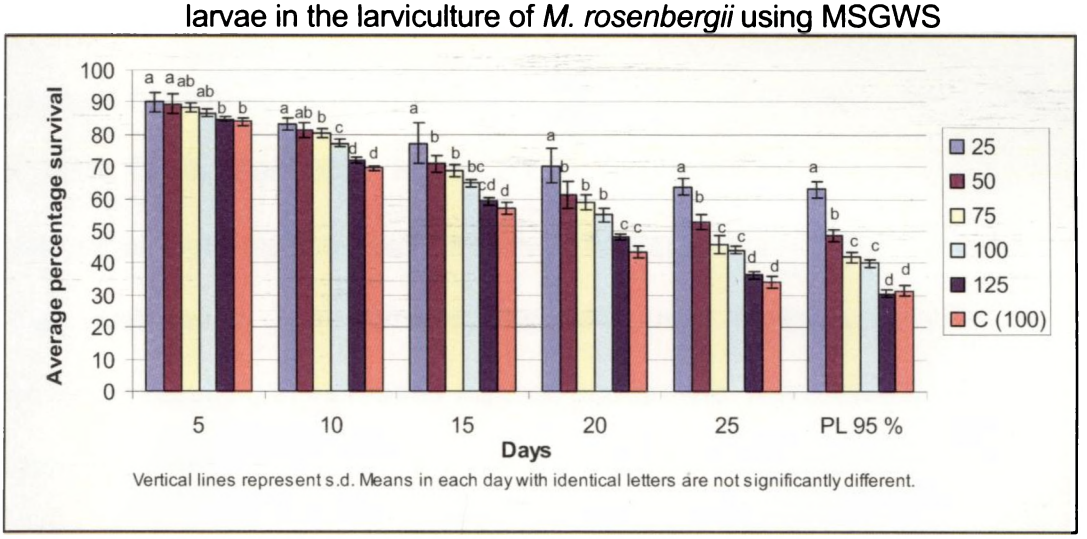


Fig 5.5 Effect of stocking density on the survival of the larvae and post larvae in the larviculture of *M. rosenbergii* using MSGWS



Chapter 6

EVALUATION OF VARIOUS ALGAE IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII* USING MODIFIED STATIC GREEN WATER SYSTEM

6.1 Introduction

Phytoplankton comprises the base of food chain in the aquatic ecosystem and therefore micro algae are indispensable in the commercial rearing of various species of aquatic organisms. The micro algae, besides being used as food source of diferrent growth stages of aquatic organisms, are used to make green water by directly adding into the larval rearing tanks. They are believed to play a role in stabilizing the water quality parameters, nutrition of larvae and microbial control (Lavens and Sorgeloos, 1996).

Controlled low water exchange green water cultures have been used world wide to rear larvae of fishes and crustaceans. There have been numerous studies from across the world referring to the significant advantages of adding phytoplankton to the larval rearing systems (Howell, 1973; Chiang and Liao, 1985; Naas *et al.*, 1992; Austin *et al.*,1992; Liao *et al*, 2001; Regunathan and Wesley, 2004; Faulk and Holt, 2005). Increased survival has been reported in India (Kurup, 2003) as well as Vietnam (Hai, 2003) by using the modified static green water system in the larviculture of *M. rosenbergii*. However, in all the studies so far conducted using this technology of larval rearing, *Chlorella* sp. is used as the main component of green water (Hein *et al.*, 2000; Oanh *et al.*, 2000; Kurup, 2003). Whereas there are many reports of using other algae as the media in larval rearing of many fishes as well as in other crustaceans. *Tetraselmis* sp has been used widely for rearing marine fish larvae resulting in increased survival and growth. Many studies have been conducted using the micro alga *Nannochloropsis occulata* (Palmer *et al.*, 2007) which is reported to be yielding high hatchery production of many

marine fishes. Live feed *Isochrysis galbana* is extensively used as an organism with a high DHA (12%) content (Liao *et al.*, 2001). When used as the rearing medium, *I. galbana* provided similar growth and survival to live prey, as enriched with commercial fatty acid boosting products (Faulk and Holt, 2005). In Taiwan, presently over 90 species of fin fishes are produced using GWC with *Nannochloropsis* and *Isochrysis* sp. as the micro algae and the results were encouraging in improving hygiene of system and larval survival (Liao *et al.*, 2001).

In this context a study was conducted with three algae other than *Chlorella* as the rearing media in the modified static green water system viz., *Tetraselmis chuii*, *Nannochloropsis occulata* and *Isochrysis galbana* and a mixed culture of these.

Thus the objective of the present study was

- To investigate the efficiency of algal species other than *Chlorella* as a rearing medium in the modified static green water system.

6.2 Materials and methods

6.2.1 Brood stock management, algal culture and larval rearing

Berried prawns with black coloured eggs were collected from grow out and transported in black coloured plastic cans to the hatchery at the SIF, disinfected (New, 2002) and transferred to 150 l FRP tanks, one berry each in a tank and maintained in 5 ppt saline water with continuous aeration. The tanks were covered with nets to prevent escapement of prawns and were kept undisturbed at night. The eggs hatched by next day morning.

The algae were cultured using modified Walne's medium (Lavens and Sorgeloos, 1996), the composition of the same is given in Chapter 2 (Table 2.2). Stock cultures (inoculum procured from CMFRI, Cochin and Marine Botany laboratory, CUSAT, Cochin) were maintained at salinity of 20 – 25 ppt. One day prior to introduction of larvae, the inoculum from mass culture along with required quantity of Walne's medium (modified) was added to each rearing tank in order to accomplish an initial density of 3 – 5 x 10^5 cells / ml. The algal concentration reached values ranging between 6 – 10 x 10^5 cells /ml by the end of the rearing period. Algal culture in exponential growth phase was used as the inoculum. In addition to routine filtration and water treatment, water was sterilized by heating using the immersion heater before inoculation with algae. Moderate to strong aeration was provided in all the tanks to facilitate mixing and keeping waste products in suspension for aerobic bacterial digestion. The larval rearing and feeding (Section 5.2.3 and 5.2.4) were done as explained in Chapter 5 (refer chapter 5 for details). The larvae were initially fed with BSN followed by egg custard from the 5^{th} larval stage.

6.2.2 Evaluation of results

The results of different treatments were ascertained based on the time required for larvae to reach each stage and their relative survival. Thirty larvae from each tank were randomly sampled every fifth day and the larval stages were identified following the descriptions of Uno and Kwon (1969). Mean larval stage (MLS) was used for determining the development of larvae and calculated using the formula given by Lovett and Felder (1988). MLS= $\sum$(S x Ps) where S is the larval stage number and Ps is the proportion of larvae at

stage S. Survival of the larvae were estimated by taking ten 1 liter samples from the rearing medium under strong aeration. The estimation of percentage composition of the different larval stages and survival continued till 20th and 25th day respectively. Since it was not possible to draw uniform samples, due to the benthic habit of post larvae neither staging nor estimation of survival was continued after appearance of considerable number of post larvae (Alam *et al.*, 1993a). However, all the tanks were observed for appearance of post larvae after 15 days from the start of the experiment and the day on which more than 95 % of larvae metamorphosed was noted and the experiment terminated.

Temperature, pH and salinity were measured daily using mercury thermometer, pH meter (Eutech Cyberscan model 510), and ATAGO refractometer. Dissolved oxygen was estimated twice a week by Winkler's method (APHA, 1995). The water samples were filtered through GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrite-N and total ammonia nitrogen (TAN) (Phenol hypochlorite method) (Grasshoff *et al.*, 1983). Total heterotrophic bacteria (THB) were estimated following standard methods and the results are expressed as colony forming units (cfu) / ml (APHA, 1995).

On the completion of the experiment, 50 post larvae from each treatment were randomly selected for measuring the total length from tip of rostrum to end of the telson and the wet weight of the body. Data were analysed by Analysis of variance using SPSS 16 statistical software. Duncan's multiple range test (DMRT) was used to analyse the significant difference

among means at 5 % probability level. Percentage data were normalised by arcsine transformations. Nonetheless non transformed data is presented in the Table 6.3 and Fig 6.5.

6.3 Results

Physico chemical parameters like temperature, pH, salinity and dissolved oxygen were found to be optimum for larval rearing (New, 2002) of *M rosenbergii*. The average values of the water quality parameters recorded are given in Table 6.2.

However, the nutrients like TAN and nitrite nitrogen showed a gradual increase towards the completion of the rearing period. The larvae were not found to be affected by the increase in the nitrogenous toxicants, as there was no specific increase in the mortality or retardation of growth (Fig 6.3 and 6.4) at any of the sampling days. Fig. 6.1 and 6.2 depicts the TAN and nitrite nitrogen variation in the rearing tanks during the larviculture. Although significant variation ($P<0.05$) existed between the tanks on different sampling days with respect to these parameters (Fig 6.1 and 6.2), no specific pattern of increase or decrease in any of the treatments was observed. However, a gradual build up of these toxicants could be observed in all the tanks towards the termination of the experiment. The highest TAN and nitrite nitrogen values recorded during the larval rearing period were 1.04 ± 0.21 mg/l and 0.76 ± 0.12 mg/l respectively.

The number of colony forming units of total heterotrophic bacteria was also found to increase with time and it ranged between 1.3 ± 2.65 x 10^5 to 79.67 ± 2.08 x 10^5 cfu/ml. However no significant difference was observed

with respect to THB among any of the treatments throughout the larval rearing period.

The performances of the algae in all the treatments were comparable except the significantly lower mean survival (45 ± 2.71%) recorded in the T1 with *T. chuii* as the rearing medium. No significant difference was observed among the treatments with the control (*Chlorella vulgaris*) with respect to the larval progression (Fig. 6. 4), appearance of the first PL, duration of the experiment, total length and wet weight of the post larvae. However, the highest mean survival of 49.03 ± 2 %, (very narrow difference was recorded between the treatments except T1) was recorded in the *I. galbana* medium. The appearance of the first PL was earliest (average of 19.7 ± 0.6 days) in the same medium. Same is the case with duration of the experiment which was lowest in T2 with an average value of 29.7 ± 0.6 days. However, the highest mean total length and wet weight (9.84 ± 0.36 and 10.1 ± 0.34) were observed in T4 with a mixed culture of all the four algal species. The production details of the post larvae are given in Table 6.3. The Figs. 6.4 and 6.5 show the MLS and survival respectively recorded at every 5 days interval during the experimental period.

6.4 Discussion

There are many reports of utilizing the algal species used in the present experiment for larval rearing of fish and crustaceans around the world, both as food and as well as rearing medium and for enriching the live feed (Liao *et al.*, 1983; Naas *et al.*,1992; Hernandez-Cruz *et al.*, 1994; Gulbrandsen *et al.*, 1996; Liao *et al.*, 2001; Papandroulakis *et al.*, 2001, 2002; Faulk and Holt,

2005 Thresiamma *et al.*,2006; Lober and Zeng, 2009). By using algae in larval rearing of *M rosenbergii*, Maddox and Manzi (1976) obtained 30% greater survival than the control without algae.

Among the various algae used in the present study, two are motile (*I galabana* and *T chuii*) and are widely used for developing green water cultures. *Tetraselmis suecica* was found to provide antibacterial benefits in the shrimp larviculture (Reghunathan and Wesley, 2004) by inhibiting pathogens. The alga was also found to inhibit fish pathogens (Austin *et al.*, 1992). The beneficial effects like contrast enhancement and chemical stimulation provided by the microalga *T. suecica* resulted in higher rotifer consumption rates rather than clean water in the larval rearing of green back flounder, *Rhombosolea tapirina* (Shaw *et al.*, 2006). However, a significantly lower survival of 45 % (45 PL/l) was obtained when *T. chuii* was used as the medium in the present study. This was due to the increased mortality that occurred in one of the rearing tanks with *T. chuii* as the medium before the initial sampling. No reason could be attributed for the same where as the other two tanks among the triplicate showed no such larval mortalities. However though the survival recorded was significantly low in the treatment with the alga *T. chuii*, the other growth indicators like length and weight of post larvae, appearance of PL and duration of the rearing period did not differ significantly with the other treatments.

Isochrysis galbana which was found to be ingested by the larvae of sea bass *Dicentrarchus labrax* while drinking, is thought to beneficially trigger the production of digestive enzymes in the pancreas and intestine (Cahu *et al.*,

1998). The studies conducted on the larval rearing of Cobia, *Rachycentron canadum* showed that larval survival was significantly improved in the presence of *Nannochloropsis occulata* and *Isochrysis galbana* (Faulk and Hault, 2005). The beneficial effects of using *I. galbana* in the larval rearing of *M. rosenbergii* have also been reported by Thresiamma *et al.* (2006) and Devika *et al.* (2006). The highest post larval production, best larval progression and the earliest settlement of post larvae in the present study was recorded in the treatment with *I. galbana* as the rearing medium.

Nannochloropsis occulata has been used to create green water culture system for larval rearing of marine fishes like Asian seabass (*Lates calcarifer*), Australian bass (*Macquaria nomenculata*), snapper (*Pagrus auratus*), dusky flathead (*Platycephalus fuxus*), sand whiting (*Sillago ciliata*) and zoo plankton like *Brachionus plicatilis* (Palmer *et al.*, 2007). The author has reported a considerable increase in the production of fry and fingerlings of the important cultivable finfish species by using the green water culture when compared to the clear water. Survival rates ranging from 80% to as high as 100% have been reported and in most cases it was above 95% and the entire larvae were found to be very healthy by the same authors. Better survival for larval *Sparus auratus* (Hernandez-cruz *et al.*, 1994) and *Mugil cephalus* (Tamura *et al.*, 1994) have also been reported by the use of green water. The first -feed larvae of Cod (*Gadus morhua*) were also found to ingest cells of the alga *Nannochloropsis atomus* at lower rates consistent with larval drinking of water. A recent study using the Australian strain of *M. rosenbergii* has demonstrated improved survival and growth of the prawn larvae by using the micro alga

Nannochloropsis sp. at higher concentration of 12.5 to 25 x 10^5 cells/ml (Lober and Zeng, 2009). In the present study also, the post larval production recorded by using *N. occulata* as the rearing medium was comparable with all the other treatments of the present study and well above the average survival of 30 % (Murthy, 2006) reported from commercial hatcheries in India. Thus, in the present study also, in comparison to the conventionally used *Chlorella* sp. for larval rearing in modified static green water system, the use of other algal species have also resulted in equally competent results.

The use of different algae was not found to influence the fluctuation (probably due to the instability in phytoplankton population) or the built up of the nitrogenous toxicants like TAN or nitrite nitrogen. The elevated levels of TAN or nitrite nitrogen in various treatments especially towards the end of the rearing period, however, was not found to have any specific negative impact on the larval growth and survival. TAN levels up to 2 mg/ l has been recorded by Kurup (2003) in the modified static green water system for rearing *M. rosenbergii* larva, without any harmful effects on the growth and survival of the larvae. A similar observation was also made by Phuong *et al.* (2000) who recorded TAN values ranging between 0.923 and 1.778mg/l, but did not observe any deleterious effects on the larvae.

Thresiamma *et al.* (2006) reported a survival as high as 59.7% while using *I. galbana* in the rearing of *M. rosenbergii* in comparison to the control (survival 46.7%) without alga. The *Artemia* nauplii concentration used by the authors, was however higher in comparison to that used in the present study. Moreover, the survival of 49.07 % recorded for the larvae reared in *I. galbana*

In the present study was comparable to 47.3 % reported by Thresiamma *et al.* (2006) by feeding about 4 nos /ml of BSN, which was the concentration of *Artemia* nauplii used for feeding the prawn larvae in the present experiment. The mean survival achieved by feeding *N. occulata* (48.47 %) in the present experiment is slightly lower when compared to 63.3% reported by Lober and Zeng (2009) using the alga *Nannochloripsis* sp. at concentration of 12.5 x 10^5 cells /ml in the larval rearing of Australian strain of *M. rosenbergii*; but higher than 35% reported by the same authors for an algal density of 6.25 x 10^5 cells/ml. However, both Thresiamma *et al.* (2006) and Lober and Zeng (2009) used lower initial stocking densities (50 and 30 larvae /l respectively) where in the larvae were stocked @ 100 / l in the present study.

High survival and growth were also observed in the mixed culture of algae and the length and weight of the larvae were also found to be the highest in the treatment T4 in which a combination of all the algae was used as the rearing medium. The use of a combination of two or more of complementary algal species like *N. occulata* and *I. galbana* has been recommended for enhancing the nutritional benefits of the algae to the larval fishes (Liao *et al.*, 2001). A combination of algae at different densities are also used in the feeding of penaeid larvae (Lavens and Sorgeloos, 1996; FAO, 2007).The use of extensive mesocosms utilizing wild plankton (consisting of different micro algae) has been found to be beneficial for rearing marine fish larvae (Naas *et al.*, 1992). Likewise, the use of a mixture of algae as the rearing medium resulted in the production of larger postlarvae with an equally

comparable postlarval production when compared to control with *Chlorella vulgaris* as the rearing medium.

6.5 Conclusion

No difference was observed in the overall performance of the prawn larvae in any of the algal medium or when their combinations were used except *T. chuii* in which a lower post larval survival was recorded in one of the triplicate tanks. Hence, use of any of the algae viz. *Isochrysis galbana*, *Nannochloropsis occulata* and *Tetraselmis chuii* or their combination can be recommended along with *Chlorella vulgaris* as the rearing medium in the modified static green water system of larval rearing of *M. rosenbergii*.

Table 6.1 Algal species used in different treatments in the larviculture of *M. rosenbergii* adopting MSGWS.

Treatment	Algal species
T1	*Tetraselmis chuii*
T2	*Isochrysis galabana*
T3	*Nannochloropsis occulata*
T4	Mixed culture
T5 (Control)	*Chlorella vulgaris*

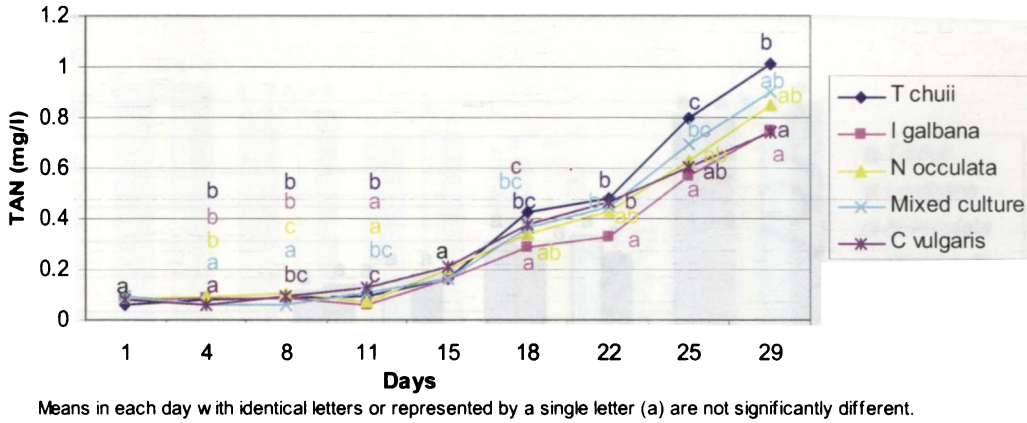
Table 6.2 Average ± s.d of various water quality parameters recorded during the experimental period in the larviculture of *M. rosenbergii* using MSGWS with different algae as the rearing medium

Parameters	Treatments				
	C	T1	T2	T3	T4
Temperature (^{0}C)	27.37 ± 0.55	27.47± 0.6	27.39 ± 0.57	27.41 ± 0.56	27.43 ± 0.52
pH	8.13 ± 0.14	8.07 ± 0.1	8.06 ± 0.13	8.17 ± 0.15	8.08 ± 0.18
Salinity (ppt)	13.09 ± 0.54	13.31 ± 0.86	13.37 ± 0.87	13.29 ± 0.85	13.15 ± 0.61
Dissolved oxygen (mg/l)	6.99 ± 0.18	7.07 ± 0.17	7.02 ± 0.18	6.96 ± 0.2	7.05 ± 0.23

Table 6.3 Effect of different algae as the rearing medium on survival, appearance of first post larvae, duration of rearing period, length and wet weight of the post larvae of *M rosenbergii* in the larviculture using MSGWS

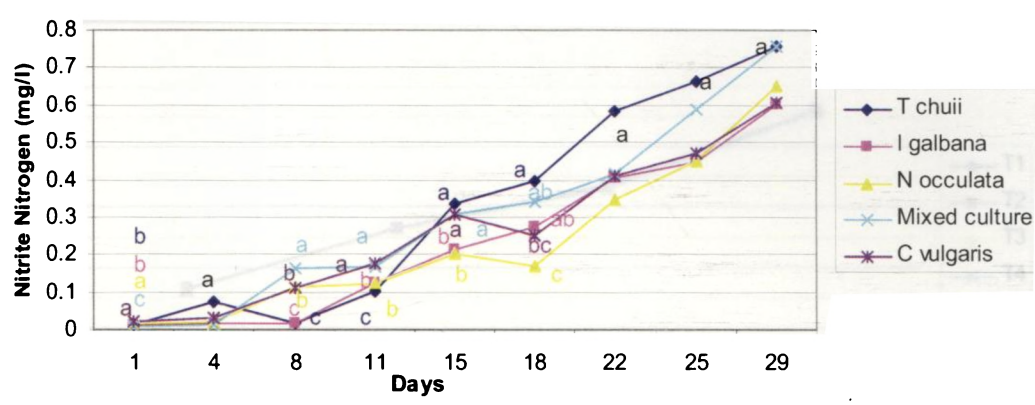
Treatments	Stocking density(larvae/litre)	% survival	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
T1	100	45 ± 2.71^{b}	$20.7 \pm .6^{a}$	$30.3 \pm .6^{a}$	9.74 ± 0.32^{a}	9.98 ± 0.36^{a}
T2	100	49.07 ± 2^{a}	$19.7 \pm .6^{a}$	$29.7 \pm .6^{a}$	9.81 ± 0.28^{c}	10.08 ± 0.27^{a}
T3	100	48.47 ± 1.7^{a}	$20.3 \pm .6^{a}$	30^{a}	9.77 ± 0.42^{a}	10.03 ± 0.46^{a}
T4	100	48.6 ± 1.2^{a}	20^{a}	30^{a}	9.84 ± 0.36^{a}	10.1 ± 0.34^{a}
T5 (Control)	100	48.93 ± 1.8^{a}	$20.3 \pm .6^{a}$	30^{a}	9.73 ± 0.41^{a}	9.96 ± 0.37^{a}
Mean ± s.d of three triplicate groups; Means sharing same same superscript letter in each column doesnot differ significantly.						

Fig 6.1 Effect of using different algae as the rearing medium on TAN in different treatments in the larviculture of *M. rosenbergii* using MSGWS



Means in each day with identical letters or represented by a single letter (a) are not significantly different.

Fig .6.2 Effect of using different algae as the rearing medium on nitrite nitrogen in different treatments in the larviculture of *M. rosenbergii* using MSGWS



Means in each day with identical letters or represented by a single letter (a) are not significantly different.

Fig 6.3 Effect of using different algae as the rearing medium on THB in different treatments in the larviculture of *M. rosenbergii* using MSGWS

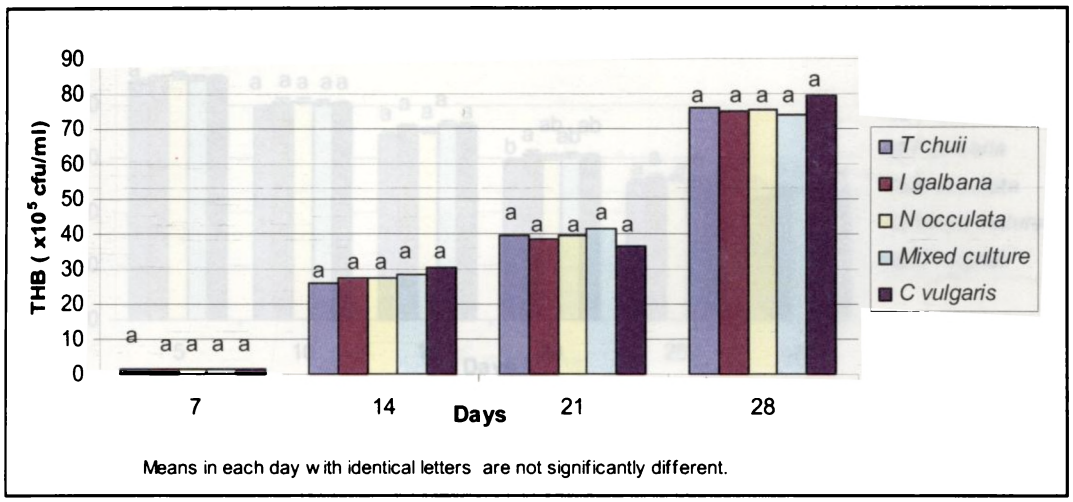


Fig 6.4 Mean Larval Stages in treatments with different algae as the rearing medium in the larviculture of *M. rosenbergii* using MSGWS

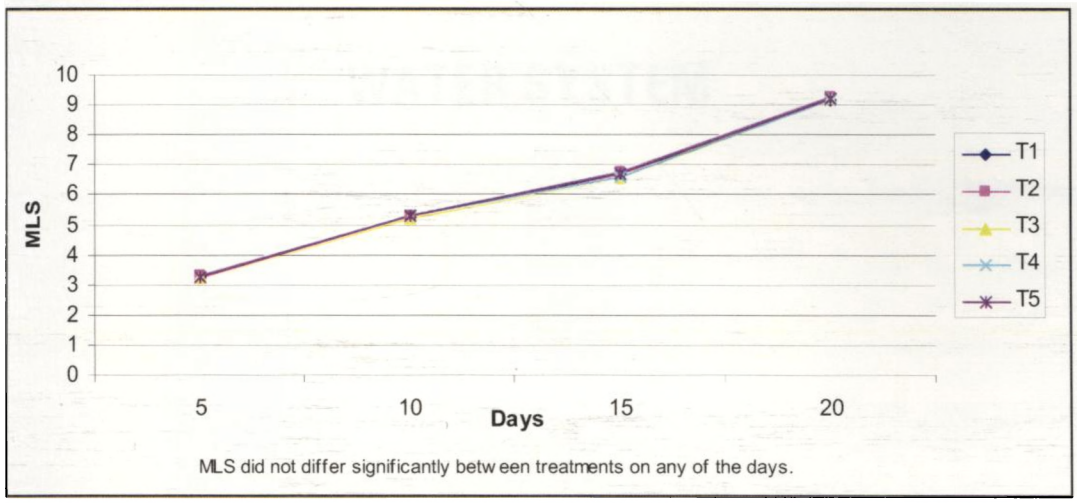
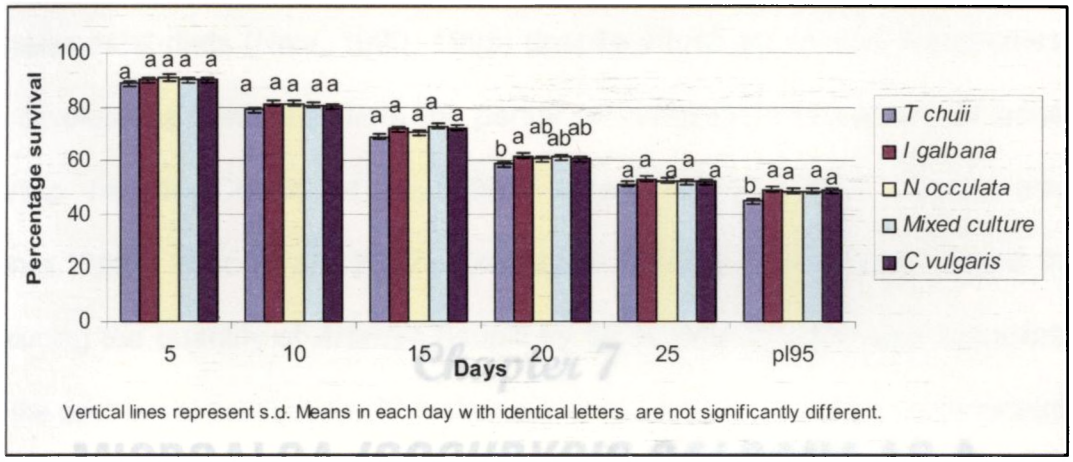


Fig 6.5 Effect of using different algae as the rearing medium on survival of larvae and post larvae in different treatments in the larviculture of *M. rosenbergii* using MSGWS



Chapter 7

MICROALGA *ISOCHRYSIS GALBANA* AS A SUBSTITUTE OF *ARTEMIA* NAUPLII IN THE LARVICULTURE OF M*ACROBRACHIUM ROSENBERGII* IN THE MODIFIED STATIC GREEN WATER SYSTEM

7.1 Introduction

The larviculture *Macrobrachium rosenbergii* is primarily dependent on the use of live feed *Artemia* nauplii either solely or in combination with supplemental diets (New, 1990, 1995) despite efforts by various researchers to develop supplemental diets for partial or complete replacement of brine shrimp nauplii (Corbin *et al.*, 1983; Cheah *et al.*, 1987; Kumlu and Jones,1995; Nair *et al.*, 2007). *Isochrysis galabana* was found useful in reducing the quantity of *Artemia* nauplii by 50 % with no significant reduction in the post larval production (Thresiamma *et al.*, 2006). Complete replacement of *Artemia* nauplii with micro particulate diets have also been reported in the larviculture of *M. rosenbergii* larvae from zoea V/ VI stages (Deru, 1990; Kumlu and Jones, 1995). The results of various trials conducted in this regard by the author with other indigenous live feeds at the fresh water prawn hatchery of SIF, CUSAT did not give encouraging results (Chapters 2, 3 and 4). However, a substitution of 25 % of *Artemia* nauplii with the alga *T. chuii* or the gradual weaning of the prawn larvae to blood worms by the X^{th} larval stage was found useful in the seed production of *M. rosenbergii* and the results so obtained did not show any significant difference with the control where in *Artemia* nauplii was given as live feed @ 100 % (4 numbers/ ml) throughout the rearing period. High survival and production of post larvae could be achieved in some of the trials conducted as a part of the study (Chapters 5 and 6) adopting the modified static green water system and the results were concurring with Kurup (2003) who got a post larval production ranging between 34 and 80 PL/ l within a period of 28 to 32 days. Among the

various algae used as larval rearing medium, the highest mean post larval production of 49.07 % was attained using the microalga *Isochrysis galbana* (refer chapter 6). Lavens and Sorgeloos (1996) listed *I. galbana* among the live micro algal diets used for larval rearing of *M. rosenbergii*. In this background, a study was conducted with the following objectives –

1. Evaluate the effectiveness of the modified static green water system supplied with the alga *Isochrysis galbana* in reducing the use of *Artemia* nauplii in the larviculture of *M. rosenbergii* and
2. Cost effectiveness of post larvae so produced.

7.2 Materials and methods

The experiment was conducted following the methodology adopted by Kurup (2003) with four treatments and a control with triplicates for each in a completely randomised design.

The different feeding schedules used in the experiment are given in Table 7.1. The larvae were stocked at density of 100/l. Four treatments (Al 10 l – *Artemia* nauplii and *I. galbana* till 10^{th} stage and no *Artemia* nauplii given from 10^{th} larval stage, Al 8l - *Artemia* nauplii and *I. galbana* till 8^{th} stage and no *Artemia* nauplii given from 8^{th} larval stage, Al 6l - *Artemia* nauplii and *I. galbana* till 6^{th} stage and no *Artemia* nauplii given from 6^{th} larval stage and *I. galbana* alone) were taken and 100 % *Artemia* nauplii throughout the rearing period was taken as control. Newly hatched *Artemia* nauplii (OSI brand) was fed at density of 4 numbers/ml in the control tanks (100 % *Artemia*) . The number in the treatment tanks were adjusted accordingly. From V^{th} larval stage onwards egg custard (Kurup, 2003) was fed, irrespective of the

treatments, four times daily while newly hatched *Artemia* nauplii was used as over night feed.

7.2.1 Brood stock management

Berried prawns with black coloured eggs were collected from grow outs and transported in black coloured plastic cans to the hatchery at SIF. They were disinfected (New, 2002) and transferred to 150 l FRP tanks, one berry / tank and maintained in 5 ppt saline water with continuous aeration. The tanks were covered with nets to prevent escapement of prawns and were kept undisturbed at night. The eggs hatched by next day morning.

7.2.2 Algal culture

Algal inoculum was purchased from Central Marine Fisheries Research Institute, Cochin which was maintained and sub cultured at the Aquaculture Laboratory, School of Industrial Fisheries using modified Walne's medium (Lavens and Sorgeloos, 1996). Large scale out door culture was carried out using this inoculum. The inoculum was maintained at a higher salinity (20-25ppt) and before adding to the rearing tanks, the salinity was gradually reduced to 15 ppt. An algal density of 3-5 x 10^5 cells /ml was maintained in the rearing medium. This differs from the methodology of Kurup (2003) wherein natural development of *Chlorella* sp was promoted using Tilapia (*Oreochromis mossambicus*).

5.2.3 Larval rearing

100 l white coloured FRP tanks were used for larviculture experiments. Brackish water with 12 ppt salinity was prepared by mixing sand filtered sea

water (25-30 ppt) and chlorinated tap water, treated with 30 ppm active chlorine and was kept with vigorous aeration for 6-7 days to remove chlorine. In addition to this, the water was sterilised by heating using immersion heaters before adding the algae. After cooling, the algal inoculum was added along with the required quantity of nutrient medium (Walne's medium) one day prior to stocking of larvae. The larvae were stocked at an initial density 100 numbers/ l. There was neither water exchange nor cleaning of tanks undertaken for green water system during the rearing cycle.

7.2.4 Feeding

Newly hatched *Artemia* nauplii (OSI brand) were fed at density of 4 numbers/ ml divided into two equal rations in the morning and evening (9 AM and 5 PM) till V^{th} larval stage in the control tanks. The quantity of BSN fed to the other tanks varied depending on the treatments. From V^{th} stage onwards egg custard, which was prepared following Kurup (2003) , was fed four times daily at 9 AM, 11 AM, 2 PM and 4PM irrespective of the treatments, while newly hatched *Artemia* nauplii was given only at 5 PM as over night feed. The aeration in the tanks was stopped before feeding with egg custard so as to enable the surfacing of the larvae. The custard feed of particular size (Kurup, 2003) was fed to the larvae judiciously using a pipette in a circular manner with out over feeding.

