APPLICATION OF BIOFLOC TECHNOLOGY (BFT) IN THE NURSERY REARING AND FARMING OF GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)

Thesis submitted to the
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in partial fulfillment of the requirements
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in
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Under the Faculty of Marine Sciences

by

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Front cover

Prawn consuming biofloc developed in the experimental tanks

Back Cover

Biofloc formed in the experimental tanks

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Certificate

This is to certify that the thesis entitled **"Application of Biofloc Technology (BFT) in the nursery rearing and farming of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man)"** to be submitted by **Mr. Prajith K. K,** is an authentic record of research work carried out by him under my guidance and supervision in partial fulfillment of the requirement of the degree of **Doctor of Philosophy** in **Aquaculture** of Cochin University of Science and Technology, under the faculty of **Marine Sciences.**

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Declaration

I, **Prajith K.K**, do hereby declare that the thesis entitled "**Application of Biofloc Technology (BFT) in the nursery rearing and farming of Giant freshwater prawn, *Macrobrachium rosenbergii* (de Man)**" is a genuine record of research work done by me under the supervision of **Prof. (Dr.) B. Madhusoodana Kurup**, Vice-Chancellor, Kerala University of Fisheries and Ocean Studies, Panangad, Kerala, India and has not been previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any university or institution.

Prajith K.K

Kochi – 682016
December 2011

Dedicated to…

All animals grown in my experimental tanks

by consuming biofloc

&

To the farmers who practice sustainable aquaculture

Words of Gratitude...

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Prajith.K.K

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Abbreviations

AAI	Aquaculture Authority of India
ADG	Average Daily weight Gain
AICRP	All India Coordinated Research Project
ANOVA	Analysis of Variance
BFFDA	Brackishwater Fish Farmers' Development Agency
BFT	Biofloc Technology
BOD	Biological Oxygen Demand
C/N Ratio	Carbon Nitrogen Ratio
CAA	Coastal Aquaculture Authority
CH	Carbohydrate
CIBA	Central Institute of Brackishwater Aquaculture
CIFA	Central Institute on Freshwater Aquaculture
CIFE	Central Institute of Fisheries Education
CIFRI	Central Inland Fisheries Research Institute
CMFRI	Central Marine Fisheries Research Institute
CRZ	Coastal Regulation Zone
CUSAT	Cochin University of Science and Technology
DNA	Deoxyribo Nucleic Acid
DO	Dissolved Oxygen
FAO	Food and Agricultural Organisation
FCR	Feed Conversion Ratio
FFDA	Fish Farmers Development Agency
FRP	Fibre Reinforced Plastic
FVI	Floc Volume Index
GMe	Green Mussel Extract

HDPE - High Density Polyethylene
ICAR - Indian Council of Agricultural Research
MPEDA - Marine Product Export Development Authority
NRCCWF - National Research Centre on Coldwater Fisheries
OC - Organic Carbon
PER - Protein Efficiency Ratio
PHA - Polyhydroxy alkanoate
PHB - Polyhydroxy butyrate
PL - Post Larvae
RAS - Recirculatory Aquaculture System
RNA - Ribo Nucleic Acid
SBR - Sequencing Batch Reactor
SCFA - Short Chain Fatty Acid
SGR - Specific Growth Rate
TAN - Total Ammonia Nitrogen
THB - Total Heterotrophic Bacteria
WAS - World Aquaculture Society

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Chapter 1

INTRODUCTION

Contents

1.1 Introduction

The human population has grown from 1.5 to 6.4 billion from 1900 till now and is predicted to increase to 9 billion by the year 2050. Not surprisingly, the fact remains that malnourishment is probably one of the challenges,. if not the biggest challenge facing the globe, with an estimated 840 million being in a state of malnourishment (UNWFP, 2005). So it is essential to ensure the health of the world population by providing nutritionally balanced, especially protein-rich food. Animal husbandry and fisheries are the two sources of animal protein for the world population (MPEDA, 1992). In the context of increasing health consciousness in the modern world, fish and fishery products are considered to be among the safest food of animal origin (MPEDA, 1992). Most importantly, fish constitute one of the main animal protein sources of the developing world, containing all essential amino acids, and is an excellent source of essential fatty acids, the highly unsaturated acids of n-3 and n-6 series (de Deckere et al., 1998; Horrocks and Yeo, 1999; Connor, 2000; Ruxton et al., 2005). But when compared to the slow growth of fish production, consumption of fishery products has been increasing in tandem with the exponential growth of world population, leaving a huge gap between production and demand. Aquaculture, growing plants and animals under controlled conditions, is the answer (FAO, 2001). The world needs an extra 40-60x10^6 tons of food fish by 2020 (De Silva and Davy, 2010). These facts point finger on the importance of aquaculture.

1.2 World aquaculture

Aquaculture in world is a fast growing industry with an average growth rate of about 12% during the past decades. With per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2008, an average annual growth rate of 6.6% was recorded. The reported global production of food fish from aquaculture, including finfishes, crustaceans, molluscs and other aquatic animals excluding plants for human consumption, reached 52.5 million tonnes in 2008 with a value of US$ 98.4 billion. Aquatic plant production by aquaculture in 2008 was 15.8 million tonnes (live weight equivalent), with a value of US$ 7.4 billion, representing an average annual growth rate in terms of weight of almost 8% since 1970 (FAO, 2010). Thus, if aquatic plants are included, total global aquaculture production in 2008 amounted to 68.3 million tonnes with a first-sale value of US$106 billion. World aquaculture is heavily dominated by the Asia– Pacific region, which accounts for 89% of production in terms of quantity and 79% in terms of value (FAO, 2010).

1.3 Indian aquaculture: Its growth and development

Aquaculture in India has a long history. There are references to fish culture in Kautilya's Arthashastra (321–300 B.C.) and King Someswara's *Abhilashitarthachinhtamani* (1127 A.D.) on growing of fish in ponds and tanks (Hora, 1951; Hora and Pillai, 1962; Jhingran, 1991). Despite having its bountiful water resources, diverse ecosystems and availability of large numbers of candidate species for culture, India has not been able to make use of its potential satisfactorily and therefore, is being often regarded as a "Sleeping giant " in aquaculture (Kurup, 2010a). The most outstanding achievements in Indian aquaculture are the historic emergence of shrimp culture based industry, remarkable advancement in carp culture techniques and cultured carp production, particularly in reservoirs. The phenomenal growth attained by shrimp culture was in fact hampered drastically by many conjecture like massive outbreak of white spot disease and consequent widespread mortality, regulation imposed by Coastal Regulation Zone Notification and Supreme Court verdicts, challenges faced in the export market front, etc. On the other hand, freshwater aquaculture in the country has been progressing positively, though it gained less attention owning to the low profit generation and the remarkable performance shown by the shrimp aquaculture industry. The development of research and adoption of technology in freshwater fish production through aquaculture was rapid. This resulted in the annual production of 3.47 million tonnes in the country, which forms more than 90% of total fish production.

India is making rapid strides with its blue revolution and today, ranks second in the world in aquaculture, with a production of 3.47 million tonnes and have an average growth rate of 7.6% (FAO, 2010). Prawns and shrimps rank as the highest foreign exchange earner among our aquaculture product exported, with farmed shrimps accounting for close to 50% of the total shrimp exports in volumes and fetching over 70% in value (FAO, 2010).

1.4 Mariculture

The development of Indian aquaculture sector showed switching over in the production from freshwater, to barckishwater and even to mariculture. The earliest attempt in mariculture in India was made at Mandapam Centre of Central Marine Fisheries Research Institute (CMFRI) in 1958–1959 with the culture of milkfish (*Chanos chanos*). Over the last four decades, CMFRI has developed various technologies in mariculture for oysters, mussels and clams as well as for shrimps and finfish. Pearl culture programme in 1972 was successful in controlled breeding and spat production of Japanese pearl oyster (*Pinctada fucata*) in 1981 and the blacklip pearl oyster (*Pinctada margaritifera*) in 1984, edible oyster farming during the 1970's, exploring culture possibilities and techniques of *Perna indica* and *Perna viridis*. production of Designer Mabe Pearls in 2008, successful operation of open sea cage farming of lobsters and captive breeding of ornamental fishes in 2009, induced breeding of cobia and developing Green Mussel Extract (GMe) in 2010 and seed production of pompano *(Trochinotous blochii)* in 2011 are some of the achievement of India in Mariculture sector.

1.5 Brackishwater aquaculture

Brackishwater farming in India is an age-old system confined mainly to the *bheries* of West Bengal and *pokkali* fields along the Kerala coast. Scientific development of brackishwater aquaculture in India started with the establishment of All India Coordinated Research Project (AICRP) on 'Brackishwater fish farming' by Indian Council of Agricultural Research (ICAR) in 1973. A phenomenal increase in the area under shrimp farming occurred between 1990 and 1994, the formation of Brackishwater Fish Farmers Development Agencies (BFFDA) in the maritime states and the implementation of various Governmental programmes to provide support to the shrimp farming sector for its further development. Demonstrations of semi-intensive farming technology were conducted with a target production levels reaching 4–6 tonnes/ha (Surendran et al., 1991). Farmed shrimp production increased from 40000 tonnes in 1991–1992 to 115000 tonnes in 2002–2003. Studies on maturation and captive breeding of shrimps were initiated by the CMFRI in the early 1970's. In the late 1980's MPEDA established the Andhra Pradesh Shrimp Seed Production and Research Centre (TASPARC) in Andhra Pradesh and Orissa Shrimp Seed Production Supply and Research Centre (OSSPARC) based in Orissa which provided assistance for the establishment of a number of private hatcheries (Ayyappan, 2006). At present about 237 shrimp hatcheries are in operation in the country with an installed capacity of 11.425 billion PL 20/year (Anon, 2002). In 1987, ICAR established Central Institute of Brackishwater Aquaculture (CIBA). Through experiments CIBA has demonstrated the potential of farming of brackishwater fishes like Asian sea bass, milkfish, pearl-spot, mullets, etc. The institute achieved a major breakthrough in the captive broodstock development, induced breeding and seed production of the Asian sea bass *Lates calcarifer* for the first time in the country.

1.6 Freshwater aquaculture

Freshwater aquaculture is by far the most ancient living resource production system known in the world. Freshwater aquaculture system differs from the brackishwater and marine aquaculture systems in several ways. It allows a strong integration of the agricultural production systems at different levels. Freshwater aquaculture production is mainly based on culture of short food-chain fish and differs basically from marine fish culture based on carnivorous fish. In India, freshwater aquaculture comprising extensive and semi-intensive aquaculture production systems, where, fertilization and supplementary feeds is the key points (Kurup, 2010a). The freshwater resources in India comprises 14 major river systems, 2.25 million hectares of ponds and tanks,1.3 ha of beels and derelict waters, 2.09 m.ha of lakes and reservoirs as also 0.12m km of irrigation canals and channels and 2.3m ha of paddy field. (Ayyappan, 2006). The state of Kerala is endowed with 44 rivers, innumerable irrigation tanks, reservoirs, streams and waterfalls, private and public ponds, quarry ponds and waterlogged paddy fields. Besides these there exist, nine freshwater lakes. The 44 rivers of Kerala have an area of about 0.85 lakh ha of which 41 are westerly flowing and three are easterly flowing. The total area of 53 reservoirs comes to around 0.43 lakh ha. (Benziger and Philip, 2010). These figures reveal the immense freshwater aquatic diversity and aquaculture potential of our country. The development of freshwater aquaculture in the country was initiated following the establishment of the Pond Culture Division at Cuttack in 1949 under Central Inland Fisheries Research Institute (CIFRI). An All India Coordinated Research Project on

Composite Culture of Indian and Exotic Fishes was initiated by the CIFRI during 1971. The late 1980's saw the dawn of aquaculture in India and transformed fish culture into a more modern enterprise. Successful breeding and larval rearing of the giant river prawn (*Macrobrachium rosenbergii*) and the monsoon river prawn (*Macrobrachium malcolmsonii*) provided scope for the farmers to diversify their culture practices. During recent years, the freshwater prawn farming sector has witnessed quite impressive growth, recording a production of over 30000 tonnes in 2002–2003 from approximately 35000 ha of water. The state of Andhra Pradesh dominates the sector with over 86% of the total production in India with approximately 60% of the total water area dedicated to prawn farming, followed by West Bengal. Mixed farming of freshwater prawn along with carp is also very much accepted as a technologically sound culture practice and a viable option for enhancing farm income. Thirty five freshwater prawn hatcheries, at present producing about 200 million seed per annum, cater for the requirements of the country (Ayyappan , 2006). Central Institute on Freshwater Aquaculture (CIFA) and the National Research Centre on Coldwater Fisheries (NRCCWF) established in 1987 are the two institutions that monitor the development of freshwater aquaculture in the country. With fisheries development being considered a state subject, each state has a full-fledged Fisheries Department. The Ministry of Agriculture of the Government of India also provides additional coordination of development programs in the different states and provides for centrally sponsored projects. For encouraging and publicising freshwater aquaculture, the Government of India introduced a scheme known as the Fish Farmers Development Agency (FFDA) during 1973–1974 at the state level, presently there are 422 FFDAs providing cover to the districts indicating major potential in the country.

1.7 Giant freshwater prawn, *Macrobrachium rosenbergii* as the candidate species for freshwater aquaculture in India

Giant freshwater prawn is scientifically known as *Macrobrachium rosenbergii* (De Man, 1879) and known as *Attukonju* or *Kuttanadan konju* in *Malayalam.* It is the indigenous crustacean species in whole of the Indo-Pacific region. It is the only freshwater crustacean with very high economic value. Freshwater prawn culture is an important aquaculture industry in many Asian countries, which together contribute over 98% of global freshwater prawn production (Asaduzzaman et al., 2008). 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 it on a mass scale. Therefore, Ling and Fujimura are considered as the 'Fathers' of freshwater prawn farming (Saritha, 2009). Freshwater prawn are preferred among the farmers due to the following reasons.

- Freshwater prawn merges perfectly with freshwater ecosystem.
- Prawn farming activity in no way comes into conflict with any other agriculture activity.
- Freshwater prawn farming can be easily integrated with paddy cultivation. In many Asian countries, a clear rotation of land based agriculture and aquaculture, especially paddy and freshwater prawn is practiced. Pokkali culture practice in central Kerala (India) is an excellent example for this. Moreover, the low lying *Kari* lands of Kuttanadu and *Kole* lands are also found to be suitable for prawn farming alternating with paddy cultivation.

- Freshwater prawn farming supports and supplements paddy cultivation, and enhances productivity and income of farmers. The nutrients and other organic residues in the fields make paddy field more fertile and increase the production of paddy. Hence, the sustainable production of both paddy and prawn is ensured.
- No adverse ecological impact due to scampi farming has been reported. When compared to the marine shrimp culture (penaeid shrimps), the ecological impact due to freshwater prawn culture is negligible.
- Lesser man power is required as management measures are minimal- e.g. : No salinity correction.
- Waste production is minimal when compared to the marine shrimp farming
- Protein requirement in the feed of freshwater prawn is also low when compared to the marine cultivable crustaceans.

1.8 Status of giant freshwater prawn culture

A very rapid global expansion in freshwater aquaculture 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 freshwater 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%. 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 freshwater prawns in 2003 was about 2,80,000 tonnes. The main contributor was China (1,80,000 tonnes) followed by India and Thailand (35,000 tonnes each).

Freshwater prawn farming in India is mainly contributed by culture of the giant freshwater prawn *M. rosenbergii*. Considering the high export potential, the giant freshwater prawn, *M. rosenbergii*, the scampi, enjoys immense potential for culture in the country. About 4 million ha of impounded freshwater bodies in the various states of India, offer great potential for freshwater prawn culture. Scampi can be cultivated for export through monoculture in existing as well as new ponds or with compatible freshwater fishes in existing ponds. It is exported to EEC countries and USA. Since the world market for scampi is expanding with attractive prices, there is great scope for scampi production and export. In the past, the culture of the species formed a part of the polyculture activity in the freshwater fish farms. Since the prawns fetch 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 (*Penaeus monodon*) aquaculture in India during mid 1990's due to outbreak of diseases, sustainability issues and implementation of Coastal Zone Regulation (CRZ) Act combined with the good demand in domestic and export markets for freshwater prawns, there was an increased interest in the culture of freshwater prawns. This ended up in a sudden spurt in the freshwater prawn farming activities from 2000 onwards (Bojan et al., 2006). Sustained production, infrastructure facilities such as hatcheries, feed units, etc, general resistance to the diseases, comparative easiness in procurement of licenses and permissions, and environmental sustainability are the various reasons attributed to the accelerated development of freshwater prawn farming/ hatchery sector (Bojan et al., 2006).

In India, the white spot disease and slow decline in price of tiger shrimp have caused coastal aquaculture to make a shift to scampi farming. It is estimated that more than 10,000 hectares of water-spread area has been brought under scampi culture in the Nellore District of Andhra Pradesh. Other districts such as Krishna, Parkas, East Godavari and West Godavari in the same state are also taking up scampi culture on a large scale. Andhra Pradesh alone accounts for 67% of the total area and over 85% of the production of the giant freshwater prawn. West Bengal, Orissa, Gujarat and Kerala are close behind in adopting scampi culture (Krishnan and Birthal, 2002)

An increase of 327% in scampi production from scientific farms was registered during the period 1999 - 2000 (7140 MT) to 2002 - 2003 (30,450 MT). 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 documented contributing to 9,264 MT to the export. 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, the production declined to 27,262 MT from an area of 50,206 ha during the year 2007- 08. It can be seen that although a 67% increase in culture area was seen during 2007 - 08, 9.47% decrease in production with a corresponding 27.5% decrease in value was recorded during the same period. This has been mainly attributed to the reduction in the productivity. In 2010-11 period also a decline in the production was recorded. In 2009-10 production was 6568 MT, whereas, it was reduced to 3721 MT in 2010-11. West Bengal is ranked first among the states with a production of 2258 MT during the year 2010-11, while, Kerala ranked fourth (150 MT). In 2008, China alone produced 1, 28,000 tonnes of giant river prawn, accounting for 61.5% of the total production of this species (FAO, 2010).

Table 1.1 Year-wise production and exports of scampi from India (MPEDA, 2010)

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
2008—2009	12806.00	4289.00
2009—2010	6568.00	3394.00
2010—2011	3721.00	2060.82

Table 1.2 State-wise details of aquaculture production of scampi (MPEDA, 2010).