7.2.5 Evaluation of results

The results of different treatments were assessed based on the time required for larvae to reach each stage and their relative survival. Thirty larvae from each tank were randomly sampled every third day and the larval stages

were identified following the descriptions of Uno and Kwon (1969). Mean larval stage (MLS) was used for determining the development of larvae and calculated using the formula of Lovett and Felder (1988). MLS= $\sum$(S x Ps) where S is the larval stage number and Ps is the proportion of larvae at stage S. Survival of the larvae were estimated by taking ten 1 litre samples from the rearing medium under strong aeration. The estimation of percentage composition of the different larval stages and survival continued till 24th day. Since it was not possible to draw uniform samples, due to the benthic habit of post larvae, neither staging nor estimation of survival was continued after appearance of considerable number of post larvae (Alam *et al.*, 1993a). However, all the tanks were observed for the appearance of post larvae after 15 days from the start of the experiment and the same was terminated on the day on which more than 95 % of larvae metamorphosed into post larvae.

Temperature, pH and salinity were measured daily using mercury thermometer, pH meter (Eutech Cyberscan model 510), and ATAGO refractometer respectively. Dissolved oxygen was estimated twice a week by Winkler's method (APHA, 1995). The water samples were filtered through GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrite-N and total ammonia nitrogen (TAN) (Phenol hypochlorite method) (Grasshoff *et al.*, 1983). Total heterotrophic bacteria (THB) were estimated following standard methods and the results are expressed as colony forming units (cfu) / ml (APHA, 1995).

On completion of the experiment, 50 post larvae from each treatment were randomly selected for measuring the total length from tip of rostrum to

and of the telson and the wet weight of the body. Data were analysed by Analysis of variance using SPSS 16 statistical software. Duncan's multiple range test (DMRT) was used to analyse the significant difference among means at 5 % probability level. Percentage data were normalised by arcsine transformations. Nonetheless, non transformed data is presented in the Table 7.3 and Fig.7.5.

7.3 Results

The mean values with their standard deviation of various physicochemical parameters like temperature, pH, salinity and dissolved oxygen during the period of experiment are given in Table 7.2. The water quality parameters were within the optimum ranges as specified by New and Singholka (1985). All the larvae in the treatment I, with no *Artemia* nauplii supply were all dead by the 7^{th} day of the experiment. Hence, for convenience of presentation and interpretation, the values recorded for the various parameters for the treatment I are not included in any of the tables and graphs.

Mean values with standard deviation of total ammonia nitrogen and NO_2 N recorded during the period in the various treatments are presented in Figure 7.1 and 7.2 respectively. These inorganic nitrogenous species were showing an increasing trend with the progression of the experiment. Though not significantly different, TAN values recorded in the control was higher than in all the treatments on most of the sampling days. Where as significantly higher values ($P<0.05$) were recorded for NO_2 - N in the control from the 18^{th} day of the experiment when compared to most of the treatments. THB was

also found ascending on the second sampling day (Fig.7.3), however, it did not differ significantly among the various treatments and the control.

The final production details of the experiment are given in Table 7.4. The mean larval stage and the percentage survival are presented in Fig. 7.4 and Table 7.5 respectively. The highest mean percentage survival of 40.2 % with an average post larva of 2010 numbers was recorded in the control in which 100 % *Artemia* nauplii was fed throughout the larval rearing period. This was followed by treatment AI 10I in which an average survival of 39.8 % was recorded. Both the control and AI 10I did not show significant difference with respect to the mean surviving larvae throughout the larval rearing period except on day 22. AI6I in which no *Artemia* nauplii were given after VIth larval stage lagged much behind all the treatments from 9th day onwards. A final average survival of 23.3 % was registered for this treatment with a corresponding average post larval number of 1165. AI8I showed significant difference from AI 10I and A from 15th day onwards. All the larvae in Treatment I in which no *Artemia* nauplii was fed died by seventh day of the experiment and never progressed beyond a mean larval stage of 2.13.

The mean larval stages also started showing significant difference among the treatments from day 9 onwards and the lowest values were recorded by AI 6I. However, control and the treatment AI 10I did not differ significantly with respect to the mean larval stages at any of the stages of the experiment except on the 15th day. The final average weight and length of the post larvae also did not show any significant variation between the treatments.

The appearance of the first post larva was observed in A and AI 10I on an average of 21.7 and 22 days respectively. In respect of AI 8I an average of 22.7 days was taken for appearance of first post larva. More than 95 % of post larval settlement was observed by day 30 in A, day 31 in AI 10I and AI 8I while it was 33 days in AI 6I.

7.4 Discussion

In control, the highest values for both TAN and NO_2^- N (1.53 and 1.015 ppm respectively) with the highest mean values (1.27 and 0.94 ppm respectively) were observed in the samples collected on 29th day which was the final sampling day. The primary component that constitutes the nitrogenous toxicants in the rearing water is the excretion by the larvae and BSN. Over feeding of BSN alone results in generation of wastes and elevated levels of toxic nitrogenous compounds (Barros and Valenti, 2003b). The lower levels of TAN and NO_2^- N recorded in the treatments where an early termination of *Artemia* nauplii was effected might be due to the lower levels of excretion by the live organisms. The highest number of biomass in the control comprising of the prawn larvae and the BSN have a direct correlation with the high levels of the toxic nitrogenous compounds in these tanks, this would explain the situation.

The lowest survival rate recorded in AI 6I, in spite of better water quality might be due to the lack of sufficient nutritional availability during the early larval stages. It appears that the larvae are able to accept inert particles well from the Vth larval stage onwards. Kumlu and Jones (1995) experimenting with use of live (*Artemia salina*) and artificial diets in caridean shrimp larvae of two

species *Palaemon elegans* and *Macrobrachium rosenbergii* concluded that the larvae are able to utilise artificial diets at stages Z4-5 in *P. elegans* and Z5-6 in *M. rosenbergii* . According to the authors Z5-6 in *M. rosenbergii* coincides with a rapid increase in the volume of hepatopancreas and formation of filter apparatus. Low trypsin activity and increased food retention times which is a characteristic of carnivorous nature and under developed gut has been observed by the same researchers in the early larval stages of this species. According to Jones *et al.* (1997), as most larvae are dependent upon enzymatic breakdown of ingested food, the development of secretory hepatopancreas dictates the type of prey consumed. They have also classified the *M. rosenbergii* larvae as carnivorous in early larval stages based on the intermediate trypsin activity. Barros and Valenti (2003a) also made a similar observation. The lower survival (33.67 %) achieved in the treatment AI8I can also be explicated as due to lack of proper nutrition. In this treatment also, *Artemia* nauplii was reduced to 50 % by V^{th} larval stage leading to increased mortality in spite of having lower levels of TAN and NO_2^- N when compared to the control.

On comparing the survival rates registered in the treatment AI 10I of the present experiment with those in a similar weaning protocol of prawn larvae from BSN to blood worms (Treatment AC10C as explained in Chapter 4 where BSN was reduced to 75 % in the III^{rd} stage, 50 % in the V^{th} stage, 25 % in the $VIII^{th}$ stage and complete replacement of BSN by chironomid larvae was effected in the tenth larval stage), the production in the former was found to be higher. The better survival in the present study can be owing to the better

general performance of the larvae in the modified static green water system (MSGWS). Though the larvae were unable to derive considerable nutrition form the algae (Cohen *et al.*, 1976; Joseph, 1977; Cook and De Baissac, 1994), the overall advantages in providing the algae in the rearing medium and the probable enrichment of the live feed by the DHA rich *I. galbana* might have resulted in a better performance in this larval rearing system when compared to the clear water system.

In the present experiment, in treatment AI10I wherein no BSN was given after the X^{th} larval stage, the larvae were showing an equally improved performance when compared to the control. The post larval production, the duration of larval rearing and the length and weight of the larvae did not differ significantly ($P>0.05$) among the control and AI10I. There was no added advantage of another feed item unlike in the previous weaning experiment wherein the BSN was substituted by blood worms (Treatment AC10C of Chapter 4), in the treatment AI10I to substitute for the reduction in the concentration of BSN. In spite of this limitation, almost the same production at par with the control could be achieved. Besides, unlike in AC10C (Chapter 4) the quantity of BSN was reduced to 50 % only by the VI^{th} larval stage in the present experiment. It may therefore be inferred that the lower TAN and NO_2 -N levels recorded in the treatment AI 10I as compared to the control might have provided a better larval rearing environment. Moreover, the superior water quality characteristics might have also helped in the efficient feed intake by the larvae (Naqvi *et al.*, 2007).

Barros and Valenti (2003b) discussed the importance of supplemental feeding in the advanced zoeal stages (zoea IX to XI) of *M. rosenbergii*. Various researchers have suggested different larval stages for the feeding of supplementary diets in the larviculture of *M. rosenbergii*. Daniels *et al.* (1992) and Aquacop (1983) have suggested supplementation between 10^{th} and 14^{th} day corresponding to VI^{th} and VII^{th} stages respectively. New and Singholka (1985) recommended egg custard feed from III^{rd} stage onwards where as Valenti *et al.* (1998) suggested the initiation of supplementary feeding between VI^{th} and VII^{th} stages. Agard (1999) observed that the beginning of effective feeding occurs at V^{th} stage when yolk reserves disappear and digestive tract is completely developed. Barros and Valenti (2003a) suggest exclusive *Artemia* nauplii diet upto stage VI, total replacement of live feed by wet diet from stage VII to VIII and a wet or dry food from stage IX onwards. The results of the feeding experiments conducted by the author as a part of this study at SIF, CUSAT (Chapters 2, 3, 4 and 7) also concurs with the findings of most of the afore mentioned authors.

The larvae in treatment I in which no brine shrimp nauplii was fed died by 7^{th} day of the experiment and did not progress beyond the II^{nd} larval stage except a few which reached the third larval stage. According to Murai and Andrews (1978) the larvae are able to survive till II^{nd} zoeal stage depending on the internal food reserves alone. Lovett and Felder (1988) reported that development of starved larvae was arrested at a mean larval stage of 2.00 and survived almost for 7 days. Conversely, Thresiamma *et al.* (2006) suggested a possible utilisation of *I. galbana* as a feed by the prawn larvae.

However, the authors could record only a very minimal production in the treatment with only the alga as feed and no *Artemia* nauplii was given, in a green water system with water exchange in the larval rearing of *M. rosenbergii*. The direct use of microalga, *I. galbana* as a food source through active uptake by the algae has been reported by Devika *et al.* (2006) as well. However, the results in the above treatment of the present experiment demonstrates the inability of the larvae to derive sufficient nutrition form the algae especially during the early larval stages.

The average post larval production attained in the present experiment in both the control (40.2 %) as well as the treatment AI10I (39.8 %) is lower than the percentage survival of 59.7 %, 59.3 % and 58 % 58 % achieved for 100 %, 75 % and 50 % of *Artemia* nauplii fed to the larvae respectively along with alga *I. galbana* reported by Thresiamma *et al.* (2006). However they used a lower initial stocking density (50 larvae / l), a higher BSN concentration (15 numbers / ml) and daily water exchange was also performed with fresh algal medium. Further more, they could ensure considerably earlier post larval settlement (20 to 24 days) as compared to the present study (average of 30.7 and 31 days in the control and AI10I respectively).

The three experiments conducted adopting the MSGWS by the author (Chapters 5, 6 and7) resulted in a percentage survival ranging between 40 and 49.07 % which readily conforms with 34 to 80 % survival reported by Kurup (2003) using the same rearing system. However it is slightly lower than 56.09 % reported by Alam *et al.* (1993a) in their studies conducted using the static system in replacing BSN with the fresh water cladoceran *Moina micrura*.

But the authors adopted a lower stocking density of 30 larvae / l (final mean production of 16.83 post larvae / litre). Hein *et al.* (2000) reported similar survival rates (45 to 51.2 %) and larval settling period (30 to 33 days) while studying the effect of vitamin C on the larvae of fresh water prawn *M. rosenbergii* employing MSGWS dominated by *Chlorella* sp., which fully concurs with the present findings.

7.5 Conclusion

The present experiment also confirms the indispensable use of *Artemia* nauplii in the larviculture of *M. rosenbergii.* However, a significant reduction in the live feed quantity without any significant reduction in the post larval production has been demonstrated (treatment AI10I where *Artemia* nauplii was reduced to 75 % at IIIrd larval stage, 50 % at VIth stage, 25 % at VIIIth stage and 0 % at Xth larval stage). Supplying the optimal amount of *Artemia* nauplii contributes considerably to reduction in production costs as well as minimisation of problems related to water quality in larviculture of *M. rosenbergii.* Moreover, by implementing the feeding protocol explained above (AI10I), the requirement of *Artemia* nauplii can be slashed by more than 50 % resulting in a considerable reduction in the cost of production.

Table 7.1 Treatments with different feeding regimes in the larviculture of *M rosenbergii* using MSGWS with *I galbana* as rearing medium

Treatments	Percentage composition of *Artemia* nauplii given to different larval stages of fesh water prawn									
	Larval stages									
	II	III	IV	V	VI	VII	VIII	IX	X	XI - PL
I	A 0	A 0	A 0	A 0	A 0	A 0	A 0	A 0	A 0	A 0
AI 6I	A 100	A 75	A 50	A 25	A 0	A 0	A 0	A 0	A 0	A 0
AI 8I	A 100	A 75	A 75	A 50	A50	A 25	A 0	A 0	A 0	A 0
AI 10I	A 100	A 75	A 75	A75	A 50	A 50	A 25	A 25	A 0	A 0
A	A 100	A 100	A 100	A 100	A 100	A 100	A 100	A 100	A 100	A 100

Table 7.2 Average ± s.d of various water quality parameters recorded during the experimental period in the larviculture of *M rosenbergii* using MSGWS with *I galbana* as rearing medium

Parameters	Treatments			
	AI 6I	AI 8I	AI 10I	A
Temperature (^{0}C)	27.64 ± 0.73	27.83± 0.78	27.74 ± 0.77	27.76 ± 0.71
pH	8.12 ± 0.15	8.08 ± 0.13	8.1± 0.21	8.16 ± 0.14
Salinity (ppt)	12.63 ± 0.49	12.71 ± 0.46	12.75 ± 0.44	12.72 ± 0.45
Dissolved oxygen (mg/l)	7.24 ± 0.15	7.23 ± 0.1	7.19 ± 0.14	7.16 ± 0.13

Table 7.3 Mean larval stages of *M rosenbergii* larvae under various overnight feeding regimes in the larviculture using MSGWS with *I galbana* as rearing medium

Treatments	Days							
	3	6	9	12	15	18	21	24
A	2.16 ± 0.12[a]	3.57 ± 0.06[a]	4.87 ± 0.06[a]	6.13 ± 0.06[a]	6.73 ± 0.06[a]	7.53 ± 0.06[a]	8.5 ± 0.1[a]	9.73 ± 0.06[a]
Al 10l	2.13 ± 0.06[a]	3.47 ± 0.12[a]	4.8[ab]	6.07 ± 0.06[a]	6.63 ± 0.06[b]	7.43 ± 0.06[ab]	8.43 ± 0.06[ab]	9.67 ± 0.06[a]
Al 8l	2.13 ± 0.06[a]	3.43 ± 0.06[a]	4.73 ±0.06[b]	6 ± 0.1[a]	6.4[c]	7.33 ± 0.06[b]	8.33 ± 0.06[b]	9.4 ± 0.1[b]
Al 6l	2.13 ± 0.06[a]	3.43 ± 0.06[a]	4.27 ± 0.06[c]	5.53 ± 0.06[b]	6.27 ± 0.06[d]	7.17 ± 0.06[c]	8.17 ± 0.06[c]	9.37 ± 0.12[b]
Mean ± s.d of three replicate groups; Means sharing same superscript letter does not differ significantly (P>0.05)								

Table 7.4 Effect of of various treatments with different feeding regimes on the survival, appearance of first post larvae, duration of the rearing period, length and wet weight of post larvae of *M. rosenbergii* in the larviculture using MSGWS with *I galbana* as rearing medium

Treatments	Stocking density(larvae/litre)	% survival	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
I	100	0	0	0	0	0
Al 6l	100	23.3 ± .64[c]	24.7 ± 0.6[c]	33.3 ± 0.6[c]	9.53 ± .43[a]	9.95 ± .43[a]
Al 8l	100	33.67 ± 1.33[b]	22.7 ± 0.6[b]	31.7 ± 0.6[b]	9.55 ± .39[a]	10.01 ±.27[a]
Al 10l	100	39.8 ±.53[a]	22[ab]	31[ab]	9.62 ± .44[a]	10.16 ±.39[a]
A	100	40.2 ± .53[a]	21.7±0.6[a]	30.7 ± 0.6[a]	9.64 ± .34[a]	10.15 ± .35[a]
Mean ± s.d of three replicate groups. Means in each column sharing a common superscript letter are not significantly different (P>0.05)						

Fig.7.1 TAN changes in the different treatments during the experimental period in the larviculture of *M. rosenbergii* using MSGWS with *I. galbana* as the rearing medium

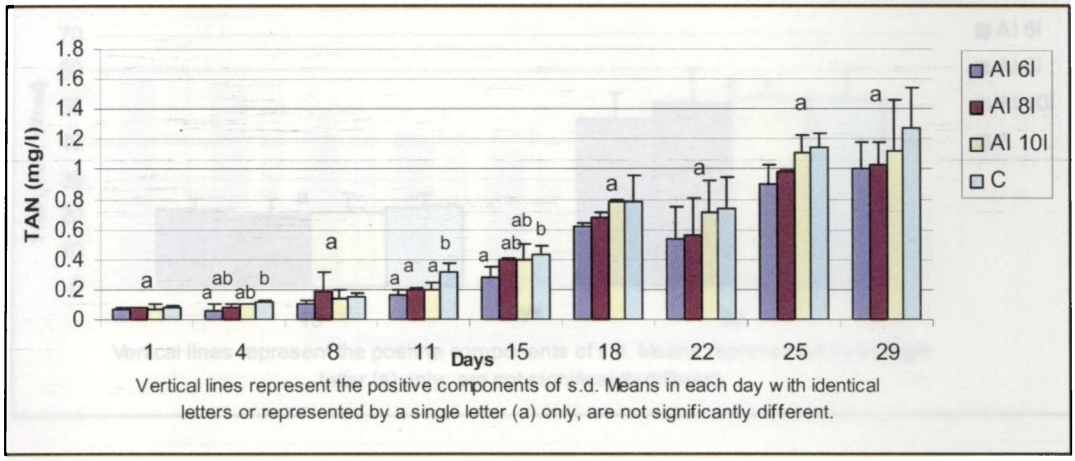


Fig 7.2 Changes in nitrite nitrogen recorded during the experimental period in the larviculture of *M. rosenbergii* using MSGWS with *I. galbana* as the rearing medium

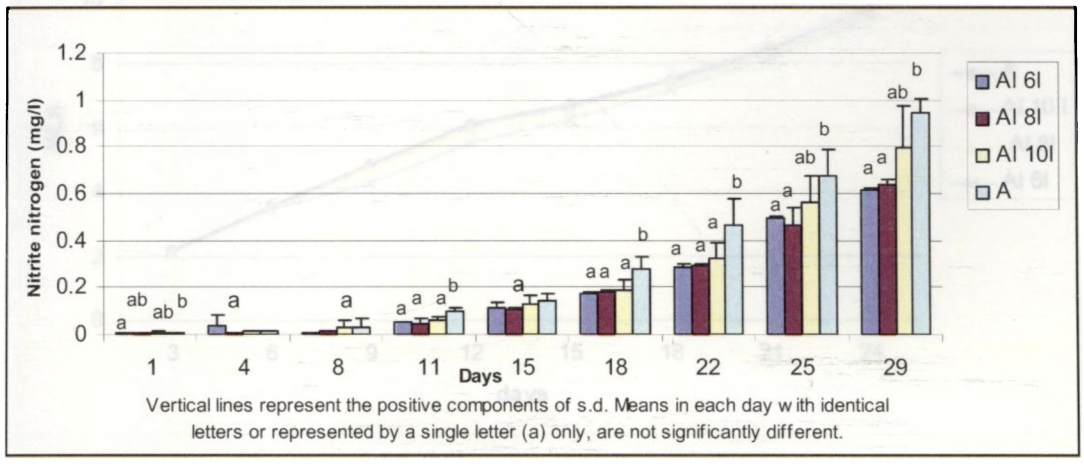


Fig.7.3 THB in different treatments during the experimental period on two sampling days in the larviculture of *M. rosenbergii* using MSGWS with *I. galbana* as the rearing medium

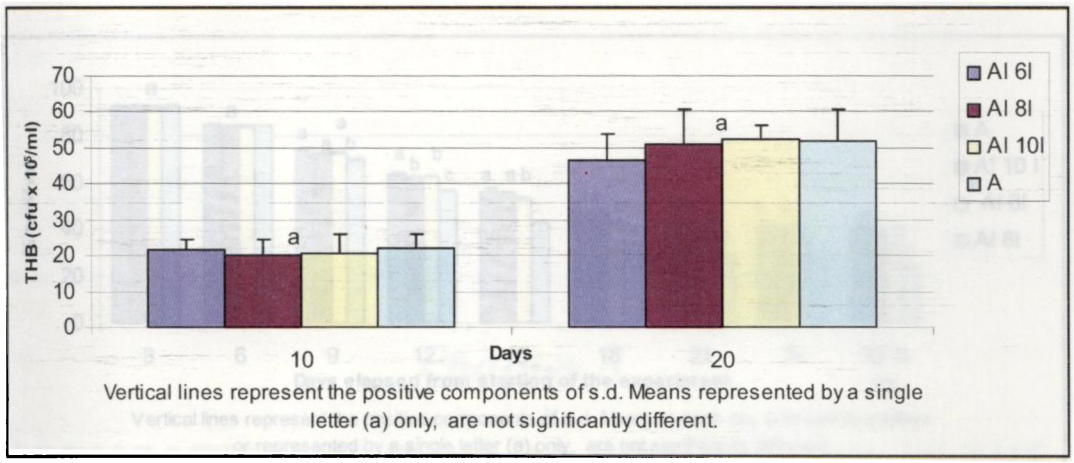


Fig.7.4 MLS in different treatments in the larviculture of *M. rosenbergii* using MSGWS with *I. galbana* as the rearing medium

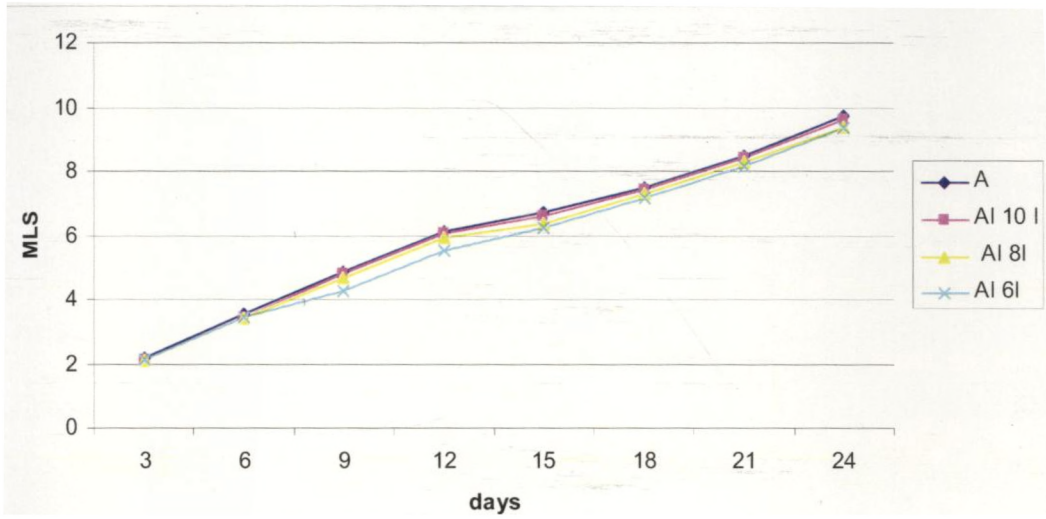
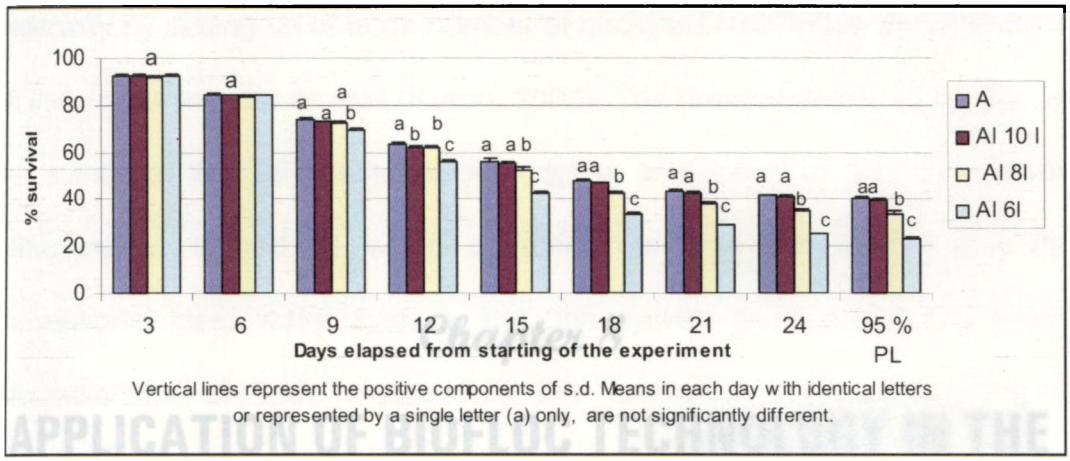


Fig 7.5 Average percentage survival in different feeding regimes in the larviculture of *M. rosenbergii* using MSGWS with *I. galbana* as the rearing medium



Chapter 8

APPLICATION OF BIOFLOC TECHNOLOGY IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII*

8.1 Introduction

The modified static green water system which has led to a tremendous increase in post larval production of the giant river prawn in Vietnam, especially by setting up of large number of backyard hatcheries, has proved to be successful in India as well (Kurup, 2003). The previous trials conducted by the author at the SIF have yielded higher production of the post larvae (Chapters 5, 6 and 7) when compared to the production using the conventional clear water system. But, the system being static, the heavy accumulations of toxic metabolites like ammonia and nitrite nitrogen, especially when the algal culture is not stable, is one of the major bottle necks faced by the farmers in the adoption of this technology at backyard level operations.

Many researchers (Ang, 1983; Phuong *et al.*, 2000; Kurup, 2003) have reported high levels of TAN and nitrite nitrogen in the static green water system of larviculture of *M. rosenbergii*. According to these authors no harmful effects or mortality of the larvae were observed due to high levels of TAN in the static system, as the bacterial consortium developed based on the algae in the green water may effectively convert ammonia and other toxicants to non-toxic products. High levels of TAN and nitrite were also recorded by the author in various experiments carried out using the modified static system at the SIF as a part of the study (refer chapters 5, 6 and 7). Although phytoplankton can adapt to higher dissolved nitrogen concentrations by increasing uptake, there is a threshold beyond which regeneration will exceed uptake and the nitrogen will start accumulating in the medium (Buford and Glibert, 1999).

Phytoplankton uptake is also reported (Hargreaves, 1998) to be insufficient to assimilate large quantity of ammonia generated as a consequence of high feeding rate and stocking density. Moreover, the variation in availability of light can also result in unpredictable fluctuation in phytoplankton biomass leading to variation in nitrogen uptake.

Nitrogenous compounds (ammonia and nitrogen) resulting from excess feed and excretory products has been reported (Chen and Lei, 1990) to cause deterioration of water quality in hatcheries. Ammonia and nitrites can cause severe mortalities in hatcheries and sub lethal concentration of these two compounds has found to cause cessation in feeding, retardation of growth and increased susceptibility to parasites and diseases in prawn larvae (Armstrong *et al.*, 1976, 1978). According to Tomasso (1994), larvae might be exposed to high ammonia and nitrite concentration when larviculture is conducted in intensive systems with high stocking density.

One of the management measures suggested for removal of ammonium from the aquaculture waters is through its assimilation into microbial proteins by addition of carbonaceous materials to the system (Avnimelech,1999), which is known as the bioflocs technology (BFT) or the active suspension ponds. The accumulation of toxic inorganic nitrogen species (NH_4, NO_2-N) is prevented in the bioflocs by maintaining a high C/N ratio and inducing uptake of ammonium by microbial community (Avnimelech *et al.*, 1994). The technology has been expansively used in fish and shrimp extensive as well as intensive culture systems by employing several carbohydrate sources like glucose, cassava meal, cellulose powder,

molasses, tapioca flour, starch and wheat flour (Avnimelech and Mokady, 1988; Avnimelech *et al.*, 1994; Avnimelech, 1998; Buford *et al.*, 2004; Hari *et al.*, 2004; Avnimelech, 2007; Azim and Little, 2008).

The modified static green water system is characterised by high aeration rates, thorough mixing and accumulation of organic substrates, the features, all of which maximise the activity of micro organisms. Therefore, the application of BFT to modified static green water system can be done at no additional expenditure other than that of the organic carbon source which is usually very cheap. The application of BFT has not been previously attempted in any of the hatchery systems. Consequently an investigation was carried out at the SIF, CUSAT to optimise the level of carbohydrate addition to the static larviculture system to maximise post larval production. Thus, the objectives of the present study are

1. Assess the effectiveness of BFT in controlling the toxic nitrogen species (TAN and NO_2^-N) by the manipulation of C/N ratio in the larviculture of *M. rosenbergii*
2. Optimise the quantity of carbohydrate addition in static larval rearing systems
3. Develop a biofloc based larviculture technology of *M. rosenbergii*.

8.2 Materials and methods

An experiment with completely randomised design with different levels of carbohydrate addition was carried out to find out the effectiveness of C/N ratio and also to optimise the quantity of carbohydrate required in controlling the ammonia and nitrite levels in the rearing system. Five different levels of

carbohydrate (Table 8.1) were applied while the rearing medium without carbohydrate addition was maintained as control. Triplicates were maintained for all the treatments and the control.

8.2.1 Experimental setup

8.2.1.1 Brood stock management and hatching

Berried prawns in the same stage of egg development (preferably with black eggs) were collected from grow outs and transported in black coloured plastic cans of 25 litre capacity with battery operated aerators. Each berry was kept in a separate fibre reinforced plastic (FRP) tank of 100 litre capacity with water having 5 ppt salinity. The tanks were covered with nets to prevent escapement of the prawns and kept undisturbed during night. The berried prawns with black eggs were hatched invariably in the next day morning. Once the hatching became completed, the mother prawns were removed, aeration turned off and the healthy larvae were siphoned out into bucket with 12 ppt saline water, utilizing phototropism. Strong aeration was given in order to ensure uniform distribution of the larvae to facilitate counting. Stocking was done in the rearing tanks at the rate of 100 larvae / litre.

8.2.1.2 Larval rearing

The experiment was carried out in 100 litre capacity fibre reinforced plastic (FRP) tanks, the inner side of which were painted white for enhancing algal growth. 50 litre of rearing water was used in all the tanks. The water used for the experiment was treated following New and Singholka (1985). The rearing tanks were filled with treated brakish water with 12 ppt salinity. The rearing water was inoculated with pure culture of *Chlorella vulgaris* which was

cultured using modified Walne's medium (Lavens and Sorgeloos, 1996) in the aquaculture laboratory of this School. The specified quantity of the medium along with the algal inoculum (to obtain an initial algal density of 5 x 10^5 cells/ml) was added to each rearing tank before introducing the larvae. No more addition of algae, water exchange or siphoning of waste was carried there after. However, the algal densities in all the rearing tanks were examined every alternate day by counting their number under a microscope.

The larvae were fed with *Artemia* nauplii at the rate of 4 numbers/ml of rearing water from the second day of hatching. Egg custard (Kurup, 2003) was given to the larvae from fifth larval stage (10^{th} day from starting of the experiment) onwards after passing through sieves of different mesh sizes. The carbohydrate selected was tapioca powder (powdered dried roots of tapioca plant, *Manihot esculenta*, Rs 20/Kg) which was locally purchased.

The quantity of tapioca powder added to assimilate the ammonium flux into microbial protein was calculated following the Avnimelech (1999).

ΔCH = Quantity of feed x % Nitrogen of feed x % Nitrogen excretion / 0.05

The average quantity of feed given / tank / day was estimated as 3g (0.06 g/l/day) and the average protein of the feed was calculated as 31% (Table 8.3). As a consequence, 1.5 g tapioca /tank/day (0.03g/l/day) was taken as the standard and two levels greater than and less than this was adopted for carrying out the experiment (Table 8.1).

The pre weighed tapioca flour was mixed with tank water in a beaker and uniformly distributed in the particular tank following administration of egg custard at 8.30 A.M. The subsequent feeding of egg custard was done at

11.30 A.M and 2.30 P.M (Daily feed ration of 3 g per tank was split into three equal feeds). Egg custard was prepared as explained in Chapter 2 (section 2.2.3) and they were passed through sieves of different mesh sizes (refer table 2.3 and table 2.4 in Chapter 2) before feeding the larvae. *Artemia* nauplii were given as overnight feed at around 5 0' clock in the evening.

8.2.2 Water quality parameters

Temperature, salinity and pH were measured daily using mercury thermometer, hand refractometer (Atago, Japan) and pH meter (Eutech instruments, Singapore) respectively. Dissolved oxygen was measured weekly twice by Winkler's method (APHA, 1995). Water samples were filtered through GF/C Whatman filter papers and the filtrate was analysed for nitrate-N (cadmium reduction), nitrite nitrogen (NO_2^-N) and total ammonia nitrogen (TAN) by phenol hypochlorite method (Grasshoff *et al.*,1983). Total heterotrophic bacteria (THB) and Total *Vibrio* bacteria (TVB) were estimated by plate counting and expressed as colony forming units (cfu) (APHA, 1995).