Sl. No	State		2009-10	2010-11
1	West Bengal	AUC	3325	3355
		EP	1725	2258
2	Orissa	AUC	448	516.81
		EP	1725	475
3	Andhra Pradesh	AUC	2823	863.5
		EP	1759	688
4	Tamil Nadu	AUC	162	455
		EP	112	141
5	Kerala	AUC	1379	301.57
		EP	399	150
6	Karnataka	AUC	0	0
		EP	0	0
7	Goa	AUC	0	0
		EP	0	0
8	Maharashtra	AUC	17	19.84
		EP	530	9
9	Gujarat	AUC	0	0
		EP	318	0
Total		**AUC**	**8154**	**5511.72**
		EP	**6568**	**3721**

AUC: Area Under Culture (HA), EP: Estimated Production (MT)

1.9 Biofloc Technology (BFT) and its application for sustainable aquaculture

The growing aquaculture industry is haunted by a number of environmental and social issues (Boyd, 1990). Aquaculture at inappropriate sites can lead to habitat conversion and ongoing operational impacts. Aquaculture potentially has several adverse effects on wild species, including disease transmission, escape and capture for brood stock or rearing among others. Production of nutrient-loaded effluent can lead to eutrophication of nearby waters (Ziemann et al., 1992). Prophylactic use of chemicals, including antibiotics can harm wildlife and the environment, and may lead to antibiotic resistance. Massive water use can result in water shortages as well as salt water intrusion and other hydrological changes or waste disposal issues. Reliance on high protein, fishmeal-based feed for carnivorous species often requires many pounds of wild fish to produce one pound of edible aquaculture product. The conflict over the use and conversion of natural resources as well as access to remaining resources and the privatization of public commons has resulted in physical conflict and even murder in some countries. Inflation in the cost of key local goods (e.g. food, labor, land or other inputs) disproportionately affects those not associated with the industry, particularly the poor. The decline in fisheries in some areas is due to direct environmental impacts of aquaculture or its indirect impacts on the market price of local catch.

Among the major problems facing the aquaculture industry, the treatment and release of farm effluent, high dependence to fishmeal for the preparation of feed and disease outbreak. Inorganic nitrogen species (NH_4^+ and NO_2^-) are the major excretory material in aquatic animals which will get accumulated in the aquaculture system (Colt and Armstrong, 1981) are the crucial issues of concern. Besides the excreta, a major source of ammonium is the typically protein-rich feed. Aquatic animals need a high concentration of protein in the feed, because of their energy production pathways depend, to a large extent, on the oxidation and catabolism of protein (Heaper, 1988). Ammonia is usually the abundant form of combined inorganic nitrogen in aquaculture ponds and it can be rather toxic to animals. Elevated concentrations of ammonia affect growth, moulting (in shellfish), oxygen consumption and even can eventually cause mortality of fish/shellfish. Increased ambient nitrite concentration negatively affects the growth performance and survival of fish/shellfish (Colt and Tchobanoglous, 1976; Colt and Armstrong, 1981; Tucker and Robinson, 1990; Mallasen and Valenti, 2006) and also inhibits the disease resistance of the cultured animals (Brock and Main, .1994). Ammonia in water exists in two forms unionized ammonia and ionised ammonium. Among this unionized ammonia is more toxic when compared to ammonium ion (Boyd and Tucker, 2009). Many researchers made attempt to find the solution for reduce or remove ammonia from aquaculture systems. There are several ways to eliminate ammonia from the aquaculture systems, like exchange and replace the water, use of bioflitration system or establishing a Recirculatory Aquaculture System (RAS), reduce or stop feeding, flush the pond with fresh water, reduce the stocking density, aerate the pond, in emergencies – reduce the pH level but these methods are expensive and some time laborious and economically not feasible or cause harm to the cultured animal (Thompson et al., 2002). The use of RAS has the ability 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).

Biofloc technology is an innovative technology identified for solving the above problems. Microbes like bacteria are generally regarded as disease-causing agents in animals and plants. However, with proper and scientific management, we can utilize the bacterial population effectively. The rapid growth of aquaculture aimed at continued expansion necessary to meet future protein demands will depend upon increasing productivity without overburdening land and water resources, applying sustainable technologies which minimize environmental effects, and developing cost-effective production systems which support economic and social sustainability. Biofloc technology can provide a major contribution towards meeting these goals while producing high quality, safe, attractive and socially acceptable products. Biofloc technologies facilitate intensive culture, while reducing investment and maintenance costs and incorporating the potential to recycle feed. The technology is based upon zero or minimal water exchange to maximize biosecurity while minimizing external environmental effects. Using artificial aeration to meet oxygen demand and suspend organic particles, the development of a heterotrophic microbial community is encouraged in the pond. This diverse microbial community functions to mineralize wastes, improve protein utilization and reduce opportunities for dominance of pathogenic strains. The BFT utilizes the co-culture of heterotrophic bacteria and algae grown in flocs under controlled conditions within the culture pond. Thus microbial biomass is grown on unconsumed feed, fish excreta and inorganic nitrogenous products resulting in the removal of these unwanted components from the water. The major driving force is the intensive growth of heterotrophic bacteria which consume organic carbon (Avnimelech, 1999; Schryver et al., 2008). A biofloc consists of a heterogeneous mixture of microorganisms (floc formers and filamentous bacteria), particles, colloids, organic polymers, cations and dead cells and can reach more than 1000 μm in size. Typical flocs are irregular by shape, have a broad distribution of particle sizes, are fine, easily compressible, highly porous and permeable to fluid. The development of BFT is achieved through sequence of motivation principles and suitable operative technologies. It always aspires for a zero or minimal water exchange, targeted to achieve maximal bio security in the pond and minimize external environmental effect of shrimp culture. In normal conditions, during the closure period of any shrimp pond there will be accumulation of residues and excessive level of organic matter and consequently there will be oxygen depletion. The overriding solution to tide over the situation is the extensive mixing of water; this also helps to minimize sludge accumulation in pond bottom.

Biofloc technology by C/N ratio control: Theory (Avnimelech, 1999).

Bacteria and other microorganisms utilise the carbohydrate source added to the aquaculture system as food, generate energy and grow.

Organic Carbon $\rightarrow$Organic Carbon $CO2$ + Energy + Cassimilation in microbial cells. ----(1)

The percentage of assimilated carbon with respect to the metabolized feed carbon is defined as the microbial conversion efficiency (E) and is in the range of 40-60%. According to the equation and definition of the microbial conversion coefficient, E - the potential amount of microbial carbon assimilation when a given amount of carbohydrate is metabolized (ΔCH), is:

$$\Delta C_{mic} = \Delta CH \times \%C \times E \text{--} (2)$$

Where ΔC_{mic} is the amount of carbon assimilated by microorganism and %C is the carbon contents of the added carbohydrates (roughly 50% for most substrates).

The amount of nitrogen needed for the production of new cell material (ΔN) depends on the C/N ratio in the microbial biomass which is about 4 (Gaudy and Gaudy, 1980).

$$\Delta N = \Delta C_{mic} /[C/N]_{mic} = \Delta CH \times \%C \times E/[C/N]_{mic} \text{-----------------} (3)$$

and (using approximate values of %C, E and $[C/N]_{mic}$ as 0.5, 0.4 and 4, respectively).

$$\Delta CH = \Delta N/(0.5\times0.4/4) = \Delta N/0.05 \text{----------------------------------} (4)$$

According to Eq. (4), and assuming that the added carbohydrate contains 50% C, the CH addition needed to reduce total ammonia nitrogen (TAN) concentration by 1 ppm N (i.e., 1 g N/m^3) is 20 g/m^3.

A different approach is to estimate the amount of carbohydrate that has to be added in order to immobilise the ammonium excreted by the fish or shrimp. It was found that fish or shrimp in a pond (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996) assimilate only about 25% of the nitrogen added in the feed. The rest is excreted as NH or as organic N in feces or feed residue. It can be assumed that the ammonium flux into the water, ΔNH_4 , directly by excretion or indirectly by microbial degradation of the organic N residues, is roughly 50% of the feed nitrogen flux:

ΔN = feed × %N feed = %N excretion ------------------------------ (5)

A partial water exchange or removal of sludge reduces the ammonium flux in a manner that can be calculated or estimated. In zero exchange ponds, all the ammonium remain in the pond. The amount of carbohydrate addition needed to assimilate the ammonium flux into microbial proteins is calculated using Eqs. (4) and (5):

ΔCH = feed × %N feed × %N excretion / 0.05 ---------------------- (6)

The C/N ratio, or the equivalent protein concentration of the feed, can be calculated using the derived Eq. (6). Assuming 30% protein feed pellets (4.65% N) and 50% of the feed nitrogen are excreted (%N excretion) we get:

ΔCH =feed x 0.0465 x 0.5/0.05 = 0.465 x feed -------------------- (7)

According to Eq. (7), the feed having 30% protein should be amended by an additional portion of 46.5% made of carbohydrates with no protein. The corrected protein percentage should accordingly be:

Corrected protein percentage = 30%/1.465 = 20.48%, ---------------(8)

and the original C/N ratio (10.75 in the 30% protein feed) should be raised to 15.75.

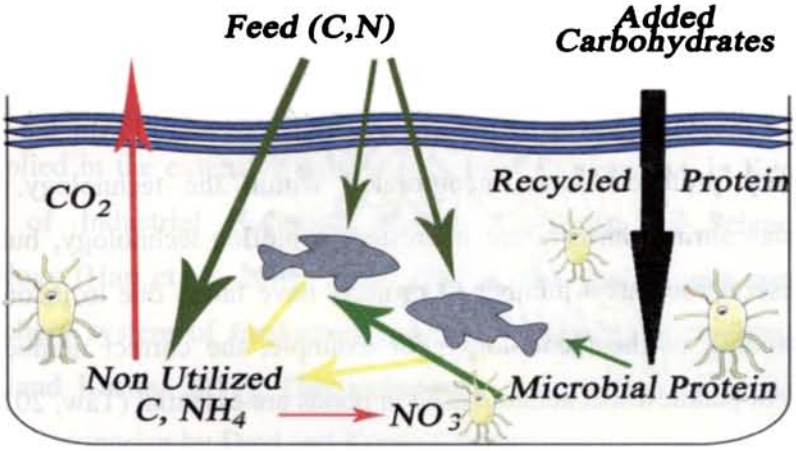

Fig.1.1 Scheme of biofloc technology (BFT) System (Avnimelech, 2009).

1.10 Status of biofloc aquaculture

Biofloc technology has become a popular technology in the farming of tilapia, *Penaeus monodon*, *Litopenaeus vannamei* and *M. rosenbergii*. It was commercially first applied in Belize by *Belize Aquaculture* (N. America). It also has been applied with success in shrimp farming in Indonesia and Australia (Taw, 2010). The combination of two technologies, partial harvesting and biofloc, has been studied in northern Sumatra, Indonesia. The number of shrimp farms currently using biofloc technology is not known, but some prominent examples are *Belize Aquaculture Ltd.*, in Belize and *P.T. Central Pertiwi Bahari* in Indonesia. The success or failure of the technology is mainly due to the degree of understanding of basic concepts of the technology in commercial application. *Belize Aquaculture* was the first commercial farm to use biofloc technology successfully. Its production of 13.5 MT shrimp/ha was quite an achievement at the time. The Belize technology was applied initially in Indonesia at *C.P. Indonesia* (now *P.T. Central Pertiwi Bahari, C.P. Indonesia*), which achieved average production over 20 MT/ha in commercial 0.5-ha lined ponds. Research trials reached 50 MT/ha. The technology combined with partial harvest was repeated in Medan, Indonesia, with better results. During 2008 and 2009, biofloc technology was used in Java and Bali successfully. In Indonesia, biosecurity protocols were incorporated within the technology. Most Indonesian shrimp farmers are interested in biofloc technology, but with some reservations, as a number of projects have failed due to incomplete understanding of the technology. For example, the correct number and position of paddlewheel aerators used in ponds are essential (Taw, 2010).

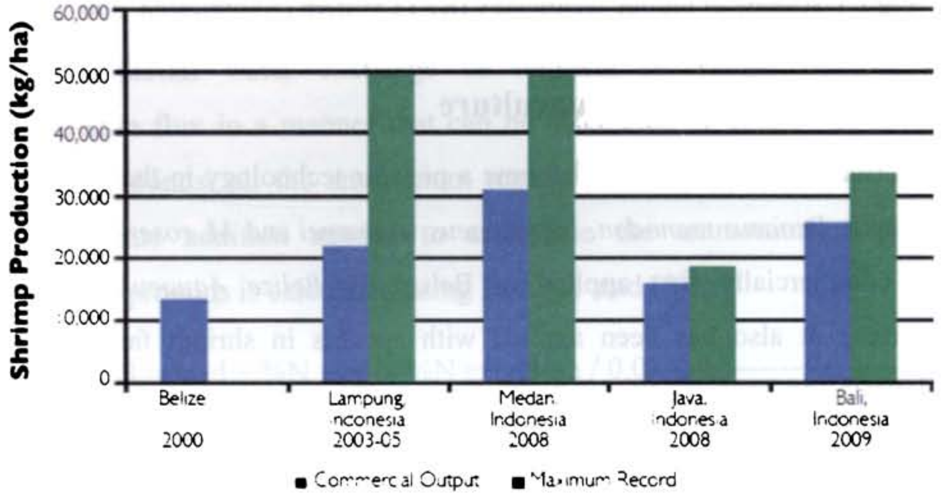

Fig 1.2 Shrimp production levels at various farms implementing biofloc technology (Taw, 2010)

Due to success stories in Indonesia and the United States, many shrimp farmers are now interested in biofloc technology. In China also a number of shrimp farmers are interested in adopting this technology. Their fully HDPE-lined, plastic-covered shrimp grow-out ponds with high-density culture are ideal for the technology. A group from Brazil is running commercial biofloc trials. Malaysia is currently initiating a 1,000-ha integrated intensive shrimp-farming project at Setiu, Terengganu by Blue Archipelago. The company also plans to use this technology.

In India, biofloc technology is not yet popular. This technology was first applied in the extensive culture system of *P. monodon*, in Kerala, by School of Industrial Fisheries, Cochin University of Science and Technology (Hari et al., 2004, 2006). BFT is also applied with success in the hatchery system of freshwater prawn, *M. rosenbergii* (Saritha, 2009; Saritha and Kurup, 2011). This technology also applied in the hatchery phase of *P.monodon* by Devi and Kurup (2011).

1.11 Hypothesis, objective and outline of the thesis

In India a study conducted by CIFE and CIBA (1997), concluded that shrimp farming does more good than harm and it is not eco-unfriendly (Krishnan and Birthal, 2002). Upsurge in coastal aquaculture activity induced by high profitability is reported to have caused adverse impacts on coastal ecosystems and social environments (Parthasarathy and Nirmala, 2000). The crustacean farming sector has received criticism for excessive use of formulated feed containing high protein, of which around 50% gets accumulated at the pond bottom as unconsumed (Avnimelech, 1999; Hari et al., 2004, 2006). The wasted feeds undergo the process of degradation and results in the release of toxic metabolites to the culture system. Reduction of protein in the feed, manipulation and utilisation of natural food in the culture system are the remedy for the above problems. But before reducing the feed protein, it should be confirmed that the feed with reduced protein is not affecting the growth and health of the cultured animal. In the present study, biofloc technology is identified as one of the innovative technologies for ensuring the ecological and environmental sustainability and examines the compatibility of BFT, for the sustainable aquaculture of giant prawn, *M. rosenbergii.*

This thesis starts with a general introduction (Chapter-1), a brief review of the most relevant literature (Chapter-2), results of various experiments (Chapter-3-6), summary (Chapter-7) and recommendations and future research perspectives in the field of biofloc based aquaculture (Chapter – 8). The major objectives of this thesis are, to improve the ecological and economical sustainability of prawn farming by the application of BFT and to improve the nutrient utilisation in aquaculture systems.

The specific objectives of the present study can be outlined as

1. Application of BFT in the nursery phase of giant freshwater prawn, *Macrobrachium rosenbergii,* and its effect on animal welfare and survival
2. Effect of application of biofloc technology in the grow-out system of giant freshwater prawn, *Macrobrachium rosenbergii*
3. Efficacy of various carbohydrate sources as biofloculating agent in the culture system of giant freshwater prawn, *Macrobrachium rosenbergii,* and its effect on water quality and production
4. Application of BFT on the polyculture system of giant freshwater prawn, *Macrobrachium rosenbergii,* with two Indian major carps

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Chapter 2

REVIEW OF LITERATURE

Contents

2.1 Sustainable aquaculture

Approximately 16% of animal protein consumed by the world's population is originated from fish, and over one billion people worldwide depends on fish as their main source of animal protein (FAO, 2000). Aquaculture offers one way to supplement the production of wild capture fisheries and it will continue to increase in importance as demand increase in future (White et al., 2004). The ever growing demand for seafood leads to the intensification of aquaculture through high stocking density and intensification of the artificial feeds, leading to the aquaculture sector as most cost-effective as well as waste promoting industry. Like other form of intensive food production, industrial-scale fish farming generates significant environmental costs (White et al., 2004). Aquaculture development should be in a sustainable manner. Sustainable development is defined as the management and conservation of the natural resource-base and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development conserve land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable and socially acceptable (FAO, 1991).

2.2 Environmental problems of aquaculture

Aquaculture production has increased at an average annual rate of 8.9% since 1970, as compared with an annual growth rate of 1.2% and 2.8% for capture fisheries and terrestrial farmed meat production over the same frame. Yet, to supply demands, aquaculture production must grow by five fold in the next five decades. This development has to overcome three major constraints: a) Produce more fish without significantly increasing the use of basic natural resource of water and land, b) Develop sustainable systems that will not damage the environment, c) Develop systems providing a reasonable cost/benefit ratio, to support the economic and social sustainability of aquaculture (Avnimelech, 2009).

Alagarswami (1995) identified adverse impacts of aquaculture on social and physical environments and emphasized the need to adopt eco-friendly technologies. Intensive aquaculture systems are used to efficiently produce dense biomasses of fish or shrimp, intensive aquaculture industry faces two major problems. The first is the water quality deterioration caused by the high concentrations of metabolites and the second is the low feed utilization in cases when high water exchange, within or outside the pond system, is practiced (Avnimelech, 2007). Artificial formulated feed is the main investment in aquaculture i.e.; feed is the largest single cost item, as it constitutes 40-60% of operational cost in prawn production (Mitra et al., 2005).

The major portion (>80%) of artificial feed is lost in the aquaculture system as uneaten feed and faeces (Daniels and Boyd, 1989; Siddiqui and Al-Harbi, 1999; Rahman, 2006). Artificial feed, which is lost in the system, has a great effect on water quality through decomposition (Horner et al., 1987; Poxton and Allouse, 1987; Cowey and Walton, 1989; Poxton and Lloyd, 1989; Wilson, 1994; Moreira et al., 2008). Higher dietary protein deteriorates the water and soil qualities in shrimp grow-out ponds (Boyd, 1989). To great extent water quality determines the success or failure of aquaculture operation. Physical and chemical characteristics such as suspended solids, temperature, dissolved gases, pH, mineral content and the potential danger of toxic metals must be considered in the selection of a suitable water source (Boyd and Tucker, 2009).

The salmon and shrimp aquaculture have proven to be destructive to the natural environment and population of aquatic animals (Gowen and Bradbury, 1987; Folke et al., 1994; Kautsky et al., 1997; Naylor et al., 2000; Milewski, 2001). Crustacean aquaculture is fraught with environmental problems that arise from: (i) the consumption of resources such as land, water, seed and feed; (ii) their transformation into products valued by society; and (iii) the subsequent release into the environment of wastes (Kautsky et al., 2000; Ronnback, 2001). The direct impacts include release of eutrophicating substances and toxic chemicals, the transfer of diseases and parasites to wild stock, and the introduction of exotic and genetic material into the environment. The environmental impact can also be indirect through the loss of habitat and niche space, and changes in food webs. Whereas traditional and extensive shrimp aquaculture uses natural production in the ponds or in the incoming waters, semi-intensive and intensive production systems are heavily dependent on formulated feeds based on fishmeal and fish oils. These latter systems use more than two times more protein, in the form of fishmeal, to feed the farmed shrimps than is ultimately harvested (Tacon, 1996). Most aquaculture systems are so-called throughput systems (Daly and Cobb, 1989). This means that resources, collected over large areas, are introduced and used in the aquaculture production site, and released back into the environment in concentrated forms as nutrients and pollutants, causing various environmental problems (Folke and Kautsky, 1992). Uneaten food, faecal and urinary wastes may lead to eutrophication and oxygen depletion, the magnitude of which is dependent on the type and size of operation as well as the nature of the site, especially size, topography, and water retention time (Kautsky et al., 2000). In semi-intensive and intensive farms, artificial feeds provide most of the nitrogen (N), phosphorus (P) and organic matter inputs to the pond system. Only 17% (by dry weight) of the total amount of feeds applied to the pond is converted into shrimp biomass (Primavera, 1993). The rest is leached or otherwise not consumed, egested as faeces, eliminated as metabolites, etc. Effluent water during regular flushing and at harvest can account for 45% of nitrogen and 22% of organic matter output in intensive ponds (Briggs and Funge-Smith, 1994). Consequently, pond sediment is the major sink of N, P and organic matter, and accumulates in intensive shrimp ponds at the rate of almost 200 t (dry weight) per ha and production cycle (Briggs and Funge-Smith, 1994). During pond preparation between cropping the top sediment is removed and usually placed on pond dikes, from where it continuously leaks nutrients to the environment. Several methods have been proposed to ameliorate the impact of shrimp pond effluents on the water quality of the recipient: improved pond design (Dierberg and Kiattisimkul, 1996); construction of waste-water oxidation-sedimentation ponds, reduction of water exchange rates (Hopkins et al., 1995); reduction of nitrogen and phosphorus input from feed (Jory, 1995); removal of pond sludge; a combination of semi-closed farming systems with settling ponds and biological treatment ponds using polycultures (Dierberg and Kiattisimkul, 1996; Troell et al., 1999) and the use of mangroves as biofilters for pond discharge prior to the release of effluent to estuarine waters (Robertson and Phillips, 1995). Furthermore, the use of fertilisers should be restricted to organic products.

In response to a public interest petition, the Supreme Court of India in 1996 directed the concerned authorities to abolish aqua-farms in the coastal regulation zone and to constitute an "Authority" to regulate aquaculture (Krishnan and Birthal, 2002). The Aquaculture Authority of India (AAI) has been constituted and guidelines on sustainable aquaculture development for regulating coastal aquaculture. By The Coastal Aquaculture Authority Act, 2005 enacted by the Central Government on 23 June 2005 the AAI was restructured to the Coastal Aquaculture Authority for regulating the activities connected with coastal aquaculture in coastal areas and matters connected therewith or incidental thereto. For making the aquaculture practices sustainable in the country, the Coastal Aquaculture Authority is giving directions, guide line and best management practices. According to Coastal Aquaculture Authority, activities such as construction of aquafarms in mangrove areas, conversion of agricultural field to aquaculture, use of ground water for aquaculture, collection and stocking of wild seeds, use of banned chemicals and drugs, releasing farm effluent into the natural aquatic environment, etc. are prohibited. CAA recommends maintenance of a buffer zone between farm and village facility, proper pre-stocking procedure, use of healthy and quality seeds from approved hatcheries, monitoring of soil and water quality at regular intervals, practicing suitable and recommended stocking density, raising seaweeds, mangrove saplings and bivalves in waste stabilization ponds and outflow canals.

2.3 Concept of biofloc technology and its application in aquaculture systems as a tool for waste management

Knoesche and Tscheu (1974) already adopted the idea of intensive heterotrophic bacteria growth in aquaculture systems and could retain 7% feed N and 6% feed P (estimated from 1% P feed, *KarpiCo Supreme-7Ex, Coppens International*, The Netherlands). They used an activated sludge process to treat water in a recirculation system, and proposed to mix produced sludge with grains for later re-use as fish feed for carps. The principles of growing fish or shrimp in limited water exchange intensive ponds were developed simultaneously for shrimp in the Waddel Mariculture Centre in the USA and for fish, mostly tilapia, in Israel (Avnimelech et al., 1989, 1994; Hopkins et al., 1993; Chemberlain and Hopkins, 1994) and practiced in the USA (Serfling, 2000), in the beginning of the 1990's. The idea of addition of carbohydrate for the immobilization of ammonia excreted by the fishes was suggested by Avnimelech and Lacher (1979), Boyd (1985), and Muthuwani and Lin (1996). But idea about the general water quality of the pond is essential before any modification or manipulation in aquaculture systems (Boyd and Tucker, 2009).

Good water quality is the key factor for the success of aquaculture and that ensures the survival, production and growth rate of the cultured animals (Boyd, 1990; Burford, 1997). Biofloc technology, BFT, (called also active suspension ponds, heterotrophic ponds, green soup and other terms) was first developed to solve water quality problems. Water quality management is based upon developing and controlling dense heterotrophic bacteria within the culture component (Avnimelech, 2007). The addition of fertilizers or carbon sources directly to the pond water is a way to augment the natural productivity (Crab et al., 2007; Uddin et al., 2007). The removal of toxic nitrogenous compounds, especially ammonium, from water through its assimilation into microbial protein by the proper addition of carbonaceous materials to the culture system is the basic principle of biofloc technology. The success of this technology mainly depend on the selection of species, because the cultured animal should have the ability to harvest the bacterial floccules developed in the system, and should have the ability to digest and utilise the microbial protein. The bacterial floc produced as the result of biofloculation serve as an important source of feed protein. Experimental trials showed that microbial flocs of different sizes can be taken up by fish or shrimp and serve as a feed source (Avnimelech et al., 1989; Beveridge et al., 1989; Rahmathulla and Beveridge, 1993; Tacon et al., 2002; Burford et al., 2004) which will help to reduce the cost of production by reducing the protein content of the artificial feed and improving the overall economics (Mc Intosh, 1999; Moss, 2002).

Avnimelech (2007) schematically represented and explained the process behind biofloc production and harvest by the fishes as

$$D[BF] / dt = BF_{production} - (BF_{harvesting} + BF_{degradation})$$

Where $D[BF] / dt$ is the bio-floc concentration change with time, as affected by production, harvesting by fish and biodegradation. The process shown in this equation depends on a verity of factors:

1) Production of biofloc depends on the supply of organic substrates to the microbial community, both external sources (feed supply, algal activity) or by the excretion of un-utilized feed components by fish. In addition bioflocs production most probably depends on the quality of the added substrates, its C/N ratio, bio-availability and other factors.

2) Uptake of the bio-flocs by fish depends most probably on the fish species and feeding traits, fish size, floc size and floc density. It is possible that bioflocs harvesting depends also on the presence and rate of formulated feed added to the pond. In addition, feed eaten by the fish may be utilized and accumulate in the fish, or excreted and serve as a substrate for the production of more bioflocs.

3) Biodegradation of the floc depends on the microbial community associated with the bioflocs, be it bacteria, protozoans or others.

4) Finally, all of these processes may be affected by environmental and operational conditions such as temperature, water salinity, water exchange rate (affecting floc mean residence time), mixing intensity and many other.

Biofloc aquaculture is a sustainable solution for the development of aquaculture and this technology is fully based on the concept of carbon nitrogen (C/N) ratio. The control of inorganic nitrogen accumulation in the pond is based upon carbon metabolism and nitrogen immobilization into microbial cells (Avnimelech et al, 1989; Avnimelech, 1999; Crab et al., 2007). Bacterial cells are composed of proteins. carbon nitrogen ratio of most microbial cell is about 4-5 (Rittmann and Mc Carty, 2001). When bacteria fed with organic substrates that contains mostly carbon and little or no nitrogen (sugar, starch, molasses, cassava meal, etc.), they have to take up nitrogen from the water in order to produce the protein needed for the cell growth and multiplication. C/N ratio in an aquaculture system can be increased by the addition of cheap carbohydrate source or reduction of protein content in the feed (Avnimelech, 1999; Hargreaves, 2006).

Avnimelech and Mokady (1988) and Avnimelech et al. (1992) suggested managing the heterotrophic food web by the development of active suspension ponds, intensive ponds with zero or limited water exchange, accumulating high amounts of organic substrates.

In summary, inorganic nitrogen accumulation is controlled through the addition of carbonaceous substrates, raising the C/N ratio and leading to the immobilization of nitrogen through the production of microbial proteins (Avnimelech et al., 1989, 1992, 1994; Crab et al., 2007).

Fish harvest the bacteria, as bacterial flocs and utilize this protein source (Avnimelech et al., 1989; Milstein et al., 2001; Mc Intosh, 2000a).The amount of carbohydrate needed for reducing the ammonium was worked by Avnimelech (1999). The equation proved its success both in indoor and farm level experiments by various researchers (Hari et al., 2004, 2006; Varghese, 2007; Saritha, 2009).

Kurup (2009) summarised the advantages of the application of BFT to shrimp culture systems in India 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 in environmentally and economically sustainable system

Minimal-exchange, intensive aquaculture systems offer an environmentally attractive means of shrimp and fish production, allowing for high density culture and little or no water exchange (Ray et al., 2010b). The basic principle of the activated suspension technique and the C/N ratio controlled systems were recently referred as biofloc technology (BFT) is the retention of waste and its conversion to biofloc as a natural food within the culture system (Azim and Little, 2008). One of the features of natural aquatic environment is the ability to recycle the nutrients. Microbial floc generally consists of floc formers, filamentous bacterial particles, protozoans, zooplankton, colloids, organic polymers, cations and dead cells surrounded by a gelatinous matrix, which contains extra-cellular polymeric substances that encapsulate the microbial cells and play a major role for binding the floc components together (Jorand et al., 1995; Avnimelech, 2007). The grouping of bacteria within the floc mainly depends upon the zeta potential and Van der Waals forces (Sobeck and Higgins, 2002). Typical bacterial flocs are irregular by shape have a broad distribution of particle size, are fine compressible, highly porous and permeable to fluids (Chu and Lee, 2004). Accumulation of toxic inorganic nitrogen species (NH_4, NO_2) is prevented in bio-flocs system by maintaining a high C/N ratio and inducing the uptake of ammonium by the microbial community (Avnimelech et al., 1994; Mc Intosh, 2000b). Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in labscale sequencing batch reactor was studied by Schryver and Verstraete (2009).

Sesuk et al. (2009) demonstrated Inorganic nitrogen control in a novel zero-water exchanged aquaculture system integrated with airlift-submerged fibrous nitrifying biofilters. The microorganisms not only remove excess nutrients, but have been implicated in nutritional provision for animals, including shrimp and tilapia, that can result in improved growth rate, feed conversion ratio (FCR) and weight gain (Moss and Pruder, 1995; Burford et al., 2004; Wasielsky et al., 2006 Azim and Little, 2008). This process was quantitatively formulated (Avnimelech, 1999), verified and practiced by farmers world-wide (Browdy et al., 2001; Panjaitan, 2004).

There are several factors that influencing floc formation and floc structure (Schryver et al., 2008). Ritvo et al. (2003) studied the salinity and pH effect on the colloidal properties of suspended particles in super intensive aquaculture systems. The mixing intensity imposed by a chosen aeration device at a certain power input will determine the steady-state floc size, this is in equilibrium between the rate of aggregation and the rate of breakage, and floc size distribution (Spicer and Pratsinis, 1996; Chaignon et al., 2002). Dissolved oxygen is another factor that influences the bioflocs. Previous studies revealed that biofloc with a higher floc volume index (FVI) are produced at lower dissolved oxygen levels. The organic carbon source of choice will to a large degree determine the composition of floc produced (Mikkelsen et al.,1996; Hollender et al., 2002; Oehmen et al., 2004). Organic loading rate, temperature and pH are the other factors that affect the biofloc formation (Schryver et al., 2008). pH level has a significant role in the floc formation both qualitatively and quantitatively in the culture of *P. monodon,* pH of 7.5 is found to be optimum for better biofloculation in shrimp aquaculture (Devi, 2009; Kurup and Devi, 2010).

Schneider et al. (2006) used molasses as carbon source for heterotrophic bacteria production on solid fish waste. Crab et al. (2010a) monitored effect of different carbon sources on the nutritional value of biofloc, in the culture system of *M. rosenbergii* post-larvae. In that experiment flocs were grown on acetate, glycerol and glucose as sources. Varghese (2007) evaluated performance of various locally available carbohydrate sources for biofloculation the culture system of *P. Monodon*; tapioca powder was the biofloculating agent selected by Hari et al. (2004, 2006), Varghese (2007), and Kurup and Varghese (2007a, 2007b).

Initial studies were conducted to optimize the addition of carbohydrate source in aquaculture systems for the process of biofloculation and standardize the quantity of carbohydrate source required for biofloculation. Mechanism and principle behind this technology was also interesting field of research (Avnimelech, 1999, 2009). Eminent Israeli scientist Yoram Avnimelech is the pioneer in this field. Species selection is one of the major factors that decide the success of the BFT aquaculture (Avnimelech, 2007; Azim and Little, 2008). BFT is proved to be beneficial for both shrimp and finfish culture (Milstein et al., 2001; Burford et al., 2003; 2004; Serfling, 2006; Wasielsky et al., 2006). Tilapia was the first studied species for the biofloc ponds, the nibbling habit and feeding behaviour were the reasons for the selection of this fish. Later, marine shrimps were also identified as the suitable species for BFT aquaculture (Burford et al., 2003, 2004; Wasielsky et al., 2006; Hari et al., 2004, 2006; Varghese, 2007; Kurup and Varghese, 2007a, 2007b; Arnold et al., 2009; Ballester et al., 2009; Kuhn et al., 2009).

Studies by Avnimelech (2007) revealed that microbial flocs developing in BFT ponds are effective potential food source for tilapia; according to his observation, fish growing in the BFT pond did not rush on to the added feed pellets, since the pond contained flocs as a potential feed, feed that is available 24 hours per day. Avnimelech et al. (1994) estimated that feed utilization is higher in BFT systems, while tilapia in such ponds is fed a ration 20% less than conventional one. Varghese (2007) studied various aspects of BFT in the culture of *P. monodon*. Optimization of protein in the feed by the application of C/N ratio, effect of various modes of carbohydrate application, on farm application of BFT, optimization of stocking density in BFT tanks, performance of carbohydrate sources, and combined application of BFT with periphyton based aquaculture were the focal theme of the study. The author summarises the study as, C/N ratio optimization by the addition of suitable carbohydrate sources is a potential method for controlling the inorganic nitrogen species in aquaculture systems.

Kurup and Varghese (2007a), and Varghese (2007) explained the suitability of various carbohydrate sources for controlling C/N ratio in shrimp culture system. They also studied the combined effect of BFT and periphyton based aquaculture. Biofloc can act as a feed for different cultured species such as Nile tilapia (*Oreochromis niloticus*) and whiteleg shrimp, *L. vannamei* (Azim and Little, 2008; Crab et al., 2009; Kuhn et al., 2009). Burford et al. (2004) suggested that "flocculated particles" rich in bacteria and phytoplankton could contribute substantially to the nutrition of *L. vannamei* in intensive shrimp ponds. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for *L. vannamei* was studied by Wasielsky et al. (2006).

Several researchers followed the uptake of microbial protein through the use of 15N enriched microbial biomass (Preston et al., 1996; Epp et al., 2002; Burford et al., 2004; Avnimelech, 2007). Avnimelech and Kochba (2009) conducted a detailed study on the uptake and utilization of microbial protein by tilapia, using N15 tagging to get data on the dynamics of the biofloc system and to critically evaluate the available experimental procedures. Authors also estimated the residence time of bioflocs. It was estimated that the residence time of bioflocs was about 8 hours, i.e. bioflocs were regenerated three times a day.

Ray et al. (2010b) characterized microbial communities in minimal-exchange, intensive aquaculture systems and studied the effects of suspended solids management. Characterisation of microbial community structure in shrimp biofloc cultures using biomarkers and analysis of floc amino acid profile was done by Ju et al. (2008). Ray et al. (2010 a) also studied the mechanism behind the suspended solids removal to improve production of *L. vannamei* and part of the same study the team has evaluated a plant based feed in minimal-exchange, super intensive culture systems. According to their study, shrimp biomass production (kg/m^{-3}) was increased 41% when biofloc concentration was managed through the use of external settling chambers. They also showed a 60% reduction in nitrate-nitrogen concentration and a 61% reduction in phosphate concentration when biofloc concentration was managed.