8.2.3 Assessment of treatments.

The efficiency of different treatments was evaluated based on the post larval production, time required for the larvae to reach each stage and their relative survival (Alam *et al.*,1993a). Larval stages were identified following Uno and Kwon (1969) and the larval development was determined using the formula given by Lovett and Felder (1998),

MLS = $\sum(S \times P_s)$, where MLS is the mean larval stage, S is the larval stage number and P_s is the proportion of larvae at stage S. Once the post larvae started appearing, due to inability in drawing uniform samples,

determination of the larval stage and survival was discontinued (Alam *et al.*, 1993a) and the experiment was terminated when more than 95 % of larvae in all the tanks got metamorphosed. On completion of the experiment, 50 post larvae were randomly taken from each tank to measure the total length (from the tip of the rostrum to the end of the telson) and wet weight. Salinity stress test (Thanuja, 2007) was also performed to assess the osmotic shock tolerating ability of the newly metamorphosed post larvae from various treatments. The cumulative mortality index (CMI) was calculated by adding the mortality noted at different time intervals.

CMI = $DX_1+DX_2+DX_3+.....DX_n$, where D is the number of dead individuals at time (in hours) X_1, X_2, X_3, ...X_n.

8.2.4 Statistical analysis.

Data analysis was done by ANOVA using SPSS 16 statistical software. Significant differences between the treatments were determined using Duncan's multiple range test (DMRT) ($P < 0.05$). Data expressed in percentages were normalised by arcsine transformation (Zar, 1984). However non transformed data is given in the table (percentage survival in table 8.5 and Fig.8.6).

8.3 Results

8.3.1 Water quality

The average values recorded for the various physicochemical parameters like temperature, salinity, pH and dissolved oxygen are presented in Table 8.2. These parameters were well with in the optimum range

suggested (New and Singholka, 1985) for the larval rearing of *M. rosenbergii* and were not found to be affected by varying levels of carbohydrate addition.

The variation in the TAN, NO_2^-N and NO_3-N in various treatments during the experimental period is given in Fig.8.1, 8.2 and 8.3 respectively. All the rearing tanks recorded almost similar TAN values during the initial days of the experiment. By day 12, the treatments started differing significantly ($P<0.05$) with respect to TAN. Once the carbohydrate addition started on the 10^{th} day, the highest average TAN (2.324 ppm on 20^{th} day) was recorded in the control. The lowering of TAN was proportional to the quantity of carbohydrate added and the treatment T5 recorded lower TAN values throughout the experiment. The treatment T4 also recorded low TAN values. The highest mean TAN concentrations recorded in T4 and T5 were 0.5 ± 0.09 and 0.38 ± 0.14 mg/l respectively. The tanks in which 1.5 g (0.03 g/l) of tapioca powder was added (T3) did not differ significantly with T4 and T5 at any stage of the experiment. However, there was a slight increase in TAN in T3 till 12^{th} day (0.612 ppm), after which it showed a declining trend. A similar tendency of slight increase in TAN for few days soon after carbohydrate addition was observed in T1 and T2 also, which continued till 20^{th} (0.99 ppm) and 16^{th} day (0.81 ppm) respectively and thereafter started decreasing. There was no significant difference ($P>0.05$) between any of the carbohydrate added treatments with respect to TAN on the last day (31) of observation. Although a wide variation (as indicated by standard deviation) was observed in the TAN values in the control, the mean value was found to be significantly higher

($P<0.05$) than all the carbohydrate added treatments from the 16th day of the experiment, 6 days after which the carbohydrate addition was initiated.

Almost the same pattern was observed in the NO_2^- - N values recorded in various treatments. The nitrite nitrogen values kept increasing and there was a steep increase after 20th day and reached value as high as 2.13 ppm on 31st day in the control. As in the case of TAN, T3, T4 and T5 recorded low nitrite nitrogen values with no significance difference observed between them. The nitrite nitrogen never recorded mean values higher than 0.45 ± 0.4, 0.18 ± 0.14 and 0.21 ± 0.16 and mg/l respectively in T3, T4 and T5. Whereas the nitrate nitrogen in all the larval rearing tanks were found to increase gradually towards the end of the experiment and no significant difference was observed among the control and the treatments ($P>0.05$). The values ranged between 0.01 to 3.93 ppm.

8.3.2 Bacterial population

The THB values ranged between 1.9 ± 0.81 x 10^5 to 1.14 ± 0.18 x 10^7 cfu /ml in the carbohydrate added treatments whereas it was between 1.96 ± 0.65 x 10^5 to 8.23 ± 1.2 x 10^6 cfu/ml in the control and it was significantly higher in the former ($P<0.05$) during the third (21st day) and fourth (28th day) sampling. THB values were directly proportional to the quantity of carbohydrate added to various treatments. An exception to this was the THB in T4 recorded on day 28 (1.14 ± 0.18 x 10^7 cfu /ml) which showed slightly higher values than in T5 (1.13 ± 0.13 x 10^7 cfu /ml). However, no significant difference ($P>0.05$) was observed among the carbohydrate added treatments. TVB was found to be almost similar in all the treatments and did not differ

significantly between any of the treatments and the control (P>0.05) throughout the rearing period. Yet, the highest average *Vibrio* bacterial count (15.33 ± 6.51 x 10^2 cfu /ml) was recorded in the control. The pattern of change in THB and TVB is presented in Fig. 8.4 and Fig. 8.5 respectively.

8.3.3 Post larval production

The highest mean survival (same as mean production since a stocking density of 100 larvae /l was adopted) was recorded in T3 (58.56 %), but the mean survival values did not differ significantly between the treatments T3, T4 and T5 (P>0.05). The lowest survival rates (46.9%) were recorded in the control. Percentage larval survival was significantly different between the treatments (P<0.05) from 18th day of the experiment, eight days after initiation of carbohydrate addition. T3, T4 and T5 recorded superior larval survival from 15th day onwards. The mean survival recorded in various treatments over the entire period of the experiment with an interval of 3 days is given in Fig.8.6.

No significant difference (P>0.05) could be observed in the mean larval stages in the treatments T3, T4 and T5 (Table 8.4). The highest mean larval stage on 24th day (9.47) was observed in T3. Despite the superior performance with respect to survival and mean larval stage during the initial days of carbohydrate addition, the larvae in treatments T4 and T5 were lagging behind those in T3 by the end of the rearing period.

Similarly, the length and weight of the larvae also did not show significant difference between the treatments in which 1.0, 1.5, 2.0 and 2.5 g of tapioca powder were added per tank. The first post larvae appeared in all the treatments on the same day except T1 and control. Also 95 % of the

larvae metamorphosed as post larvae by 28th day in T3, T4 and T5 where as it was delayed by two days in T1 and three days in the control. The final production details are presented in Table 8.5.

8.3.4 Salinity stress test

The post larvae in the treatments T2, T3, T4 and T5 showed better stress resistance at 0 ppt and 7 ppt. The highest average percentage mortality (79.5 % in 0 ppt and 15.05% in 7 ppt) over a period of 24 hours was recorded in the control. The lowest percentage mortality over the same period in 0 ppt was recorded in T3 (45.16%) while in 7 ppt, it was recorded in T5 (2.15 %). The highest mean CMI for 0 ppt and 7 ppt (1008 and 112 respectively) was recorded in control whereas the lowest values (472.5 and 24 respectively) were recorded in T3. The percentage mortality at 0 ppt salinity in the different treatments is given in Fig.8.6. The highest mean percentage reduction in stress at 0 ppt was observed in T3 (43.24 %) while the maximum stress reduction at 7 ppt was observed in T5 (85.71 %).

8.4 Discussion

8.4.1 Water quality

Different methods have been suggested by various researchers to reduce the toxic metabolites in larval rearing systems and nurseries (Liu and Han, 2004; Khatoon *et al.*, 2007; Palmer *et al.*, 2007) as well as grow outs (Avnimelech,1999; Buford *et al.*, 2004; Hari *et al.*, 2004; Hari *et al.*, 2006; Avnimelech, 2007; Azim and Little, 2008) , thereby improving water quality and increasing production. In the present experiment the addition of tapioca

powder was found to be positively influencing the hatchery production of seeds of *M. rosenbergii* using modified static green water system.

There was a reduction in TAN and NO_2^- - N in all the tanks in which tapioca powder was added. Hari *et al.* (2006) reported a significant reduction in TAN and NO_2^- -N concentrations in the water column of culture tanks of *Penaeus monodon* due to the addition of carbohydrate. However, in the present study, the reduction was inversely proportional to the quantity of carbohydrate added. With the increase in the quantity of tapioca powder from 0.01 to 0.05 g/l, a proportionate reduction in TAN and NO_2^- -N levels was observed in various treatments (Fig.8.1 and Fig.8.2). TAN in T3 and T4 was found similar to that in T5. It can be seen that there was a reduction of TAN and NO_2^- -N concentration in the treatments wherein 0.01 and 0.02 g/l of carbohydrate were added, however, the reduction could be accomplished over a longer period of time. Where as, in T5, there was a sudden lowering of TAN followed by NO_2^- -N, but the changes in the other treatments were gradual and T3 and T4 recorded TAN and NO_2^- -N values very much nearer to T5 by 31st day. Armstrong *et al.* (1976,1978) and Valenti (1985) reported that the larvae of *M. rosenbergii* can tolerate the level of 1.0mg/l for both ammonia and nitrite. The average TAN and NO_2^- -N values registered in all the carbohydrate added treatments have remained lower than 1 mg/l throughout the experiment. However many authors have recommended lower levels for both ammonia nitrogen in the order of < 0.5 mg/l (Valenti *et al.*,1998) and < 0.1mg/l (Lee and Wickins, 1992) and nitrite nitrogen of <0.1 mg/l (Correia *et al.*, 2000 ; New, 2002) for the larviculture *Macrobrachium rosenbergii*. Even the sub lethal

concentration of these two nitrogenous species has been reported (Armstrong *et al.*, 1976, 1978) to cause cessation in feeding, retardation of growth or increased susceptibility to parasites and diseases. On the other hand, Mallasen and Valenti (2006) did not observe any significant effect on larval development, growth rate and survival at nitrite levels below 2 mg/l.

Higher levels of TAN was also reported by Ang (1983), Phuong *et al.*(2000) and Kurup (2003) with no significant effect on larval growth and post larval production. The previous experiments conducted at the SIF also resulted in higher post larval production using the modified static green water system compared to the clear water system in spite of the higher TAN and nitrite nitrogen recorded in the former.

The TAN includes both unionised ammonia (NH_3) as well as the ionised form (NH_4^+) of which the unionised form is more toxic since it is uncharged and lipid soluble and consequently traverses the biological membranes more readily than the charged and hydrated NH_4^+ ions (Korner *et al.*, 2001). Ammonia-N is toxic to cultured fish at concentrations above 1.5 mg N/l (Crab *et al.*, 2007), but mostly the accepted level of unionised ammonia in aquaculture systems is only 0.025 mg N/l (Chen *et al.*, 2006). Colt and Tchobanoglous (1978) had demonstrated a linear reduction of channel catfish growth over the range of 0.05 to 1.0 mg/l. Straus *et al.* (1991) suggested that *M. rosenbergii* post larvae and juveniles should not be exposed to a pH greater than 9 or to ammonia nitrogen higher than 1.0 mg/l in a pH range of 8.5 to 9.0. The authors have also mentioned a tolerance of less than 2 mg/l at pH 8.5 for *M. rosenbergii* juveniles. A progressive lowering of feed intake in

juvenile *M. rosenbergii* was demonstrated by Naqvi *et al.* (2007) on increasing the NH_3-N concentrations from 0.05 to 1.5 mg/l. However, ammonia is more toxic to fish at elevated pH and temperature, which shifts the equilibrium toward the toxic unionised form (Hargreaves, 1998). The addition of chloride ions to the culture water is reported (Wise and Tomasso, 1989) to increase nitrite tolerance more than 30 fold in the red drum, *Sciaenops ocellatus*. On the other hand it has been recommended (Correia *et al.*, 2000) to keep the levels of nitrite nitrogen below 0.1 ppm in *M. rosenbergii* hatcheries. In the present study, the water pH rarely exceeded 8 and the water temperature was always below 30^0C. This must have kept the troubles related to elevated levels of potentially toxic ammonia nitrogen and nitrite nitrogen under check in the control. Nevertheless a better larval rearing environment in terms of lower levels of toxic nitrogenous compounds has been created by the addition of carbohydrate to the rearing tanks.

A gradual build up of nitrate nitrogen was observed (Fig. 8.3) irrespective of carbohydrate addition, in all the larval rearing tanks with no significant difference among the treatments and the control ($P>0.05$). This might be due to the nitrification process that must have been continuing by the nitrifiers which might have established in the system before the addition of carbohydrates. A progressive accumulation of NO_3 was also observed by Valenti and Daniels (2000) in closed recirculation hatchery systems of *M. rosenbergii*. In extensive culture systems of *Penaeus monodon* too, there was a gradual increase in the levels of nitrate nitrogen with time and no significant effect of carbohydrate addition in the accumulation of NO_3 was noticed (Hari *et*

al., 2004). Accumulation of nitrate in BFT systems has also been noticed by Azim and Little (2008). Although nitrification is reported to be inhibited by addition of organic carbon (Zhu and Chen, 2001), the nitrification rates registered in high-intensity zero-exchange shrimp ponds in Belize by Buford *et al.* (2003) were similar to those recorded in the pilot scale, high-intensity, low-waste shrimp systems (Bratvold *et al.*, 1999; Bratvold and Browdy, 2001). Both intensive heterotrophic activity and nitrification has also been reported (Avnimelech, 2006) to take place simultaneously in the biolflocs. Although there was a gradual build up of nitrate nitrogen towards the final days of larval rearing, the values recorded in the present study never exceeded 4 mg/l. However, levels of up to 20 mg/l have been suggested by New (1990) as optimal for *M. rosenbergii* hatcheries.

8.4.2 Bacterial population

The heterotrophic bacterial population was also found to increase proportionately with the quantity of tapioca flour added (Fig. 8.4) and it was significantly higher ($P<0.05$) in the carbohydrate added treatments compared to the control on the third and fourth sampling. Floc volume was found to increase when high levels of starch was added to the BFT tanks (Avnimelech, 2007). According to the author, when higher level of substrate was available, the bacterial proliferation was found to be better. This explains the proportionate increase in the THB with the quantity of carbohydrate addition observed in the present experiment. Anderson *et al.* (1989) recorded total viable cell counts ranging between 2.3×10^4 to 5.6×10^6 / ml in two prawn hatcheries producing *M. rosenbergii* post larvae adopting the modified static

green water system in Malaysia. The authors have also observed an increase in the bacterial counts with larval age as well as total suspended solids. While Phatarpekar *et al.* (2002) recorded values ranging between 1.1 ± 0.6 x 10^4 to 9.8 ± 1.5 x 10^6 cfu /ml in the rearing water of *M. rosenbergii* when larval rearing was conducted using clear water system with batch water exchange. Aquacop (1977) reported water counts of up to 4.9 x 10^5 bacteria /ml whereas the values ranged between 6 x 10^0 to 1 x 10^6 cfu/ml in Hawaiian *M. rosenbergii* hatcheries (Miyamoto *et al.*, 1983). Conversely, higher counts has been reported by Vici *et al.*, 2000 (1.6x 10^7 to 4.8 x 10^9 cfu/ml.) and Al-Harbi and Uddin (2004) (2.1 ± 1.3 x 10^5 to 2.2 ± 0.8 x 10^7 cfu /ml) in the larval rearing water of *M. rosenbergii*. The THB count recorded in the present experiment is much similar to those recorded by most of the afore mentioned authors. However, the counts did not reach high values as those observed by Vici *et al.* (2000) even in the carbohydrate added treatments. This may be due to the probable grazing of the bacterial cells in flocs by the prawn larvae, at least in the later larval stages when they become more omnivorous (Barros and Valenti, 1997) in their feeding habit due to which the bacterial numbers were kept under control. This aspect probably requires further research.

However, the intake of bacterial flocs has been reported in post larve and adult *M. rosenbergii* (Crab *et al.*, 2009; Asaduzzaman *et al.*, 2008; 2009), *P. monodon* (Hari *et al.*, 2004 ; 2006) and tilapia (Avnimelech,1999; 2007; Azim and Little, 2008). Lowering of feed application by up to 30 % of conventional feeding ration was not found to affect shrimp growth (Panjaitan, 2004) in BFT grow out ponds. This was attributed to the partial replacement of

fed by the microbial flocs. However, it is not possible to confirm the grazing of the bacterial flocs by the prawn larvae in the current experiment. Nevertheless, in natural populations of aquatic animals, the microflora of the gut might reflect that of the aquatic environment (Gomez-Gil *et al.*, 2000). It has also been reported (Al-Harbi and Uddin, 2004) that the aquatic animals take up various kinds of bacteria from food, water and sediments, which may in turn become the constituents of the bacterial flora of the digestive tract. Therefore, even if the larvae were not able to directly feed on the flocs, it is possible that the beneficial bacteria must have acted as a probiotic by colonising the gut of the larvae and rendering the beneficial effects of probiotics. The possible benefits linked to the administration of probiotics has been summarised by Balcàzar *et al.* (2006) as (1) competitive exclusion of pathogenic bacteria; (2) source of nutrients and enzymatic contribution to digestion; (3) direct uptake of dissolved organic material by bacteria; (4) enhancement of immune response against pathogenic micro organisms and (5) antiviral effects. Even though all of these benefits of probiotics might have worked in combination resulting in a high post larval production in the present experiment, only the lowering of the toxic nitrogenous compounds by the THB could be clearly demonstrated.

TVB count in all the treatments and the control remained low through out the experiment with no variation in relation to tapioca powder addition. It should be assumed that green water system itself, even without addition of carbohydrate must be effective in controlling the pathogenic bacteria. Tendencia and Pena (2003) have reported the ability of green water system

with the alga *Chlorella* in controlling *Vibrio harveyi*. The inhibition of shrimp pathogens by microalgae like *Tetraselmis* sp. has been reported by Regunathan and Wesley (2004). Furthermore, other than competitive exclusion of pathogens, the heterotrophic bacteria are also suspected to have a controlling effect on pathogenic bacteria (Michaud *et al.*, 2006). In the present experiment also the TVB was found to be very low when compared to the THB. The poly-β-hydroxybutyrate (PHB) produced by the mixed bacterial cultures in bioflocs in specific conditions has been suggested to confer a probiotic advantage for aquaculture (Schryver *et al.*, 2008). The depolymerisation of PHB is shown to act as a preventive or curative protector of *Artemia franciscana* against *Vibrio* infections (Defoirdt *et al.*, 2007).

8.4.3 Development, growth and Survival of prawn larvae

The MLS and percentage survival was significantly higher ($P<0.05$) in the treatments T2, T3, T4 and T5 than the control in which no carbohydrate was added from the 18th day of the experiment. Although the TAN was lowered to a level in T1 (with 0.01g/l of carbohydrate addition) which was not significantly different ($P>0.05$) to the other carbohydrate added treatments by the end of the rearing period, the higher levels of toxic nitrogenous compounds during the earlier period and the lower production of THB (which was lowest among the treatments with carbohydrate addition) might have resulted in an inferior mean production of 49.88 ± 1.33 post larvae/l which was not significantly different ($P<0.05$) with that of 46.9 ± 1.81 recorded in the control. The highest production of 58.56 ± 2.14 was recorded in T3 followed by T4 and T5 with no significant difference ($P>0.05$) among them. The

appearance of the post larvae were earliest and the duration of the rearing period was shortest in the very same treatments. The growth of the newly settled post larvae in terms of the total length and wet weight were also found to be highest in the treatments T3, T4 and T5. The stress tolerating ability of the post larvae on exposure to lower salinities (0 ppt and 7 ppt) was the best in the treatments T3, T4 and T5. The lowest mortalities were recorded in these treatments with no significant difference ($P>0.05$) among them. A considerable reduction in stress with respect to the control was also observed in the treatments T3, T4 and T5 with 0.03. 0.04 and 0.05 g/l of carbohydrate addition per day.

Tapioca powder was added to the rearing systems as a carbohydrate source for bacterial proliferation on the tenth day of larviculture when custard feeding was commenced. The variation shown by the larvae with respect to the survival rates and mean larval stages in the different treatments and the control must be due to the effect of carbohydrate addition, since that was the only variable in the entire system. The better quality of the rearing water was ensured in all the larval rearing tanks as a result of carbohydrate addition. The increase in the levels of TAN and nitrite nitrogen must have led to lower feed intake by the larvae in the control as observed by Naqvi *et al.* (2007) in *M rosenbergii* juveniles. This must have resulted in wastage of feed which in turn elevated the levels of toxic nitrogenous compounds due to decomposition of excess feed in the static system. At increased organic loading rates the phytoplankton may not be able to assimilate all the TAN produced ultimately increasing its levels in the rearing tanks. Where as in the carbohydrate added

tanks (especially T3, T4 and T5), the TAN and nitrite nitrogen was assimilated in the presence of Carbon (from the added carbohydrate) into new bacterial cells which must probably have formed the feed of the larvae in addition to the better larval rearing environment (lower TAN and nitrite) which also might have promoted the uptake of what ever feed that was given (lower feed wastage).

Despite the fact that the most favourable water quality parameters recorded in the treatments T5 followed by T4, the treatment T3 most often exhibited a superior performance with respect to post larval production, mean larval stage, length and weight of the larvae and stress tolerating ability and no significant difference could be observed between these treatments (T3, T4 and T5) with respect to the above mentioned parameters. The quantity of carbohydrate added in T4 and T5 may not have been fully utilised by the bacteria for its proliferation, because there might have been some other limiting factor for the growth of these bacteria in these tanks other than carbon and nitrogen, due to which some residual carbohydrate may get accumulated especially towards the end of the experiment. Alexander (1999) has recognised five categories of nutrients needed by micro organisms as Carbon source, Nitrogen source, Phosphorous source, inorganic salt and growth factors. Bacterial growth can be possibly limited by the nutrients imbalance in the medium, since the dynamics of living bacterial growth are linked to the quantity of available nutrients (Reay *et al.*, 1999; Leonard *et al.*, 2002). Thus, the accumulation of residual carbohydrate continues which might have negatively affected the growth of the larvae, since there is a possibility for the

accumulation of inherent toxic compounds in tapioca in the rearing system if they are continuously added at a higher rate. Sastry (1983) reported a delay in larval development as a generalised response of decapod larvae exposed to some type of pollutant. Moreover, it results in an unnecessary additional expenditure although tapioca powder is cheap and easily procurable in this part of India, when the same or a better production with superior quality larvae can be accomplished at a lower rate of addition of the organic carbon source.

Unlike observed by Azim and Little (2008) in tilapia culturing tanks, it could be demonstrated that the equation proposed by Avnimelech (1999) with respect to the quantity of carbohydrate required to contain and immobilise the ammonium flux to the aquaculture systems as bioflocs holds good in modified static green water hatchery systems, as a lowering of nitrogenous compounds (TAN and nitrite nitrogen but not nitrate) could be observed on the addition of calculated quantities of carbohydrate. A quantity of 0.03 g/l/day (approximately half the quantity of the custard feed fed to the larvae daily) can be taken as an average level of addition in cases of regular usage in hatcheries, and a higher quantity can be recommended in case of urgent situations like high TAN in hatcheries following a static method of larviculture. The addition of carbohydrate at higher levels can also be taken up as an emergency action when the level of the nitrogenous toxicants goes up as in case of crash of phytoplankton blooms when the addition of tapioca powder or any other potential carbohydrate source is not done on a regular basis. Starch addition was done in tilapia ponds whenever the TAN concentration rose to values above 7 mg/l (Avnimelech, 2007). Thus a consistent post larval production

irrespective of the fluctuations in the phytoplankton population can be ensured by the application of biofloc technology which is very stable and active independent of light conditions (Avnimelech, 2006).

New (1995) has reported typical production of 10 to 20 PL per litre of larval rearing water in *M. rosenbergii* hatcheries. However Correia *et al.* (2000) reported production rates of 40 to 50 PL/l. The post larval production recorded in the present study (Table 8.5) is higher than those reported by the above mentioned authors. It is also very high when compared to the average survival of 30 % (Murthy, 2006) in the commercial hatcheries in India with 25 to 40 days required for the completion of one rearing cycle. The post larval settlement in the present experiment was observed as early as 28 days in the best treatments and it took 3 more days for the completion of rearing in the control without carbohydrate addition. A comparison has been made on the production of seed of *M. rosenbergii* following different methods of larval rearing (Table 8.7). It can be observed that the production of around 58 PL/l (in T3, T4 and T5) in the treatments with carbohydrate addition, although lower than the highest values of 60 (Aquacop, 1983) and 80 PL/l (Kurup,2003), is well above when compared to the other authors who followed mostly the conventional systems. The larval rearing period was either lower than or comparable with those observed by most of the other workers, although a shorter period for settlement of post larvae was recorded by Thresiamma *et al.* (2006). Therefore, it can be summarised that an improved post larval production at no additional cost can be achieved by adopting the

modified static green water system of larviculture with carbohydrate addition and therefore it is a path breaking finding in the larviculture of *M. rosenbergii.*

The construction of backyard hatchery and its operation with the biofloc technology explained in the present chapter can be done with nominal investment cost. A hatchery of the same type was constructed at the SIF, CUSAT for conducting experiments using modified static green water system. An area of about 40 m^2 adjoining the University building was developed and the expenditure involved has been presented in Table 8.8. The economic analysis carried out on the expenditure involved in adopting the new technology has shown benefit cost ratio of 4.79 with variable cost (Table 8.8) and a pay back period of approximately 99 days. This proves that the technology in addition to being simple and highly productive with lower requirement of labour and water, is economically more feasible and highly cost-effective.

8.5 Conclusion

In the carbohydrate added tanks, in addition to the beneficial effects of green water, the bacterial assimilation of the toxic inorganic nitrogen compounds for production of new bacterial cells utilising the added carbon source has resulted in improved water quality. The ability of tapioca powder in lowering TAN and NO_2^--N and improving THB populations in static hatchery systems is illustrated in the present experiment. However, the use of microbial protein as a food source by the larvae requires further research. The addition of carbohydrate source has been proved to be an effective and inexpensive management method in the larval rearing of *M. rosenbergii* by (1) reducing the

concentration of potentially toxic TAN and NO_2^--N (2) providing a better larval rearing environment (3) improved post larval production without the use of antibiotics or probiotics and (4) reduced discharge of nutrient rich waters to the environment. The carbohydrate addition has also been optimised at roughly half the quantity of the inert feed fed daily and the quantity can be varied depending on the management strategies followed in the hatchery.

Table 8.1 Levels of carbohydrate added in different treatments in the larviculture of *M. rosenbergii* applying biofloc technology in the MSGWS

Treatments	Quantity of carbohydrate added (g/l)
T1	0.01
T2	0.02
T3	0.03
T4	0.04
T5	0.05
Control	No carbohydrate added

Table 8.2 Average ± s.d of various water quality parameters recorded during the experimental period in the larviculture of *M. rosenbergii* applying biofloc technology in the MSGWS

Parameters	Treatments					
	T1	T2	T3	T4	T5	C
Temperature (^{0}C)	28.11 ± 0.41	28.23 ± 0.55	28.18 ± 0.5	28.17 ± 0.43	28.13 ± 0.4	28.32 ± 0.53
pH	7.86 ± 0.1	7.84 ± 0.11	7.87 ± 0.11	7.89 ± 0.08	7.88 ± 0.10	7.85 ± 0.09
Salinity (ppt)	12.53 ± 0.7	12.58 ± 0.71	12.4 ± 0.53	12.56 ± 0.67	12.39 ± 0.55	12.57 ± 0.68
Dissolved oxygen (mg/l)	7.16 ± 0.18	7.13 ± 0.22	7.16 ± 0.17	7.04 ± 0.21	7.1 ± 0.22	7.23 ± 0.17

Table 8.3 Proximate composition (% ± s.d) of egg custard and tapioca flour used in the larval rearing of *M rosenbergii* applying biofloc technology

	Protein	Lipid	Ash
Tapioca	1.67 ± 0.21	0.67 ± 0.15	2.53 ± 0.35
Egg custard	31 ± 0.92	20.9 ± 0.3	8.9 ± 0.2

Table 8.4 Effect of carbohydrate addition on the mean larval stage of *M rosenbergii* larvae in different treatments in the larviculture applying biofloc technology in the MSGWS

Mean larval stages (MLS)								
Treatments	**Days elapsed from starting of the experiment**							
	3	6	9	12	15	18	21	24
T1	2.17 ± 0.12	3.57 ±0.06	4.87 ± 0.06	6.23 ± 0.06	6.67 ± 0.15	7.27 ± 0.06^{c}	8.13 ± 0.12^{b}	9.23 ± 0.06^{bc}
T2	2.13 ± 0.06	3.5 ± 0.10	4.87 ± 0.06	6.20	6.67 ± 0.06	7.4 ± 0.10^{b}	8.37 ± 0.15^{a}	9.43 ± 0.12^{ab}
T3	2.13 ± 0.06	3.50	4.80	6.2 ± 0.10	6.70 ± 0.10	7.43 ± 0.06^{ab}	8.40 ± 0.10^{a}	9.47 ± 0.06^{a}
T4	2.1 ± 1.0	3.57 ± 0.06	4.87 ± 0.06	6.13 ± 0.06	6.73 ± 0.12	7.50^{ab}	8.43 ± 0.06^{a}	9.40 ± 0.10^{abc}
T5	2.13 ± 0.06	3.60	4.83 ± 0.06	6.17 ± 0.06	6.73 ± 0.06	7.53 ± 0.06^{a}	8.40 ± 0.10^{a}	9.40 ± 0.17^{abc}
Control	2.17 ±0.06	3.60	4.90	6.2 ± 0.10	6.63 ± 0.06	7.2^{c}	8.13 ± 0.06^{b}	9.20 ± 0.10^{c}

Mean ± s.d. of 3 replicate groups. Means in each column sharing a common superscript letter are not significantly different (P<0.05) ; Means upto 15th day were not significantly different

Table 8.5 Effect of various levels of carbohydrate addition on the post larval production, appearance of first post larvae, duration of the experiment, length and weight of post larvae of *M rosenbergii* in various treatments in the larviculture applying biofloc technology in the MSGWS

Treatments	Stocking density(larvae/litre)	% survival	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
T1	100	49.88 ± 1.33^{cd}	19 ± 1^{ab}	30 ± 1^{ab}	8.96 ± 0.33^{bc}	9.24 ± 0.48^{b}
T2	100	53.66 ± 1.35^{bc}	18^{a}	29 ± 1^{bc}	9.24 ± 0.33^{ab}	9.49 ± 0.36^{a}
T3	100	58.56 ± 2.14^{a}	18^{a}	28^{c}	9.29 ± 0.26^{a}	9.77 ± 0.44^{a}
T4	100	58.00 ± 3.61^{ab}	18^{a}	28^{c}	9.23 ± 0.43^{ab}	9.68 ± 0.57^{a}
T5	100	57.45 ± 3.72^{ab}	18^{a}	28^{c}	9.29 ± 0.54^{a}	9.75 ± 0.53^{a}
Control	100	46.90 ± 1.81^{d}	20 ± 1^{b}	31 ± 1.73^{a}	8.60 ± 0.47^{c}	9.08 ± 0.57^{b}

Mean ± s.d. of 3 replicate groups. Means in each column sharing a common superscript letter are not significantly different (P<0.05)

Table 8.6 Cumulative Mortality Index (CMI) values and final survival of newly metamorphosed *M rosenbergii* post larvae in treatments with different levels of carbohydrate application and control exposed to low salinity stress (0 and 7 ppt) in the larviculture carried out using MSGWS

Treatments	0 ppt			7 ppt		
	CMI	Final mortality (%)	Reduction in stress (%)	CMI	Final mortality (%)	Reduction in stress (%)
Control	1008 ± 106.76^{b}	79.57 ± 4.93^{b}	0	112 ± 36.66^{c}	15.05 ± 4.93^{c}	0
T1	888.58 ± 121.92^{b}	72.04 ± 6.72^{b}	9.46	88 ± 36.66^{bc}	11.82 ± 4.93^{bc}	21.46
T2	548.5 ± 97.89^{a}	50.54 ± 6.729^{a}	36.48	48 ± 24^{ab}	6.45 ± 3.23^{ab}	57.14
T3	472.5 ± 35.52^{a}	45.16 ± 3.23^{a}	43.24	24 ± 24^{a}	3.23 ± 3.23^{a}	78.54
T4	517.58 ± 143.59^{a}	48.39 ± 11.63^{a}	39.19	32 ± 13.86^{a}	4.3 ± 1.86^{a}	71.43
T5	481.42 ± 78.54^{a}	46.24 ± 8.12^{a}	41.89	24^{a}	2.15 ± 1.86^{a}	85.71

Table 8.7 Post larval production of *M rosenbergii* in different experiments conducted by various authors applying different systems of larviculture

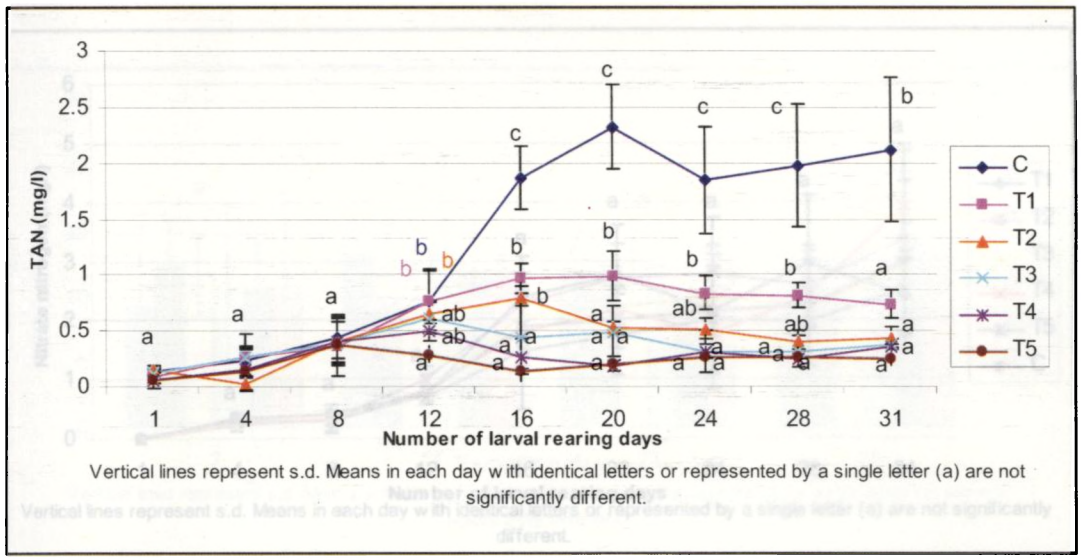
Rearing systems	Production (post larvae/liter)	Duration (days)	Reference
Clear water	32 - 60	30-35	Aquacop (1983)
Clear water	25 - 30	37- 40	Pramanik and Halder (1996)
Clear water	8 - 37	33 - 36	Kurup *et al.* (1998)
Clear water	7 - 25	40-45	Mohanta and Rao (2000)
Clear water	8 - 13	36*	Murthy and Yogeshbabu (2006)
Clear water	21 - 42	32*	Nair *et al.* (2007)
Green water	15 - 35	25 - 35	Fast and Leung (2003)
Green water	5 - 30	22 - 26	Thresiamma *et al.* (2006)
M.S.G.W.**	8 - 19	42 - 53	Ang and Cheah (1986)
M.S.G.W.	10 - 17	35*	Alam *et al.* (1993)
M.S.G.W.	28 - 50	30	Phuong *et al.* (2000)
M.S.G.W.	34 - 80	28 - 32	Kurup (2003)
M.S.G.W.	11 - 34	30 - 34	Hein *et al.* (2005)

The values given as % survival has been converted to post larvae / l. The decimals are corrected to whole numbers. The table presents the lowest and highest production recorded in each experiment. Similarly duration shows the shortest and longest time for post larval settlement. *Larval rearing terminated on the particular day. ** Modified static green water system.