Michaud et al. (2006) studied the effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Addison et al. (2010) studied the affect of biofloc replaced fish meal and soybean meal in semi-purified shrimp diets. Ju et al. (2008) studied the enhanced growth effect on shrimp (*L. vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. The results suggest that inclusion of the floc material in shrimp diets could enhance the shrimp growth. They observed enhanced growth when compared with that of the control ($P<0.05$); the diet preference and pellet stability study were also positive. Shrimp preferred the experimental diets over commercial diet and the feed stability was same as that of commercial diet. Addison et al. (2010) reported increased growth of *L. Vennamei* by replacing fishmeal and soybean with biofloc. Microbial flocs produced in suspended growth bioreactors could offer the shrimp industry a novel alternative feed. Study by Kuhn et al. (2009) showed that bioflocs harvested from the sequencing batch reactors (SBRs) and membrane biological reactors proved to be a suitable and often superior ingredient, to soybean protein and fishmeal in lab-scale feeding trials with *L. vannamei.* High quality control diets were compared against experimental diets in 35-day feeding trials. Result was that experimental diets were varied greatly and notable independent variables included complete replacement of soybean protein, two-thirds replacement of fishmeal, and no fish oil. Biofloc inclusion always increased growth rates and ranged from a low average increase of 4% to a high average of 67% over the control diets; the latter percent increase was significant at $P < 0.01$. It seems that biofloc technology represents a promising option for sustainability of the aquaculture industry. Ballester et al. (2010) compared the performance of *Farfantepenaeus paulensis* reared with partial diet with different protein levels under zero exchange microbial floc intensive system. Forty five days experimental trial concluded that shrimps fed with a protein percentage of 35 which are maintained in biofloc ponds showed maximum growth parameters. It was significantly greater when compared to the feed with 25, 30, 40 and 45% of crude protein. Kuhn et al. (2010) compared the possibilities of incorporating two types of microbial flocs in the feed of *L. vannamei* derived from biological treatment of fish effluent. Logan (2010) presented the possibilities of commercializing the biofloc feeds in recent World Aquaculture Society conference held at California, the product is expected to be available in the market by 2012 as the trade name *Obron SCP ingredient.*

In addition to being a tool for water quality and feed management, biofloc technology also has potential to protect the cultured organisms from infections with pathogenic bacteria, which are responsible for major economic losses in aquaculture (Crab et al., 2010b). Biofloc can be a novel strategy for disease management on a long-term basis, in contrast to conventional approaches such as antibiotic, probiotic and prebiotic application (Sinha et al., 2008). Recent research questions focus on the possible extra added value of bioflocs, more specifically regarding pathogen control, because infectious diseases burden the aquaculture industry. It was observed that the regular addition of carbon to the culture is known to select for polyhydroxyalkanoate (PHA) accumulating bacteria (Salehizadeh and Von Loosdrecht, 2004) such as *Alcaligenes eutrophus, Azotobacter vinelandii, Pseudomonas oleovorans* and others that synthesise PHA granules. Such granules are synthesised under conditions of nutrient stress, that is, when an essential nutrient like nitrogen is limited in the presence of an excess carbon source (Avnimelech, 1999). These PHAs are polymers of β-hydroxy short chain fatty acids and if degraded in the gut, they could have antibacterial activity similar to short chain fatty acids (SCFAs) or organic acids. The breakdown of PHA inside the gastrointestinal tract can be carried out via enzymatic and chemical hydrolysis (Yu et al., 2005). Apart from inhibiting the growth of pathogenic bacteria by lowering the pH of surrounding milieu, SCFA have also been shown to specifically down-regulate virulence factor expression and positively influence the gut health of animals (Teitelbaum and Walker, 2002). Moreover, these compounds are capable of exhibiting bacteriostatic and/or bacteriocidal properties, depending on the physiological status of the host and the physiochemical characteristics of the external environment (Ricke, 2003). Defoirdt et al. (2006) reported increased survival of artemia nauplii when fed formic, acetic, propionic, butyric and valeric acid and challenged with a luminescence pathogenic *Vibrio campbellii* strain. In another study, the same authors (Defoirdt et al., 2007) reported that commercial polyhydroxy butyrate (PHB) particles or PHB accumulating bacteria offered a preventive and curative protection to artemia against luminescent vibriosis. Crab et al. (2010b) studied the use of bioflocs as new bio-control agents for aquaculture using a model system with gnotobiotic brine shrimp (*Artemia franciscana*). They found that the bioflocs can also be used by brine shrimp (*A. franciscana*) nauplii as a feed. The technique has also proved to be beneficial in the nursery rearing of *P. monodon*, wherein 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; Anjalee and Kurup, 2011).

2.4 Application of biofloc technology in giant freshwater prawn aquaculture

Eventhough more than 25 species of freshwater prawns have been reported from Kerala, *M. rosenbergii* is the only species with aquaculture importance. There is a vast potential for the development of prawn culture and nearly 65,000 ha comprising ponds, tanks and check dams are found suitable for freshwater prawn farming in monoculture or polyculture with carps (Kumar and Velayudhan, 2006). 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 potential areas for culture purposes as denoted by the increase in the area brought under the culture. Raising of freshwater prawn production through expansion of pond area would demand large additional quality of water and land area, both are very scarce resources. The most practical way to raise freshwater prawn production is by increasing pond productivity per unit land area and water (Asaduzzaman et al., 2008). *M. rosenbergii* is omnivore in the feeding behaviour and is more or less similar to the feeding of the tiger shrimp, *Peanaeus monodon*. The benefits of BFT has been described extensively for shrimp culture (Buford et al., 2003, 2004; Hari et al., 2004, 2006; Wasielsky et al., 2006) and for finfish culture (Avnimelech, 1999; Avnimelech, 2007; Milstein et al., 2001; Serfling 2006). There are only few works on its applicability and advantages of the technology to *M. rosenbergii* grow outs (Asaduzzaman et al., 2008, 2009a, 2009b 2010a, 2010b) and in larviculture (Saritha, 2009; Saritha and Kurup, 2011).

Asaduzzaman et al. (2008, 2009a, 2009b, 2010a, 2010b) conducted a series of experiments on diversified application of C/N ratio control in giant freshwater prawn culture. In 2009, the research team studied the effects of addition of tilapia, *Oreochromis niloticus*, and substrates for periphyton developments on pond ecology and production in C/N-controlled *M. rosenbergii* farming systems. In the same year the team investigated effects of stocking density of *M. rosenbergii* and addition of different levels of *Oreochromis niloticus* on production in C/N controlled periphyton based system. Asaduzzaman et al. (2010a) explained the effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. Asaduzzaman et al. (2010b) investigated the possibilities of combining C/N ratio control, fish-driven re-suspension and periphyton based aquaculture in freshwater prawn culture with varying stocking densities of tilapia and *Labeo rohita*. They also studied the effect of two carbohydrate sources for controlling the C/N ratio in the culture pond. In the same year, the team scanned various carbohydrate sources for maintaining a high C/N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems.

The biofloc technology has been widely used in shrimp culture for reducing the accumulation of toxic metabolites like ammonia by its conversion to heterotrophic bacterial biomass, especially in static systems. The modified static green water system of seed production of *M. rosenbergii* faces the problem of elevated levels of metabolites like ammonia and nitrite. Saritha (2009), Kurup and Saritha (2010) and Saritha and Kurup, 2011) made an attempt to evaluate the effectiveness of biofloc technology in larval rearing of the giant freshwater prawn wherein the quantity of carbohydrate addition has been optimised to assimilate the toxic metabolites generated and consequently converting them into bacterial floccules.

Application of BFT making culture more sustainable by 54% increase of total revenue from the harvested shrimp, reduce water based nitrogen species discharge to the environment and also reducing the protein content of the feed substantially (Kurup, 2009). Enhanced pond productivity through stimulation of suspended and attached algal development and by using them to improved water quality, provide additional food, higher carrying capacity in combination with reduced nutrient discharge, improving environmental sustainability. This technology is simple and cheap, making it also socially and economically sustainable, even for small scale or poor farmers (Hari et al., 2004, 2006; Varghese, 2007; Kurup, 2009).

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Chapter 3

APPLICATION OF BIOFLOC TECHNOLOGY IN THE PRIMARY NURSERY REARING OF GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* IN VARYING STOCKING DENSITY: EFFECT ON ANIMAL WELFARE AND SEED QUALITY

Contents

3.1 Introduction

The aquaculture of giant freshwater prawn comprising hatchery, nursery and growout system. Each stage has its own important role for the success in production. The intermediate stage between the larval rearing and growout is referred as nursery system (Ling, 1969; New and Valenti, 2000). This stage generally results in higher production and/or a potentially higher value of harvested prawns (Sandifer and Smith, 1975; Ra'anan and Cohen, 1982, 1983). Studies showed that nursery system helps to ensure the optimal use of growout ponds for larger prawns and to increase the efficiency of food use (New and Singholka, 1985). In order to achieve higher survival and reduce the grow-out period an intermediate nursery phase is essential (Soundarapandian and Kannan, 2008). Nursery rearing is beneficial because the farmer has more control over water quality, feeding schedule, diet and predators (Alson, 1989).

The production costs in the inland nursery are usually higher due to the frequent water exchange. In order to reduce both production cost and environmental pollution, the strategy of wastewater reuse should be encouraged in the inland hatchery and nursery (Liu and Han, 2004). Application of biofloc technology will solve the problem of water exchange (Avnimelech, 1999; Kurup, 2009). There are mainly two types of contaminants that emerge in the nursery rearing systems of prawns, viz., dissolved organics leaching from faeces or uneaten feed and ammonia-nitrogen excreted by shrimps, rotifers or *Artemia sp.* (Burford and Glibert, 1999; Burford and Williams, 2001; Jackson et al., 2003). BFT already has proven its capacity to reduce toxic metabolite compounds in the freshwater prawn hatchery and growout systems (Varghese, 2007; Saritha, 2009; Asaduzzaman et al., 2008, 2009a, 2009b, 2010a, 2010b). When the biofloc technology is applied, it is necessary to ensure that animals are not under any stress or they sustain and survive well in the manipulated pond conditions. Assessment of the health and fitness of the crustacean larvae has become of increasing importance, particularly for shrimp and prawn farming industry. The quality of the animal stocked initially can significantly affect pond yields (Tayamen and Brown, 1999). There are many ways to assess the animal welfare. Biochemical indicators are valuable tools in the evaluation of organism health and environmental quality of its associated habitat (Dahlhoff, 2004; Gilliers et al., 2004; Amaral et al., 2008). The RNA/DNA ratio has been especially useful as an index of physiological condition of marine invertebrates and fish (Wright and Hetzel, 1985; Gilliers et al., 2004; Amaral et al., 2008).

Stocking density is another important factor that decides the success of aquaculture practices. In primary nursery phase of giant freshwater prawn, stocking the prawns at optimum number will be beneficial for ensuring higher production. Post-larval nursery culture can be achieved in four different systems, viz, indoor, outdoor, cages and multiphase nurseries. In general, nursery systems may be one-phase two-phase or multiphase operations. One and two phase operations may combine outdoor and indoor systems (New and Valenti, 2000). Primary nursery system (indoor) of giant freshwater prawn, which is referred as the extended larval rearing phase (Cohen and Ra'anan, 1989) was selected for the present study.

Biofloc technology proved success in the growout and hatchery production of freshwater prawn, but there is no work on the effect of this technology in the nursery phase of prawns. The present experiment is the pioneer attempt to apply BFT in the nursery phase of freshwater prawn. The experiment was framed with the following objectives.

- To find out the effect of BFT in the indoor primary nursery rearing of *M. rosenbergii*
- To optimize the stocking density in the BFT applied nursery rearing of giant freshwater prawn
- Study the influence of BFT in the animal welfare, seed quality and survival

3.2 Materials and methods

3.2.1 Tank allocation

Completely randomized designed experiment with three different levels of stocking density under biofloc system and non-biofloc system was carried out to find out the effectiveness of BFT under varying stocking density in the primary nursery rearing system of giant freshwater prawn. Triplicates were maintained for all the treatments. Experiment was carried out in 100 litre capacity Fiber Reinforced Plastic (FRP) tanks, inner side of which was painted white. Tanks were filled with tapwater with a depth of 50 cm. All tanks were facilitated with 2 air stone-hoses type of diffuser system which is fitted to 5HP blower. Aeration was provided 24 hours throughout the experiment for ensuring better biofloculation. Tanks kept one week for dechlorination, BFT tanks were prepared as per Avnimelech (2009), and 75L of dechlorinated tapwater was inculcated with 25L of water collected from tilapia biofloc tank, which was maintained separately. Tapioca flour, (flour of dried root of tapioca plant *Manihot esculenta*) was selected as carbohydrate source. Raw tapioca was processed and powder was prepared (details of processing of carbohydrate sources is described in Chapter 5, section 5.2.2). The BFT tanks with stocking density 2, 6 and 10 PL/L were represented as 200BFT, 600BFT and 1000BFT respectively. Whereas the normal culture systems with same stocking densities were denoted as 200, 600 and 1000. Details of tank allocation and experimental code given in the Table 3.1. All the systems were maintained for 30 days without any water exchange.

Fig: 3.1 Experimental tanks maintained in the indoor hatchery complex of School of Industrial Fisheries, CUSAT

Table: 3.1 Experimental design and stocking density of freshwater prawn postlarvae in the BFT applied primary nursery phase.

SL No	Treatment	Experimental code
1	2 postlarvae/L without biofloc application	200
2	2 postlarvae/L with biofloc application	200BFT
3	6 postlarvae /L without biofloc application	600
4	6 postlarvae /L with biofloc application	600BFT
5	10 postlarvae /L without biofloc application	1000
6	10 postlarvae /L with biofloc application	1000BFT

3.2.2 Larval maintenance and assessment

Postlarvae of *M. rosenbergii* were purchased from the Kerala Government shrimp hatchery, Azheekod, Thrissur. Prawns having an individual weight of 0.0254±0.0085 g and length of 1.36±0.24 cm were strong, healthy with bright colouration. They were active and uniform size with a translucent body and tend to swim against water swirl. The seeds were transported in oxygenated double-layered polythene bags. The condition index of the seed was evaluated before and after the experiment as per Tayamen and Brown (1998, 1999). Initial samples were taken immediately after reaching postlarvae in hatchery, and final sample is taken from each tank at the end of experiment. Details are given in Table 3.2. RNA/DNA ratios of the animal were also estimated (Tri Reagent, Sigma) before stocking, 15th and 30th day of the experiment. Animals were fed 15% of their body weight with a commercial crumble, sinking starter feed having a crude protein of 32% (*Grow best* scampi feeds). The ration was divided and distributed twice daily with similar portions between 0900 and 1000 h and between 1700 and 1800 h. Pre-weighed carbohydrate source was mixed in a glass beaker with the water taken from the corresponding culture tanks, 49.6 g tapioca flour for 100 g of feed was added in the tanks and poured directly to the water column after first feeding (Avnimelech, 1999). Final assessment was done by draining the tank to a scoop net fitted to the rim of the tank. Average weight gain, survival and condition index of the prawn juveniles were compared.

3.2.3 Water quality

Temperature, and pH were measured daily using mercury thermometer, and pH scan (Eutech instruments, Singapore) respectively. Dissolved oxygen was measured weekly by Winkler's method (APHA, 1995). Water samples were filtered through GF/C Whatman filter papers and the filtrate was analyzed for nitrate-N (Resorcinol method), nitrite nitrogen (NO_2^-N) and total ammonia nitrogen (TAN) by phenol hypochlorite method (Grasshoff et al., 1983). Total heterotrophic bacteria (THB) was estimated and expressed as colony forming units (cfu) (APHA, 1995). Chlorophyll-*a* in non-filtered water column samples were analyzed following standard methods (APHA, 1995). Biological oxygen demand (5 day BOD) of water samples was estimated following APHA (1995).

3.2.4 Statistical analysis

All non repeatedly measured variables (Prawn weight, survival RNA/DNA ratio) were analyzed by one way ANOVA Tukey HSD programme using SPSS 17 software. If a main effect was significant, the ANOVA was followed by Tukey's test at $P<0.05$ level of significance. Water and sediment quality parameters were analyzed following two way ANOVA.

3.3 Results

The water quality parameters like temperature, pH and dissolved oxygen in all the rearing tanks were found to be well within the accepted range for nursery rearing for *M. rosenbergii* (New. 2002).The TAN and NO_2-N concentrations were found to increase proportionately with increase in stocking density. It was significantly low in BFT tanks. The average values recorded for various physical and chemical parameters are given in Tables 3.3-3.4. The variation in the levels of TAN, nitarite and nitrate are depicted in Fig. 3.2, 3.3 and 3.4 respectively. The total heterotrophic bacteria in the non BFT ($42.2 \pm 26 \times 10^3$ to $81.2 \pm 74 \times 10^3$) were also found to be significantly lower than the BFT treatments ($79.4 \pm 35 \times 10^3$ to $131.8 \pm 71 \times 10^3$). Comparing the bacterial counts in the treatments, it can be seen that the number of bacteria increased in direct relation to the stocking density. The highest THB on all sampling days was recorded in the treatment with a stocking density of 1000 PL/L with biofloc application. The variation in the THB recorded during the experimental period is given in Table 3.5. Chlorophyll *a* showed significantly higher values in BFT tanks (32.7±8.9-36.2±4.1 μg/l)

The highest survival rate of 60% was envisaged at the lowest stocking density of 2 PL/l with biofloc application (200BFT), whereas the lowest of 21.8% was recorded at a larval density of 1000 PL/l. The survival rate recorded in the 600 and 600 BFT was 52 and 53% respectively (Table 3.6). But the prawns with significantly higher weight were recorded from 600BFT tanks. Condition index from all the treatments were same both in initial and final period. A score of 18 out of 20 was recorded from all tanks (Table 3.2). Highest RNA/DNA ratio was recorded from the treatment 1000 and significantly lower was from treatments 200 and 200BFT (Table 3.6).

Table 3.2 Condition index used for the evaluation of larval quality of *Macrobrachium rosenbergii* and score obtained to sampled animals at the end of of the experiment. © Tayamen and Brown (1998)

CRITERIA TO CHECK No:		SCORE			Sample									
		0	1	2	1	2	3	4	5	6	7	8	9	10
1	GUT FULLNESS	Gut empty	Moderately full (30%-60%)	Full gut with faecal strands	1	1	1	1	1	1	1	1	1	1
2	GUT LIPID CONTENT (STATE OF HEPATOPANCREAS)	No lipid vacuoles	Very small vacuoles (10% - 40%)	Relatively full (60%-90%)	2	2	2	1	2	2	2	1	2	2
3	PIGMENTATION (STATE OF CHROMATOPHORE)	No colour pigments (fully contracted chromatophores)	Moderate chromatophores in one area	Well dispersed chromatophores (pinkish/amber colouration)	2	2	2	2	2	2	2	2	2	2
4	BODY COLOURATION	Grey/dark/bluish on abdominal segments	Moderate light orange on abdominal segments	Tan/orange/red blend on abdominal segments	1	1	1	1	1	1	1	1	1	1
5	SETATION	Disfigured/damaged setae on rostrum, periopods, telson, uropods	Curled/kinked setae on rostrum, peropods, pleopods, telson and uropod	Straight/whole setae no deformalities on rostrum, periopods, pleopods, telson, uropods	2	2	2	2	2	2	2	2	2	2
6	MUSCLE TO GUT RATIO	Gut appears wide, muscles narrow on VI abdominal segment	Gut appears narrow and slightly wider muscle	Gut appears narrow and muscle appears thick and wider on VI abdominal segment	2	2	2	2	2	2	2	2	2	2
7	MUSCLE APPEARANCE OF ABDOMEN (APPEARANCE OF ABDOMINAL MUSCLE)	Opaque/grainy	Slightly transparent	Clear/transparent smooth	2	2	2	2	2	2	2	2	2	2
8	MELANIZATION (PRESENCE OF BLACK SPOT)	Appendages and part of the body	Very minor necrosis	No necrosis, absence of black spots	2	2	2	2	2	2	2	2	2	2
9	FOULING ORGANISMS	Major part of the body affected	Minor part of the body affected	Body clean/absence of protozoans, ciliate organisms	2	2	2	2	2	2	2	2	2	2
10a	SWMMING BEHAVIOUR (BETWEEN STAGE VIII - X)	Sluggish/circular motion, erratic movement	Moderate movement with head upside down	Very active tail first lateral motion/jump-like towards the side										
10b	PHOTO POSITIVE RESPONSE	Negative response	Slow positive response	Fast positive response	2	2	2	2	2	2	2	2	2	2
				Total Score (Out of 20)	18	18	18	18	18	18	18	18	18	18
				Larval Stage	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL

Score rating 0=Poor, 1= fair, 2=Excellent

Table 3.3 Daily water quality variations in the primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

Treatment	200	200BFT	600	600BFT	1000	1000BFT
Temperature (C^{0})	29.8±0.00^{a}	29.8±0.00^{a}	29.8±0.00^{a}	29.8±0.00^{a}	29.8±0.00^{a}	29.8±0.00^{a}
pH	7.7±0.94^{a}	7.6±0.88^{a}	7.7±0.59^{a}	8.0±0.44^{a}	7.8±0.51^{a}	7.9±0.64^{a}

Table 3.4 Biweekly water quality variations in the primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

Treatment	200	200BFT	600	600BFT	1000	1000BFT
DO (mg/l)	6.7±0.5^{a}	6.7±0.5^{a}	7±0.4^{a}	6.9±0.4^{a}	6.9±0.3^{a}	6.9±0.3^{a}
BOD (mg/l)	3.8±0.37^{a}	3.6±0.62^{a}	3.8±0.63^{a}	3.9±0.62^{a}	3.6±0.58^{a}	4±0.53^{a}
Nitrite (mg/l)	1.59±0.81^{ab}	1.40±1.27^{ab}	1.56±1.21^{ab}	1.08±0.85^{a}	2.04±1.60^{ab}	1.59±1.23^{b}
Nitrate (mg/l)	9.7±5.3^{ab}	10.1±7.1^{ab}	10.2±5.7^{ab}	7.4±5.3^{a}	10.7±6^{ab}	13±9^{b}
Ammonia (mg/l)	0.50±0.39^{ab}	0.19±0.12^{a}	0.91±0.68^{bc}	0.29±0.25^{ab}	1.40±0.84^{c}	1.15±1.53^{c}

Table 3.5 Monthly THB and chlorophyll a variation in the primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

Treatment	200	200BFT	600	600BFT	1000	1000BFT
THB X 10^{3} cfu	42.2±26^{a}	79.4±35^{ab}	58±46^{ab}	100±50^{bc}	81.2±74^{ab}	131.8±71^{c}
Chlorophyll *a* (µg/l)	4.4±1.5^{a}	32.7±8.9^{b}	5.4±1.8^{a}	30.1±8.9^{b}	7.4±2.6^{a}	36.2±4.1^{b}

Table 3.6 Prawn average weight gain, Survival and RNA/DNA ratio in the primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

Treatment	200	200BFT	600	600BFT	1000	1000BFT
Average weight gain (g)	0.29 ± 0.13^{a}	0.46 ± 0.26^{ab}	0.47 ± 0.41^{ab}	0.74 ± 0.37^{b}	0.25 ± 0.08^{a}	0.50 ± 0.19^{ab}
Survival (%)	45.3 ± 8.3^{ab}	60 ± 23^{b}	51.9 ± 10^{ab}	53 ± 8^{ab}	21.8 ± 3^{a}	24.3 ± 9^{a}
RNA/DNA ratio	0.169 ± 0.016^{a}	0.147 ± 0.011^{a}	0.300 ± 0.46^{ab}	0.467 ± 0.54^{ab}	0.620 ± 0.30^{b}	0.524 ± 0.34^{ab}

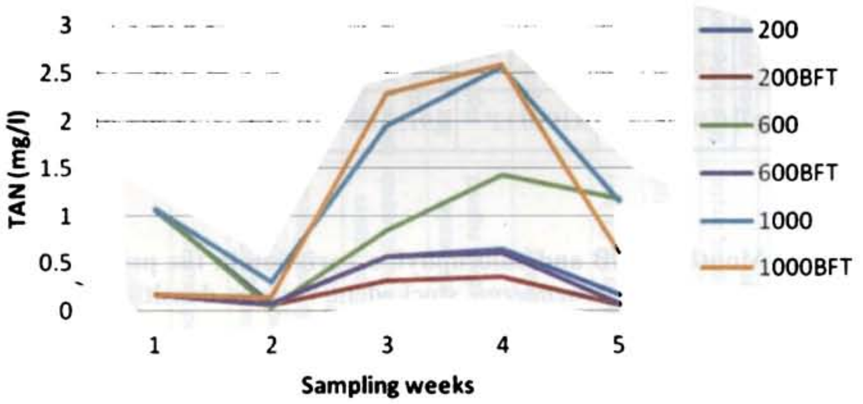

Fig. 3.2 Weekly variation of TAN in the water column of primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

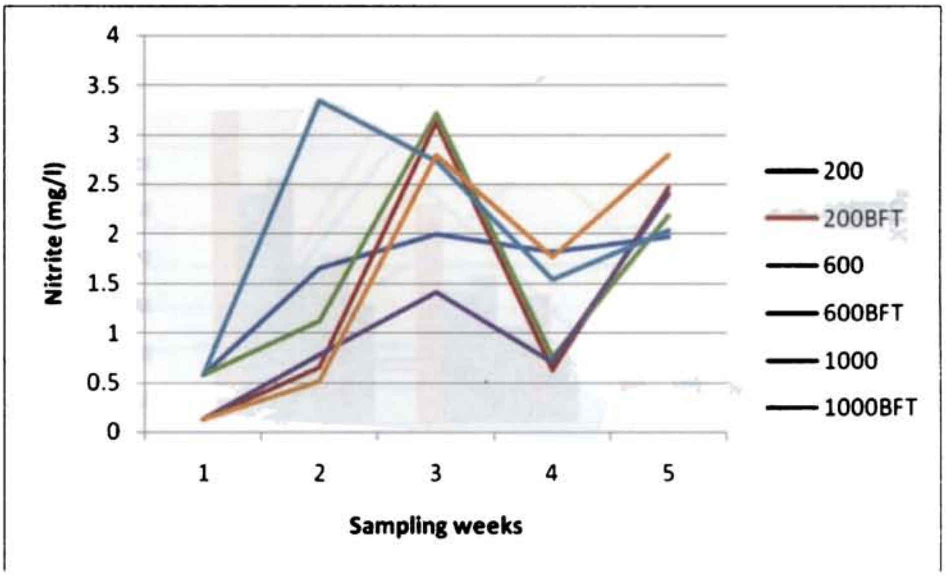

Fig. 3.3 Weekly variation of nitrite in the water column of primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

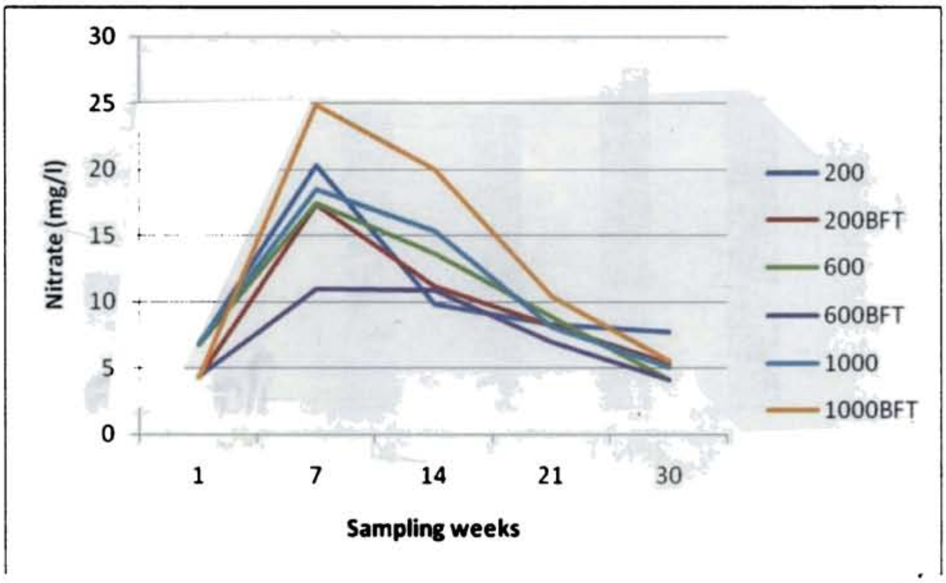

Fig. 3.4 Weekly variation of nitrate in the water column of primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

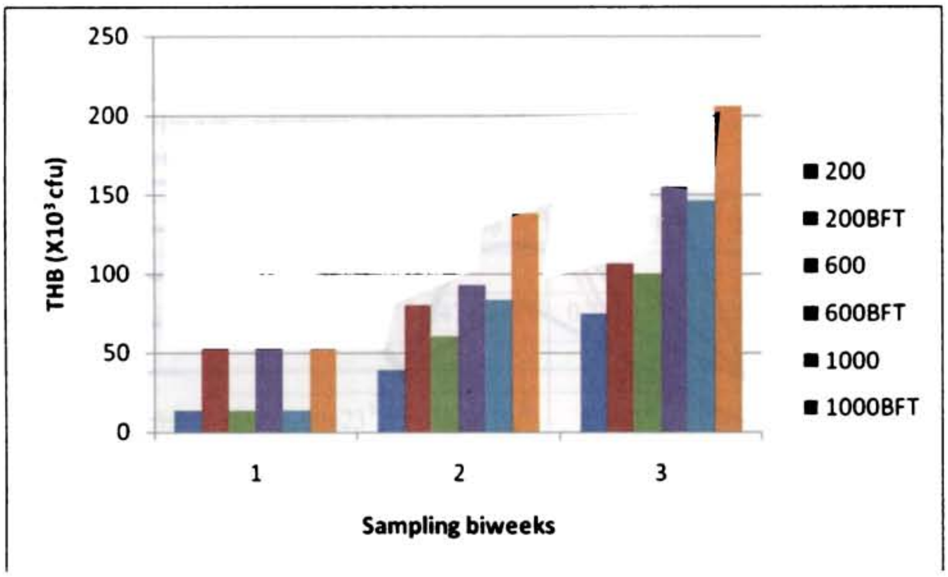

Fig. 3.5 Bi-weekly variation of THB in the water column of primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

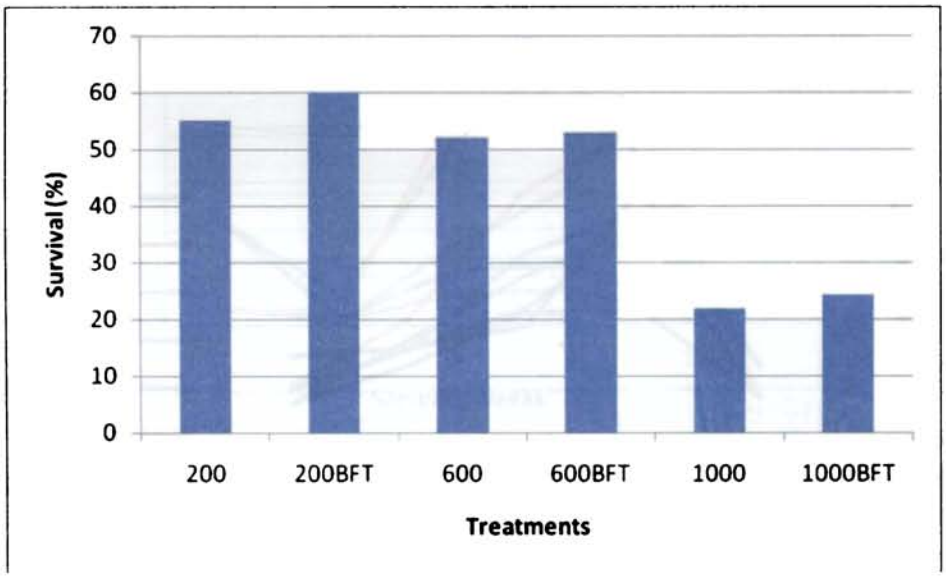

Fig. 3.6 Prawn survival recorded from the primary nursery phase of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks

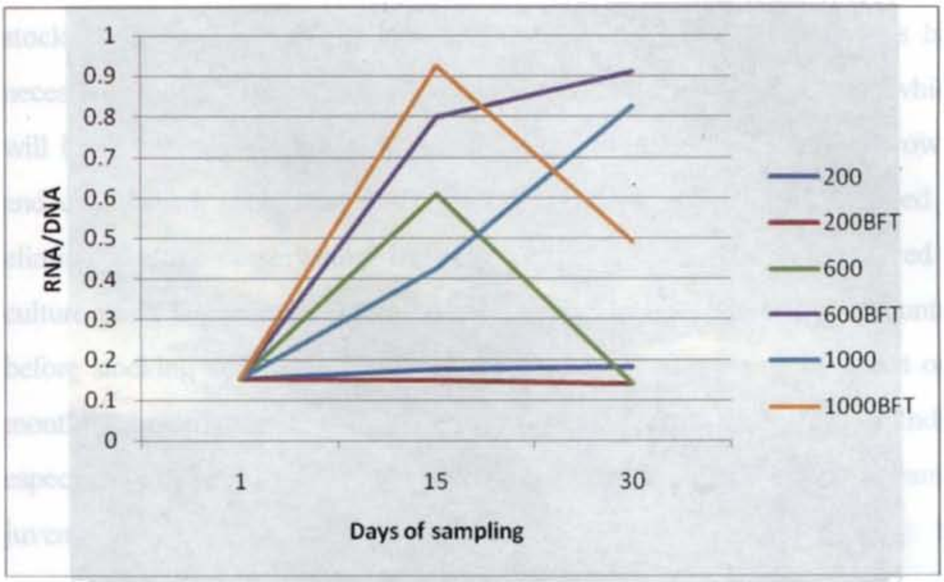

Fig. 3.7 **Variation of RNA/DNA in prawn muscle during the 30 day primary nursery phase experiment of *M. rosenbergii* stocked in various densities in non-BFT and BFT applied tanks**

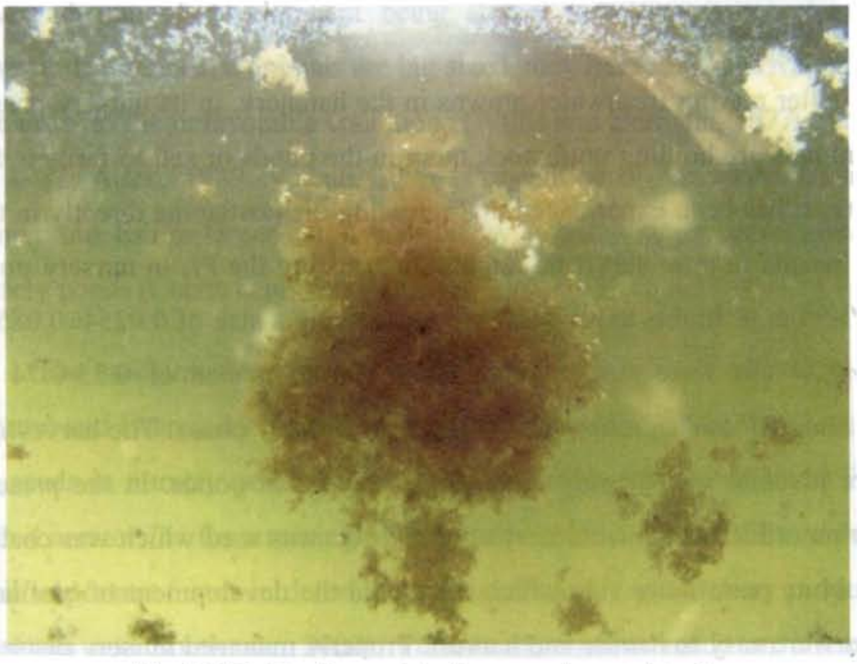

Fig. 3.8 Biofloc formed in the experimental tanks

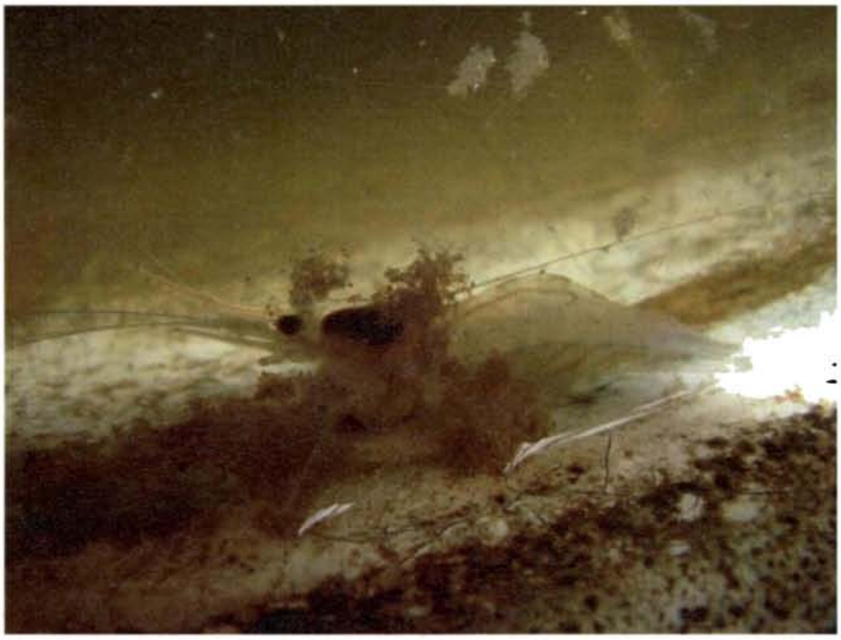

Fig. 3.9 Prawn consuming biofloc developed in the experimental tanks

3.4 Discussion

After rearing freshwater prawns in the hatchery, in its nursery phase, the animals are holding until stock them in the ponds or sell to farmers for culture. It has been experienced that releasing the postlarvae directly in the pond results in poor survival, emphasizing raising the PL in nursery pond till 30-50 mm. In this experiment animals having a size of 0.0254±0.085 g 1.36±0.24 cm were stocked and nursed up to a size of 0.25-0.74 g. Concrete/FRP tanks are usually used for the nursery phase. The harvesting of the juvenile will be very easy in these types of ponds. In the present experiment FRP tanks with a capacity of 100L was used which was coated with white paint inner side which facilitated the development of biofilim. Tanks were easy to handle and harvest. Properly managed nursery phase is one of the important stages which determine the survival and quality of the seed. Parameswaran et al. (1992) recommended nursery rearing before stocking in growout system such as pond, tanks and reservoirs. This has necessitated the development of indoor and outdoor nursery systems, which will be reduce the mortality of postlarvae resulting from differential growth and cannibalism (Sebastian et al., 1992). The weak and unhealthy seed is eliminated at the nursery stage itself, hence the survival of seed transferred to culture pond increases. Bigger and robust seed can be selected and counted before stocking and also the culture period may be shortening by about one month (Ramakrishna and Rao, 2000). Some of the entrepreneurs in India, especially in the south Indian states, sell the nursery reared male scampi juveniles around the weight of 3-5 g to the farmers (MPEDA, 2003). So nursery rearing not only fulfills the seed requirements of individual farmers but also creates an opportunity to sell male population for good price (Soundarapandian and Kannan, 2008). Parado-Estepa (1988) reported the greater degree of development being shown indicating better chance of survival. If the stocked animals are big sized, they have high survival and will be hardier to the unfavorable conditions (Willis and Berrigan, 1977; Sarver et al., 1982; Alson, 1989; Valenti, 1996). Polyculture of *Macrobrachium* with carnivorous fish or larger fish is possible by stocking larger prawns from the nursery ponds (Cohen et al., 1981).