Table 8.8 Economics of a backyard fresh water prawn hatchery (*M rosenbergii*) trial conducted at SIF, CUSAT adopting modified static green water system with BFT application.

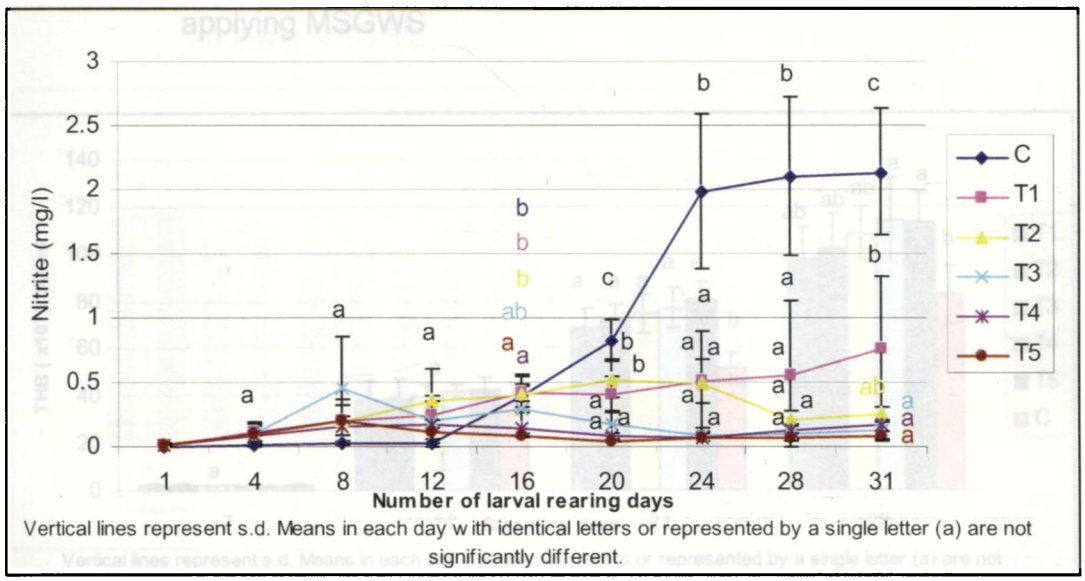
No.	Fixed costs	Cost (Indian Rupees)
1	Hatchery construction (Independent shed with iron frame and silpaulin roof)	**28,000**
2	Equipments	92000
	Salinometer	10,000
	Microscope	10,000
	Air compressor	17,000
	Pump	5,000
	Generator	50,000
3	Tanks (400 litre capacity - 8 numbers)	**32,000**
4	Miscellaneous	**8,000**
	Total fixed costs	**160,000**
	Operating costs	
5	Berried prawns	600
6	Artemia cysts	3600
7	Egg custard	1000
8	Labour	3000
9	Electricity	2500
10	Miscellaneous	1000
	Total variable costs	**11700**
	Depreciation of fixed assets*	2630.14
	Interest on fixed capital at 12 % level for 30 days	1600
	Total cost per production cycle	**15930.14**
	Total gross income (Income realised from sale of seed @ 60 paisae)	**72000**
	(Assuming a production of 50 post larvae/ liter with a total production of 1,20,000 post larvae)	
	Net income per production cycle	**56069.86**
	Economic analysis	
	Benefit cost ratio with variable cost	4.79
	Benefit cost ratio with total cost	3.52
	Percentage of profit over capital for one production cycle	35.04%
	Pay back period (assuming 10 production cycles per year)	98.61 days
	*Depreciation of fixed assets at 20 % level for 30 days	

Fig.8.1 Effect of different levels of carbohydrate addition on total ammonia nitrogen in the larviculture of *M. rosenbergii* applying MSGWS



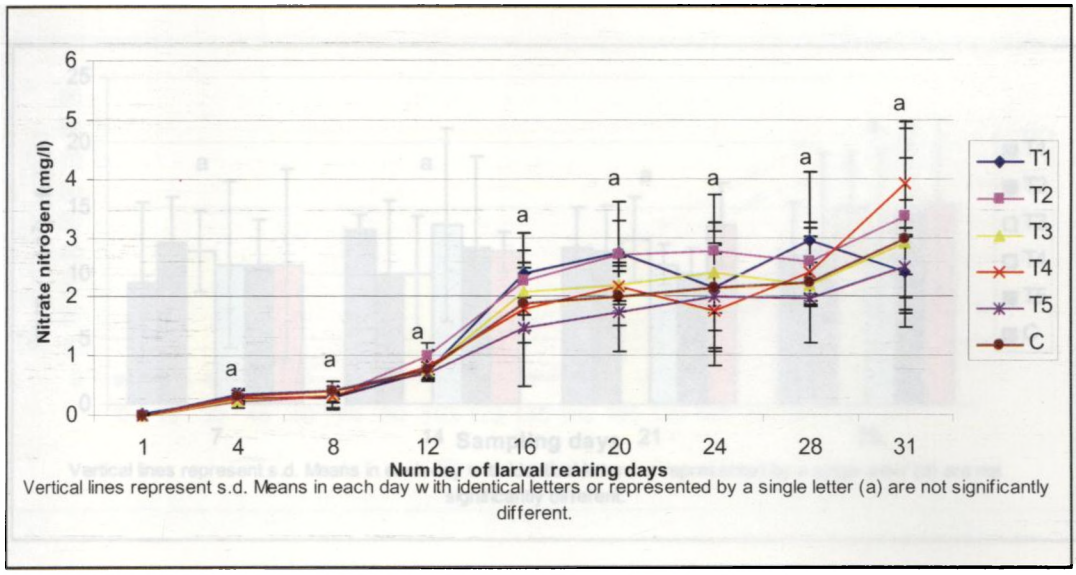
Vertical lines represent s.d. Means in each day with identical letters or represented by a single letter (a) are not significantly different.

Fig.8.2 Effect of different levels of carbohydrate addition on nitrite nitrogen in the larviculture of *M. rosenbergii* applying MSGWS



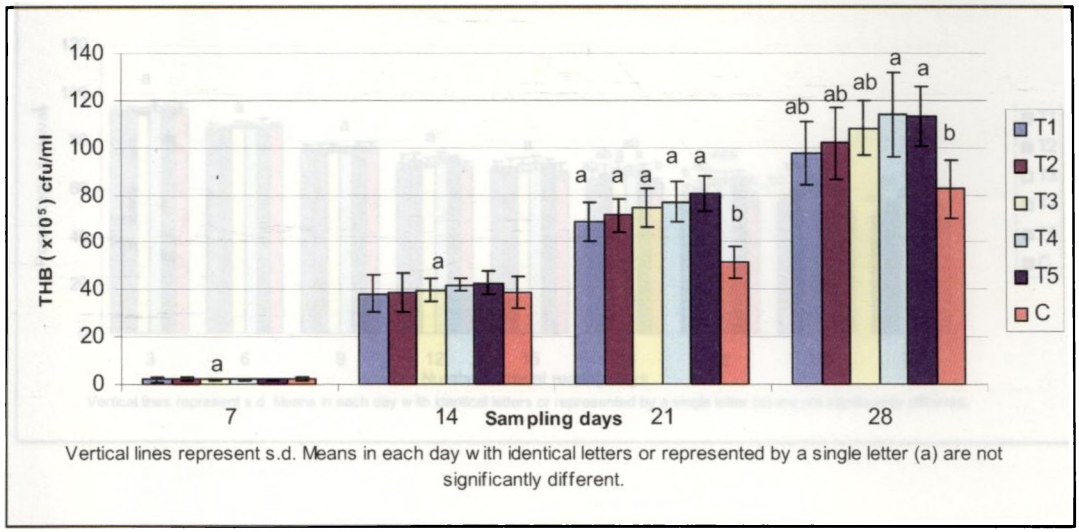
Vertical lines represent s.d. Means in each day with identical letters or represented by a single letter (a) are not significantly different.

Fig.8.3 Effect of different levels of carbohydrate addition on nitrate nitrogen in the larviculture of *M. rosenbergii* applying MSGWS.



Vertical lines represent s.d. Means in each day with identical letters or represented by a single letter (a) are not significantly different.

Fig. 8.4 Effect of different levels of carbohydrate addition on total heterotrophic bacteria in the larviculture of *M. rosenbergii* applying MSGWS



Vertical lines represent s.d. Means in each day with identical letters or represented by a single letter (a) are not significantly different.

Fig.8.5 Total *Vibrio* bacteria in various treatments with different levels of carbohydrate addition in the larviculture of *M. rosenbergii* using MSGWS

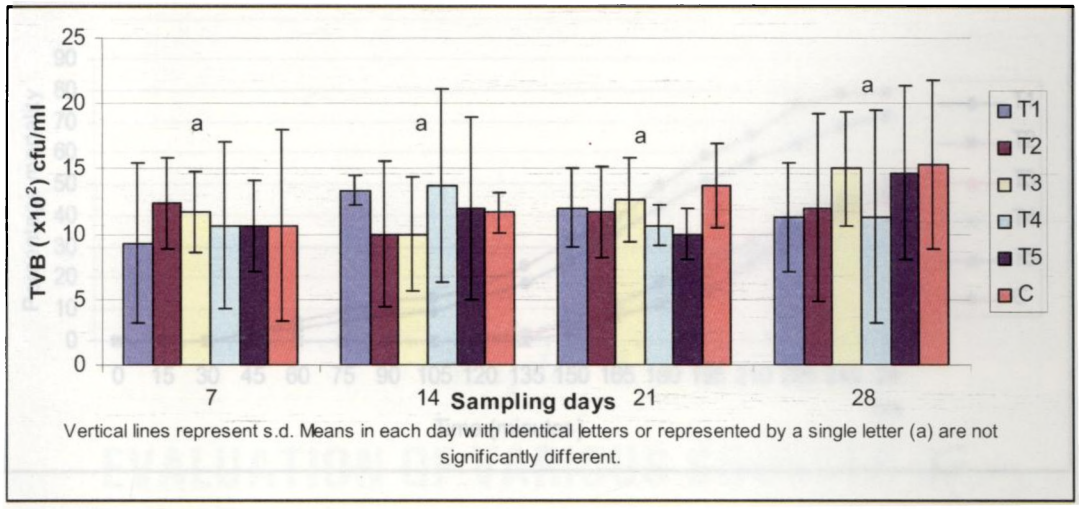


Fig.8.6 Effect of various levels of carbohydrate addition on the survival of *M. rosenbergii* larvae and post larvae in the larviculture adopting MSGWS

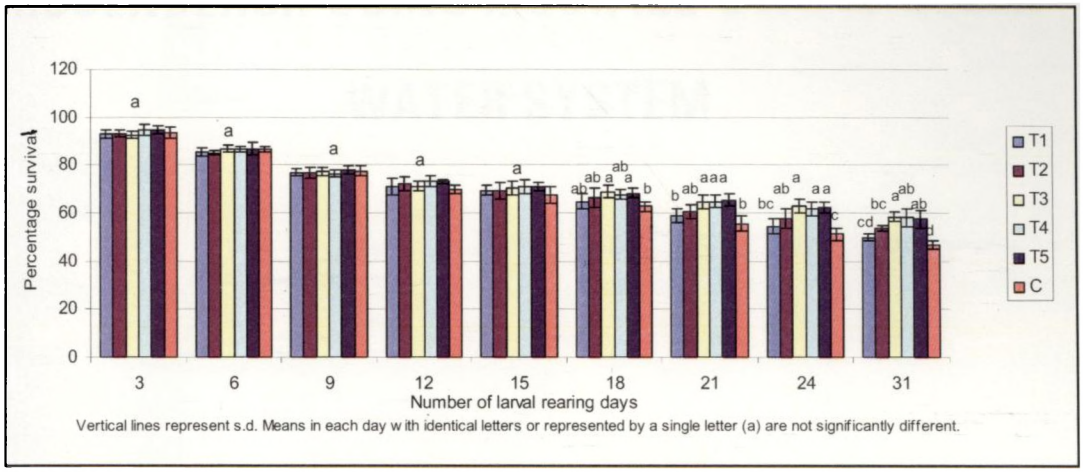
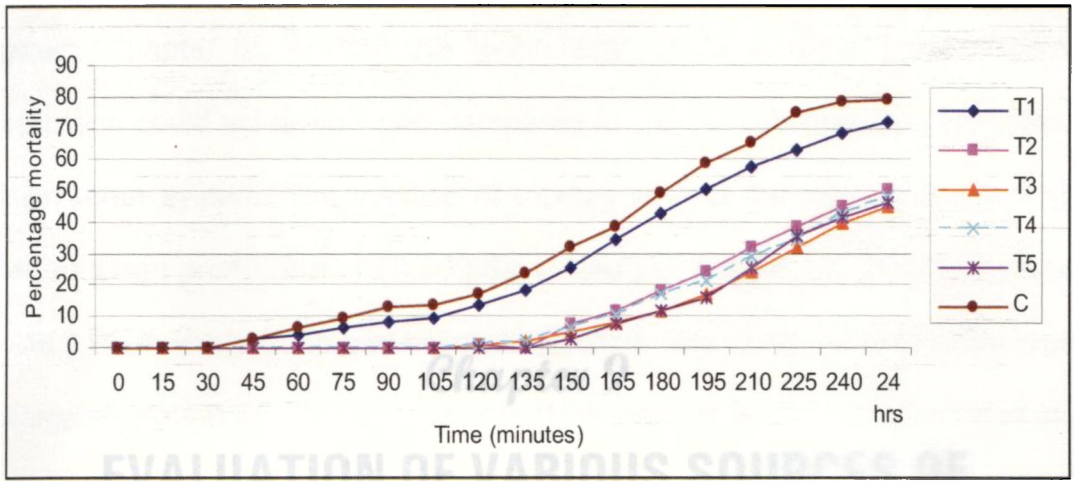


Fig.8.7 Percentage mortality of the post larvae from treatments with different levels of carbohydrate application exposed to salinity stress test (0 ppt)



Chapter 9

EVALUATION OF VARIOUS SOURCES OF CARBOHYDRATE IN CONTROLLING C/N RATIO IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII* USING MODIFIED STATIC GREEN WATER SYSTEM

9.1 Introduction

A new system of larviculture of *M. rosenbergii* applying biofloc technology has been standardized using the modified static green water system (Chapter 8). Abiding this technology, 25 % increase in post larval production could achieved when compared to the conventional modified static green water system. The addition of tapioca flour at the rate of 0.03 g/l/day yielded mean production of 58.56 post larvae per liter (PL /l) when compared to 46.9 PL /l where in no carbohydrate addition was done. However, the type of organic carbon source is reported to (Hollender *et al.*, 2002; Oehmen *et al.*, 2004) determine, to a large extend, the composition of flocs produced especially with respect to type and amount of storage polymers which are supposed to play an important role in combating pathogens. Moreover, the bioflocs production depends on the quality of added carbon substrate and its C/N ratio (Avnimelech, 2007). Crab *et al.* (2009) had also reported variation in the quality and quantity of bioflocs produced by the application different substrates as organic carbon source. Different carbohydrate sources have been employed by various workers to enhance the bacterial production like glucose, cassava meal, cellulose powder, molasses, tapioca flour, starch and wheat flour (Avnimelech and Mokady, 1988; Avnimelech *et al.*, 1994; Avnimelech, 1999; Buford *et al.*, 2004; Hari *et al.*, 2004; Avnimelech, 2007; Azim and Little, 2008) in fish and shrimp extensive as well as intensive culture systems. It can be hypothesised that the larvae being more fragile and sensitive, the added carbohydrate can affect its development and survival, finally influencing the postlarval production while controlling the toxic nitrogen

species. Thus, the present study was undertaken using 5 locally available carbohydrate sources such as Potato flour (P), Yam flour (Y), Rice flour (R), Wheat flour (W) and Tapioca flour (T) with specific objectives to

1. Evaluate their efficiency in controlling carbon / nitrogen ratio, there by reducing the level of TAN and nitrite nitrogen
2. Examine their effectiveness in enhancing heterotrophic bacterial population and finally
3. Assess the post larval production in each carbohydrate source.

9.2 Materials and methods

The experiment was carried out in 100 l capacity FRP tanks painted white inside. Five different carbohydrate sources were selected (Table 9.1) for the evaluation of their performance in controlling the inorganic nitrogen production by adjusting C/N ratio. The tank allocation for each treatment was done in a completely randomized way and triplicate tanks were maintained for each treatment. Carbohydrate source were purchased from local market. Potato flour (P), Yam flour (Y), Rice flour (R), Wheat flour (W) and Tapioca flour (T) were sieved through 35 µ and used as carbohydrate source. The quantity of carbohydrate added was calculated following the theory of Avnimelech (1999) as explained in Chapter 8 (section 8.2.1.2). Consequently, the various carbohydrates were added at the rate of 0.03 g /l/day which was found to be the optimal quantity when egg custard with an average protein content of 31 % was used as the larval feed. The brood stock management,

hatching, stocking the larvae, feeding, application of egg custard and carbohydrate were carried out in the similar way as explained in Chapter 8.

9.2.1. Water quality parameters

Temperature, salinity and pH were measured daily using mercury thermometer, hand refractometer (Atago, Japan) and pH meter (Eutech instruments, Singapore) respectively where as dissolved oxygen was measured weekly twice by Winkler's method (APHA, 1995). Water samples were filtered through GF/C Whatman filter papers and the filtrate was analysed for nitrite nitrogen (NO_2^-N) and total ammonia nitrogen (TAN) by phenol hypochlorite method (Grasshoff *et al.*,1983). Total heterotrophic bacteria (THB) and Total vibrio bacteria (TVB) were estimated using standard methods (APHA, 1995). TAN and NO_2^-N were estimated twice a week where as the bacterial counts were estimated once weekly.

9.2.2 Assessment of treatments.

The evaluation of the different carbohydrates was done based on the post larval production, time required for the larvae to reach each stage and their relative survival (Alam *et al.*,1993a) as explained in Chapter 8 (Section 8.2.2). However, the sampling to assess the MLS and survival was done every 5^{th} day. Since it was difficult to draw uniform samples due to settling of considerable number of post larvae, no sampling for MLS and survival was done after 20^{th} and 25^{th} day respectively (Alam *et al.*,1993a). Salinity stress test at lower salinities was conducted to measure the stress tolerating ability of the newly metamorphosed post larvae in the different treatments and control following Thanuja (2007).

9.2.3 Statistical analysis.

Data analysis was done by ANOVA using SPSS 16 statistical software. Significant differences between the treatments were determined using Duncan's multiple range test (DMRT) ($P < 0.05$). Data expressed in percentages were normalised by arcsine transformation (Zar, 1984). However, non transformed data is given in the Table 9.4 and Fig. 9.5.

9.3 Results

9.3.1 Water quality

The average values recorded for the various physicochemical parameters like temperature, salinity, pH and dissolved oxygen are presented in Table 9.2. These parameters were well with in the optimum range suggested (New and Singholka, 1985) for the larval rearing of *M. rosenbergii* and were not found to be affected by different sources of carbohydrate added to the system.

The TAN and NO_2^-N in all the carbohydrate (CH) added tanks were found to record values considerably lower than the control (Fig.9.1 and 9.2), once the addition of CH was initiated on the 10^{th} day of the experiment. The highest average values of TAN recorded in the CH added treatments was 0.76 ppm as compared to 1.72 ppm in the control where as it was 0.55 and 1.6 ppm respectively for NO_2^-N. From the 12^{th} day of the experiment, the TAN recorded significantly higher values ($P<0.05$) in the control. The NO_2^-N started showing significant difference in the control as compared to the CH added treatments from the next sampling date, that is the 16^{th} day. Another

interesting observation made was that none of the CH added treatments differed among themselves (P>0.05) with respect to lowering of TAN and NO_2^-N on any of the days subsequent to CH addition.

9.3.2 Bacterial population

The THB did not show significant difference (P>0.05) among treatments and the control on the first and second sampling on 7th and 14th day respectively which was done just before and after the initiation of CH addition (Fig.9.3). On the 21st day, the control recorded significantly lower (P<0.05) THB than the treatments in which potato, tapioca, rice and wheat flour were added, where as no significant difference could be observed between the control and the treatment T1 in which yam flour was added as the carbohydrate source. Again, on the next sampling in the 28th day, no significant difference was observed among the CH added treatments and the control. Nevertheless, the lowest mean THB was recorded in the control. The THB counts ranged between $1.7 \pm 0.36 \times 10^5$ to $1.11 \pm 0.19 \times 10^7$ cfu/ml.

The TVB recorded values ranged between $5 \pm 1 \times 10^2$ to $20.6 \pm 0.35 \times 10^2$ cfu/ml and did not differ significantly (P>0.05) among the treatments and the control on any of the sampling days. However, the highest TVB counts after the CH addition was observed in the control (Fig.9.4). A general increasing trend both in the case of THB and TVB could also be observed with time.

9.3.3 Post larval production

The MLS of the control was significantly lower (P>0.05) than the treatments only on the 20th day of larval rearing. There was no significant difference among the treatments with respect to MLS on any of the sampling days (Table 9.3).

Similarly the mean percentage survival also started showing significantly lower values from the 20th day in the control where as there was no significant difference among the treatments with different CH sources at any stage of the experiment. The highest mean survival (production) of 53.47 ± 1.36 % was recorded in the treatment with rice as the organic carbon source followed by tapioca flour (52.53 ± 1.5 %), wheat flour (52.4 ± 2.55 %), potato flour (51.97 ± 2.37 %) and finally yam flour (50.53 ± 2.9 %). A significantly lower mean survival of 41.6 ± 1.8 % was recorded in the control. On an average there was 25.43 % increased production compared to the control in the CH added treatments. The appearance of PL was the earliest in wheat flour (18 days) followed by potato and rice flour (18.3 ± 0.6 days) added treatments. Whereas the duration of larval rearing was shortest in tapioca flour added treatments (28.3 ± 0.6 days) whereas it took a significantly longer time (31 ± 1 days) in the control. The total length and wet weight of the post larva also did not differ significantly (P>0.05) among the treatments where as it was significantly lower (P<0.05) in the control. The production details in the different treatments and the control has been presented in Table 9.4.

9.3.4 Salinity stress test

None of the treatments differed among them (P>0.05) with respect to the CMI (0 and 7 ppt) and percentage final mortality (0 and 7ppt). An average of 36 to 40 % reduction in stress was observed in all the treatments with respect to control at 0 ppt. The percentage stress reduction was in the range of 66.67 to 75.04 % in the treatments as compared to control at 7 ppt salinity. The results of salinity stress test are presented in Table 9.5.

9.4 Discussion

In the present experiment, the addition of different carbohydrate sources was found to be positively influencing the hatchery production of *Macrobrachium rosenbergii* post larvae using modified static green water system. There was a reduction in TAN and NO_2^--N in all the tanks in which the organic carbon source was added. Several authors (Avnimelech, 1999; 2006; 2007; Buford *et al.*, 2003; Hari *et al.*, 2004; 2006; Asaduzzaman *et al.*, 2008; 2009; Crab *et al.*, 2009) have reported a significant reduction in TAN and NO_2^--N concentrations in the water column on the addition of different carbohydrate sources to the aquaculture production systems. Shi-Yen and Chun-Yang (1992) compared three carbohydrate sources, viz. glucose, dextrin and corn starch in favor of substituting the dietary protein. Varghese (2007) has also attempted the use of different CH sources in the extensive culture systems of *P. monodon*. The author has used the very same CH sources as in the present experiment and he too observed no difference among the various CH sources in keeping the levels of TAN and NO_2^--N under control.

The larvae have been reported (Armstrong *et al.*, 1976, 1978; Valenti, 1985) to tolerate levels in the order of 1.0mg/l for both ammonia and nitrite. The average TAN and NO_2^--N values recorded for all the carbohydrate added treatments has remained lower than 0.76 and 0.55 mg/l respectively throughout the experiment. The effect of elevated levels of these nitrogenous toxicants on the development and survival of diverse fishes and shrimps have been discussed in detail in the previous Chapter (Chapter 8, section 8.4.1). The tolerance level of the *M. rosenbergii* as observed by many researchers has also been discussed under the same section in the previous chapter. However the TAN and nitrite nitrogen values recorded in the control in the present experiment (Fig. 9.1 and 9.2) is in the range suggested as deleterious to the prawn larvae in most of the previous studies (Armstrong *et al.*, 1976, 1978; Lee and Wickins, 1992; Valenti *et al.*, 1998; Correia *et al.*, 2000; New, 2002). On the other hand, higher levels of TAN has been reported by Ang (1983), Phuong *et al.* (2000) and Kurup (2003) with no significant effect on larval growth and post larval production. They recorded TAN values as high as 5.5, 1.778 and 2 ppm respectively and did not observe any detrimental effects on the growth and survival of the larvae grown in static green waters. However, the post larval production recorded in the control in the present experiment (average of 41.6 PL/l) was considerably higher than that achieved in the larval culture carried out previously (Chapters 2, 3 and 4) applying the clear water system (which was around 30 PL/l). The toxicity of ammonia is reported to increase with increase in pH and temperature (Hasgopian and Riley, 1998; Hargreaves, 1998). In the present experiment the water pH was

always below 8.37 and the values above 8 were not recorded frequently. The water temperature was always below 30^0C. As in the previous experiments using the modified static green water system, the problems related to elevated levels of potentially toxic ammonia nitrogen and nitrite nitrogen must have been kept under control due to these reasons. Nevertheless, it appears that the addition of all types of carbohydrates was useful in the control of TAN and NO_2^-N which concurs with the findings of Avnimelech (1998), Avnimelech and Lacher (1979) and Avnimelech and Mokady (1988). In consequence, a better larval rearing environment was provided by the addition of various carbohydrate sources. The lower TAN level in rearing water might have also influenced positively the food intake and health of the larvae. Avnimelech *et al.* (1995), Avnimelech (1999) and Hari *et al.* (2004) have observed improved feed uptake at lower TAN levels. The feed intake of *M. rosenbergii* juveniles was also found to be considerably higher at TAN concentrations less than 0.5 ppm (Naqvi *et al.*, 2007).

The heterotrophic bacterial population was found to fluctuate in the treatments with different carbohydrate sources (Fig. 9.3). However, no significant difference ($P<0.05$) among the treatments and the control was observed on any of the sampling days. However, a general increase in the mean THB values with time was observed in all the treatments and the control. Anderson *et al.* (1989) have also observed an increase in the bacterial counts with larval age as well as total suspended solids. The bacterial counts observed in the present experiment was very much comparable to the total viable counts recorded by several others (Aquacop, 1977; Miyamoto *et al.*,

1983; Anderson *et al.*,1989; Phatarpekar *et al.*, 2002) in the larval rearing waters of *M. rosenbergii* in various culture systems. On the other hand, higher counts has been reported by other workers (Vici *et al.*, 2000; Al-Harbi and Uddin, 2004) in the larval rearing water of *M. rosenbergii*. However, the THB recorded in the present experiment did not reach high values as those observed by Vici *et al.* (2000) in the control or in any of the carbohydrate added treatments. This can be attributed to the probable grazing of the bacterial cells in flocs by the prawn larvae, at least in the later larval stages when they become more omnivorous in their feeding habit. Many researchers (Kovalenko *et al.*, 2002; Barros and Valenti, 2003a) have demonstrated the ability of *M. rosenbergii* larvae to feed on wet or dry inert diets during the later larval stages. Hence, there is a very high probability favouring the use of bacterial flocs as feed by the prawn larvae. Crab *et al.* (2009) demonstrated the intake of bacterial flocs in the post larve *M. rosenbergii*. However, it is not possible to confirm the grazing of the bacterial flocs by the prawn larvae in the current experiment and this aspect probably requires further research.

Nevertheless, the probability of gut microbiota of aquatic animals being constituted by indigenous microbiota jointly with high levels of allochtonous microorganisms so maintained by their constant ingestion from the surrounding water has been reported by Hansen and Olafsen (1989). For that reason, even if the larvae were not able to directly feed on the flocs, it is possible that the beneficial bacteria must have acted as a probiotic by colonising the gut of the larvae or by competitive exclusion of pathogenic bacteria in addition to maintenance of water quality and probable antibiotic

effect. The indiscriminate use of antimicrobial compounds in fresh water prawn hatcheries in India leading to the development of antibiotic resistant organisms, multiple antibiotic resistance, resistance transfer to pathogenic bacteria and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens has been reported by Sahul Hameed *et al.* (2003). The post larvae grown in the relatively sterile environment of hatcheries has been reported (Gomez-Gil *et al.*, 2000) as more susceptible to diseases when exposed to environmental stress and potentially pathogenic bacteria. Therefore, the adoption of modified static green water system with addition of any of the carbohydrate sources currently investigated can be suggested as a solution to many of the above mentioned problems.

TVB count in all the treatments and the control was also observed to remain low when compared to the THB through out the experiment with no variation in relation to carbohydrate source. The proliferation of THB in all the tanks must have kept the numbers of potentially toxic *Vibrio* bacteria under control either by competitive exclusion or by producing substances which inhibit the growth of pathogens (Schryver *et al.*, 2008).

The MLS and percentage survival was significantly higher ($P<0.05$) in all the carbohydrate added treatments irrespective of the organic carbon source than the control. The appearance of the post larvae were the earliest and the duration of the rearing period was shortest in the very same treatments. The growth of the newly settled post larvae in terms of the total length and wet weight were also found to be highest in the carbohydrate added treatments. This could have been due to the over all well being of the

larvae in more favourable environmental conditions attained as a result of lowering of toxic metabolites which in turn must have enhanced the intake of feed in the larvae, finally resulting in a higher growth in the carbohydrate added treatments. The stress tolerating ability of the post larvae on exposure to lower salinities (0 ppt and 7 ppt) was also found to be significantly higher in the biofloc tanks than the control. The lowest mortalities were recorded in these treatments with no significant difference ($P>0.05$) among them. A considerable reduction in stress with respect to the control was also observed in these treatments with 0.03 g/l of carbohydrate addition per day. Conversely while establishing the use of bioflocs as feed by *M. rosenbergii* PL, Crab *et al.* (2009), reported that the biofloc quality was significantly influenced by the carbonaceous substrate added. The authors observed the highest protein content of the flocs as well as high survival in the PL fed flocs grown on glycerol+Bacillus compared to those grown on acetate or glucose.

However, the various carbohydrate sources used in the present experiment did not show any significant difference in their ability to reduce the concentration of TAN and nitrite nitrogen while augmenting the post larval production. Besides, no attempt was made in the present experiment to assess the nutritional quality of the flocs produced in the treatments with the addition of different organic carbon sources or the contribution of floc to the nutrition of the prawn larvae in the various treatments. Nevertheless, a consistent post larval production was ensured by the application of biofloc technology with various carbohydrate sources. The post larval production attained in the present experiment was either higher than or comparable with

the production earlier reported (Aquacop, 1983; New, 1995; Correia *et al.*, 2000; Kurup, 2003; Murthy, 2006; Thresiamma *et al.*, 2006; Nair *et al.*, 2007) by various workers in the hatcheries of *M. rosenbergii* adopting different larviculture techniques.

9.5 Conclusion

The ability of various locally available, comparatively cheap carbohydrate sources (Potato flour, Yam flour, Rice flour, Wheat flour and Tapioca flour) in lowering TAN and NO_2^--N and improving THB populations in static hatchery systems has been demonstrated in the present experiment. Therefore, it can be concluded that (1) carbon source material have the ability to reduce the inorganic nitrogen accumulation, (2) the five carbohydrate sources viz. potato, yam, rice, wheat and tapioca powder are equally effective in controlling the nitrogen production with no significant difference in post larval production and (3) in order to make the larvicultue more cost effective, any of the cheap carbohydrate source presently tried can be used.

Table.9.1 Sources of carbohydrate in different treatments in the larviculture of *M rosenbergii* using MSGWS

Treatment	Carbohydrate source
T1	Potato flour
T2	Yam flour
T3	Rice flour
T4	Wheat flour
T5	Tapioca flour
C	No carbohydrate

Table 9.2 Average ± s.d of daily water quality parameters of various carbohydrate added treatments stocked with *M. rosenbergii* larvae

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Temperature (^{0}C)	28.5 ± 0.66	28.67 ± 0.74	28.69 ± 0.77	28.59 ± 0.7	28.7 ± 0.76	28.57 ± 0.68
pH	8.02 ± 0.12	7.97 ± 0.11	7.98 ± 0.12	8.06 ± 0.15	7.98 ± 0.17	8.04 ± 0.13
Salinity (ppt)	12.8 ± 0.69	12.98 ± 0.7	13 ± 0.71	12.96 ± 0.68	12.99 ± 0.62	13.01 ± 0.66
Dissolved oxygen (mg/l)	7.09 ± 0.17	7.12 ± 0.14	7.12 ± 0.15	7.07 ± 0.21	7.09 ± 0.16	7.17 ± 0.14

Table 9.3 Effect of different carbohydrate sources on the mean larval stages of *M.rosenbergii* larvae.

Treatments	Days			
	5	10	15	20
T1	3.37 ± 0.06a	5.16± 0.06a	6.7 ± 0.1a	8.93 ± 0.12a
T2	3.3 ± 0.1a	5.16± 0.06a	6.67 ±0.06a	8.97 ± 0.15a
T3	3.3a	5.1± 0.1a	6.7 ± 0.1a	9 ± 0.1a
T4	3.37 ± 0.06a	5.16± 0.06a	6.73 ± 0.12a	9.03 ± 0.06a
T5	3.4a	5.13± 0.06a	6.73 ± 0.06a	9 ± 0.1a
Control	3.4a	5.2a	6.63 ± 0.06a	8.73 ± 0.06b
Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)				

Table 9.4 Survival, duration of rearing period, length and wet weight of *M rosenbergii* post larvae in various treatments with different carbohydrate sources

Treatments	Stocking density(larvae/litre)	% survival	Post larva/liter	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
Y	100	50.53 ± 2.9^{a}	50.53 ± 2.9^{a}	19^{ab}	29 ± 1^{a}	9.64 ± 0.05^{a}	9.92 ± 0.05^{a}
P	100	51.97 ± 2.37^{a}	51.97 ± 2.37^{a}	18.3 ± 0.6^{a}	29 ± 1^{a}	9.65 ± 0.09^{a}	9.93 ± 0.09^{a}
T	100	52.53 ± 1.5^{a}	52.53 ± 1.5^{a}	19 ± 1^{ab}	28.3 ± 0.6^{a}	9.75 ± 0.09^{a}	10.03 ± 0.09^{a}
R	100	53.47 ± 1.36^{a}	53.47 ± 1.36^{a}	18.3 ± 0.6^{a}	28.6 ± 0.6^{a}	9.67 ± 0.24^{a}	9.95 ± 0.24^{a}
W	100	52.4 ± 2.55^{a}	52.4 ± 2.55^{a}	18^{a}	29 ± 1^{a}	9.73 ± 0.13^{a}	10.02 ± 0.13^{a}
Control	100	41.6 ± 1.83^{b}	41.6 ± 1.83^{b}	20 ± 1^{b}	31 ± 1^{b}	9.29 ± 0.16^{b}	9.62 ± 0.16^{b}

Mean ± s d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)

Table 9.5 Effect of different carbohydrate sources on Cumulative Mortality Index (CMI) values and final survival of newly metamorphosed *M rosenbergii* post larvae in different treatments and control exposed to low salinity stress (0 and 7 ppt)

Treatments	0 ppt			7 ppt		
	CMI	Final mortality (%)	Reduction in stress (%)	CMI	Final mortality (%)	Reduction in stress (%)
Control	896 ± 34.84^{b}	80.65 ± 3.23^{b}	0	96 ± 24^{b}	12.90 ± 3.23^{b}	0
Y	468.08 ± 64.26^{a}	48.39 ± 6.45^{a}	40	24^{a}	3.22^{a}	75.04
P	484.33 ± 85.45^{a}	50.54 ± 3.72^{a}	37.33	32 ± 13.86^{a}	4.3 ± 1.86^{a}	66.67
T	457.25 ± 51.36^{a}	48.39 ± 3.23^{a}	40	24 ± 24^{a}	3.23 ± 3.23^{a}	74.96
R	472.33 ± 84.37^{a}	51.61 ± 8.53^{a}	36	24^{a}	3.23^{a}	74.96
W	452.58 ± 47.28^{a}	49.46 ± 3.72^{a}	38.67	32 ± 13.86^{a}	4.3 ± 1.86^{a}	66.67

Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)

Fig.9.1 Effect of addition of various carbohydrate sources on total ammonia nitrogen in various treatments during the experimental period in the larviculture of *M. rosenbergii*

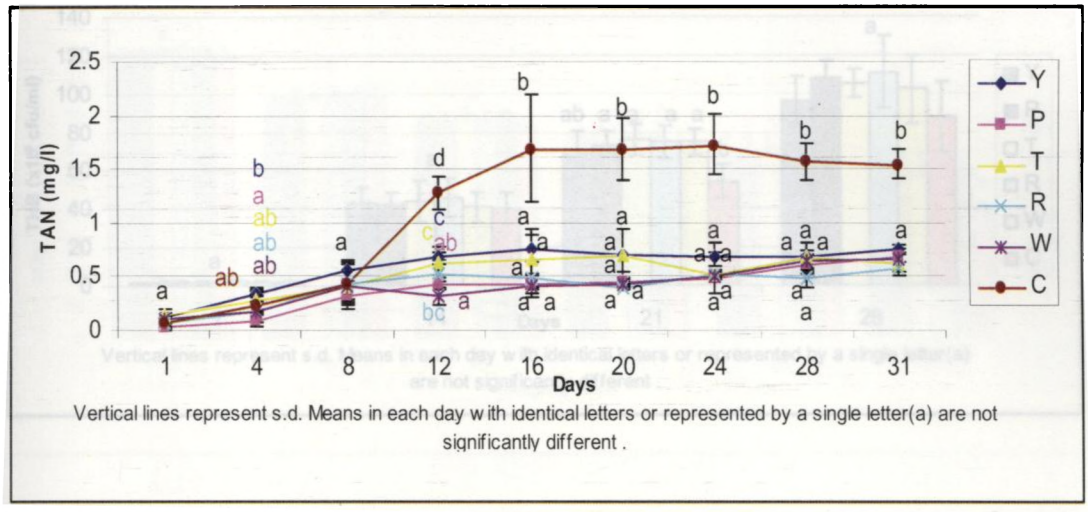


Fig.9.2 Effect of addition of various carbohydrate sources on nitrite nitrogen in various treatments during the experimental period in the larviculture of *M. rosenbergii*

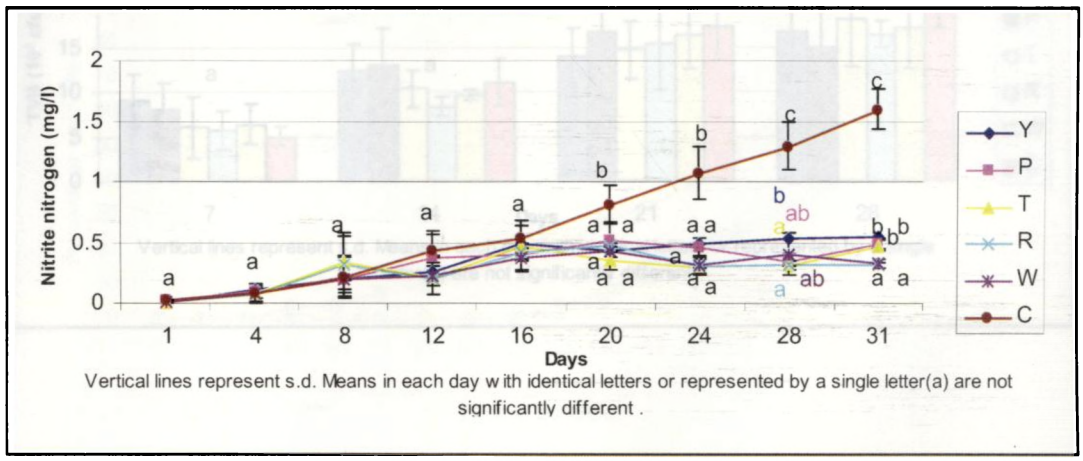


Fig.9.3 Average THB in treatments with different carbohydrate sources in the larviculture of *M. rosenbergii*

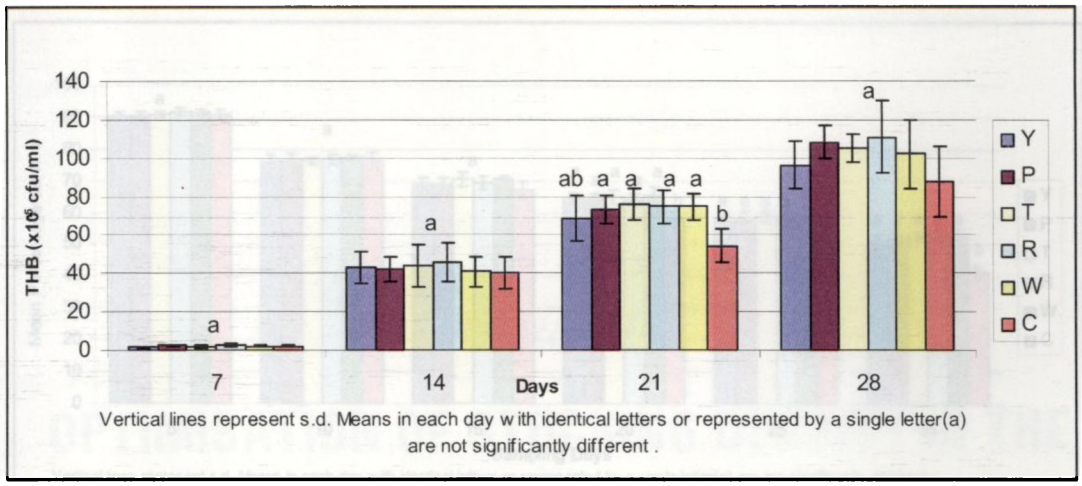


Fig.9.4 Average TVB in treatments with different carbohydrate sources in the larviculture of *M. rosenbergii*

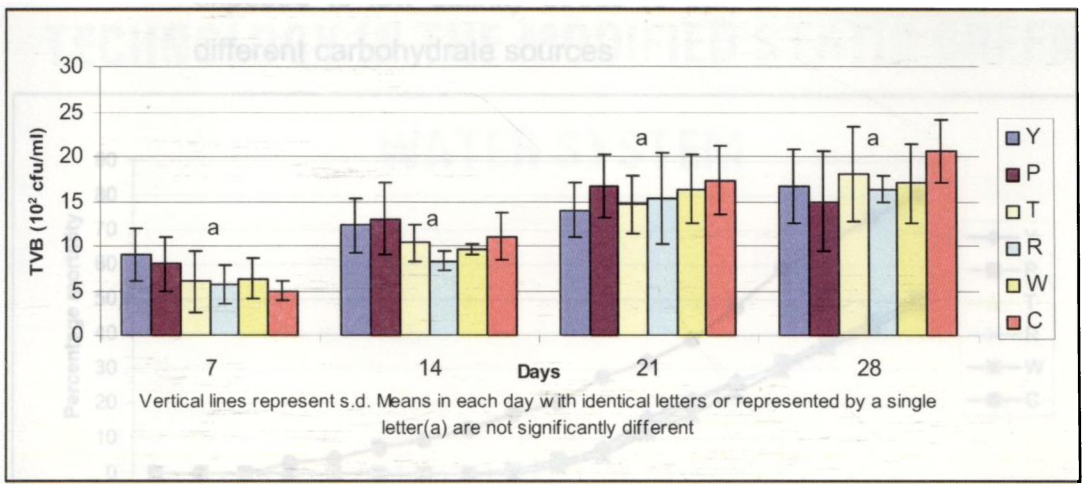


Fig 9.5 Effect of addition of various carbohydrate sources on the survival of *M rosenbergii* in the larviculture carried out using MSGWS

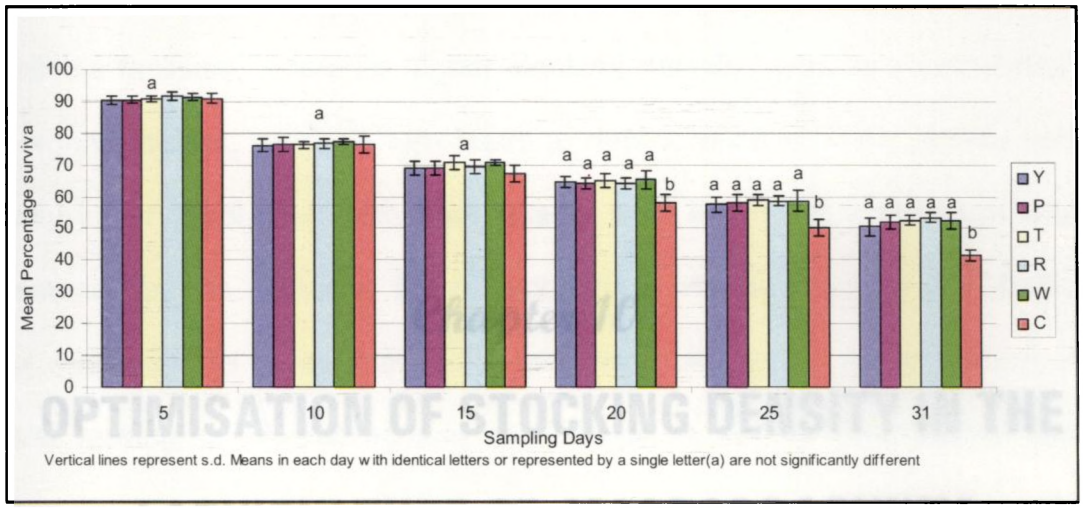
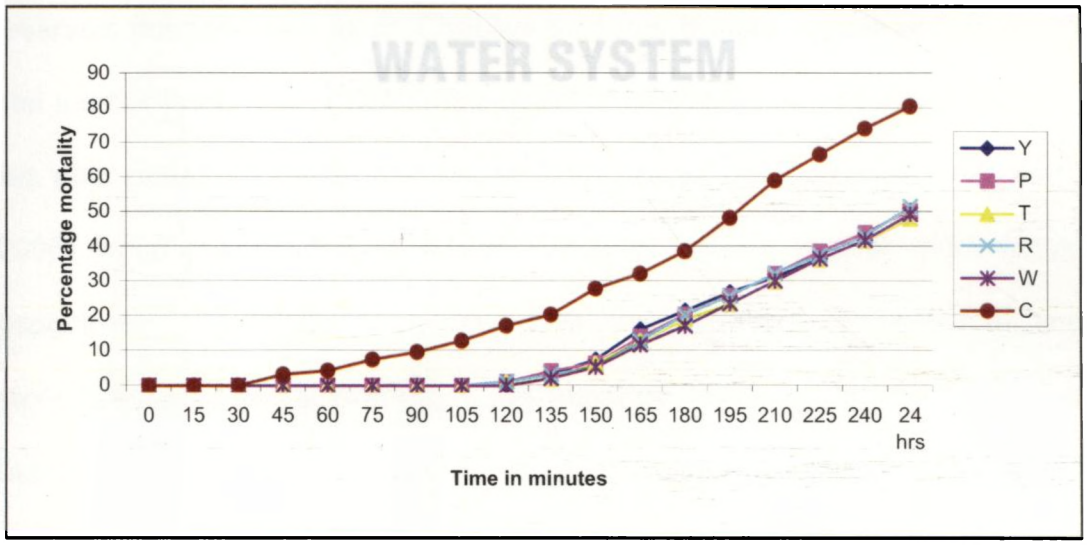


Fig.9.6 Mean percentage mortality of *M rosenbergii* postlarvae exposed to low salinity stress (0 ppt) in treatments with different carbohydrate sources



Chapter 10

OPTIMISATION OF STOCKING DENSITY IN THE LARVICULTURE OF *MACROBRACHIUM ROSENBERGII* WITH AND WITHOUT BIOFLOC TECHNOLOGY IN THE MODIFIED STATIC GREEN WATER SYSTEM

10.1. Introduction

Initial stocking density is a crucial factor in the process of post larval production. The lower stocking density results in under utilization of available facilities, where as higher stocking density leads to accumulation of organic matter, which in turn leads to deterioration of water quality and finally lower production with poor quality post larvae. In a previous trial conducted by the author at SIF (Chapter 5), a stocking density of 100 PL/ liter was found to be optimal in the modified static green water system of larval rearing of *Macrobrachium rosenbergii.* A similar observation was made by Phuong *et al.* (2000) who recommended a stocking density ranging between 90 and 120 larvae per liter following the same larval rearing system. On the other hand, the increase in stocking density was accompanied by elevated levels of toxic nitrogenous compounds (as observed from the results of Chapter 5 of this thesis). Increased levels of total ammonia nitrogen (TAN) as a result of intensification of initial stocking was also observed by Emmerson and Andrews (1981) and Phuong *et al.* (2000) while rearing the larvae of *Penaeus indicus* and *M. rosenbergii* respectively. Therefore, it seems that the foremost limitation in the intensification of larval rearing is the elevated levels of toxic nitrogenous metabolites. The management of such system depends on developing methods to remove these compounds from the larval rearing tanks. If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. The proper manipulation of microbial biomass enables to control water quality, mostly through the

conversion of potentially toxic inorganic nitrogen species to microbial protein (Avnimelech, 2006). The use of a carbohydrate source was also found to control the levels of TAN and nitrite nitrogen in the larviculture of *M. rosenbergii* in the two previous experiments conducted at the SIF. Hence, it was hypothesized that, higher post larval production can be realized by increasing the initial stocking density in the larviculture systems of *M. rosenbergii* with the help of regular addition of carbohydrates, which encourages the proliferation of total heterotrophic bacteria resulting in lower levels of toxic nitrogenous compounds. Thus, the objectives of the present experiment were

1. To find out the effectiveness of C / N ratio in the control of inorganic nitrogen accumulation during the rearing period in fresh water prawn larviculture systems at different initial stocking densities.
2. To compare the effect of various stocking densities on the postlarval production with and without carbohydrate addition.

10.2 Materials and methods

The experiment tanks allocation for each treatment followed a completely randomized design with or without carbohydrate addition directly to the water column. The larval prawns were stocked at a density of 100, 150 and 200 per liter. The treatments without carbohydrate addition are abbreviated as 100, 150 and 200 while the treatments with carbohydrate addition as 100 + CH, 150 + CH and 200 + CH (Table 10.1).

10.2.1 Experimental setup

The brood stock management, hatching and larval rearing was carried out as explained in Chapter 8 (refer section 8.2.1.1 and 8.2.1.2).

The larvae were fed with *Artemia* nauplii at the rate of 4 numbers/ml of rearing water from the second day of hatching. Since the amount of BSN required at any time depends primarily on tank volume and not on the number of larvae present (New, 2002), no change was made in the quantity of BSN which was fed irrespective of the initial stocking density. Egg custard (Kurup, 2003) was given to the larvae from fifth larval stage onwards after passing through sieves of different mesh sizes. The carbohydrate selected was tapioca powder which was locally purchased.

The quantity of tapioca powder added to assimilate the ammonium flux into microbial protein was calculated following the Avnimelech (1999).

ΔCH = Quantity of feed x % Nitrogen of feed x % Nitrogen excretion / 0.05

The average quantity of feed given / tank (50 l) / day was estimated as 3g (0.06 g/l/day) at a stocking density of 100 larvae / liter. This was increased to 4.5 and 6 g / l/ day at stocking densities of 150 and 200 larvae / liter. As a consequence, 1.5, 2.25 ad 3 g tapioca /tank/day (0.03, 0.045 and 0.06 g/l/day) was added to immobilize the nitrogen into microbial protein.

The pre weighed tapioca flour was mixed with tank water in a beaker and uniformly distributed in the particular tank following administration of

egg custard at 8.30 A.M. Two more feedings of egg custard was done at 11.30 A.M and 2.30 P.M (Daily feed ration was split into three equal feeds). Egg custard was prepared as explained in Chapter 2 (section 2.2.3) and they were passed through sieves of different mesh sizes (Table 2.3 and Table 2.4 in Chapter 2) before feeding the larvae. BSN were given as overnight feed at around 5 0' clock in the evening.

10.2.2 Water quality parameters

Temperature, salinity and pH were measured daily using mercury thermometer, hand refractometer (Atago, Japan) and pH meter (Eutech instruments, Singapore) respectively. Dissolved oxygen was measured weekly twice by Winkler's method (APHA, 1995). Water samples were filtered through GF/C Whatman filter papers and the filtrate was analyzed for nitrite nitrogen (NO_2^-N) and total ammonia nitrogen (TAN) by phenol hypochlorite method (Grasshoff *et al.*,1983). Total heterotrophic bacteria (THB) and Total *Vibrio* bacteria (TVB) were estimated following standard methods (APHA, 1995).

10.2.3 Assessment of treatments.

The evaluation of different treatments was done based on the post larval production, time required for the larvae to reach each stage and their relative survival (Alam *et al.*,1993a) (section 8.2.2. of chapter 8).

10.2.4 Statistical analysis.

Data analysis was done by ANOVA using SPSS 16. (section 8.2.3).

10.3 Results

10.3.1 Water quality

The average values recorded for the various physicochemical parameters like temperature, salinity, pH and dissolved oxygen are presented in Table 10.2. These parameters were well with in the optimum range suggested (Correia *et al.*, 2000; New, 2002) for the larval rearing of *M. rosenbergii* and were not found to be affected by varying levels of stocking density or carbohydrate addition.

However, a significant difference could be observed in the TAN and NO_2^-N in various treatments during the experimental period which are shown in Fig.10.1 and 10.2. respectively. From the second sampling onwards, elevated TAN levels were recorded in the treatments with higher stocking densities. Nevertheless, once the addition of carbohydrate (CH) started on the 10^{th} day, all the CH added treatments recorded significantly lower values than the non-CH added treatments. The highest mean TAN value of 3.1 ± 0.18 ppm was recorded in 200, whereas the TAN value in 200+CH on the same day was 1.12 ± 0.15 ppm. Similarly, in the treatment T1 (100 larvae/l), the TAN reached values as high as 2.24 mg/l on the 34^{th} day of larval rearing. While in 100+CH, TAN value reached a maximum of 0.64 ± 0.04mg/l on the 12^{th} day and there after it was reduced to as low as 0.35 ± 0.12 ppm on 28^{th} day and it again got elevated to 0.41 ± 0.04 ppm on the 34^{th} day. In the treatment T3 with a stocking density of 150 larvae / liter, the TAN value was the highest on the 34^{th} day as in the case of other treatments without CH

addition. Where as in the same stocking density with carbohydrate addition, the TAN on the 34th day was recorded as 0.67 ± 0.07 mg/l.

Likewise, the NO_2^- - N values recorded in treatments without CH on the 34th day was 1.92 ± 0.49, 2.39 ± 0.42 and 2.57 ± 0.63 mg /l for stocking densities of 100, 150 and 200 larvae/ liter respectively. At the same time, the nitrite nitrogen values in the same stocking densities with CH addition were 0.14 ± 0.04, 0.21 ± 0.16 and 0.89 ± 0.08 mg/l respectively.

10.3.2 Bacterial population

Generally THB recorded in the carbohydrate added treatments was higher, so also the THB showed a positive relation with stocking density (Fig 10.3). The highest mean THB on the 4th sampling day was recorded in 200+CH (1.27± 0.36 x 10^7 cfu/ml), although it was not significantly different from ($P>0.05$) 200 (1 ± 0.14 x 10^7 cfu/ml), 150(1 ± 0.14 x 10^7 cfu/ml), 150+CH (1.14 ± 0.16 x 10^7 cfu/ml) or 100+CH (1.08 ± 0.17 x 10^7 cfu/ml). The THB recorded values ranged between 2.2 ± 0.96 x 10^5 to 8.5 ± 2.1 x 10^6 cfu /ml in the treatment T1 (100) and it was significantly lower ($P<0.05$) than all the other treatments on the 28th day. TVB did not differ significantly between any of the treatments ($P>0.05$) throughout the rearing period. Yet the highest average *Vibrio* bacterial count (21.67 ± 3.51 x 10^2 cfu /ml) was recorded in the treatment 200 without CH addition followed by 150 (19 ± 5.29 x 10^2 cfu /ml) and 100 (17 ± 4.16 x 10^2 cfu /ml) As in case of THB, a positive relation was also observed between TVB and stocking density. The weekly changes in the TVB are presented in Fig. 10.4.

10.3.3 Post larval production

The treatments started showing significant difference ($P<0.05$) in the mean larval stages from as early as 5th day (Table 10.3). The highest mean larval stage on all the days was recorded in T2 (100+CH) following the addition of CH. The treatments 150+CH and 200+CH showed a delayed growth in terms of MLS during the early sampling days similar to 150 and 200. However, the addition of CH was found effective to speed up the larval growth in the CH added treatments, and in spite of the higher stocking density, did not differ significantly with T1 (100) on the 20th day. More over the larvae in 150+CH recorded a better growth than a lower stocking density (100) without CH addition.

The highest mean survival was recorded in T2 (55.3 ± 1.03 %), followed by T1 (42.47 ± 0.5 %) and T4 (41.82 ± 1.47 %). The lowest mean survival (16.37 ± 0.57 %) was observed at the highest stocking density of 200 larvae / liter without CH addition. Percentage larval survival was significantly different between the treatments ($P<0.05$) from 5th day of the experiment, and the superior survival was recorded in the lower stocking density. The mean survival recorded in various treatments over the entire period of the experiment with an interval of 5 days is presented in Fig.10.6.

Nevertheless, the highest mean post larval production of 62.73 ± 2.21 post larvae / liter was attained in 150+CH followed by 100+CH (55.3 ± 1.03) and 200+CH (54.07 ± 1.81). All the treatments without CH addition, irrespective of stocking density, recorded significantly lower post larval production.

The total length and wet weight of the larvae also did not show significant difference among the treatments 100+CH and 150+CH. The appearance of the first post was the earliest in 100+CH (19 ± 1 days) followed by 100 (20 days) and 150+CH (21.3 ± 1.5 days). Also 95 % of the larvae metamorphosed as post larvae by an average of 29.6 ± 0.6 days in T2 followed by T1 (30.3 ± 0.6 days) and T4 (31 days), where as it took an average of 34 days in T5. The final production details are presented in Table 10.4.

10.3.4 Salinity stress test

The highest stress tolerance at 0 ppt as well as 7 ppt (as indicated by lowest CMI) was exhibited by the post larvae in the treatment 100+CH followed by 150+CH and 200+CH (Table 10.5). Similarly the lowest average percentage mortality on exposing the post larvae to lower salinities (0 ppt and 7 ppt) was recorded in the carbohydrate added treatments (T2, T4 and T6) when compared to the treatments without CH addition (T1, T3 and T5).

10.4 Discussion

10.4.1 Water quality

There was an elevation in the levels of TAN and NO_2^- -N with an increase in the stocking density. This could be due to increased accumulation of organic matter in form of excess feed or excretion of higher number of organisms at higher stocking densities. Another factor that could have added to the accumulation of toxic nitrogenous compounds might be the increased larval mortalities in the tanks with higher stocking densities as compared to

those with lower stocking densities. According to Tomasso (1994), larvae might be exposed to high ammonia and nitrite concentration when larviculture is conducted in intensive systems with high stocking density.

However, the application of a CH source was effective in containing the levels of TAN and NO_2^- -N in all the treatments irrespective of the stocking density. Lowering of potentially toxic nitrogenous nutrients by the application of CH source in intensive aquaculture systems has been reported by many authors (Avnimelech, 1999; 2006; 2007; Buford *et al.*, 2003; 2004; Crab *et al.*, 2007; Azim and Little, 2008; Ballester *et al.*, 2009). Nevertheless, a gradual rise in the levels of both TAN and NO_2^- -N was observed in the treatment 200+CH towards the end of the experiment. This could be due to the higher mortalities that occurred in the tanks with higher stocking densities, which in turn resulted in the release of more nitrogenous compounds by the decomposition of dead organisms. Moreover, a lot of feed remains unconsumed due to comparatively larger mortalities at higher densities of the larval stocking. This also leads to the accumulation of toxic nitrogenous compounds.

According to Varghese (2007), in the extensive culture systems of *Penaeus monodon*, TAN concentrations of water and sediment showed a cumulative increase in control tanks without CH addition with stocking density of 3, 7 and 12 post larvae / m^2 when compared with treatments 3 + CH, 7 + CH and 12 + CH with carbohydrate addition. The lower TAN concentration observed in carbohydrate added treatments irrespective of stocking density was attributed by the author to the effect of carbohydrate addition in reducing

the concentration of inorganic nitrogen. Thus the addition of CH seems to be a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in the static larviculture systems as in culture ponds. Avnimelech and Mokady (1988) reported that the addition of carbohydrate is an effective method to reduce concentrations of inorganic nitrogen in intensive aquaculture practices and the results of the present study strongly corroborate with their findings.

The results of present study also revealed that there was an increase in the level of toxic inorganic nitrogen commensurate with the increase in stocking density. This might be due to the higher feed quantity supplied to the larvae (quantity of unconsumed feed increases with increased mortality rates at higher stocking densities) and the higher excretion of the larger biomass at higher stocking densities. Ghosh and Mohanty (1981), Brady (1990), Phillips *et al.* (1993), Ayub *et al.* (1993) and Csavas (1994) reported that the toxic inorganic nitrogen concentration in culture systems increase by the rate of feeding, unconsumed feed and the rate of excretion and the present findings show full agreement with the above.

10.4.2 Bacterial population

The heterotrophic bacterial population was also found to increase proportionately with stocking density (Fig. 10.4) especially towards the end of the larval rearing period. This must be due to the additional quantity of nutrients released into the tanks with higher stocking densities. The addition of CH might have helped in the proliferation of THB and at the time of 4^{th} sampling, the highest THB was recorded in the CH added treatments.

Aikyama *et al.* (1989) reported that carbohydrate source (glucose and starch) application to the culture system provided better environment for multiplying bacterial population. An increase in the bacterial numbers with time was also noticed in the present experiment. A similar trend of increased bacterial numbers with increased age of the larvae was also observed by Sahul Hameed *et al.* (2003) in the larviculture of *M. rosenbergii.*

However as observed by the author in the previous experiments conducted at SIF (Chapter 8 and 9), the THB recorded in the present study was comparable with the number of colony forming units of bacteria recorded by several researchers (Aquacop, 1977; Miyamoto *et al.*, 1983; Anderson *et al.*, 1989; Vici *et al.*, 2000; Phatarpekar *et al.*, 2002; Al-Harbi and Uddin, 2004) in the rearing water of *M. rosenbergii* hatcheries. As discussed in the previous chapters (8 and 9), this could be due to the probable grazing of the bacterial floccules by the prawn larvae. Nogami and Maeda (1992) ascribed the grazing by protozoa as the reason for bacterial cell level not exceeding 10^6 cells/ml in spite of repeated inoculation of probiotic bacteria. According to the available reports (Schroeder, 1987; Beveridge *et al.*, 1989; Rahmatulla and Beveridge, 1993 and Avnimelech, 1999), the heterotrophic bacteria produced single cell protein that might be utilized as a food source by carp and tilapia whereby lowering the demand for supplemental feed protein. Utilization of microbial protein depends on the ability of the target animal to harvest the same (Avnimelech, 1999). Intake of bacterial flocs have been reported in post larvae and adult *M. rosenbergii* (Crab *et al.*, 2009; Asaduzzaman *et al.*, 2008; 2009),

P. monodon (Hari *et al.*, 2004 ; 2006; Buford *et al.*, 2003; Panjaitan, 2004) and tilapia (Avnimelech,1999; 2007; Azim and Little, 2008).

Several studies on the feeding habits of larval *M. rosenbergii* indicated they become more omnivorous in advanced larval stages and were able to select and digest food as they grow older (Deru, 1990; Jones *et al.*, 1993; Barros and Valenti, 1997; 2003a; Kovalenko *et al.*, 2002). The probable contribution of probiotic bacteria which develop within the system to larval nutrition has been reported by Sebastin (2006) in the larviculture of *M. rosenbergii* in large outdoor tanks. The ingestion of microflora in the aquatic environment by the organisms living in it has also been reported by several authors (Gomez-Gil *et al.*, 2000; Al-Harbi and Uddin, 2004; Izquierdo *et al.*, 2006). It is, therefore, possible that the bacteria may have directly provided essential nutrients not present in the feed supplied, if ingested by prawn larvae as food, or indirectly improved larval digestion by contributing enzymes. A similar observation was made by Douillet and Langdon (1994) in the larval rearing of oyster *Crassostrea gigas*.

The reduction of TAN and corresponding increase of THB observed in the present study can be attributed to the utilization of TAN from the water column as reported by Avnimelech and Mokady (1988), Avnimelech *et al.* (1989, 1994) and Avnimelech (1999). The heterotrophic bacteria may utilize TAN, and organic carbon and convert them to microbial protein. Microorganisms present in shrimp culture systems has been seen to take up significant amounts of NH_4^+, from the water column (Pomeroy *et al.*, 1965).

TVB count in all the treatments and the control remained low through out the experiment with no significant difference (P>0.05) in relation to stocking density or CH addition although an increase in number was observed commensurate with time. *Vibrios* are found to be pathogenic in fresh water prawn hatcheries (Anderson *et al.*, 1989). Mortalities in finfish and shell fish has also been reported as a result of increase in *Vibrio* populations (Sung *et al.*, 2001). However the TVB recorded in the present experiment (8.33 ± 3.06 x 10^2 to 21.67 ± 3.51 x 10^2 cfu /ml) was low as compared to 0.1 x 10^4 to 35.4 x 10^4 cfu/ml recorded by Sahul Hameed *et al.* (2003) from the fresh water prawn hatchery following intensive recirculating clear water larval rearing where antibiotics are being used daily as a prophylactic measure to control bacterial population. The THB recorded in the same study (0.2 x 10^4 to 74.4 x 10^4 cfu/ml) was on the lower side as compared to the present experiment. The authors have therefore suggested that eliminating bacteria from rearing tanks or controlling bacterial populations by employing antimicrobials can lead to severe problems. However, the TVB recorded from the rearing water following the modified static green water system of larval rearing of *M. rosenbergii* without the application of antibiotics or probiotics was found to vary between 2.56 x 10^2 to 6.48 x 10^3 cfu/ml (Phuong *et al.*, 2000). This is very much comparable with the TVB recorded in the present experiment. Therefore, it may be inferred, that green water system itself, even without addition of carbohydrate might be very effective in controlling the pathogenic bacteria. Further more, other than competitive exclusion of pathogens, the heterotrophic bacteria are also suspected to have a controlling effect on

pathogenic bacteria by producing antimicrobial compounds (Michaud *et al.*, 2006; Defoirdt *et al.*, 2007; Schryver *et al.*, 2008). The addition of 'microbially matured' water or probiotics has been reported to control pathogenic bacteria in hatching or nursing prawn larvae either by competitive exclusion (Verdonck *et al.*, 1994; Riquelme *et al.*, 2001) or by production of antibiotic substances that inhibits the growth of undesired cells (Garriques and Arevalo, 1995; Moriarty, 1998; Riquelme *et al.*, 2001).