The stocking density may cause detrimental effects on fish growth (Barton and Iwama, 1991). MPEDA (2003) recommend stocking density of the seeds in nursery ponds as 25 PL/m^2 without aerators, up to 50 PL/m^2 with 4 aerators/ha for a rearing period of 40-45 days. Larval culture density is an important factor in determining the efficiency of hatchery production (Doroudi and Southgate, 2000). Present experiment made an attempt to evaluate the efficacy of biofloc technology in the indoor (first phase) nursery system of giant freshwater prawn, *M. rosenbergii* under varying stocking densities. Usually a stocking density for primary nursery phase is 5-10 PL/Litre. FAO recommend a stocking density of 2000-5000/m^2 (2-5 PL/Litre). So in this experiment stocking densities of 2, 6 and 10 PL/L with and without BFT were selected. 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 was better in low stocking densities when compared to high stocking densities (Forster and Beard, 1974; Sandifer and Smith, 1976). Results of this experiment are fully concurring with the above findings. In this study, survival was found to be more at lower stocking density (200BFT). Marques et al. (2000) also reported similar observations. Many studies reported an inverse relationship between the growth of cultured animal and stocking density (Lee et al., 1986; Sandifer et al., 1987; Ray and Chien, 1992; Daniels et al., 1995). However in the present experiment, lower average prawn weight gain was reported from treatment 200 and 1000. Reduced growth and survival at higher densities are attributed to a number of factors like 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 deterioration of water quality (Nga et al., 2005) and accumulation of undesirable sediment (Arnold et al., 2005, 2006).

Paul and Smith (1977) conducted experiment on the intensive rearing of postlarvae of *M. rosenbergii* in closed cycle nursery system with very high stocking densities, with animals having a weight of 0.31g used in the experiment. While comparing the animal size in the experiments of Paul and Smith (1977) and present experiments, initial prawn stocking size was small (0.025 gm) in the present study. The experiment was with 24,150 juvenile prawns with stocking densities of 1,617 prawns/m^2 of tank floor and density of 1,078 prawns/m^2 with gravel biological filter in tank. Mulla and Rouse (1985) compared the techniques for the nursery rearing of *M. rosenbergii.* average weight 0.04 g and the stocking density was 40 PL/m^2 the experiment duration was 35 days. After a 35-day nursery period, average survival and weight in fertilized pools with additional substrate were 65% and 0.301 g, but in this experiment from 200BFT and 600BFT recorded a survival of 60 and 53% respectively and treatments were without substrate and the experiment duration was 30 days and weight gain was in the range of 0.25-0.74 g. It is better when compared with the data of Mulla and Rouse (1985). Many workers have tried nursery rearing with different time duration. Normally newly metamorphosed PL is stocked in indoor nurseries for 2 to 8 weeks. In the present experiment the animal weight is 0.0254±0.085 g. The stocking density selected was comparatively higher than that of the above experiments and the duration of the experiment was 30 days.

The biofloc technology has been found very useful for improving both the ecological and economic sustainability in shrimp aquaculture. The technology helped in reduction of inorganic nitrogenous products in the farms and also to reduce the protein content of the feed substantially. BFT is found to be effective in controlling the elevated levels of metabolites like ammonia and nitrite in the modified static green water system of seed production of *M. rosenbergii* (Saritha, 2009). This shows that this technology may well work in the nursery phase of the giant freshwater prawn.

M. rosenbergii larviculture is commonly conducted in intensive system with high stocking density. In this situation, larvae might be exposed to high nitrite and ammonia concentrations (Tomasso, 1994). Biofloc technology has excellent capacity to reduce toxic metabolic compounds such as TAN, nitrite which develops in the culture tanks as result of animal metabolism and feed degradation. TAN, nitrite and nitrate in water is significantly low in BFT treatments except in 1000BFT. This showed that due to the higher stocking density the production of inorganic nitrogenous compounds in the tank may be very high and bioflocuation in this tank is not adequate to control the harmful metabolites developed in the system. The properly adjusted and added carbohydrate was found useful in reducing the inorganic nitrogen concentration in the culture pond by the proper utilization of heterotrophic bacterial population (Avnimelech, 1999). In the previous studies conducted in this connection, several other carbohydrate sources like glucose, cellulose powder (Avnimelech and Mokady, 1988; Avnimelech et al., 1989, 1994; Avnimelech, 1999), molasses (Burford et al., 2004) and tapioca (Hari et al., 2004, 2006) were used in fish and shrimp ponds to reduce the inorganic nitrogen concentration for increasing the yield and getting high survival rate. Tapioca powder which is cheaply available in the local market was used in the present study.

Naing et al. (2008) evaluated the efficiency of three types of probiotics (powder form) *viz*, beer spent grain powder + concentrated probiotics, ceramic powder + concentrated probiotics, beer spent grain powder + ceramic powder + concentrated probiotics. Then they were utilized in three earthen nursery ponds of giant freshwater prawn postlarvae with the stocking density of 10000 pieces per pond of 0.25 acre. After 16 weeks of nursery experiment, the survival rates of 81-84% and mean growth weights of 15.42-18.25 g was obtained. The studies recommend the use of powdered probiotics in the nursery system of giant freshwater prawn for ensuring the environmental safety and production. In the above experiment, researchers applied probioitics with carbonaceous substance, beer spent grain powder that may resulted in the better flocculation of the microbes present in the formulated probiotics and the good water quality parameters obtained may be due to the bacterial metabolism. Hirono (2011) employed biofloc technology to rear PL to small juvenile of *L. vannamei* in high density. The densities of 5,000, 10,000 and 20,000/m^3 were evaluated. The high and low density tanks had a similar survival rate over 75% and inexplicable result in the medium density tank with 33.7% survival rate. Microbial flocs produced in this study could offer the shrimp industry a novel alternative feed, and reduction in the dependency on fish oil and fishmeal in feeding freshwater prawns.

During the time of feeding, when the aeration was stopped assembling of bioflocs in the tank surface was observed. It can be seen that the prawns in all the biofloc treatments was moving towards the suspended flocs and they were able to consume the flocs. Scrapping and ingestion of flocs with chelate legs was observed. Besides this, the floc consumption was also confirmed visually by observing the colour of the digestive tract of the prawn. The prawns from biofloc tanks showed greenish or brownish digestive tracts similar to the colour of the bioflocs.

Higher chlorophyll-*a* content in BFT treatment indicates phytoplankton production, which is an indication of the positive effect on plankton nutritional quality (Azim et al., 2002). 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 support larval survival (Regunathan and Wesley, 2004). Several other studies have shown that active compounds in fresh and processed microalgae like *Phaeodactylum tricornutum*, *Skeletonema costatum*, *Chaetoceros* sp., *Tetraselmis suecica* and *Dunaniella tertiolecta* (Cooper et al., 1983; Viso et al., 1987; Austin and Day, 1990; Marques et al., 2006) and this in turn, reduces the potential for viral infection (Wang, 2003). Synergistic actions between microalgae and bacterial flora have also been suggested by several authors (Avendano and Requelme, 1999; Vine et al., 2006). Hence, Palmer et al. (2007) 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. Use of biofloc represents a viable and more sustainable feed option due to cost, the manner in which it is generated, and the potential that it can ease the pressure on wild fisheries by reducing at least some of the demand for fishmeal (Megahed, 2010). Biofloc is thought to provide a packaging of microbial proteins and nutrients that directly accessible to culture animals (Burford et al., 2004; Avnimelech, 2009). Algae contain more protein than macrophytes, presumably because macrophyte structure requires more carbohydrate fibre (Bowen et al., 1995). Zooplankton consumes algae and bacteria, and can play an important role in the transfer of nutrients from primary producers to secondary consumers. Zooplankton such as rotifers can contribute significantly to the protein and energy requirements of shrimp (Focken et al., 1998). The results of Ju et al. (2008) suggest that inclusion of the floc material in shrimp diets could enhance the shrimp growth and survival.

Moriarty (1997) reported that most of the bacteria in a pond are saprophytic heterotrops, which use organic matter for growth and energy by decomposing detritus. Higher THB count from BFT tanks indicated bacterial proliferation and biofloculation. Numerous studies demonstrated that shrimp reared in clear water using initial stocking densities above 300/m^3 have a significantly lower growth rate than those reared in dirty water using much lower initial stocking densities. This becomes increasingly evident when shrimp tested in clear water are compared directly to shrimp in systems with a high productivity of natural organisms (Tacon et al., 2002; Izquierdo et al., 2006; Moss et al., 2006; Mc Lean et al., 2006).

The profitability of prawn farming depends on the feed cost; most of the farmers use commercial feed with a protein range of 28-32% (Nagarajan and Chandrasekar, 2002). Commercial feed with a crude protein percentage of 32 was used in the present experiment and feeding was twice in a day in the experiment. New (2002) reported feeding once or twice per day is sufficient for better prawn production. Many researchers studied the nursery and growout system of giant freshwater prawn. Thailand (Menasveta and Piyatiratitvokul, 1982; Singholka and Suk- sucheep, 1982), in Hawaii (Stanley and Moore, 1983), in Israel (Sagi et al., 1986; Gofer, 1991) and in Bangladesh (Angell, 1994). Soundarapandian and Kannan (2008) studied the technology for the better survival and production of giant freshwater prawn, the experiments were conducted in four different farms with varying stocking densities 5, 11, 15 and 20 prawns/m^2 and recorded survival was 78, 83, 80, 81% respectively. The authors concluded that semi-intensive type of culture with duration of 70 days was more profitable for the male scampi selling farmers. Successful nursery and grow-out culture of *M. rosenbergii* in cages already tested in India by Panicker and Kadri (1981), who did not find significant differences between weight increases of juveniles reared in conventional concrete tanks and in cages placed in dams. Marques et al. (1996) stocked *M. rosenbergii* postlarvae in cages at low densities of 50 and 100 PL/m^2. In the nursery rearing conducted for 60 days, better growth and survival recorded and this was much better when compared to postlarvae nursed in concrete tanks. The author also reported that higher stocking density will result in lesser survival. Marques et al. (2000) studied the effects of high-density on growth and survival of *M. rosenbergii* postlarvae reared in cages in 20 day primary nursery phase and in 60 days secondary nursery phase. The experiment was also conducted in cages. The study suggests that stocking post-larvae in cages, at high densities, 10 PL/L for the primary nursery and 800 PL/m^2 for the secondary nursery phase would be ideal. It can be a good strategy to reduce costs of the nursery phase.

The condition index proposed by Tayamen and Brown (1999) was selected for the sensory evaluation of the seed quality. The score was allocated to the individual after the careful examination of external characteristics. The average score obtained for the prawns revealed that they are good for the culture. Out of 20, 18 score was obtained both in the initial and at the end on the nursery period (Table 3.2). RNA/DNA analysis was done to assess the wellbeing of the prawns in the treatments. This method was proposed 41 years ago as a biochemical indicator of the physiological and nutritional state of the aquatic organisms in natural environment (Holm-Hansen et al., 1968). RNA content correlates with the synthesis of new proteins, which is usually interpreted as beneficial to the organism, and reflects active metabolic rates related to growth and reproduction. As DNA content remains relatively constant in an individual, as a function of chromosome number, RNA/DNA ratio is expected to increase when environmental conditions are favourable (Dahlhoff, 2004). The quantity of ribonucleic acid, expressed as mg RNA mg"1 tissue, mg RNA mg"1 protein, or mg RNA mg"1 DNA, has been used as a biochemical index of growth in a variety of contexts for both freshwater and marine fishes (Bulow, 1987). The RNA/DNA ratio has been used to investigate critical life stages and resource limitations of larval fish, seasonal growth characteristics within fish populations and as an indicator of fish health after exposure to toxicants (Bulow et al., 1981; Barron and Adelman, 1984; Mohapatra and Noble, 1992; Steinhart and Eckmann, 1992). RNA/DNA values were calculated in the first day, 15th day and at the time of harvest (30th day), higher values were recorded from higher stocking densities (1000 and 1000BFT), but the recorded survival rate was significantly low from both treatments. Since scampi juveniles exhibit heterogeneous growth and cannibalism during the nursery phase (Soundarapandyan and Kannan, 2008), the lower survival from the tanks with higher stocking density may due to the competition between the animals, as a result only strong animals may survived. This may be the reason for the higher RNA/DNA values from tanks stocked with higher stocking densities.

Table: 3.7 Data on the culture period, stocking density and survival of giant freshwater prawn, *M. rosenbergii* in the primary nursery system reported by various researchers

SL No	Researcher	Culture period (Days)	Stocking density	Survival (%)
1	Marques et al. (2000)	20	10PL/L	62.8
2	Ang (1989)	4week	450-1000PL/m^2	60-80
3	Prasad (1993)	61days	100PL/m^2	71
4	Marx et al. (2000)	70 days	50PL/m^2	80
5	Nagarajan and Chandrashekar (2002)	40-45 days	20-25PL/m^2	-
6	Reddy (1996)	25-30days	200-300PL/m^2	-
7	Present study	30 days	2-10PL/L	21-60

3.5 Conclusion

An intermediate nursery phase is essential in the aquaculture of giant freshwater prawn, *M. rosenbergii.* High stocking density was evaluated in the present experiment. BFT application in the nursery system found to be useful in increasing the animal growth. The results showed that higher survival and animal quality were observed in 200N and 200BFT. Even though higher production obtained from the lower stocking densities, when considering the economics of the culture, it is better to select the next stocking density (600) with biofloc application. Therefore, the study recommends a stocking density of 6 PL/L for the biofloc applied primary nursery rearing of giant freshwater prawn. Further research is required to estimate the application of BFT in substrate-added system, and development of low-cost and long lasting substrates for reducing the initial investment in the nursery rearing of *M. rosenbergii.*

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Chapter 4

APPLICATION OF BIOFLOC TECHNOLOGY IN THE GROW-OUT SYSTEM OF GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII:* EFFECT ON WATER QUALITY AND PRODUCTION

Contents

4.1 Introduction

All forms of food production like any other human activity affect environment in one way or another (Pillai, 1992). It is true in the case of aquaculture also. Land-based fish farms produce effluents that may have, if not properly handled, a negative impact on the quality of water courses, rivers and soil (Acierno and Zonno, 2010). Excessive accumulation of toxic inorganic nitrogen from feed, feaces and other external factors in the aquaculture ponds is always posing major threat to pond ecology, thus not only deteriorating pond environment but also the environment of the surrounding aquatic ecosystem (Kurup, 2010b). In aquaculture, the accumulated waste must be removed continuously to maintain optimal growth conditions and the health of the cultured organism. The commercial success of fish farms and the quality of aquaculture product will depend increasingly on their success in either minimizing the production of waste or utilizing waste as inputs to other production processes. Therefore, the management of solid wastes and dissolved substances is one of the most important aspects in the aquaculture industry today. In order to make aquaculture industry more successful, it is imperative to develop technology that will increase economic and environmental sustainability (Kuhn et al., 2010).

Among the freshwater crustaceans, giant freshwater prawn, *Macrobrachium rosenbergii* (scampi), has received remarkable attention as a candidate species for intensive culture in freshwater throughout the world (Hari, 2000; Kurup and Ranjeet, 2005). Scampi farming is an important aquaculture industry in many Asian countries, which together contributes to over 98% of global freshwater prawn production (Asaduzzaman et al., 2008). Scampi farming under mono and polyculture in good arable lands, low-lying water logged areas, saline and alkaline soils, tanks, reservoirs, coastal flood plains, etc. is fast expanding in India. Recent setbacks experienced in the land-based agriculture in Kerala and the prevailing socio-economic conditions have further tempted many farmers to switch over to farming of scampi in the derelict water bodies and fallow polders by resorting to either monoculture of this species or polyculture in combination with freshwater fishes. The intricate coconut garden channels and homestead ponds, which are available in plenty in Kerala, also provide a conducive environment for the successful farming operations (Hari, 2000). Being a hardy species compared to tiger prawn, the culture of this species is ecofriendly with least aquaculture wastes, utility of used water/soil for integrated aquaculture, effective utilization of water column, with fish and prawns besides helping in recharge of aquifers and ground water. The promotion of this important sector of inland aquaculture as a diversification measure with utmost care to achieve sustainability in terms of resource utilization, environmental safety through sustainable scampi production systems assumes significance (Anon, 2007). But many of the prawn farmers release the pond waste water into the ambient environment without any treatment and with least regard to environment. This causes lots of damages to the environment, and already tiger shrimp farming witnessed the environmental damage and consequent impacts of salinization and depletion of ground water, and recurring attack of viral disease through horizontal transmission via water (Ilaiah, 2003).