10.4.3 Development, growth and Survival of prawn larvae

A lower MLS and mean survival was observed with an increase in the initial stocking density. However, the MLS and percentage survival was significantly higher ($P<0.05$) in the CH added treatments when compared to the controls without CH addition. The growth of the newly settled post larvae in terms of the total length and wet weight were also found to be highest in the treatments with CH addition or at the lowest stocking density (100 larvael/ liter). Higher stocking density resulted in lower survival of *M. rosenbergii* larvae cultured in closed recirculating larval rearing systems (Menasveta and Piyatiratitivokul, 1980). Likewise, higher stocking densities resulted in lower growth and survival in the larval rearing of spiny lobster, *Jasus edwardsii* (Smith and Ritar, 2006). Many works show an inverse relationship between the growth and stocking density (Ray and Chien, 1992; Lee *et al.*, 1986; Daniels *et al.*, 1995; Sandifer *et al.*, 1987) in shrimp growouts. High mortality of 40 - 50% has been reported in dense culture systems (8 kg m^{-2}) (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996) due to the ammonium excreted by the fish or shrimp in the ponds. However, no

correlation between stocking density and survival was perceived in the larvae of matrinxa, *Brycon cephalus* (Gomes *et al.*, 2000). Nevertheless, increased stocking density was associated with reduced growth and homogeneity.

In the present experiment, increase in stocking density was followed by lowering of growth and survival in the control without CH addition. High mortalities at higher stocking densities can be related to elevated levels of toxic nitrogenous compounds. But the addition of CH even at elevated stocking densities was found to improve the survival and growth of the larvae. Thus, a higher post larval production was achieved in CH added treatments by providing a more favorable larval rearing medium for the larvae with lower levels of toxic nitrogenous compounds. That was probably the added advantage of the floc acting as feed for the prawn larvae, but this effect is not confirmed in the present experiment. Increased production was attained by increasing the stocking density as well as by the addition of tilapia to fresh water prawn grow out ponds to maximize the utilization of the periphyton and THB produced as a result of addition of shoots of bamboo and CH (Asaduzzaman *et al.*, 2009). Although stocking density was negatively correlated to survival and growth of larval kuruma prawn, *Penaeus japonicus*, the increase in stocking density resulted in increased post larval production (Lim and Hirayama, 1993). Furthermore, in the present study, the stress tolerating ability of the post larvae on exposure to lower salinities (0 ppt and 7 ppt) was the best in the treatments with CH addition.

The post larval production attained in the present experiment (average of 62.73 PL/ l in 150+CH) is very much comparable to a maximum of 60 PL/

liter reported by Aquacop (1983), on the contrary lower than a maximum of 80 PL/l reported by Kurup (2003). It is also higher than 40 to 50 PL / l reported for Thai backyard hatcheries (Correia *et al.*, 2000) and 50 PL / l reported for Brazilian flow through systems (Parseval *et al.*, 1989). Still higher stocking densities than experimented in the present study has been advocated in the multistage rearing system (300 to 1000 larvae / l in 500 l tanks as reported by Hsieh *et al.* (1989). The larvae are transferred to larger tanks as they grow. Stocking of 300 to 500 larvae /litre in pre-stocking tanks has been practiced in some Brazilian hatcheries (Correia *et al.*, 1988) up to around 10 days after which the density is reduced to 60 to 100 / l. The system is supposed to result in efficient feeding of the larvae. On the other hand, it results in excessive handling of the larvae which can result in damages and stress to the larvae and physical losses during transfer operations (New, 2002). A two-phase open clear water system has also been explained by Kurup *et al.* (1998) in the larval rearing of *M. rosenbergii*. However, the water requirements in such systems is very high and the use of such systems was recommended by the authors in hatcheries where both sea water and fresh water were available to the desired level. However, the present system envisages low water requirement, less handling of the larvae, low labour requirement and finally increased post larval production of healthy larvae. The possibility of increasing stocking density thus further increases the post larval production.

Unlike carbon dioxide, which is released to the air by diffusion or forced aeration, there is no effective mechanism to release the nitrogenous metabolites out of the pond (Avnimelech, 1999). Thus, intensification of

aquaculture system is inherently associated with enrichment of the water with respect to ammonium and other inorganic nitrogenous species. The management of such system depends on developing methods to remove these compounds from the system. The strategy which is presently getting more attention is the removal of ammonium from water through its assimilation into microbial proteins by the addition of carbonaceous materials to the system (Avnimelech, 1999). If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. Although not confirmed in this study, a further important aspect of this process is the potential utilization of microbial protein as a source of feed protein for shrimp or fish.

10.5 Conclusion

By the manipulation of C / N ratio by carbohydrate addition it is possible to increase the stocking density up to 150 larvae / litre, thereby maximizing post larval production without incurring any additional expenditure (except for the higher number of brooders and higher amount of egg custard required, both of which are not very expensive) and therefore found suitable for adoption in the commercial hatcheries of the Giant fresh water prawn.

Table10.1 Initial stocking density in various treatments in the larviculture of *M rosenbergii* with and without application of biofloc technology

Treatments	Stocking density (No / l)
T1	100
T2	100 + CH
T3	150
T4	150 + CH
T5	200
T6	200 +CH
CH represents carbohydrate added treatments	

Table 10.2 Average ± s.d of various water quality parameters recorded during the experimental period in the larviculture of *M. rosenbergii* with and without application of biofloc technology at various stocking densities

Parameters	Treatments					
	T1	T2	T3	T4	T5	C
Temperature (^{o}C)	28.62 ± 0.72	28.75 ± 0.74	28.78 ± 0.8	28.74 ± 0.7	28.82 ± 0.76	28.64 ± 0.71
pH	7.92 ± 0.11	7.89 ± 0.1	7.9 ± 0.12	7.95 ± 0.16	7.9 ± 0.17	7.96 ± 0.16
Salinity (ppt)	12.87 ± 0.84	12.9 ± 0.79	12.95 ± 0.8	12.85 ± 0.8	12.96 ± 0.74	12.93 ± 0.82
Dissolved oxygen (mg/l)	7.05 ± 0.15	6.99 ± 0.14	6.96 ± 0.14	6.94 ± 0.17	6.93 ± 0.16	6.89 ± 0.12

Table 10.3 Effect of stocking density and carbohydrate addition on the Mean Larval Stages of *M. rosenbergii* in different treatments

Treatments	Days			
	5	10	15	20
100	3.4 ± 0.1ab	5.3± 0.1a	6.67 ±0.06b	8.97 ± 0.06bc
100 + CH	3.43 ± 0.06a	5.3± 0.1a	6.8 ±0.1a	9.1 ± 0.1a
150	3.3abc	5.13± 0.06b	6.6 ± 0.1b	8.87 ± 0.06c
150 + CH	3.37 ± 0.06abc	5.13± 0.06b	6.63 ± 0.06b	9.03 ± 0.06ab
200	3.26 ± 0.12bc	5.1b	6.43 ± 0.06c	8.63 ± 0.06d
200 +CH	3.23 ± 0.06c	5.13± 0.06b	6.46 ± 0.06c	8.86 ± 0.06c
Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)				

Table.10.4 Effect of stocking density and carbohydrate addition on the survival, duration of rearing period, length and wet weight of post larvae of *M rosenbergii* in various treatments

Treatments	Stocking density(larvae/litre)	% survival	Post larvae/ liter	Appearance of first post larvae(days)	Duration(Number of days for more than 95 % post larval settlement)	Total length(mm)	Wet weight (mg)
100	100	42.47 ± 0.5^{b}	42.47 ± 0.5^{c}	20^{ab}	30.3 ± 0.6^{ab}	9.42 ± 0.22^{b}	9.62 ± 0.22^{b}
100 + CH	100	55.33 ± 1.03^{a}	55.33 ± 1.03^{b}	19 ± 1^{a}	29.6 ± 0.6^{a}	9.89 ± 0.11^{a}	10.12 ± 0.13^{a}
150	150	28.22 ± 0.89^{c}	42.33 ± 1.33^{c}	22.3 ± 0.6^{c}	31.3 ± 0.6^{cd}	9.25 ± 0.04^{bc}	9.45 ± 0.08^{bc}
150 + CH	150	41.82 ± 1.47^{b}	62.73 ± 2.21^{a}	21.3 ± 1.5^{bc}	31^{bc}	9.70 ± 0.11^{a}	9.94 ± 0.13^{a}
200	200	16.37 ± 0.57^{d}	32.73 ± 1.14^{d}	23 ± 1^{c}	34^{e}	9.10 ± 0.07^{c}	9.26 ± 0.06^{c}
200 +CH	200	27.43 ± 0.55^{c}	54.07 ± 1.81^{b}	21.7 ± 0.6^{bc}	32^{d}	9.21 ± 0.14^{bc}	9.43 ± 0.14^{bc}
Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)							

Table.10.5 Cumulative Mortality Index (CMI) values and final survival of newly metamorphosed *M rosenbergii* post larvae in treatments with different stocking densities with and without the addition of carbohydrate, exposed to low salinity stress (0 and 7 ppt)

Treatments	0 ppt		7 ppt	
	CMI	Final mortality (%)	CMI	Final mortality (%)
100	842.92 ± 41.79^{bc}	65.59 ± 3.72^{c}	88 ± 13.86^{bc}	11.83 ± 1.86^{bc}
100 + CH	567.83 ± 72.65^{a}	49.46 ± 4.93^{a}	24^{a}	3.22^{a}
150	893.33 ± 67.05^{cd}	67.74 ± 5.59^{c}	112 ± 13.86^{c}	15.05 ± 1.86^{c}
150 + CH	772.67 ± 15.43^{b}	58.06^{b}	48 ± 24^{a}	6.45 ± 3.23^{a}
200	959.5 ± 38.87^{d}	70.97 ± 3.23^{c}	144^{d}	19.35^{d}
200 +CH	840.33 ± 2.67^{bc}	64.52^{bc}	80 ± 13.86^{b}	10.75 ± 1.86^{b}
Mean ± s.d of 3 replicate groups. Means in each column not sharing a common superscript letter are significantly different (P<0.05)				

Fig 10.1 Effect of different stocking densities with and without addition of carbohydrate on total ammonia nitrogen in various treatments in the larviculture of *M. rosenbergii*

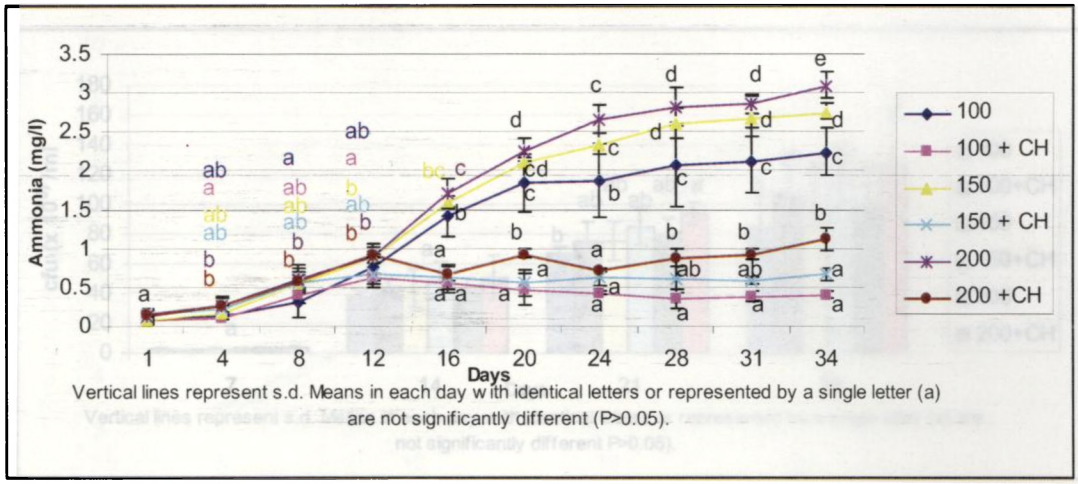


Fig.10.2 Effect of different stocking densities with and without addition of carbohydrate on nitrite nitrogen in various treatments in the larviculture of *M. rosenbergii* using MSGWS

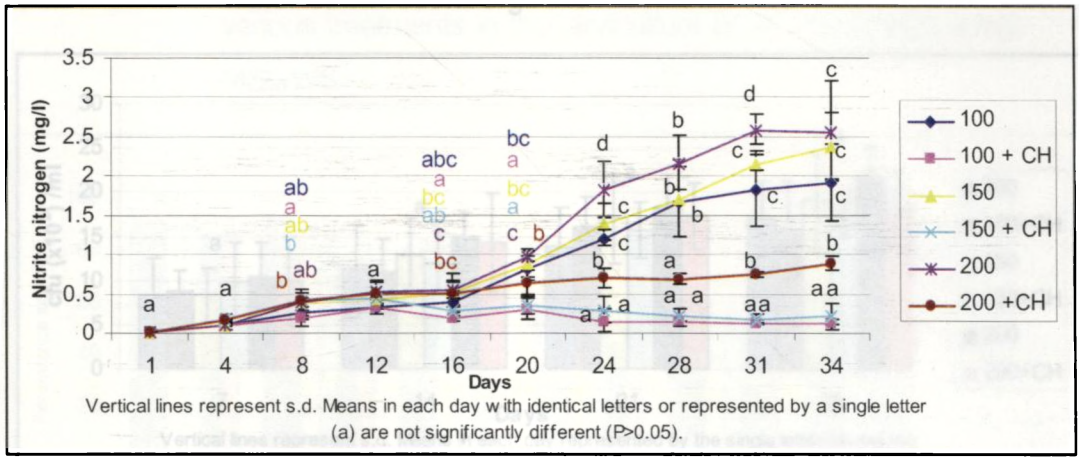


Fig.10.3 THB in treatments with different stocking densities with and without carbohydrate addition in the larval rearing water of *M. rosenbergii*

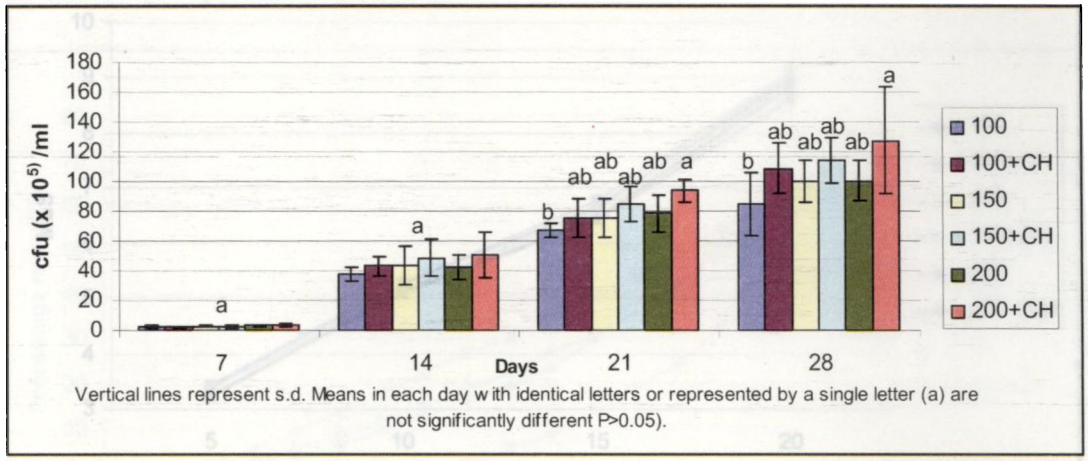


Fig.10.4 TVB in treatments with different stocking densities with and without carbohydrate addition in the larval rearing water of *M. rosenbergii*

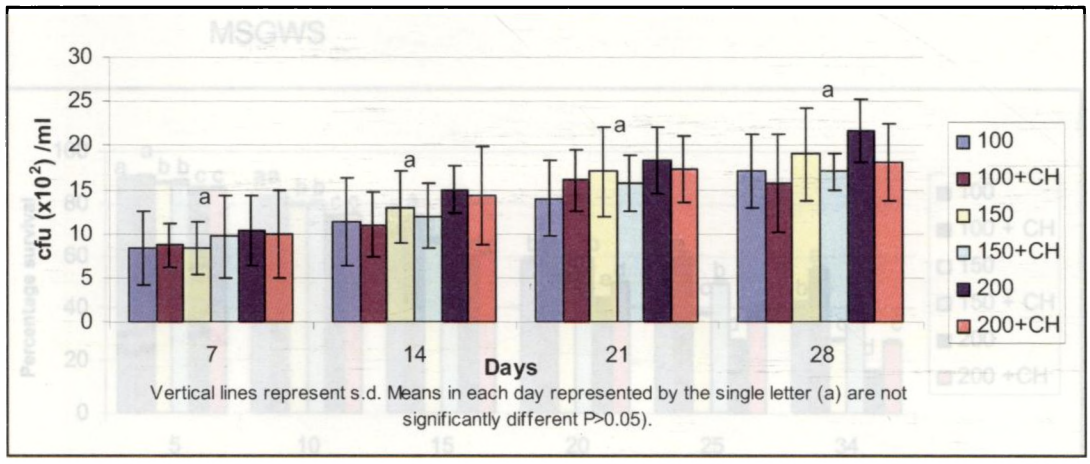


Fig.10.5 Mean larval stage in treatments with different stocking densities with and without addition of carbohydrtae in the larviculture of *M. rosenbergii* using MSGWS

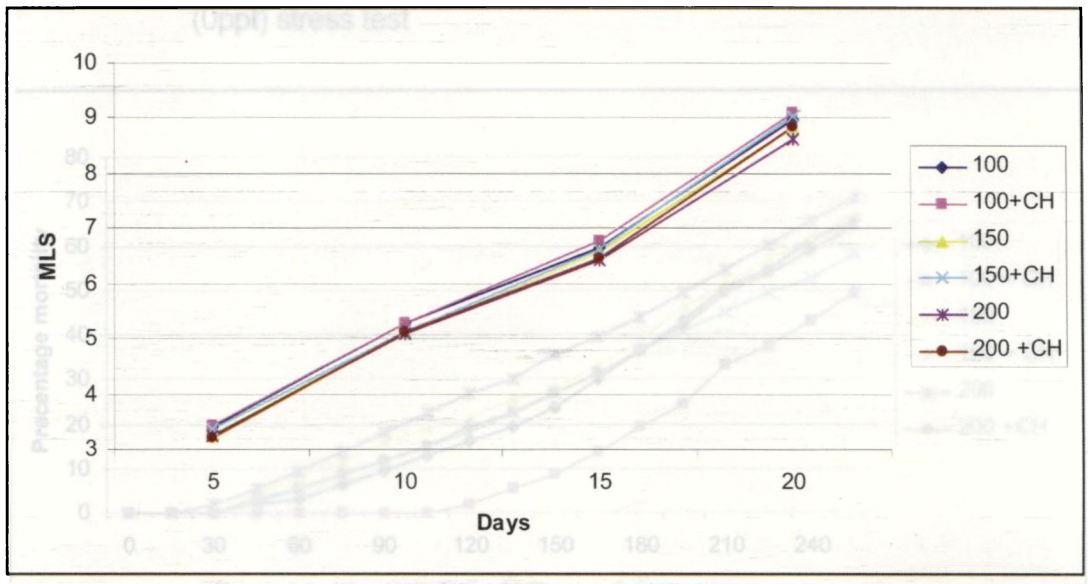


Fig.10.6 Effect of different stocking densities with and without addition of carbohydrate on survival of larvae and post larvae in various treatments in the larviculture of *M. rosenbergii* using MSGWS

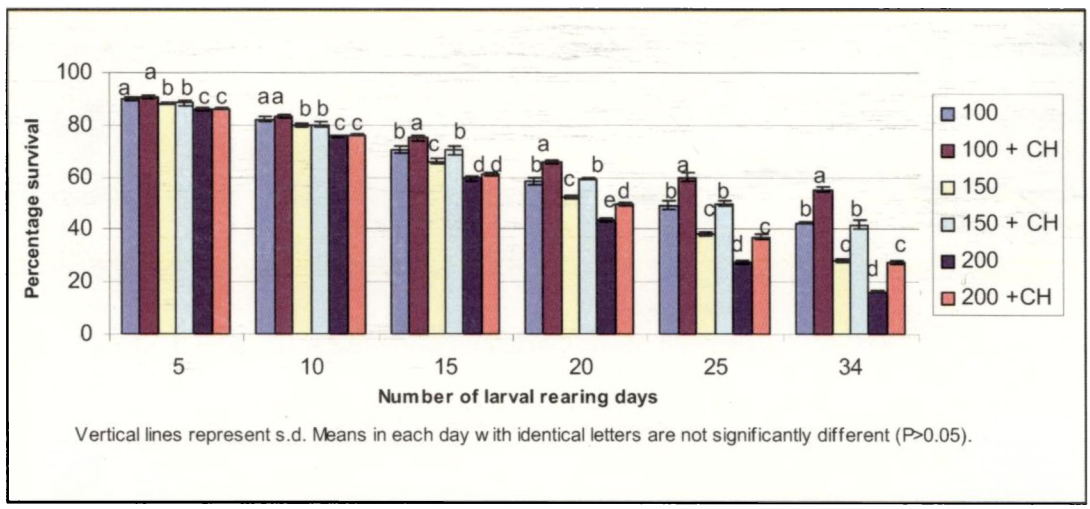
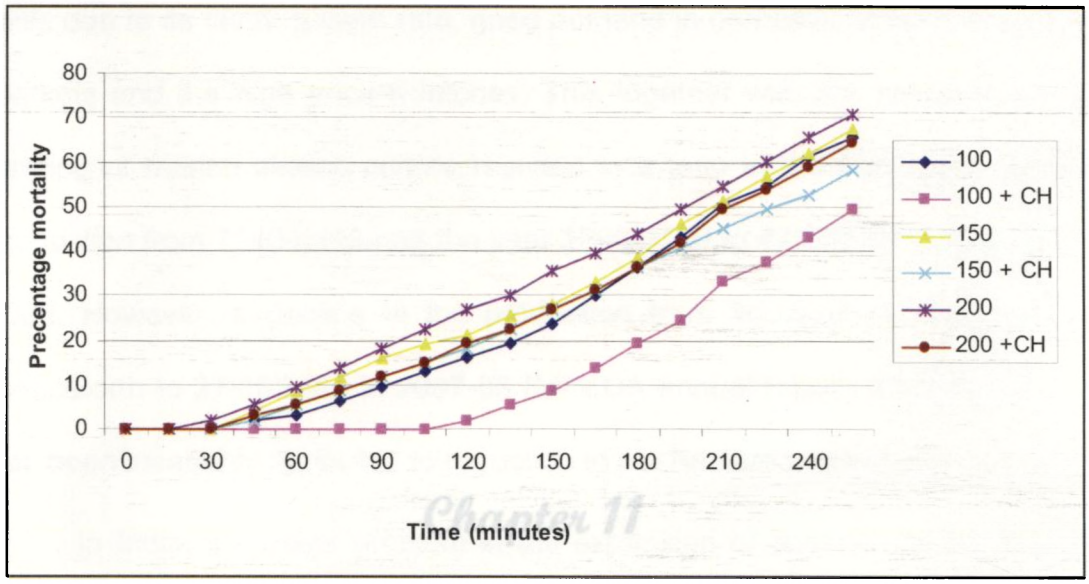


Fig.10.7 Percentage mortality of newly metamorphosed *M. rosenbergii* post larvae in treatments with different stocking densities with and without carbohydrate addition, exposed to low salinity (0ppt) stress test



Chapter 11

SUMMARY AND RECOMMENDATIONS

Macrobrachium rosenbergii, the giant freshwater prawn with trade name `Scampi ' is one of the prime candidate species used for fresh water aquaculture. It is locally known as `Kutttanadan konchu ' or `Attukonchu'. In recent years a growing interest has been noticed in farming of this species in India due to its faster growth rate, good demand in domestic as well as export markets and the high price it fetches. This together with the collapse of the farming of marine shrimp culture resulted in a leap in the freshwater prawn production from 7140 mt during the year 1999-2000 to 42,820 mt during 2005-2006. However, a decline in the production from aquaculture was noticed thenceforth to 27,262 MT in 2007-08 (MPEDA annual report, 2007-08) which has been invariably attributed to reduction in per hectare productivity.

In India, the major problem in the expansion of commercial farming of *M. rosenbergii* is the non availability of quality seed in the required number at the right time of stocking (Kurup, 2003). Though success was achieved in seed production of *M. rosenbergii* during late 1990's (Sebastin and Nair, 1995; Kurup *et al.*, 1998) and a few freshwater prawn hatcheries could be established, none of them could achieve the installed capacity due to various inherent problems and the average survival achieved in the commercial hatcheries in the country was reported to be 30 % (Murthy, 2007). The non availability of quality seed remains the major bottleneck in the promotion of commercial culture and also implementation of various fishery rehabilitation programmes.

Artemia is an essential component of all the freshwater prawn hatcheries, irrespective of systems of larviculture followed. *Artemia* is rather

costly, sometimes scarce and nutritionally inadequate. Cost of feeding accounts for as much as 60 % of the total production costs in *M. rosenbergii* hatcheries (Hagood and Willis, 1976). Therefore, in the present study, an effort was made to explore the possibility of replacing *Artemia* nauplii either completely or partially, with other indigenous live feeds thereby reducing the total cost of production and avoiding its overdependence.

The larviculture of *Macrobrachium rosenbergii* is most widely carried out using the clear water system. Green water system is difficult to be managed consistently and is not in use except in Malaysia (New, 1991). During 80's another system was developed in Vietnam - the **Modified static green water system** which is the refined version of Green water system developed by Ang and Cheah in the year 1986 (Kurup, 2003) . The system became very popular throughout Vietnam and more than fifty backyard hatcheries came to be established and a large number of stake holders are getting trained in the new system of larviculture (Kurup, 2003). The system though less expensive and less sophisticated, is characterized by a high percentage of survival, however, the same is not yet popular in India. In the present study, an attempt is made to standardize the modified static green water system in India and also to improve its efficiency by incorporating the biofloc technology.

The MSGWS being static, another hitch associated was the possible accumulation of toxic nitrogenous compounds like ammonia. The addition of properly adjusted carbohydrates could potentially eliminate the problem of inorganic nitrogen accumulation (Avnimelech, 1999). Thus, in the present study, a pioneer attempt was also made to develop a technology for the

reduction of inorganic nitrogen in the rearing water of static green water systems by the addition of carbohydrate to enhance the growth of heterotrophic bacteria (biofloc technology). The development of simple, eco-friendly, economically sustainable and scientific rearing practice incorporating innovative management strategies without the use of antibiotics will be useful in enhancing post larval production and quality of the seed.

Against this background, this thesis encompasses the results of nine experiments carried out in different aspects of larviculture of *M. rosenbergii* which are presented in 9 chapters. The topic is adequately introduced in the first chapter. Together with this, an exhaustive review of the relevant literature on the subject is also made in Chapter 1.

Chapter 2 contains the details of a preliminary experiment conducted to evaluate the substitution of brine shrimp nauplii with an alga, *Tetraselmis chuii* in the larviculture of *Macrobrachium rosenbergii.* Four treatments were experimented. The live feed given in various treatments were T1 – 75 % *Artemia* + 25 % *T. chuii*, T2 - 50 % *Artemia* + 50 % *T. chuii*, T3 – 25% *Artemia* + 75 % *T. chuii*, T4 - 100 % *T. chuii* and the control in which 100 % *Artemia* was used. The control with *Artemia* alone as the live feed gave the best results with an average survival of 24.4 %. The average number of days required for the metamorphosis of 95 % of the larvae into post larvae was 31 days. The treatment T1 with 75 % *Artemia* nauplii and 25 % *T. chuii* as live feed showed an average survival of 22.6 % with the very same 31 days required for the settlement of PL. *T. chuii* also being a live feed, additional facilities are required for maintaining its culture which is also incurring

additional cost, and there is no added advantage of using this alga other than the slight reduction in the quantity of BSN which is often expensive, that too by compromising final post larval production. Hence, the use of the alga *T. chuii* as feed for the *M. rosenbergii* larvae is not recommended. In the tanks in which only *T. chuii* was given as the live feed, the larvae did not survive beyond the fifth day. Therefore, it can be concluded that the prawn larvae were not able to derive required nutrition from the algae at least during its early larval stages.

The third chapter deals with the substitution of *Artemia* with chironomid larvae in the seed production of the giant fresh water prawn, *M. rosenbergii.* Five different overnight feeding regimes were selected viz., 100 % *Artemia* (control), 75 % *Artemia* and 25 % *Chironomous* larvae, 50 % each of *Artemia* and *Chironomous* larvae, 25 % *Artemia* and 75 % *Chironomous* larvae and finally 100 % *Chironomous* larvae. Clear water system of seed production with a stocking density of 100 larvae/ liter was adopted. The different water quality parameters like salinity, temperature, pH, dissolved oxygen and ammonia were found to be within optimum levels. The larval progression and survival were noted every third day. The results showed that *Artemia* cannot be fully replaced in the seed production of *M. rosenbergii* since the significantly higher percentage survival (32.69 %) and the desired levels of larval progression was observed only in the control in which only *Artemia* nauplii was given as the live feed. A 25 % substitution of *Artemia* nauplii by the blood worms lowered the mean percentage survival to 29.94 % while a 50 % reduction resulted in the plummeting of mean survival down to 22.48 %. However, the lower

percentage mortality in the treatments fed *Chironomous* larvae after stage V and above suggests a possible replacement of *Artemia* nauplii with *Chironomous* larvae in the larval stage VI and above. The replacement of *Artemia* nauplii @ 25 % from stage VI and above can bring down the cost of post larval production to a substantial level.

The results of the consequences of weaning of *M. rosenbergii* (de Man) larvae from *Artemia* to chironomid larvae in the seed production of the giant fresh water prawn are presented and discussed in Chapter 4. The larvae were weaned to the blood worms by gradually reducing the quantity of *Artemia* nauplii with a corresponding increase in the amount of blood worms and finally effecting the complete replacement of *Artemia* nauplii. The results showed that there was no significant difference between AC 10C (in which a complete substitution of *Artemia* nauplii was effected at the X^{th} larval stage) and A (the control with only brine shrimp nauplii), showing survival rates of 31.7 % and 32.3 % respectively. There was also no significant difference among these two feeding regimes with respect to development and growth of the larvae as indicated by mean larval stage of the larvae and total length and wet weight of the post larvae. It is also worth while to state that the larvae fed a mixed diet performed well towards the later stages than those fed *Artemia* alone.

Modified static green water system of seed production was used in the subsequent experiments. In this system no siphoning of waste or water exchange was done after stocking the larvae and the custard feed was given only from the fifth larval stage onwards. Experiment no.4 was targeted for optimising the stocking density suitable for the system, the results are given in

Chapter 5. The stocking densities selected were 25, 50, 75, 100 and 125 larvae per liter of the rearing medium. Total ammonia nitrogen and nitrite nitrogen was found to be significantly elevated in higher stocking densities where as other water quality parameters did not differ significantly. Total heterotrophic bacteria (THB), estimated by plate counting and expressed as colony forming units (cfu) (APHA, 1995) was also more in higher stocking densities. The following were the survival rates obtained in various treatments – 62.93 %, 48.53 %, 41.87 %, 40 % and 30.56 % respectively. However, the highest post larval production of 40 larvae/ liter was accomplished with an initial stocking density of 100 larvae /liter without compromising the metamorphosis and the growth of larvae. The post larval settlement was delayed only by 2.7 days as compared to the mean duration of 27 days recorded for an initial stocking density of 25 larvae /litre. Thus, an initial stocking density of 100 larvae/ liter is recommended in modified static green water system. In spite of having the most favourable water quality parameters, the control which adopted the clear water system could produce only an average of 31.46 post larvae /l with an extended period of 33 days. The results of the study showed a superior performance of the modified static green water system over the clear water system in the larviculture of *M. rosenbergii.*

In the fifth experiment (Chapter 6), the possibilities of replacing the larviculture medium, *Chlorella* with other algae were investigated. The algae in different larval rearing medium in the four separate treatments were *Isochrysis galbana*, *Nannochloropsis occulata*, *Tetraselmis chuii* and mixed culture of all

the four species and *Chlorella* water as the control. In this experiment also the different water quality parameters were checked which did not differ significantly between the various treatments. In all the treatments the first post larvae appeared by the 20th day and more than 95 % of the larvae metamorphosed by the 30th day, when the experiment was terminated. It could be observed that the treatments with the algae *Isochrysis*, *Nannochloropsis*, and mixed culture of all the four species as the rearing medium performed equally well. Only *Tetraselmis* medium recorded a slightly lower survival rate of 45 %. The highest survival rate was attained in *Isochrysis* medium (49.07 %) followed by *Chlorella* (48.93 %), mixed culture (48.6 %) and *Nannochloropsis* (48.47 %). The larval progression was very much similar in almost all the treatments and the control. The results revealed that, the use of any of the algal species used in this experiment can be recommended for prawn larval rearing.

The use of *Isochrysis galbana* as a partial substitute of *Artemia* nauplii in the seed production of *Macrobrachium rosenbergii* was evaluated in the sixth experiment by gradually reducing the quantity of *Artemia* nauplii fed to the larvae in the modified static green water system, the details are presented in Chapter 7. Al 10 I – *Artemia* nauplii and *I. galbana* till 10th stage and no *Artemia* nauplii given from 10th larval stage, Al 8I - *Artemia* nauplii and *I. galbana* till 8th stage and no *Artemia* nauplii given from 8th larval stage, Al 6I - *Artemia* nauplii and *I. galbana* till 6th stage and no *Artemia* nauplii given from 6th larval stage and *I. galbana* alone were the treatments while 100 % *Artemia* nauplii (4 nos / ml) throughout the rearing period was used as control. Al 10I in

which no *Artemia* nauplii was given after 10th larval stage performed equally well (Survival rate – 39.8 %) as the treatment in which *Artemia* nauplii was given throughout the rearing period (Survival rate – 40.2 %). The levels of the toxic nitrogenous metabolites were found to be significantly low in treatments where the brine shrimp nauplii feeding was terminated at an earlier stage. In conclusion, the quantity of *Artemia* nauplii can be gradually reduced and finally fully stopped without any effect on the final post larval production in a larval rearing medium constituted by *I. galbana* in the MSGWS.

Chapters 8, 9 and 10 encompass the results of experiments carried out by applying biofloc technology in the modified static green water system of larval rearing of the giant fresh water prawn. The modified system being static, there is a possibility of the toxicants like ammonia getting accumulated in the system. A strategy that is presently getting attention is the removal of ammonium from the water through its assimilation in to microbial protein by addition of carbonaceous material to the system (Avnimelech, 1999). The addition of properly adjusted carbohydrates could potentially eliminate the problem of inorganic nitrogen accumulation. The optimum quantity of carbohydrate addition in the larval rearing system was found out by applying various levels of tapioca flour (T1-0.01, T2-0.02, T3-0.03, T4-0.04, and T5-0.05 grams/liter) in the experimental tanks (Chapter 8). Pre weighed carbohydrate source (calculated using the equation given by Avnimelech, 1999) was mixed with tank water and applied to the water column uniformly followed by feeding in the morning. Once the carbohydrate addition started on the 10th day, the highest average total ammonia nitrogen (2.324 mg/l on 20th

day) was recorded in the control. The lowering of total ammonia nitrogen was found to be proportional to the quantity of carbohydrate added. Almost the same trend was also observed in the NO_2^- - N values recorded in various treatments. The nitrite nitrogen values kept increasing and there was a steep increase after 20th day and reached value as high as 2.13 mg/l on 31st day in the control. Both TAN and nitrite nitrogen recorded low values in the carbohydrate added treatments T3, T4 and T5 with no significant difference among them. THB also recorded the highest value in T5 followed by T4 and T3; however there was no significant difference among these three treatments. The tanks in which 0.02, 0.03 and 0.04 g /l of tapioca powder was added were found to give a final survival of 53.66%, 58.56% and 58 % respectively when compared to control (without carbohydrate addition) with an average survival of 46.9%. The larval progression did not show any difference between the treatments. The experiment was terminated when more than 95 % post larval settlement was observed. Thus addition of tapioca powder @ 0.03 g/ l of rearing water can be recommended as optimum for maximum post larval production in modified static green water system which is about 50 % of the quantity of egg custard fed to the larvae.

The effect of addition of different carbohydrate sources such as potato flour, yam flour, rice flour, wheat flour and tapioca flour to the modified static green water system was evaluated and the results are presented in Chapter 9. The highest survival of the 53.47 % was recorded in the treatment in which rice powder was used as the carbohydrate source where as it was lowest of 41.6 % in the control with no carbohydrate addition. However, the different

carbohydrate sources did not show significant difference among them with respect to their efficiency in lowering of total ammonia nitrogen or nitrite nitrogen. The five carbohydrate sources viz. potato, yam, rice, wheat and tapioca powder were found to be equally effective in controlling the nitrogen production with no significant difference in post larval production. Thus, it can be concluded that for cost effective larviculture of *M. rosenbergii* any of the cheap carbohydrate sources tried in this study can be used in the biofloc technology.

An experiment was conducted to optimise the stocking density with and without the biofloc technology in the larviculture of *M. rosenbergii* (de Man) using the modified static green water system and the results are given in chapter 10. The larval prawns were stocked at a density of 100, 150 and 200 per liter. The treatments without carbohydrate addition are abbreviated as 100, 150 and 200 while the treatments with carbohydrate addition as 100 + CH, 150 + CH and 200 + CH. The highest percentage survival was obtained in 100 + CH (55.33 %) followed by 100 (42.47 %) and 150 + CH (41.82 %), but the highest mean post larval production of 62.73 PL / liter was recorded in 150+CH. The higher levels of total ammonia nitrogen and nitrite nitrogen were recorded in treatments with no carbohydrate addition compared to the ones with carbohydrate addition. Properly adjusted and added carbohydrate potentially reduced the level of toxic inorganic nitrogen in the system even at increased stocking densities. The possibility of increasing the stocking density from 100 to 150 larvae / l without compromising the post larval production or

health of the larvae by the addition of carbohydrate has been demonstrated which in turn leads to more efficient utilization of the available facilities.

Based on the results of the present study, the following recommendations are made:

- The microalga *T. chuii* is not a suitable substitute for *Artemia* nauplii in the larviculture of *M. rosenbergii*.
- *Chironomous* larvae can be used as a partial substitute (upto 25 % replacement) of *Artemia* nauplii in the larval rearing of *M. rosenbergii*, especially from stage VI and above larval stages.
- Gradual weaning of prawn larvae from BSN to blood worms with a complete substitution by X^{th} larval stage can be effectively used to reduce the quantity of BSN by more than 50 % without compromising post larval production of *M. rosenbergii*.
- An initial stocking density of 100 larvae / liter is ideal in the larval rearing of *M. rosenbergii* using modified static green water system, which can be adopted by commercial hatcheries.
- Microalgae like *Nannochloropsis occulata* and *Isochrysis galbana* are equally suitable like *Chlorella* sp. as the rearing medium in the MSGWS of larviculture of *M. rosenbergii*. A mixed culture of *I. galbana*, *N. occulata*, *C. vulgaris* and *T. chuii* is also found equally effective.
- More than 50 % reduction in the cost of production can be achieved by reducing the quantity of *Artemia* nauplii to 75 % at III^{rd} larval stage, 50 % at VI^{th} stage, 25 % at $VIII^{th}$ stage and no BSN at X^{th} larval stage in a

larval rearing medium constituted by *Isochrysis galbana*. Supplying the optimal amount of *Artemia* nauplii also resulted in minimisation of problems related to water quality in static larviculture systems of *M. rosenbergii*.

- Daily application of tapioca powder @ 0.03 g/l commencing with the feeding of egg custard can result in the lower levels of toxic nitrogenous compounds like ammonia nitrogen and nitrite nitrogen in the MSGWS of larviculture of *M. rosenbergii*. This type of larviculture system also resulted in high post larval production which are characterized by good health and vibrancy. The system is also eco friendly without the use of probiotics and antibiotics. The adoption of MSGWS also minimize the use of sea water thus facilitating the setting up of hatcheries at places far away from the sea. The technology being simple, with low labour and capital requirement, is recommended for setting up hatcheries at backyard level.
- Any of the carbohydrate sources such as potato flour, yam flour, rice flour or wheat flour are equally effective as tapioca flour in the biofloc technology applied static larval rearing systems of *M. rosenbergii*.
- An initial stocking density of 150 larvae /liter can be adopted in biofloc technology applied MSGWS of larval rearing of *M. rosenbergii* which ensures effective utilization of available facilities and higher post larval production per liter and is therefore recommended for commercial hatcheries.

REFERENCES

Abdussamad, E.M., Thampy, D.M., 1994. Cannibalism in the tiger shrimp *Penaeus monodon* Fabricius in nursery rearing phase. J. Aquacult. Trop. 9 (1), 67 - 75.

Abraham, T.J., Manely, R., Palaniappan, R., Devendaran, K., 1997. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. J. Aquat. Trop. 12 (1), 1 – 8.

Agard, J. B. R.,1999. A four-dimensional response analysis of the ontogeny of physiological adaptation to salinity and temperature in larvae of the palaemonid shrimp *Macrobrachium rosenbergii* (de Man). J. Exp. Mar. Biol. Ecol. 236, 208-233.

Aikyama, D.M., Coelho, S.R., Lawerence, A.L., Robinson, E.H., 1989. Apparent digestability of feedstuffs by the marine shrimp *Penaeus vannamei* Boone. Nippon Suisan Gakkaishi, 55 (1), 91 – 98.

Alam, M. J., Ang, K. J., Cheah, S. H., 1993a. Use of *Moina micrura* (Kurz) as an *Artemia* substitute in the production of *Macrobrachium rosenbergii* (de Man) post larva, *Aquaculture* 109, (3 – 4): 337 – 349.

Alam, M. J., Ang, K. J., Cheah, S. H., 1993b. Weaning of *Macrobrachium rosenbergii* larvae from *Artemia* to *Moina micrura* (Kurz) Aquaculture 112 (2 – 3): 187 – 194.

Alexander, M., 1999. Biodegradation and bioremediation, 2nd ed. Academic Press, San Diego.

Al-Harbi, A. H., Uddin, M. N., 2004. Quantitative and qualitative study of the bacterial flora of farmed freshwater prawn (*Macrobrachium rosenbergii*) larvaeJ. Appl. Ichthyol. 20, 461–465.

Anderson, I.G., Shamsudin, M. N., Nash, G., 1989. A preliminary study on the aerobic heterotrophic bacterial flora in giant freshwater prawn, *Macrobrachium rosenbergii*, hatcheries in Malaysia. Aquaculture 81, 213– 223.

Ang, K.J.,1983. The evolution of an environment friendly hatchery technology for Udang Galah, the king of fresh water prawn and a glimpse into the future of aquaculture of 21st century. University Pertanian, Malaysia.

Ang, K. J., Cheah, S. H., 1986. Juvenile production of Malaysian giant fresh water prawn (*Macrobrachium rosenbergii* de Man) using modified static green water system. Proc. Intl. Conf. Dev. Manage. Trop. Living Aquat. Resources, Serdang, Malaysia, 2-5 Aug.1983.UPM Publ., Serdang, pp. 141-144.

Ang, K. J., Komilus, C. S., Cheah, S.H., 1992. Culture of *Macrbrachium rosenbergii* in cages. In: Proceedings of Third Asian Fisheries Forum, Abstract OSC-07, Manila, Philippines, pp.127.

Aniello, M. S., Singh, T., 1982. Some studies on the larviculture of the giant prawn (*Macrobrachium rosenbergii*), In: New, M.B.(Ed.), Giant prawn farming. Elsevier, NewYork, NY, pp.225-232.

APHA (American Public Health Association), 1995. Standard Methods for the Examination of Water and Waste Water (19th ed.). American Public Health Association, Washington, D C. 1082 pp.

Aquacop, 1977. Observations on diseases of crustacean cultures in Polynesia. Proc. World. Maric. Soc. 8, 685–703.

Aquacop, 1983. Intensive larval rearing in clear water of *Macrobrachium rosenbergii* (De Man, Anuenue stock) at the Centre Oce´anologique du Pacifique, Tahiti. In: Mcvey, J.P., Moore, J.R. (Eds.). CRC Handbook of Mariculture, Crustacean Aquaculture, vol. 1. CRC, Florida, pp. 179– 187.

Araujo de, M, C., Valenti, W.C., 2007. Feeding habit of the Amazon river prawn *Macrobrachium amazonicum* larvae. Aquaculture 265, 187-193.

Armstrong, D. A., Stephenson, M. J., Knight, A. W., 1976. Acute toxicity of nitrite to larvae of giant Malaysian prawn, *Macrobrachium rosenbergii. Aquaculture* 9, 39 – 46.

Armstrong, D. A., Chippendale, D., Knight, A. W., Colt, J. E. 1978. Interaction of ionized and un-ionised ammonia on short-term survival and growth of larvae *Macrobrachium rosenbergii.* Biological Bulletin 154, 15-31.

Arnold, S. A., Sellars, M. J., Crocos, P. J., Coman, G. J., 2005. Response of juvenile brown tiger shrimp (*Penaeus esculentus*) to intensive culture conditions in a flow through tank system with three-dimensional artificial substrate. Aquaculture 246, 231 - 238.

Arnold, S. A., Sellars, M. J., Crocos, P. J., Coman, G. J., 2006. An evaluation of stocking density on the intensive production of juvenile brown tiger shrimp (*Penaeus esculentus*). Aquaculture 256, 174 - 179.

Arnold, S. J., Coman, F. E., Jackson, C. J., Groves, S. A., 2009. High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: An evaluation of artificial substrates and stocking density. Aquaculture 293, 42-48.

Asaduzzaman, M., Wahab, M. A., Verdegem, M. C. J., Huque, S., Salam, M. A., Azim, M. E., 2008. C/N ratio control and substrate addition for periphyton development jointly enhance fresh water prawn *Macrobrachium rosenbergii* production in ponds. Aquaculture 280 (1-4), 117-123.

Asaduzzaman, M., Wahab, M. A., Verdegem, M. C. J., Mondal, M. N., Azim, M. E., 2009. Effects of stocking density of fresh water prawn *Macrobrachium rosenbergii* and addition of different levels of tilapia *Oreochromis niloticus* on production in C/N controlled periphyton based system. Aquaculture 286, 72-79.

Austin, B., Day, J.G., 1990. Inhibition of prawn pathogenic *Vibrio* spp. by a commercial spray-dried preparation of *Tetraselmis suecica*. Aquaculture 90, 389–392.

Austin, B., Baudet, E., Stobie, M., 1992. Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. J. Fish Dis. 15, 55–61.

Avendaño, R.E., Riquelme, C.E., 1999. Establishment of mixed culture probiotics and microalgae as food for bivalve larvae. Aquac. Res. 30, 893–900.

Avnimelech, Y., 1998. Minimal discharge from intensive fish ponds. World Aquacult. 29, 32 – 37.

Avnimelech, Y., 1999. C/N ratio as a control element in aquaculture systems. Aquaculture 176, 227–235.

Avnimelech, Y., 2006. Bio-filters. The need for a new comprehensive approach. Aquacultural Engineering 34, 172–178.

Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture 264, 140–147.

Avnimelech, Y., Lacher, M., 1979. A tentative nutrient balance for intensive fish ponds. The Israeli J. Aquacult. – Bamidgeh, 31: 3 – 8.

Avnimelech, Y., Mokady, S., 1988. Protein biosynthesis in circulated ponds. In: Pullin, R.S.V., Bhukaswan, Tonguthai, K., Maclean, J.J. (Eds.), The Second International Symposium on Tilapia in aquaculture. ICLARM Conference Proceedings, vol. 15, pp. 301 – 309.

Avnimelech, Y., Mokady, S., Schroder, G.L. 1989. Circulated ponds as efficient bioreactor for single cell protein production. Isr. J. Aquac.-Bamidgeh. 41, 58 – 66.

Avnimelech, Y., Kochva, M., Diab, S., 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted C to N ratio. Isr. J. Aquac.-Bamidgeh. 46, 119 – 131.

Avnimelech, Y., Noam Mozes., Shaher Diab., Malka Kochba., 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture 134, 211 – 216.

Ayub, M., Boyd, C. E., Teichert-Coddington, D., 1993. Effects of urea application, aeration and drying on total carbon concentrations in pond bottom soils. Progressive Fish-Culturist. 55, 210 – 213.

Azim, M. E., Little, D. C., 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283, 29-35.

Balca´zar, J.L., 2003. Evaluation of probiotic bacterial strains in *Litopenaeus vannamei*. Final Report, National Center for Marine and Aquaculture Research, Guayaquil, Ecuador.

Balca´zar, J.L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., Muzquiz, J. L., 2006. The role of probiotics in aquaculture. Veterinary microbiology. 114. 173-186.

Ballester, E. L. C., Abreu, P. C., Cavalli, R. O., Emerenciano, M., de Abreu, L., Wasielesky JR, W. 2009. Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. Aquacult. Nutrition 15(2), 117-126.

Barahona-Fernandes, M. H., 1982. Effet de la présence d'algues dans l'élevage du bar (*Dicentrarchus labrax* L.) au Centre Océanographique de Bretagne. J. Rech. Océanogr. 6 (3-4), 13–16 (in French with English abstract).

Barros, H.P. and Valenti, W. C., 1997. Comportamento alimentar do camarao de agua doce *Macrobrachium rosenbergii* (De Man) (Crustacea, Palaemonidae) durante a fase larval: analise quailtiva. Revista Brasileira de Zoologia 14: 785-793.

Barros, H.P., Valenti, W.C., 2003a. Food intake of *Macrobrachium rosenbergii* during larval development. Aquaculture 216, 165–176.

Barros, H.P., Valenti, W.C., 2003b. Ingestion rates of *Artemia* nauplii for different larval stages of *Macrobrachium rosenbergii*. Aquaculture 217, 223–233.

Beveridge, M. C. M., Begum, M., Frerichs, G. N., Millar, S., 1989. The ingestion of bacteria in suspension by the tilapia *(Oreochromis niloticus).* Aquaculture. 81, 373 – 378.

Body, A. G. C., Murai, T., 1986. Prawn culture in Japan. Australian Fisheries April/1986, pp. 6–10.

Bojan, J., Vishnu Bhat, B., Vinod, P. N., 2006. Role of fesh water prawn farming in coastal aquaculture diversification. In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and

marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi.20-23 Aug, 2003. 52-60.

Boyd, C. E., 1985. Chemical budget for channel catfish ponds. Trans. Am. Fish. Soc. 114, 291 – 298.

Boyd, C. E., 1990. Water quality in ponds for aquaculture. Agriculture Experiment Station, Auburn University, Auburn.

Brady, N. C., 1990. The nature and properties of soils, 10th edition. McMillan Publishing Company, New York, USA.

Bratvold, D., Lu, J., Browdy, C. L., 1999. Disinfection, microbial community establishment and shrimp production in a prototype biosecure pond. J. World Aquac. Soc. 30, 422-432.

Bratvold, D., Browdy, C. L., 2001. Effect of sand sediment and vertical surfaces on production, water quality and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture 195, 81-94.

Bronk, D.A., 2002. Dynamics of DON. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, San Diego, pp. 153 – 247.

Burford, M., 1997. Phytoplankton dynamics in shrimp ponds. Aquaculture Research 28, 351 – 360.

Burford, M.A., Glibert, P.M., 1999. Short-term N uptake and regeneration in early and late growth phase shrimp ponds. Aquacult. Res. 30, 215–227.

Burford, M. A., Thompson, P. J., McIntosh, Bauman, R. H., Pearson, D. C., 2003. Nutrient and microbial dynamics in high- intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 393 – 411.

Burford, M. A., Thompson, P. J., McIntosh, Bauman, R. H., Person, D. C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-water exchange system. Aquaculture 232, 525 – 537.

Cahu, C. L., Zambonino Infante, J. L., Péres,A., Quazuguel, P., Le Gall, M. M., 1998. Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing: effect on digestive enzymes. Aquaculture 161, 479–489.

Campbell, J. W., 1991. Excretory nitrogen metabolisam. In: Comparative Animal Physiology (ed. By C.J. Prosser). Wiley-Liss Inc., New York, NY.

Cheah, S. H., Ang, K. J., Arshad, A., 1987. Larval development of *Macrobrachium rosenbergii* fed P40, egg custard, cockles and *Macrobrachium lanchestri*. Proc. 10th Annu. Conf. MSAP, Pahang, Malaysia, 2-4 April 1987. The Malaysian Society of Animal production, UPM, Serdang, pp. 181-186.

Chen, J. C., Lei, S. C.,1990. Toxicity of ammonia and nitrite to *Penaeus monodon* juveniles. J. World. Aquac. Soc. 21, 300-306.

Chen, S., Ling, J., Blancheton, J. P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquac. Eng. 34, 179–197.

Chiang, P., Liao, I. C., 1985. The practice of grass prawn (Penaeus monodon) culture in Taiwan from 1968 to 1984. J. World Maric. Soc. 16, 297–315.

Chuntapa, B., Powtongsook, S., Menasveta, P., 2003. Water quality control using *Spirulina platensis* in shrimp culture tanks. Aquaculture 220, 355-366.

Cohen, D., Finkel, A., Sussman, M., 1976. On the role of algae in the larviculture of *Macrobrachium rosenbergii* . Aquaculture 8: 199 – 207.

Colt, J., Tchobanoglous, G., 1978. Chronic exposure of channel catfish, *Ictalurus punctatus*, to ammonia: effects on growth and survival. Aquaculture 15, 353–372.

Colt, J., Armstrong, D., 1981. Nitrogen toxicity to fish, crustaceans and molluscs. Bio-engineering Symposium for Fish Culture. American Fisheries Society, Betesda, MD, pp. 34 – 47.

Cook, H. L., Murphy, M. A., 1969. The culture of larval Penaeid shrimp. Trans. Am. Fish. Soc. 4, 751–754.

Cook, P. A., De Baissac, P., 1994. Does phytoplankton play a role in the nutrition of the larvae of the prawn, *Macrobrachium rosenbergii* (De Man)? Water SA. 20(1), 85-90.

Cooper, S., Battat, A., Marsot, P., Sylvestre, M., 1983. Production of antibacterial activities by two Bacillariophyceae grown in dialysis culture. Can. J. Microbiol. 29, 338–341.

Corbin, J. S., Fujimoto, M. M., Iwai, Jr. T. Y., 1983. Feeding practises and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P., Moore, J.R. (Eds.), CRC Handbook of Mariculture, vol. 1. CRC Press, Boca Raton, FL, USA, pp. 391-442.

Corre, V. L., Janeo, R., Caipang, C. M., Calpe, A. T., 2000. Use of probiotics and reservoirs with green water. Aquaculture Asia V (2), 34-38.

Correia, E. S., Martins, M. M. R., Maia Filho, M. A., Cavalcanti, M. A. A. U.,1988. Modelo artesanal de producao de pos-larvas do camarao *Macrobrachium rosenbergii.* In: Nascimento, p., Rodrigues, J.B.R., Muller, Y. M., Andreatta, E., Gomes, S. Z., Magalhaes, M. R. M. (Eds.). Anais do VI Simposio Latino Americano Aquicultura e V Simposio Brasileiro de Aquicultura, 17-22 abril 1988, Florianopolis. Associacao Brasileira de Aquicultura (ABRAq), Florianopolis. 652-656.

Correia, E.S., Suwannatous, S., New, M.B., 2000. Flow-through hatchery systems and management. In: New, M. B., Valenti, W. C. (Eds.), Freshwater Prawn Culture. Blackwell, Oxford, pp. 52– 68.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal in aquaculture towards sustainable production. Aquaculture 270 (1–4), 1–14.

Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W., 2009. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquacult. Res. 40(10), 1103-1112.

Csavas, I., 1994. Important factors in the success of shrimp farming. World Aquaculture. 25, 34 – 56.

Daniels, W. H., D'abramo, L. R., De Parseval, L., 1992. Design and management of a closed, recirculating "clearwater" hatchery system for freshwater prawns, *Macrobrachium rosenbergii*, De Man, 1879. J. Shellfish Res. 11, 65–73.

Daniels, W. H., D'Abramo, L. R., Fondren, M. W., Durant, M. D., 1995. Effects of stocking density and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as size-graded juveniles. J. World Aquacult. Soc. 26 (1), 38 – 47.

De La Noue, J., Choubert, G., 1985. Apparent digestibility of invertebrate biomass by rainbow trout. Aquaculture 50, 103-112.

Defoirdt, T., Halet, D., Vervaeren, H., Boon, N., Van de Wiele, T., Sorgeloos, P., Bossier, P., Verstraete, W., 2007. The bacterial storage compound poly-β-hydroxybutyrate protects *Artemia franciscana* from pathogenic *Vibrio campbellii*. Environ. Microbiol. 9 (2), 445–452.

Deru, J., 1990. Studies on development and nutrition of caridean prawn, *Macrobrachium rosenbergii* (De Man) (Crustacea:Decapoda). PhD Thesis, University of Wales.

Devika Pillai., Thresiamma, J., Aneykutty, J., Vadher, K. H., 2006. Enrichment of live feed with HUFA: Effects on *Macrobrachium rosenbergii* larval performance. In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi.20-23 Aug, 2003. 541-545.

Diab, S., Shilo, M., 1986. Transformation of nitrogen in sediments of fish ponds in Israel. Bamidgeh 38, 67–88.

Doroudi, M. S., Southgate, P. C., 2000. The influence of algal ration and larval density on growth and survival of blacklip pearl oyster *Pinctada margaritifera* (L.) larvae. Aquac. Res. 31, 621-626.

Douillet, P. A., Langdon, C. J., 1994. Use of a probiotic for the culture of larvae of the Pacific oyster *Crassostrea gigas* Thunberg. Aquaculture 119, 25–40.

Emmerson, W. D., Andrews, B., 1981. The effect of stocking density on the growth, development and survival of *Penaeus indicus* Milne Edwards larvae. Aqaculture 23 (1-4), 45-57.

FAO, 2007. Improving *Penaeus monodon* hatchery practices. Manual based on experience in India. FAO Fisheries Technical paper No.446. Rome. pp.59.

FAO, 2009. Fishstat Plus (V.2.32) issued on 02.03.2009. FAO, Rome.

Fast, A. W., Leung, P. S., 2003. Rise and Fall of Fresh water prawn (*Macrobrachium rosenbergii*) culture in Hawaii: 1965-1999. Reviews in Fisheries Science. 11(3), 243-290.

Faulk, C. K., Holt, G. J., 2005. Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: live prey enrichment and green water culture. Aquaculture 249, 231–243.

Fermin, A. C., 1991. Fresh water cladoceran *Moina macropoda* (Strauss) as an alternative live food for rearing sea bass *Lates calcarifer* (Bloch) fry. J. Appl. Ichthyol., 7; 8-14.

Fernando, C. H., 1994. Zooplankton, fish and fisheries in tropical fresh waters. Hydrobiologia 272, 105-123.

Fontenot, Q., Bonvillain, C., Kilgen, M., Boopathy, R., 2007. Effects of temperature, salinity, and carbon:nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. Bioresource Technology 98, 1700–1703.

Forster, J. R. M., Beard, T. W., 1974. Experiments to assess the suitability of nine species of prawns for intensive cultivation. Aquaculture 3, 355-368.

Fox, S. M., 1988. Probiotics: intestinal inoculants for production animals. Vet. Med. 83, 806–830.

Frappaolo, P.J., Guest, G.B., 1986. Regulatory status of tetracyclines, penicillin and other antimicrobial microbials drugs in animal feeds. J. Anim. Sci. 62, 86– 92.

Fujimura, T., Okamoto, H., 1972. Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. In: T.V.R. Pillai (Ed.), Coastal Aquac. in Indo-pacific Region, Fishing New Books, Blackwell Science, pp.313-327.

Garriques, D., Arevalo, G., 1995. An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of Penaeus vannamei postlarvae in Ecuador. In: Browdy, C.L., Hopkins, J.S. (Eds.), Swimming through troubled water. Proceedings of the Special Session on Shrimp Farming, Aquaculture'95. Baton Rouge, World Aquaculture Society, pp. 53–59.

Ghosh, S.R., Mohanty, A.R., 1981. Observations of the effect of aeration on mineralization of organic nitrogen in fish pond soil. Bamidgeh. 33, 50 – 56.

Gomes, L. C., Baldisserotto, B., Senhorini, J. A., 2000. Effect of stocking density on water quality, survival and growth of larvae of the matrinxa, *Brycon cephalus* (Characidae) in ponds. Aquaculture 183(1-2), 73-81.

Gomez-Gil, B., Roque, A., Turnbull, J. F., 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 191, 259–270.

Grasshoff, K., Ehrharddt, M., Kremling, K., 1983. Methods of seawater analysis. Verlag Chemie, Weinheim, Germany.

Gruffyd, L. D., Beaumont, A. R., 1972. A method of rearing *Pecten maximus* larvae in the laboratory. Mar. Biol., 15: 350-355.

Gulbrandsen, J., Lein, I., Holmefjord, I., 1996. Effects of light administration and algae on first feeding of Atlantic halibut larvae, *Hippoglossus hippoglossus* (L.). Aquac. Res. 27, 101–106.

Hagwood, R. W., Willis, S. A., 1976. Cost comparisons of rearing larvae of fresh water shrimp, *Macrobrachium acanthurus* and *M. rosenbergii*, to juveniles. Aquaculture 7: 59-74.

Hai, T. N., Phuong, N. T., Hein, T. T. T., Bui, T. V., Son, V. N., Wilder, M. N., 2003. Research, development and economics of seed production of giant fresh water prawn (*Macrobrachium rosenbergii*) ; a review. Proc. Of the final workshop of the JIRCAS Mekong Delta Project, Nov.23-25, 2003, Cantho, Vietnam, pp. 298-306.

Hansen, G. H., Olafsen, J. A., 1989. Bacterial colonisation of cod (*Gadus morhua* L.) and halibut (*Hippoglossus hippoglossus*) eggs in marine aquaculture. Appl.Environ. Microbiol. 55, 1435-1446.

Hanson, J. A., Goodwin, H. L., 1977. Shrimp and prawn farming in the Western Hemisphere. Dowden, Hutchinson, and Ross, Stroudsburg, PA, 439 pp.

Hargreaves, J. A., 1997. A simulation model of ammonia dynamics in commercial catfish ponds in the southeastern United States. Aquacult. Eng. 16, 27–43.

Hargreaves, J. A., 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166, 181-212.

Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering 34, 344–363.

Hari, B., Madhusoodana Kurup, B., Varghese, J. T., Schrama, J. W., Verdegem, M. C. J., 2004. Improved sustainability in extensive shrimp culture systems: control of carbon nitrogen ratio through addition of carbohydrate to the pond. Aquaculture 241, 179 – 194.

Hari, B., Madhsoodana Kurup, B., Varghese, J. T., Schrama, J. W., Verdegem, M. C. J., 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture system. Aquaculture. 252, 248 – 263.

Harikrishnan, M., Kurup, B. M., 2006. On the sustainability issues of fishery of the giant fresh water prawn *Macrobrachium rosenbergii* (de Man) in the Vembanad lake, South India. In: Kurup, B. M., Ravindran, K. (Eds.) Sustain Fish- Proc. of Intl. Symp. on improved sustainability of fish prdn. sys. And app. Tech. for utilzn., 16-18 Mar. 2005, Cochin, 502-510.

Hart, P. R., Purser, G. J., 1996. Weaning of hatchery-reared green back flounder (*Rhombosolea tapirina* Gunther) from live to artificial diets: effects of age and duration of the changeover period. Aquaculture 145, 171-181.

Hasgopian, D. S., Riley, J. G., 1998. A closer look at the bacteriology of nitrification. Aquacult. Enngineering. 18. 223-244.

Hein, T. T. T., Hai, T. N., Phuong, N. T., Ogata, T., Wilder, M. N., 2000. The effects of vitamin C on the larvae of fresh water prawn *Macrobrachium rosenbergii.* In: Proceedings of annual workshop of JIRCAS Mekong Delta Project ô development of new technologies and their practices for sustainable farming systems in the Mekong Delta. 179-183.

Hein, T. T. T., Hai, T. N., Phuong, N. T., Ogata, H. Y., Wilder, M. N., 2005. The effects of dietary lipid sources and lecithin on the production of giant fresh water prawn *Macrobrachium rosenbergii* larvae in the Mekong delta region of Vietnam. Fisheries Science 71, 279-286.

Helm, M. M., 1977. Mixed algal feeding of *Ostrea edulis* larvae with *Isochrysis galbana* and *Tetraselmis suecica.* J. Mar. Biol. Assoc. UK, 57: 1019-1029.

Hernández-Cruz, C. M., Salhi, M., Fernández-Palacios, H., Izquierdo, M. S., 1994. Improvements in the culture of *Sparus aurata* L. larvae in relation to the use of antibiotics, phytoplankton and rearing system. Aquaculture 124, 269–274.

Hollender, J., van der Krol, D., Kornberger, L., Gierden, E., Dott, W., 2002. Effect of different carbon sources on the enhanced biological phosphorus removal in a sequencing batch reactor. World J. Microbiol. Biotechnol. 18 (4), 355–360.

Holme, M, H., Zeng, C., Southgate, P. C., 2009. A review of recent progress toward development of a formulated microbound diet for mud crab, *Scylla serrata*, larvae and their nutritional requirements. Aquaculture 286, 164-175.

Howell, B. R., 1973. The effect of unicellular algae on the growth of early larvae of the turbot (*Scophthalmus maximus* L.). International Council for the Exploration of the Sea, C. M. 1973/E:21, pp. 1–7.

Hsieh, C. H., Chao, N. H., Gomes, L. A. O., Liao, I. C., 1989. Culture practices and status of the giant fresh water prawn, *Macrobrachium rosenbergii*, in Taiwan. . In: Martins, M. M. R., Correia, E. S., Cavalheiro, J. M. (Eds). Anais do III Simposio Brasileiro sobre Cultivo de Camarao, 15-20 outubro 1989, Joao Pessoa, Vol.2: Camarao de Agua Doce e Outros. MCR aquaculture, Joao Pessoa. 85-109.

Hudinaga, M., 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. Jpn. J. Zool. 10, 305–424.

Ismael, D., New, M. B., 2000. Biology. In: New, M. B., Valenti, W. C. (Eds.), Freshwater Prawn Culture. Blackwell, Oxford, pp. 18-40.

Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O., Tacon, A., 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. Aquac. Nutrition 12, 192-202.

Jensen, F. B., 2003. Nitrite disrupts multiple physiological functions in aquatic animals. Comp. Biochem. Physiol. 135, 9-24.

Jones, D. A., Kamarudin, M. S., LeVay, L. L., 1993. The potential for replacement of live feeds in larval culture. Journal of World Aquaculture Society 24: 199-210.

Jones, D. A., Yule, A. B., Holland, D. L., 1997. Larval nutrition. In : D'Abramo, L. R., Coklin, D. E., Akiyama, D. M. (Eds), Crustacean nutrition. Advances in world aquaculture. World aquaculture society, Baton Rouge, pp.356-389.

Joseph, J. D., 1977. Assessment of nutritional role of algae in the culture of larval prawns *Macrobrachium rosenbergii*. Proceedings of the World Mariculture Society 8, 853-861.

Kamarudin, M. S., Jones, D. A., Vay, L., Abidin, A. Z., 1994. Ontogenetic change in digestive enzyme activity during larval development of *Macrobrachium rosenbergii*. Aquaculture 123, 323–333.

Karunasagar, I., Pai, R., Malathi, G. R., 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture 128, 203– 209.

Khatoon, H., Yusoff, F. Md., Banerjee. S., Shariff, M., Mohamed, S., 2007. Use of periphytic cyanobacterium and mixed diatoms coated substrate for improving water quality, survival and growth of *Penaeus monodon* Fabricius postlarvae. *Aquaculture* 271, 196-205.

Khatoon, N., Chattopadhyay, P., Mukhopadhyay, A., Mukhopadhyay, M., Pal, R., 2009. Algal diet in prawn aquaculture. Fishing Chimes 28 (10/11), 44-47.

King, N. J., Howell, W. H., Huber, M., Bengtson, D. A., 2000. Effects of larval stocking density on laboratory scale and commercial scale production of summer flounder *Paralichthys dentatus*. J. of the World Aquac. Soc. 31(3), 436 - 445.