Feed is a major input which plays a decisive role in freshwater aquaculture. One of the major problems in prawn culture is the cost of feed. Optimizing feed efficiency and reducing feed cost is key to the aquaculture of freshwater prawn (Kumar and Velayudhan, 2006). High protein content in the feed become unutilized and subjected to microbial decomposition which leads to the production of toxic inorganic nitrogenous compounds like ammonia, which is detrimental to the organism in the culture ponds (Avnimelech and Ritvo, 2003). Many scientists have suggested innovative ideas for solving these problems. Application of biofloc technology by the addition of suitable cheap carbohydrate source is found to be effective for the control of toxic metabolites from the culture system. Biofloc technology is the retention of waste and its conversion to biofloc as a natural food within the culture system (Avnimelech et al., 1986; Hargreaves, 2006). Microbial consortium associated with a matrix of extracellular polymeric substances bound to any submersed surfaces is responsible for many biogeochemical cycles in aquatic ecosystems, especially nitrogen cycling (Decho, 1990; Meyer-Reil, 1991). Since pond aquaculture systems depend mainly on the exploitation of microbial community (Azim et al., 2008), application of BFT facilitate the effective utilization of microbial food-web by the cultured organism. This novel technique is found to be effective in both penaied and non-penaied crustacean culture systems (Hari et al., 2004, 2006; Varghese, 2007; Asaduzzaman et al., 2008, 2009a, 2009b, 2010a, 2010b). Present study is an attempt to apply the BFT in the semi-intensive monoculture system of *M. rosenbergii* with following objectives,

- To estimate the efficiency of BFT in the semi-intensive culture system of giant fresh water prawn
- Optimization of protein percentage in the feed of scampi by the application of BFT
- Comparison of the production of giant freshwater prawn in BFT applied and normal culture systems

4.2 Materials and methods

4.2.1 Tank facilities and design

Indoor experiments were conducted in FRP tanks with 1200 L capacity and with an effective bottom area of 1.86 m^2. Six treatments with triplicate were maintained in the prawn hatchery complex of School of Industrial Fisheries, CUSAT. Juveniles of *M. rosenbergii* were used for the experiment. Prawns were stocked at a rate of 250/m^2 for one week and they were fed with a commercial crumble, sinking starter feed with a crude protein of 32% (*Grow best* scampi feeds). All tanks were provided with sand collected from the upper streams of Vembanad lake system and filled with municipal water with a depth of 60 cm. All tanks were provided with 2 air-stone hoses type of diffuser system for 24 hours throughout the experiment for increasing biofloculation rate and kept for one week for dechlorination. Urea and super phosphate were added as fertilizers at a dosage of 4 and 1 g m^2/ week, during the first three weeks (Varghese, 2007). After one week, all tanks were stocked with juvenile prawns at the rate of 15/m^2 (New, 2002). Before stocking initial weight of the organism (0.184±0.1 g), water and sediment parameters were recorded. Commercial palletized sinking prawn feed with three dietary protein levels, viz., 32, 28 and 24% were selected as experimental feeds. Feed with 32% protein was in crumble form, 28 and 24% was in pellet form. For initial feedings 28 and 24% protein feeds repelletized to smaller size. For the final feeding the 32% protein containing crumble sized feed was repelletized to larger size. Before and after replelletization, the protein percentage of the feed was estimated, there was no considerable difference in the protein content of the feed. Tapioca flour (flour of dried roots of tapioca plant *Manihot esculenta*) was selected as carbohydrate source. Prawns were fed with experimental feed at 15% of initial weight (1-90 days) and adjusted gradually to 6% at the end of the culture (91-180 days). The daily feeding ration for each treatment was calculated and adjusted by estimating the monthly sampled mean biomass. The ration was divided and distributed twice daily with similar portions between 0900 and 1000 hrs and between 1700 and 1800 hrs. Pre-weighed carbohydrate source was mixed in a glass beaker with the water taken from the corresponding culture tanks, 49.6g, 43.3g and 37.2g for 100g of feed was added in the tanks fed with 32, 28 and 24% protein feed respectively, and poured directly to the water column after first feeding (Avnimelech, 1999).

In this experiment the tanks were fed with the protein percentages 32, 28 and 24 and represented with the acronym 32P, 28P and 24P. While the BFT applied tanks were abbreviated as 32BFT, 28BFT and 24BFT respectively. Proximate composition of experimental feed and tapioca powder is presented in Table 4.2. The C/N ratio of the treatments was calculated using the formula of Avnimelech (2009) and given in Table 4.2. The systems were maintained for 180 days without any water exchange. Water loss due to evaporation was compensated by adding dechlorinated water as per requirement.

Table 4.1 Proximate composition of experimental feed & tapioca powder

Feed code	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Moisture (%)
24	24	3	18	12	12
28	28	3	18	12	12
32	32	3	18	12	12
Tapioca powder	1.67	0.67	2.52	4.6	13

4.2.2 Calculation of quantity of carbohydrate required for biofloculation

Tapioca powder was purchased from local market. The quantity of carbohydrate added to the BFT system was calculated using the equation of Avnimelech (1999) and assuming that the added carbohydrate contains minimum of 50% carbon, the CH addition needed (ΔCH) to reduce the total ammonia nitrogen concentration by 1g N/m^3 is 20 g/m^3.

$$\Delta CH = \Delta N/0.05 \text{ --- } (1)$$

It can be assumed that the ammonium flux into water, ΔNH_4+, directly by excretion or indirectly by microbial degradation of the feed residues, is roughly around 50% of the feed nitrogen (Avnimelech, 1999):

$$\Delta N = \text{quantity of feed} \times \%\,N \text{ in feed} \times \%N \text{ excretion} \text{ -------------- } (2)$$

The amount of carbohydrate addition needed to assimilate the ammonium flux into microbial protein is calculated using equations (1) and (2):

$$\Delta CH = \text{quantity of feed} \times \%\,N \text{ in feed} \times \%\,N \text{ excretion}/0.05 \text{ ------ } (3)$$

According to equation (3), 496 g, tapioca flour is required for each kg of 32% dietary protein and 433 g for feed with 28% protein and 372 g for each kg of 24% dietary protein.

Table 4.2 C/N ratio of the feed used for the experiment and quantity of carbohydrate required for biofloculation

Experimental Feed Protein (g/kg)	Carbohydrate required for biofloculation (g/kg of feed)	C/N
320	496	10.08
280	433	11.5
240	372	13.4

4.2.3 Assessment of water and sediment quality parameter

Water quality parameters, temperature (mercury thermometer), dissolved oxygen (APHA, 1995) and pH (desktop pH meter) were measured in-situ at 9.00 hrs on daily basis. Water samples were collected using a horizontal water sampler from three locations of each tank and pooled together. Sediment samples were collected from three locations using PVC pipes. Both water and sediment samples were transported to the laboratory within two hours after collection and analyzed. Sediment and water samples were collected on bi-weekly basis between 9.00 and 10.00 hrs. Composite water column samples were filtered through GF/C Whatman glass fiber filter, the filtrate was analyzed for nitrate-N (No_3^- -N) (Cadmium reduction), nitrite-N (No_2^- -N), TAN (phenol hypochlorite method), chlorophyll-*a* in non-filtered water column samples was estimated following standard methods (APHA, 1995). Biological oxygen demand (five day BOD) was estimated following (APHA, 1995). The organic carbon in the sediment was determined following EI Wakeel and Rilley (1957). Exchangeable

TAN, No_2^- -N, No_3^- -N in the sediment were measured (Mudroch et al., 1996). Total heterotrophic bacteria (THB) count in the water and sediments was estimated following the standard procedure (APHA, 1995) and expressed as colony forming units (cfu).

4.2.4 Prawn yield parameters

Harvesting was done by hand picking after complete draining of the culture tanks. Individual length and weight were recorded. individual prawn weight gain, net prawn yield (g/m^2), mean weight gain, net prawn yield, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), average daily weight gain (ADG) and survival rate, protein intake were calculated as follows.

- Mean weight gain (g) = Mean final weight - Mean initial weight weight (Zaid and Sogbesan, 2010)
- Net prawn yield (g/m^2) = Total biomass at harvest - total biomass at stocking (Varghese, 2007)
- Specific growth rate ($SGR\%d^{-1}$) = [(Log final weight - log initial weight) x100] / rearing period in days (Raj et al., 2008)
- Feed conversion ratio (FCR) = Dry weight of feed given (g)/ weight gain (g) (Raj et al., 2008)
- Protein efficiency ratio (PER) = Live weight gain (g) / Protein intake (g) (Raj et al., 2008)
- Average daily weight gain (ADG) (g) = (Final mean weight (g) – Initial mean weight (g)) / rearing period in days (Varghese, 2007).

- Survival rate (%) = 100 × (Number of prawns stocked - Number of prawns died) / Number of prawns stocked (Raj et al., 2008)
- Protein Intake (PI) = Feed intake × Crude protein of feed (Zaid and Sogbesan, 2010)

4.2.5 Statistical analysis

All non repeatedly measured variables (prawn growth, yield, FCR,SGR and PER, survival) were analyzed by one-way ANOVA Tukey HSD programme using SPSS 17 software. If a main effect was significant, the ANOVA was followed by Tukey's test at $P<0.05$ level of significance. Water and sediment quality parameters were analyzed with two- way ANOVA.

4.3 Results

4.3.1 Water and sediment quality

The mean values of the water quality parameters such as temperature and water pH are shown in Table 4.3. No significant difference ($P>0.05$) was observed among the treatments in temperature (27.9 – 28 °C) and water pH (6.48 - 6.59). However, dissolved oxygen (6.84 - 7.1 mg/l), showed significant variation ($P<0.05$) among treatments, with higher value in treatment 32P and 28BFT (7.14 and 7.10 mg/l), while it was lower in treatment 32BFT (6.84 mg/L). The dietary protein level and carbohydrate addition had no significant effect BOD and soil pH, the values being in the range 3.71 – 3.91.mg/l and 6.48 – 6.58 respectively. The organic carbon concentration in the sediment from various treatments showed that higher values were recorded from the BFT tanks THB population in water and sediment were observed in carbohydrate added treatments 24BFT, 28BFT and 32BFT. The organic carbon was higher in the 28BFT tanks and lower was in 32P.

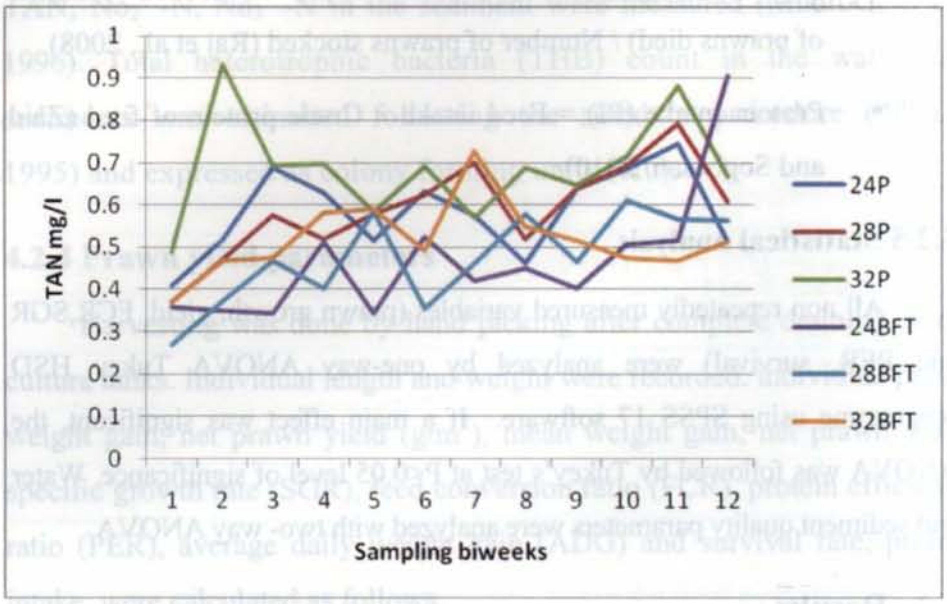

Fig. 4.1 Effect of biofloc technology on the water TAN in indoor tanks stocked with Giant freshwater prawns, *M. rosenbergii*

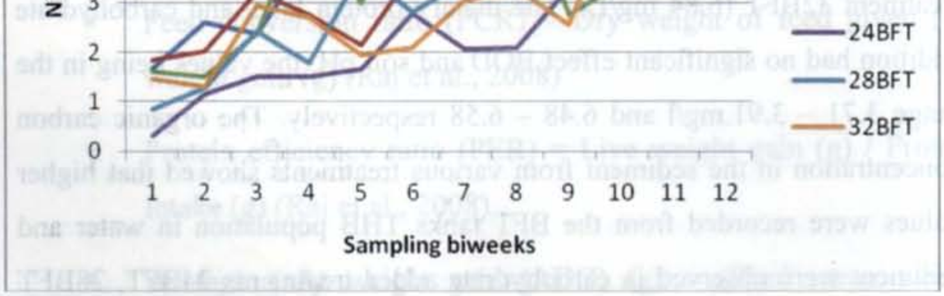

Fig. 4.2 Effect of biofloc technology on the water Nitrate in indoor tanks stocked with Giant freshwater prawns, *M. rosenbergii*

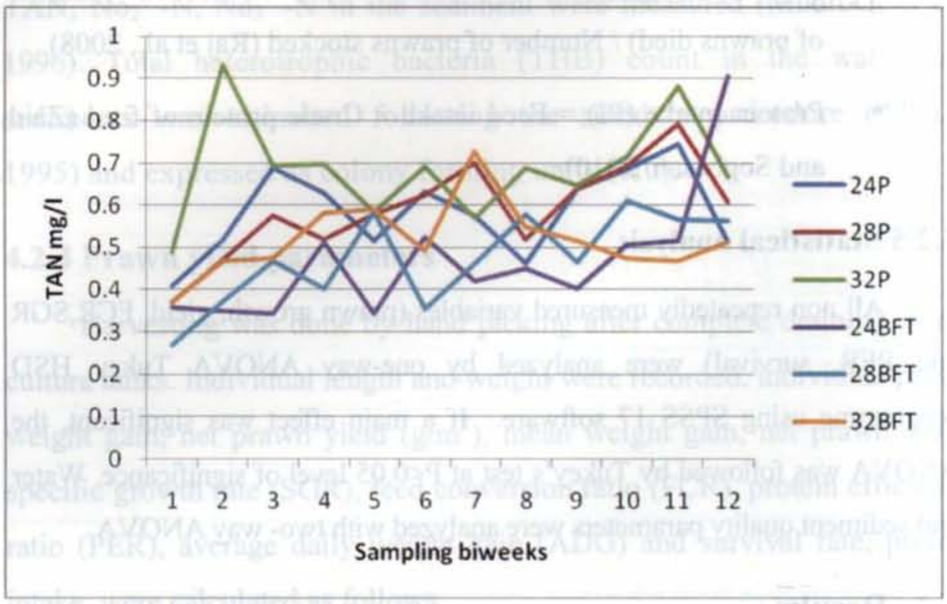

Fig. 4.1 Effect of biofloc technology on the water TAN in indoor tanks stocked with Giant freshwater prawns, *M. rosenbergii*

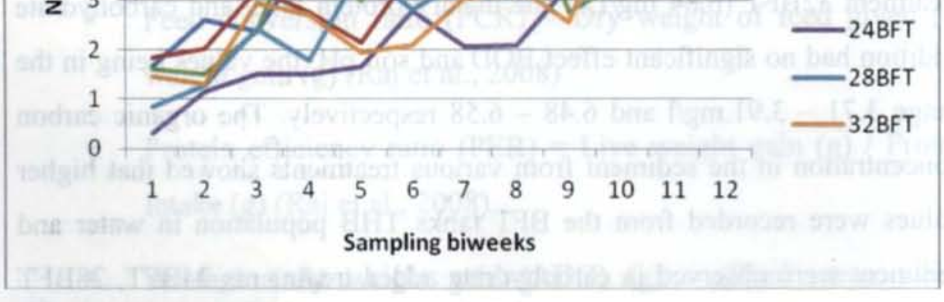

Fig. 4.2 Effect of biofloc technology on the water Nitrate in indoor tanks stocked with Giant freshwater prawns, *M. rosenbergii*

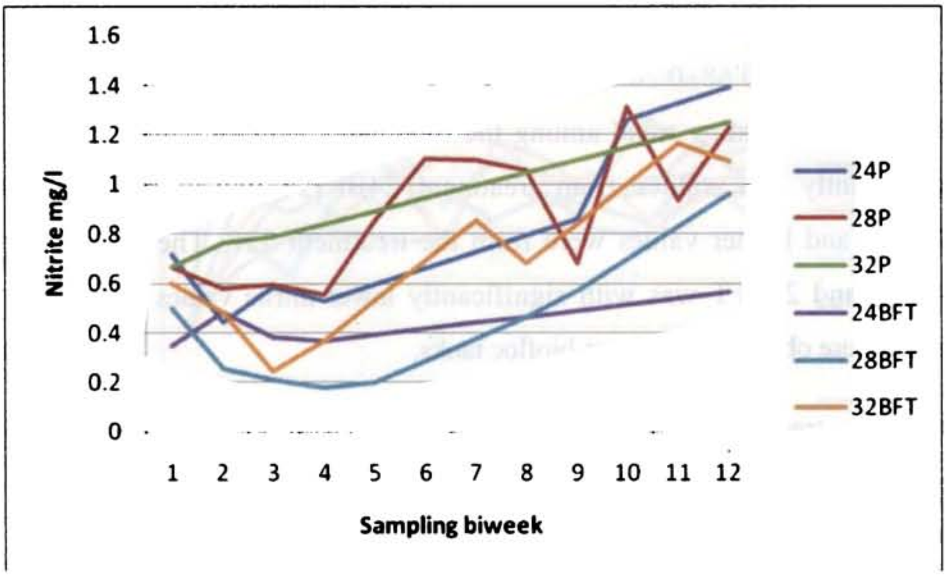

Fig 4.3 Effect of biofloc technology on the water Nitrite in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

The mean values of biweekly water and sediment TAN, nitrite-N, nitrate-N and THB results are summarized in Table 4.4 - 4.6. The treatment with carbohydrate addition showed significant reduction ($p<0.05$) in inorganic nitrogen production in water and sediment. The treatment 32P showed significantly ($p<0.05$) higher water TAN, nitrite-N and nitrate-N concentrations while lower values were reported from treatment 24BFT and 28BFT. The ANOVA results showed that protein level in the diet have significant effect ($p<0.05$) in the production of inorganic nitrogen in water and sediment. The effect of carbohydrate addition and dietary protein levels on water and soil quality of treatments during the culture period is shown in Fig. 4.1 - 4.6 respectively. The results revealed that addition of carbohydrate to water column is effective in reducing ($p<0.05$) the TAN and nitrite-N levels during the rearing period. Among treatment significantly lower TAN values were observed from 24BFT, 28BFT, 32 BFT. In treatments 24P and 28P, there was no considerable variation.