Kolkovski, S., Tandler, A., Izquierdo, M. S., 1997a. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass (*Dicentrarchus labrax*) larvae. Aquaculture 148, 313-322.

Kolkovski, S., Koven, W., Tandler, A., 1997b. The mode of action of *Artemia* in enhancing utilisation of microdiet by gilthead seabream *Sparus aurata* larvae. Aquaculture 155, 193-205.

Körner, S., Das, S.K., Veenstra, S., Vermaat, J. E., 2001. The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*. Aquat. Bot. 71, 71–78.

Kovalenko, E. E., D'Abramo, L., Ohs, C. L., Buddington, R. K. 2002. A successful microbound diet for the larval culture of fresh water prawn *Macrobrachium rosenbergii*. Aquaculture 210, 385-395.

Kumlu, M., 1999. Feeding and digestion in larval decapod crustaceans. Tr. J. of Biology 23, 215-229.

Kumlu, M., Jones, D. A., 1995. Feeding and digestion in the caridean shrimp larva *Palaemon elegans* Rathke and *Macrobrachium rosenbergii* (DeMan) (Crustacea, Palaemonidae) on live and artificial diets. Aquac. Nutr. 1, 3–12.

Kurmaly, K., Jones, D. A., Yule, A. B., East, J., 1989. Comparative analyses of the growth and survival of *Penaeus monodon* (Fabricius) larvae, from protozoea 1 to postlarvae 1, on live feeds, artificial diets and on combination of both. Aquaculture 81, 27– 45.

Kurup, B. M., 2003. On the standardisation of Vietnamese Technology of Modified static green water system of larviculture of *Macrobrachium rosenbergii* (de Man) in India .Fishing chimes 22 (10 and 11): 46-51.

Kurup, B. M., 2009. Biofloc in shrimp aquaculture. Fishing Chimes 29 (1), 23-28.

Kurup, B. M., Sureshkumar, S., Prince, S., 1998. Preliminary observations on two phase larval rearing of *Macrobrachium rosenbergii* (de Man) by adopting clear water system. In: Hameed, M. S., Kurup, B. M. (Eds). Technological advancements in Fisheries. Publn. No.1. School of Indl. Fish., Cochin University of Science and Technology, Cochin. 1-6.

Lanari, D., Ballestrazzi, R., Tibaldi, E., 1989. Effects of fertilization and stocking rate on the performance of *Penaeus japonicus* Bate in pond culture. Aquaculture 83, 269-279.

Langdon, C. J., Levine, D. M., Jones, D. A., 1985. Microparticulate feeds for marine suspension feeders. J. Microencapsulation 21(1), 1-11.

Lavens. P., Leger, P., Sorgeloos, P., 1989. Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. In: De Pauw, N., Jaspers, E., Ackefors, H., Wilkins, N. (Eds), Aquaculture - A Biotechnology in progress, European Aquac. Soc., Bredene, pp.731-739.

Lavens, P., Sorgeloos, P. 1996. Manual on the production and use of live food for aquaculture. FAO Fish . Tech . Pap . (361).

Lavens, P., Thongrod, S., Sorgeloos, P., 2000. Larval prawn feeds and the dietary importance of *Artemia*. In: New, M.B., Valenti, W.C. (Eds.),

Freshwater Prawn Culture: The Farming of *Macrobrachium rosenbergii*. Blackwell, Oxford, United Kingdom, pp. 91– 111.

Le´onard, N., Guiraud, J. P., Gasset, E., Caille`res, J. P., Blancheton, J. P., 2002. Bacteria and nutrients – nitrogen and carbon – in a recirculating system for sea bass production. Aquacult. Eng. 26, 111–127.

Lee, C. S., Sweeney, J. N., Richards, W. K. Jr., 1986. Marine shrimp aquaculture: a novel waste treatment system. Aquacult. Eng. 5, 147 – 160.

Lee, D. O'C., Wickins, J. F.,1992. *Crustacean farming*. Blackwell Science, Oxford.

Leger, P., Bieber, G. F. and Sorgeloos, P. 1985. International study on *Artemia* .XXXIII. Promising results in larval rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for *Artemia* enrichment. J. of the Worl. Aquac. Soc.. 16 : 354-367.

Leger, P., Bengtson, D. A., Simpson, K. L., Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. Oceanographic and Marine Biology Annual Review. 24 : 521-623.

Lewis, C. A., 1975. Some observations on factors affecting embryonic and larval growth of *Pollicipes polymerus* (Cirripedia : Lepado morpha) in vitro. Mar. Biol., 32:127-139.

Liao, I. C., Su, H. M., Lin, J. H., 1983. Larval foods for Penaeid prawns In: McVey, J.P. (Ed.), CRC Handbook of Mariculture. Crustacean Aquaculture, vol. 1. CRCPress, Inc., Boca Raton, Florida, pp. 43–69.

Liao, I. C., Su, H. M., Chang, E. Y., 2001. Techniques in finfish culture in Taiwan. Aquaculture 200, 1–31.

Lim, B. K., Hirayama, K., 1993. Effect of stocking density on the yield of larval mass production of Kuruma Prawn. Nippon Suisan Gakkaishi 59(2), 229-235.

Ling, S. W., 1969 b. The general biology and development of *Macrobrachium rosenbergii* (de Man). FAO Fish. Rep., 57(3). 589-606.

Ling, S. W., Merican, A. B. O., 1961. Notes on the life and habits of the adults and larval stages of *Macrobrachium rosenbergii* (De man). Proc. Indo-Pacific Fish. Council, FAO, Bangkok, 9(2), 55-60.

Liu, F., Han, W., 2004. Reuse strategy of waste water in prawn nursery by microbial remediation. Aquaculture 230, 281-296.

Lober, M., Zeng, C., 2009. Effect of microalage concentration on larval survival, development and growth of an Australian strain of giant fresh water prawn. Aquaculture 289, 95-100.

Lovett, D. L., Felder, D. L.,1988. Evaluation of the rotifer *Brachionus plicatilis* as a substitute for *Artemia* in feeding larvae of *Macrobrachium rosenbergii*. Aquaculture 71, 331 – 338.

Loya-Javellana, G.N., 1989. Ingestion saturation and growth responses of *Penaeus monodon* larvae to food density. Aquaculture 81, 329–336.

Maddox, M, B., Manzi, J. J., 1976. The effects of algal supplements on static system culture of *Macrobrachium rosenbergii* (De Man) larvae. Proceedings of the World Mariculture Society 7, 677-698.

Maeland, A., Rosenlund, G., Stoss, J., Waagbo, R., 1999. Weaning of Atlantic halibut *Hippoglossus hippoglossus* L. using formulated diets with various levels of ascorbic acid. Aquac. Nutr. 5, 211-219.

Maguire, G. B., Leedow, M. I., 1983. A study of the optimum stocking density and feed rate for school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackishwater farming ponds. Aquaculture 30, 285 - 297.

Mallasen, M., Valenti, W. C., 2006. Effect of nitrite on larval development of giant river prawn *Macrobrachium rosenbergii*. Aquaculture 261, 1292-1298.

Manzi J. J., Maddox M. B., Sandifer P. A., 1977. Algal supplement enhancement of *Macrobrachium rosenbergii* larviculture. Proceedings of the World Mariculture Society 8, 207-223.

Marques de A, H. L., Lombardi, J. V., Boock, M. V., 2000. Stocking densities for nursery phase culture of fresh water prawn *Macrobrachium rosenbergii* in cages. Aquaculture 187, 127-132.

Marques, A., Thanh, T.H., Sorgeloos, P., Bossier, P., 2006. Use of microalgae and bacteria to enhance protection of gnotobiotic *Artemia* against different pathogens. Aquaculture 258, 116–126.

Martin, L., Arenal, A., Fajardo, J., Pimentel, E., Hidalgo, L., Pacheco, M., Garcia, C., Santiesteban., 2006. Complete and partial replacement of *Artemia* nauplii by *Moina micrura* during early postlartval culture of white shrimp (*Litopenaeus schmitti*). Aquac. Nutr. 12, 89-96.

Mclarney, W.O., Henderson, S., Sherman, M. M., 1974. A new method for culturing *Chironomous tentans* Fabricius larvae using burlap substrate in fertilized pools. Aquaculture 4, 267-276.

Menasveta, P., Piyatiratitivokul, S., 1980. A comparative study on larviculture techniques for the giant fresh water prawn, *Macrobrachium rosenbergii* (DE MAN). Aquaculture 20, 239-249.

Meyers, S. P., Hagwood, R. W., 1984. Flake diets and larval crustacean culture. Prog. Fish-Cult. 46, 225-229.

Michaud, L., Blancheton, J. P., Bruni, V., Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquac. Eng. 34, 224–233.

Milstein, A., Avnimelech, Y., Zoran, M., Joseph, D., 2001. Growth performance of hybrid bass and hybrid tilapia in conventional and active suspension intensive ponds. Israeli Journal of Aquaculture – Bamidgeh 53 (3–4), 147–157.

Miyamoto, G., Brock, J., Nakamura, R., Nakagawa, L., Shimoja, R., Sato, V., Akita, G., 1983. A preliminary microbiological and water quality survey of two Hawaiian prawn (*Macrobrachium rosenbergii*) hatcheries. In: Rogers, G. L., Day, R., Lim, A. (Eds.), Proc. First Int. Conf. Warm water Aquaculture-Crustacea. Brigham Young University Hawaii Campus, Laie, HI, pp. 429– 458.

Mohanta, K . N., Rao , K . J., 2000. Comparative performance of three types of larval diet in seed production of giant fresh water prawn and a trial for partial or complete substitution of brine shrimp. J. of Aquac. in the Tropics 15(2): 145 – 152.

Moller, T. H., 1978. Feeding behaviour of larvae and post larvae of *Macrobrachium rosenbergii*. J. Exp. Mar. Biol. Ecol., 35: 251-258.

Moriarty, D. J. W., 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164, 351–358.

MPEDA annual report, 2007-08. Annual report, Marine products Export Development Agency, Cochin.

Munilla-Moran R., Stark, J. R., Barbour, A., 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scopthalmus maximus* L). Aquaculture 88, 337-350.

Murai, T., Andrews, J. W., 1978. Comparison of feeds for larval stages of giant prawn (*Macrobrachium rosenbergii*). Proc. World Maricult. Soc., 9: 189-193.

Murthy, H. S., 1996. Hatchery production of giant fresh water prawn *M rosenbergii* seed in India- present status and future prospects. Fishing Chimes 16(7), 27-28.

Murthy, H. S., 2006. Recent advances in fresh water prawn farming and hatchery seed production in India – An insight. In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi. 20-23 Aug, 2003. 61-65.

Murthy, H. S., Yogeshbabu, M. C., 2006. Replacement of *Artemia* by *Brachionus plicatilis* in the larval rearing of fresh water prawn (*Macrobrachium rosenbergii*). In: Kurup, B. M., Ravindran, K. (Eds.) Sustain Fish- Proc. Of Intl. Symp. On improved sustainability of fish prdn. Sys. And app. Tech. for utilzn., 16-18 Mar. 2005, Cochin, 272-278.

Murthy, H. S., Yogeshbabu, M. C., Thanuja, K., Prakash, P., Shankar, R., 2008. Evaluation of formulated inert larval diets for giant fresh water prawn, *Macrobrachium rosenbergii* weaning from *Artemia*. Mediterranean Aquac. J.1(1), 21-25.

Muthuwani, V., Lin, C. K., 1996. Water quality and nutrient budget in intensive shrimp culture ponds. Proceedings of the World Aquaculture Society Meeting, Bangkok, 1996, p. 270.

Naas, K. E., Naess, T., Harboe, T., 1992. Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus* L.) in green water. Aquaculture 105, 143–156.

Nair, C. M., Salin, K. R., Ashok Kumar, K., 2007. Use of Cyclop-eeze as a substitute for *Artemia* nauplii in larval rearing of giant fresh water prawn *Macrobrachium rosenbergii* . Aquacult. Nutr. 13(2), 88-93.

Naqvi, A. A., Adhikari, S., Bindu, R, P., Sarangi, N., 2007. Effect of ammonia-N on growth and feeding of juvenile *Macrobrachium rosenbergii* (De-Man). Aquacult. res. 38, 847-851.

New, M. B., 1990. Freshwater prawn culture: a review. Aquaculture 88, 99–143.

New, M. B., 1995. Status of freshwater prawn farming: a review. Aquaculture Research 26,1-54.

New, M. B., 2000. History and global status of freshwater prawn farming. In: New, M. B., Valenti, W. C. (Eds). Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*, Blackwell Science, Oxford, UK. pp.1-11.

New, M. B., 2002. Farming Freshwater Prawns: A Manual for the Culture of the Giant River Prawn (*Macrobrachium rosenbergii*), vol. 428. FAO, Rome.

New, M. B., 2005. Freshwater prawn farming: global status, recent research and a glance at the future. Aquac. Res. 36, 210–230.

New, M. B., Singolka, K., 1985. Freshwater prawn farming . A manual for the culture of *Macrobrachium rosenbergii* . FAO fish. Tech. Pap. (225): 188.

Nga, B. T., Lurling, M., Peters, E. T. H. M., Roijackers, R., Scheffer, M., Nghia,T. T., 2005. Chemical and physical effects of crowding on growth and survival of *Penaeus monodon* Fabricus post larvae. Aquaculture, 246, 455 - 465.

Nogami, K., Maeda, M., 1992. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus*. Can. J. Fish. Aquat. Sci. 49, 2373–2376.

Oanh, D. T. H., Hoa, T. T. T., Phuong, N. T., 2000. The effects of probiotics on culture conditions of fresh water prawn larvae. In: Proceedings of annual workshop of JIRCAS Mekong Delta Project ô development of new technologies and their practices for sustainable farming systems in the Mekong Delta, 200-206.

Oehmen, A., Yuan, Z., Blackall, L. L., Keller, J., 2004. Short-term effects of carbon source on the competition of polyphosphate accumulating organisms and glycogen accumulating organisms. Water Sci. Technol. 50 (10), 139–144.

Ong, K. S., Ng, F.O., Wonk, Y. K., 1977. Experiences and problems in the design and operation of a *Macrobrachium rosenbergii* hatchery. Joint FAO-UNDP/SCSP and SEAFDEC Region Workshop on Aquaculture engineering (with emphasis on small scale projects). Tigbauan, Iloilo, Philippines, 27 Nov-3Dec.1977.5pp (SCSP-SFDC/77/Can/CP/32).

Palmer, P. J., Bruke, M. J., Palmer, C. J., Bruke, J. B., 2007. Developments in controlled green water larval culture technologies for estuarine fishes in Queensland, Australia and elsewhere. Aquaculture 272, 1-21.

Panjaitan, P., 2004. Field and laboratory study of *Penaeus monodon* culture with zero water exchange and limited water exchange model using molasses as a carbon source. Ph.D. thesis, Charles Darwin University, Darwin, NT, Australia.

Papandroulakis, N., Divanach, P., Anastasiadis, P., Kentouri, M., 2001.The pseudo-green water technique for intensive rearing of sea bream (*Sparus aurata*) larvae. Aquac. Int. 9 (3), 205–216.

Papandroulakis, N., Divanach, P., Kentouri, M., 2002. Enhanced biological performance of intensive sea bream (*Sparus auratus*) larviculture in the presence of phytoplankton with long photophase. Aquaculture 204, 45–63.

Parseval, L., Malecha, S.R., Cotsapas, L., D'Abramo, L., MacMichael, R.E., Desmond, T. 1989. Potential application to Brazil of recent advances in the culture of marine shrimp and fresh water prawns. In: Martins, M. M. R., Correia, E. S., Cavalheiro, J. M. (Eds). Anais do III Simposio Brasileiro sobre Cultivo de Camarao, 15-20 outubro 1989, Joao Pessoa, Vol.2: Camarao de Agua Doce e Outros. MCR aquaculture, Joao Pessoa. 71-83.

Person Le Ruyet, J., Alexandre, J. C., Thebaud, L., Mugnier, C., 1993. Marine fish larvae feeding: formulated diets or live prey. J. World Aquacult. Soc. 24, 211-224.

Peterson, J. J., Griffith, D. R. W., 1999. Intensive nursery systems. Glob. Aquacult. Advoc., 2 (6): 60 - 61.

Phatarpekar P. V., Kenkre, R. A. Sreepada, U. M., Desai, C. T., Achuthankutty. 2002. Bacterial flora associated with larval rearing of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture 203, 279–291.

Phillips, M., Lin, C.K., Beveridge, M. C. M., 1993. Shrimp culture and the environment: lesson from the world's most rapidly expanding warm water aquaculture sector. In: Pullin, R.S.V., Rosenthal, H., Maen, S.N. (Eds.), Conf. Proc. Environment and Aquaculture in Developing Countries. ICLARM, 312, 171 - 197.

Phuong, N. T., Hai, T. N., Yen, N. L. H., Cuong, N. C., 2000. The effects of stocking densities on the growth and survival rate of fresh water prawn larvae reared under modified green water system and re-circulating systems. In: Proceedings of annual workshop of JIRCAS Mekong Delta Project ô development of new technologies and their practices for sustainable farming systems in the Mekong Delta.193-199.

Phuong, N. T., Hai, T. N., Wilder, M. N., 2006. Development of giant fresh water prawn farming in Vietnam. In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi.20-23 Aug, 2003. 41-51.

Pitipornchai, S., 1998. Experiment on rearing giant prawn, *Macrobrachium rosenbergii* (De Man), larvae with different feeds. Technical Paper No. 11/1998. Inland Fisheries Division. Department of Fisheries, Bangkok.

Pomeroy, L. R., Smith, E. E., Grant, C. M., 1965. The exchange of phosphate between estuarine water and sediments. Limnology and oceanography. 10, 167 – 172.

Pramanik, M. W. A., Halder, G. C., 1996. Development of backyard *Macrobrachium* hatchery system and larval rearing technique in different stocking density. J. Inland Fish. Soc. India 28, 21-27.

Prasad, R. P., 1996. Present status of fresh water prawn (*Macrobrachium rosenbergii*) culture. Fishing Chimes 16(1), 17-22.

Rahmatulla, S. M., Beveridge, M. C. M., 1993. Ingestion of bacteria in suspension by Indian major carp (*Catla catla, Labeo rohita*) and Chinese carps (*Hypophthalmichthys molotrix, Aristichthys nobilis*). Hydrobiol. 264, 79 – 84.

Ray, W. M., Chien, Y. H., 1992. Effects of stocking density and aged sediment on tiger prawn, *Penaeus monodon*, nursery system. Aquaculture 104, 231 – 248.

Reay, D., David, B., Nedwell, J., Priddle, J., Cynan, E. E., 1999. Temperature dependence of inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl. Environ. Microbiol., 2577–2584.

Reddy, A.K., 1997. Management of fresh water prawn hatcheries and culture possibilities. Fishing Chimes 16(10), 32-39.

Regunathan, C., Wesley, S. G., 2004. Control of *Vibrio* spp. In shrimp hatcheries using the green algae *Tetraselmis suecica*. Asian Fish. Sci. 17, 147-157.

Riquelme, C. E., Jorquera, M. A., Rojas, A. I., Avendan˜o, R. E., Reyes, N., 2001. Addition of inhibitor-producing bacteria to mass cultures of *Argopecten purpuratus* larvae (Lamarck, 1819). Aquaculture 192, 111 – 119.

Rodgers, L. J., Barlow, C. C., 1987. Better nutrition enhances survival of barramundi larvae. Australian Fisheries 46(7), 30-32.

Rosenlund, G., Stoss, J., Talbot, C., 1997. Co-feeding in marine fish larvae with inert and live diets. Aquaculture 155, 183-191.

Russo, R. C., Thurston, R. V., 1991. Toxicity of ammonia, nitrite and nitrate to fishes. In: Brune, D. E., Tomasso, J. R. (Eds.), Aquaculture and water quality. The World aquaculture Society, Baton Rouge, pp.58-89.

Sahul Hameed, A. S., Balasubramanian, G., 2000. Antibiotic resistance in bacteria isolated from *Artemia* nauplii and efficacy of formaldehyde to control bacterial load. Aquaculture 183, 195– 205.

Sahul Hameed, A. S., Rahaman, K. H., Alagan, A., Yoganandhan, K., 2003. Antibiotic resistance in bacteria isolated from hatchery-reared larvae and post-larvae of *Macrobrachium rosenbergii*. Aquaculture 217, 39-43.

Samocha, T. M., Uziel, N., Browdy, C. L., 1989. The effect of feeding two prey organisms , nauplii of *Artemia* and rotifers *Brachionus plicatilis* (Muller), upon survival and growth of larval marine shrimp, *Penaeus semisulcatus* (de Haan). Aquaculture 77, 11-19.

Sandifer, P. A., Smith, T. L. J., 1976. Effects of population density on growth and survival of *Macrobrachium rosenbergii* reared in recirculating water management systems. In: Avault, J.W., Miller, R.(Eds), Proc. 6th Annu. Workshop World Maricult. Soc. Louisiana State University Press, Baton Rouge. Pp. 43-53.

Sandifer, P. A., Hopkins, J. S., Smith, T. L. J., 1977. Production of juveniles. In: Hanson,J. A., Goodwin, H. L. (Eds), Shrimp and prawn farming in the

Western Hemisphere. Dowden, Hutchinson, and Ross, Stroudsburg, PA, pp. 220-229.

Sandifer, P. A., Hopkins, J. S., Stokes, A. D., 1987. Intensive culture potential of *Penaeus* Õ*annamei*. J. World Aquacult. Soc. 18 (2) , 94 – 100.

Sastry, A. N., 1983. Pelagic larval ecology and development. In : Bliss, D. E. (Ed.), The biology of Crustacea. Academic Press, New York, pp.214-283.

Schmidt-Nielsen, K., 1983. Animal Physiology: Adaptation and Environment. Cambridge University Press, Cambridge.

Schneider, O., Sereti, V., Eding, E. H., Verreth, J. A. J., 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. Aquac. Eng. 32 (3–4), 379–401.

Schroeder, G. L., 1987. Carbon and Nitrogen budget in manured fish ponds on Israel's coastal plain. Aquaculture 62, 259 – 279.

Schryver De, P., Crab, R., Defoirdt, T., Boon, N., Verstraete., 2008. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 277, 125-137.

Scott, A. P., Baynes, S. M., 1979. The effect of unicellular algae on survival and growth of larvae of (*Scophthalmus maximus* L.). In: Halver, J.E., Tiews, K. (Eds.), Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology, Hamburg 20–23 June 1978, Vol. I. Berlin, pp. 423–433.

Sebastin, C. D., 2006. Observations on the production of giant fresh water prawn post larvae in large outdoor tanks. In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi.20-23 Aug, 2003. 349-352.

Sebastin, M. J., Nair, C. M.,1995. Seed production and culture of Giant Fresh Water Prawn *Macrobrachium rosenbergii*. Souvenir INDAQUA ' 95

Serfling, S.A., 2006. Microbial flocs. Natural treatment method supports freshwater, marine species in recirculating systems. Global Aquac. Adv., June 2006; pp. 34–36.

Shaw, G. W., Pankhurst, P. M., Battaglene, S. C., 2006. Effect of turbidity, prey density and culture history on prey consumption by greenback flounder *Rhombosolea tapirina* larvae. Aquaculture 253, 447–460.

Shilo, M., Rimon, A., 1982. Factors which affect the intensification of fish breeding in Israel: 2. Ammonia transformation in intensive fish ponds. Bamidgeh 34, 101–114.

Shi-Yen, S., Chun-Yang, P., 1992. Utilisation of different carbohydrates at different dietary protein levels in grass prawn, *Penaeus monodon*, reared in seawater. Aquaculture 101, 241 – 250.

Sick, L. V., Beaty, H., 1975. Development of formula foods designed for *Macrobrachium rosenbergii* larval and juvenile shrimp. Proc. World Maricult. Soc., 6: 89-102.

Silva, F. M., Rodrigues, J. B. R., 1997. Effect of the replacement of the *Artemia* sp. for the nematode *Panagrellus redivivus* on the larval growth and survival of the fresh water prawn (*Macrobrachium rosenbergii*). Boletim-do-Instituto-de-Pesca-Sao-Paulo 24, 35-48.

Smith, G. G., Ritar, A.J., 2006. The influence of animal density and water turbulence on growth and survival of cultured spiny lobster (*Jasus edwardsii*) larvae. Aquaculture 258 (1-4), 404-411.

Sorgeloos, P., Bossuyt, E., Lavens, P., Leger, P., Vanhaecke, P., Versichele, D., 1983. The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. In: McVey, J.P., Moore, J.R. (Eds.), CRC Handbook of Mariculture, vol. 1. CRC Press, Boca Raton, FL, USA, pp. 71– 96.

Sorgeloos, P., Van Stappen, G., 1995. Artemia cyst shortage: the ARC's point of view. Larviculture and Artemia newsletter 35, pp.19.

Stappen, G.V., 1996. Introduction, biology and ecology of *Artemia*. In: Lavens, P., Sorgeloos, P. (Eds). Manual on the production and use of live food for aquaculture. FAO Fish . Tech . Pap . (361).

Stone, C. J., 1988. Test of sequential feeding regimes for larvae of *Elminius modestus* Darwin (Cirripedia : Balanomorpha). J. Exp. Mar. Biol. Ecol., 115: 41-52.

Straus, D. L., Robinette, H. R., Heinen, J. M., 1991. Toxicity of unionized ammonia and high pH to post larval and juvenile fresh water shrimp *Macrobrachium rosenbergii*. J. of the Worl. Aquac. Soc. 22, 128-133.

Sugden, L. G., 1973. Feeding ecology of Pintail, Gadwall, American Wigeon and lesser Scaup ducklings. Canadian Wildlife Services report 24. pp.45.

Suharto, H. H., Ismail, A., Poernomo, A., 1982. Breeding technique of *Macrobrachium rosenbergii* in conical fiberglass tanks. In: New, M. B. (Ed.). Giant prawn farming. Amsterdam, Elsevier, 115-122.

Sung, H. H., Li, H.C., Tsai, F.M., Ting, Y. Y., Chao, W.L., 2001. Changes in the composition of *Vibrio* communities in pond water during tiger shrimp cultivation and in the hepatopancreas of healthy and diseased shrimp. J. Exptl. Mar. and Ecol. 236, 261-271.

Suresh Kumar, K., Velayudhan, T. D., 2006. Status of fresh water prawn farming in Kerala, India. In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi. 20-23 Aug, 2003. pp.72-80.

Tacon, A. G. J., Cody, J. J., Conquest, L. D., Divakaran, S., Forster, I. P., Decamp, O. E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquacult. Nutr. 8, 121–137.

Tamura, C. S., Murashige, R., Cheng-Sheng, L., 1994. The paradox of using background phytoplankton during the larval culture of striped mullet (*Mugil cephalus* L.). Aquaculture 119, 167–174.

Tendencia, E. A., Pena, M dela., 2003. Investigations of some components of the greenwater system which makes it effective in the control of luminous bacteria. Aquaculture 272, 115 –119.

Thanuja, K., 2007. Nutritive enrichment of *Artemia* and its production as a source of larval feed for fresh water prawn *Macrobrachium rosenbergii* (De Man). PhD Thesis. Mangalore University, Mangalore, Karnataka.

Thomaz, L. A., Oshiro, L. M. Y., Bambozzi, A. C., de Seixas Filho, J. T., 2004. Larval performance of the fresh water prawn (*Macrobrachium rosenbergii*) · R. Bras. Zootec 33(6/2), 26·

Thompson, F. L., Abreu, P. C., Wasielesky, W., 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203, 263–278.

Thresiamma, J., Aneykutty, J., Devika Pillai., Nair, C. M., Susheela Jose., 2006. The microalgae *Isochrysis galbana* culture in the larval rearing of *Macrobrachium rosenbergii* (De Man). In: Nair, C. M., Nambudiri, D. D., Susheela J., Sankaran, T, N., Jayachandran, K. V., Salin, K.R. (Eds.). Fresh water prawns: Advances in biology, aquaculture and marketing. Proc. Intl. Symp. on fresh water prawns, College of Fisheries, KAU, Kochi. 20-23 Aug, 2003. 535-540.

Tidewell, J. H., Schulmeister, C. M., Coyle, M., 1997. Growth, survival and biochemical composition of fresh water prawns *Macrobrachium rosenbergii* fed natural food organisms under controlled conditions. J. World Aquac. Soc. 28(2), 123-132.

Tomasso, J. R., 1994. Toxicity of nitrogenous wastes to aquaculture animals. Rev. Fish. Sci. 2, 291- 314.

Tucker, C. S., 1996. The ecology of channel cat fish culture ponds in north west Mississippi. Rev. in Fish. Sci. 4, 1-55.

Tunsutapanich, A., Sanggontangit, T., Thongrod, S., Omanee, C., 1996. Improved techniques for seed production and nursing giant prawn, *Macrobrachium rosenbergii* larvae in earthen pond in closed system. In: Creswell, I.R.(Ed.) Abstracts of World Aquaculture, 29 Jan- 2 Feb, 1996, World aquac. Soc. Baton Rouge, p.398.

Tunsutapanich, A., Thongrod, S., Sanggontangit, T., Omanee, C., 1997. Research points to improve commercial production technique of juvenile prawn in earthen pond. Aquaculture Asia II (2), 47-51.

Uno, Y., Kwon, C. S., 1969. Larval development of *Macrobrachium rosenbergii* reared in the laboratory. J. Tokyo Univ. Fish. 55, 179– 190.

Valenti, W. C., 1985. *Cultivo de Camaroes de Agua Doce*. Nobel, Sao Paulo.pp.82.

Valenti, W.C., 1993. Fresh water prawn culture in Brazil. World Aquaculture 24(1), 30-34.

Valenti, W.C., 1995. Situacao atual e perspectives da carcinicultura no Brasil e no mundo. In: Anais do I Simposio Internacional sobre Notricao de Peixes e Crustaceos, 8-10 novembro 1994, Campos do Jordao. Colegio Brasileiro de Nutricao Animal (CBNA), Campinas., pp. 8-18.

Valenti, W. C., Mallasen, M., Silva, C. A., 1998. Larvicultura em sistema fechado dina^mico. In: Valenti, W.C. (Ed.), Carcinicultura de A ´ gua Doce: Tecnologia para a Produc_a˜o de Camaro˜es. FAPESP e IBAMA, Sa˜o Paulo.

Valenti, W. C., Daniels, W. H., 2000. Recirculation hatchery systems and management. In: New, M.B., Valenti, W.C. (Eds.), Freshwater Prawn Culture. Blackwell, Oxford, pp. 69– 90.

Van der Meeren, T., 1991. Algae as first food for cod larvae, *Gadus morhua* L.: filter feeding or ingestion by accident? J. Fish Biol. 39 (2), 225–237.

Varghese, J. T., 2007. Carbon / nitrogen ratio optimization and periphyton development on the production and sustainability of *Penaeus monodon* (fabricius) in extensive culture system. PhD thesis. Cochin University of Science and Technology, Cochin, India.

Verdonck, L., Swings, J., Kersters, K., Dehasque, M., Sorgeloos, P., Leger, P., 1994. Variability of the microbial environment of rotifer *Brachionus plicatilis* and *Artemia* production systems. J. World Aquac. Soc. 25, 55–59.

Vici, V., Bright Singh, I. S., Sarita, G. B., 2000. Application of bacterins and yeast *Acremonium dyosporii* to protect the larvae of *Macrobrachium rosenbergii* from vibriosis. Fish and Shellfish Immunology 10, 559-563.

Vine, N. G., Leukes, W. D., Kaiser, H., 2006. Probiotics in marine larviculture. FEMS Microbiol. Rev. 30, 404–427.

Viso, A. C., Pesando, D., Baby, C., 1987. Antibacterial and antifungal properties of some marine diatoms in culture. Bot. Mar. 30, 41–45.

Wahab, M. A., Bergheim, A., Braaten, B., 2003. Water quality and partial mass budget in extensive shrimp ponds in Bangladesh. Aquaculture 218, 413 – 423.

Wais, A. W., Wolfram, G., Aver, B., Mikschi, E., Hain, A., 1999. Feeding habits of two introduced fish species (*Lepomis gibbosus*, *Pseudorasbora parva*) in Neusiedler sea (Australia), with special reference to chironomid larva. Hydrobiologia 408/409, 123-129.

Wang, J. K., 2003. Conceptual design of a microalgae-based recirculating oyster and shrimp system. Aquac. Eng. 28, 37–46.

Wasielsky Jr.,W., Atwood, H., Stokes, A., Browdy, C. L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei.* Aquaculture 258, 396–403

Wayban, J. A., Lee, C. S., Sato, V. T., Sweeney, J. N., Richards Jr, W. K., 1987. Effect of stocking density on shrimp growth rates in manure-fertilized ponds. Aquaculture 61, 23-32.

Wise, D. J., Tomasso, J. R., 1989. Acute toxicity of nitrite to red drum, *Sciaenops ocellatus*: Effect of salinity. J.World Aquaculture Soc. 20 (4), 193-198.

Yusoff, M. F., Om, A. D., Cheah, S. H., 1996. Use of agro-industrial effluent in augmenting microalgae production and fish fry growth in hatchery tanks. J. Aquac. in the tropics 11, 119-126.

Zar, J. H., 1984. Biostatistical Analysis, 2nd edn. Prentice Hall Inc. New York. 718 pp.

Zhu, S., Chen, S., 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. Aquacult. Eng. 25, 1–11.

Ziemann, D. A., Walsh, W. A., Saphore, E. G., Fulton-Bennett, K., 1992. A survey of water quality characteristics of effluent from Hawaiian aquaculture facilities. J. World Aquacult. Soc. 23, 180–191.

LIST OF PUBLICATIONS

Saritha, T., Kurup, B.M., 2005. Substitution of *Artemia* with chironomid larvae in the seed production of the giant fresh water prawn, *Macrobrachium rosenbergii.* Abstracts of Indian Fisheries Forum, 8-12 Nov., 2005. Asian Fisheries Society, Banglore.

Saritha, T., Kurup, B.M., 2007. The use of microalga *Isochrysis galbana* in the seed production of *Macrobrachium rosenbergii* (de man) using the modified static green water system. Abstracts of Eighth Asian Fisheries Forum, 20-23 Nov., 2007. Asian Fisheries Society, Kochi, p.169