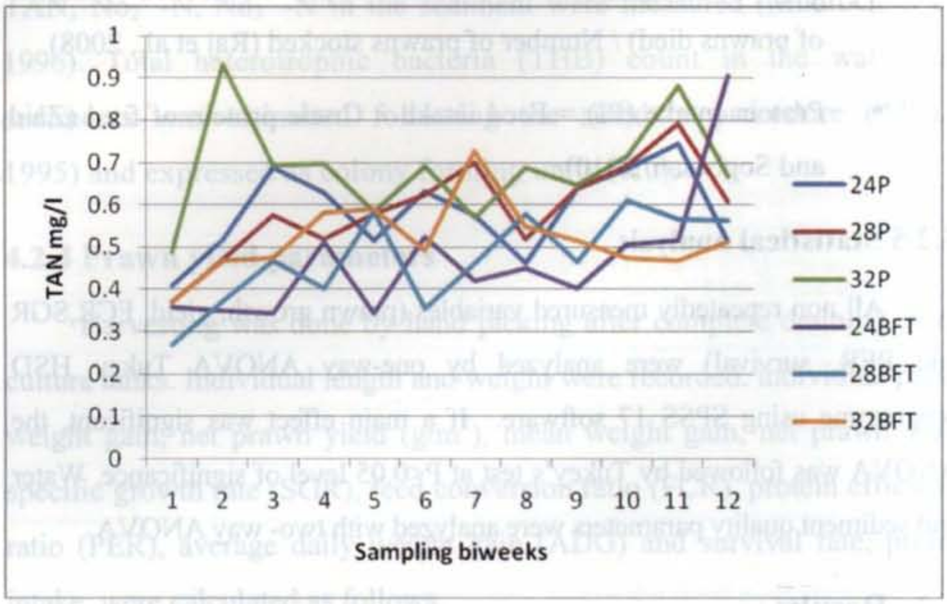

Fig. 4.1 Effect of biofloc technology on the water TAN in indoor tanks stocked with Giant freshwater prawns, *M. rosenbergii*

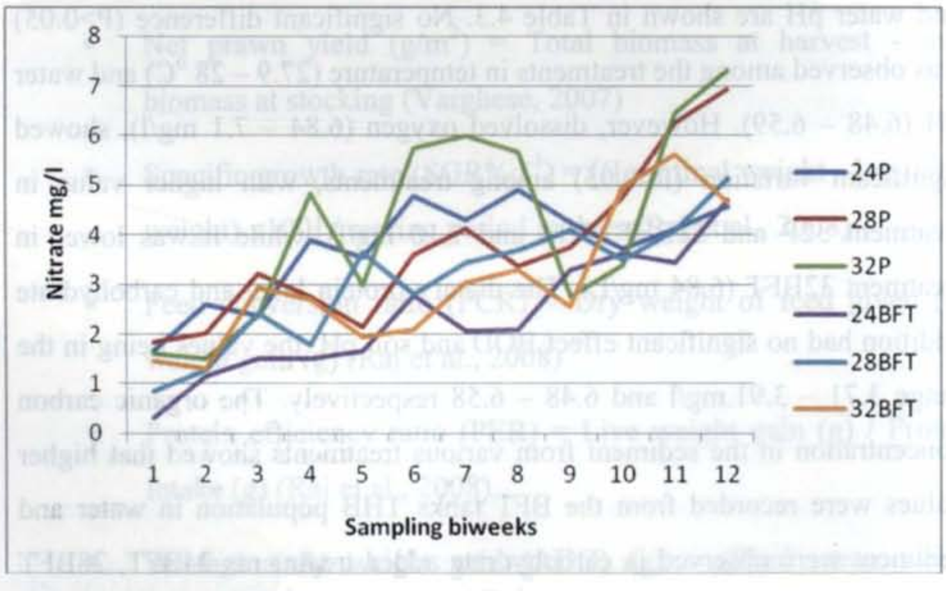

Fig. 4.2 Effect of biofloc technology on the water Nitrate in indoor tanks stocked with Giant freshwater prawns, *M. rosenbergii*

Treatment 32P was significantly varied from other treatments with a higher value of 0.68±0.26 mg/l. Biweekly recorded nitrate values in water showed a fluctuating trend among the treatments. Nitrate values showed significantly low values from treatment 24BFT followed by 32BFT, 28BFT, and higher values were from the treatment 32P. The treatments 24BFT and 28BFT was with significantly lower nitrite values and higher values were observed from non-biofloc tanks.

The treatments 24BFT, 28BFT and 28P were significantly differs from other tanks. Treatment 32P showed significantly higher TAN values from sediment. Nitrate concentration of sediment from the treatment 24BFT showed significantly lower values and higher values were from the treatments 32P and 24P.

The chlorophyll-*a* values showed fluctuating trend among treatments. Significantly higher chlorophyll-*a* value was recorded in BFT tanks. The THB population during the culture period in water ranged from 126 – 306 x 10^3 cfu ml^{-1} while in sediment, it ranged from 101.38 – 469 x 10^3 cfu ml^{-1}. During the culture period, treatment 32BFT showed higher THB concentration followed by treatment 28BFT and 24BFT. Results showed that the addition of carbohydrate source had a significant effect on ($p < 0.05$) the THB count and it was useful in promoting the growth of THB population both in water and sediment.

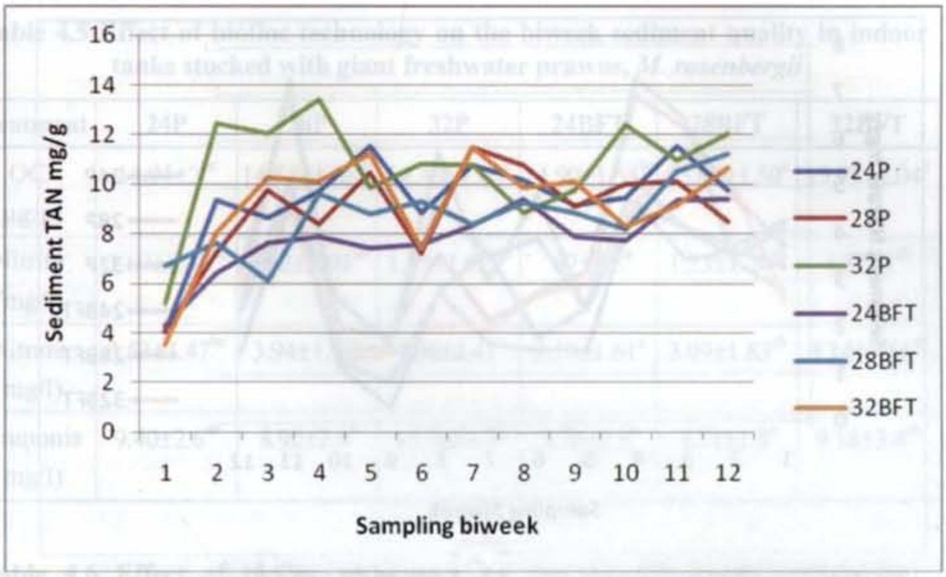

Fig 4.4 Effect of biofloc technology on the sediment TAN, in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

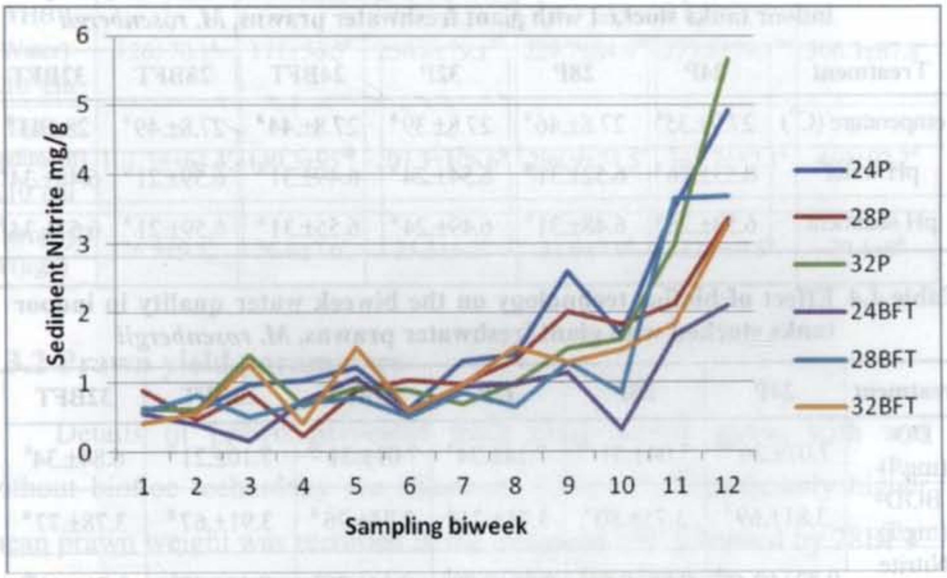

Fig. 4.5 Effect of biofloc technology on the sediment nitrite in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

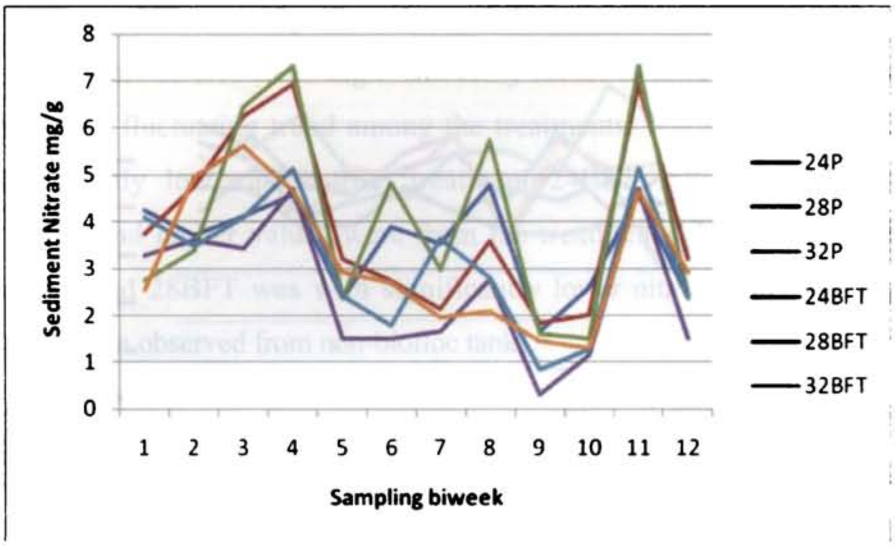

Fig 4.6 Effect of biofloc technology on the sediment nitrate in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Table 4.3 Effect of biofloc technology on the daily water and sediment quality in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Treatment	24P	28P	32P	24BFT	28BFT	32BFT
Temperature (C^0)	27.9±.35^a	27.8±.46^a	27.8±.39^a	27.8±.44^a	27.8±.49^a	28±.43^a
pH water	6.53±.26^a	6.52±.31^a	6.54±.24^a	6.49±.31^a	6.59±.21^a	6.48±.34^a
pH sediment	6.58±.26^a	6.48±.31^a	6.49±.24^a	6.55±.31^a	6.59±.21^a	6.57±.34^a

Table 4.4 Effect of biofloc technology on the biweek water quality in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Treatment	24P	28P	32P	24BFT	28BFT	32BFT
DO (mg/l)	7.07±.26^{ab}	7.04±.31^{ab}	7.14±.24^b	7.01±.31^{ab}	7.10±.21^b	6.84±.34^a
BOD (mg/l)	3.81±.69^a	3.75±.80^a	3.71±.71^a	3.74±.76^a	3.91±.67^a	3.78±.77^a
Nitrite (mg/l)	0.823±0.48^b	0.88±0.84^b	0.96±0.70^b	0.44±.30^a	0.46±.42^a	0.71±.41^{ab}
Nitrate (mg/l)	3.72±1.52^{bc}	3.72±1.77^{bc}	4.21±2.31^c	2.33±1.41^a	3.05±1.69^{ab}	3.03±1.50^{ab}
Ammonia (mg/l)	0.584±0.20^{ab}	0.59±0.18^{ab}	0.68±0.26^b	0.46±0.18^a	0.479±0.17^a	0.51±0.13^a

Table 4.5 Effect of biofloc technology on the biweek sediment quality in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Treatment	24P	28P	32P	24BFT	28BFT	32BFT
OC (µg/g)	14.91 ± 2^{bc}	14.58 ± 1.88^{ab}	14.48 ± 1.66^{a}	14.90 ± 1.54^{bc}	15.03 ± 1.50^{c}	15.75 ± 2.04^{d}
Nitrite (mg/l)	1.69 ± 1.46^{ab}	1.32 ± 1.09^{ab}	$1.53 \pm 1.62a^{b}$	$.87 \pm .68^{a}$	1.23 ± 1.34^{ab}	1.25 ± 1^{ab}
Nitrate (mg/l)	3.53 ± 1.47^{bc}	3.94 ± 1.97^{c}	4.04 ± 2.41^{c}	2.50 ± 1.61^{a}	3.09 ± 1.83^{ab}	3.14 ± 1.51^{ab}
Ammonia (mg/l)	9.40 ± 2.6^{ab}	8.92 ± 2.9^{a}	10.70 ± 2.7^{b}	7.78 ± 2.8^{a}	8.71 ± 1.8^{a}	9.18 ± 3.4^{ab}

Table 4.6 Effect of biofloc technology on the monthly total heterptrophic bacterial count in water and sediment and chlorophyll a content in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Treatment	24P	28P	32P	24BFT	28BFT	32BFT
THB (Water) X10^{3} cfu	126 ± 70.1^{a}	171 ± 56.5^{a}	250.8 ± 79.1^{ab}	229.7 ± 84.9^{ab}	273.5 ± 79.1^{bc}	306.3 ± 87.8^{c}
THB (Sediment) X10^{3} cfu	101.38 ± 64.4^{a}	140.3 ± 95^{ab}	207.3 ± 108.6^{b}	298.9 ± 74.5^{c}	365.7 ± 82.1^{c}	469 ± 92.2^{d}
Chlorophyll a (µg/l)	26.5 ± 9.5^{a}	26.6 ± 7.6^{a}	25.5 ± 6.2^{a}	31.9 ± 7.9^{b}	32.8 ± 9.5^{b}	29.1 ± 9^{b}

4.3.2 Prawn yield parameters

Details of prawn harvested from experimental ponds with and without biofloc technology are shown in Table 4.7. Significantly higher mean prawn weight was recorded in the treatment 24P followed by 28BFT. 24BFT and 32P (6.18, 5.51, 5.47, 5.18) than in 32BFT and 28P treatment (2.70 and 3.94 g). Higher prawn yield was recorded from all BFT tanks and also from the 32P and 28P and comparatively very low yield was recorded from 24P (24.86 g/m^2). The biofloc tanks 24BFT and 28BFT and non-biofloc tank 24P and 32P showed significantly ($p<0.05$) higher SGR value than the other treatments. Protein intake of the treatments fed with 32, 28 and 24% of the feed were calculated as 13.5, 11.74 and 10.06, respectively. Significantly lower FCR values (0.56-1.36) were recorded from all treatments except 24P (1.36). The results of one-way ANOVA showed that the ADG values of 24P, 32P, 24BFT and 28 BFT (0.035-0.037 g) were significantly different ($p<0.05$) from other treatments. PER results did not show any significant difference among treatments. Survival of the prawns did not vary significantly ($p>0.05$). But raw data and direct observation showed relatively lower survival from 24P. The lowest and highest survival was recorded from the treatment 24P (40%) and 32BFT (86.9%), respectively.

Fig. 4.7 Prawns harvested from with and without biofloc technology applied system fed with varying protein level

Table 4.7 Effect of biofloc technology on weight, prawn yield, SGR, FCR, PER, ADG and survival of *M. rosenbergii* in indoor trials

	24P	28P	32P	24BFT	28 BFT	32 BFT
Mean prawn weight gain	6.18 ± 1.68^{c}	3.94 ± 0.24^{ab}	5.18 ± 0.37^{bc}	5.47 ± 0.16^{bc}	5.51 ± 0.58^{bc}	2.70 ± 0.38^{a}
Net prawn yield (g/m^2)	24.86 ± 1.44^{a}	45.63 ± 2.39^{b}	50.70 ± 3.25^{b}	50.50 ± 3.20^{b}	45.76 ± 3.51^{b}	43.19 ± 8.87^{b}
FCR	1.36 ± 0.34^{b}	0.86 ± 0.23^{a}	1.36 ± 0.45^{a}	0.77 ± 0.20^{a}	0.56 ± 0.39^{a}	0.90 ± 0.15^{a}
SGR	0.87 ± 0.04^{b}	0.81 ± 0.00^{ab}	0.86 ± 0.01^{b}	0.87 ± 0.00^{b}	0.87 ± 0.02^{b}	0.76 ± 0.01^{a}
PER	3.47 ± 0.90^{a}	4.3 ± 1.45^{a}	4.2 ± 1.31^{a}	5.6 ± 1.72^{a}	5.34 ± 1.45^{a}	3.47 ± 0.54^{a}
ADG (g)	0.037 ± 0.00^{b}	0.029 ± 0.00^{ab}	0.035 ± 0.00^{b}	0.036 ± 0.00^{b}	0.036 ± 0.00^{b}	0.023 ± 0.00^{a}
Survival rate (%)	40 ± 8.80^{a}	73.3 ± 23.09^{a}	68.8 ± 26.96^{a}	64.7 ± 19.74^{a}	73.86 ± 23.61^{a}	82.2 ± 15.41^{a}

Results from Tukey one-way ANOVA

Treatments with mean values in same row with different superscripts differ significantly ($p<0.05$).

4.4 Discussion

4.4.1 Efficiency of BFT in the grow-out system of giant fresh water prawn and optimization of protein in the feed

According to New (2002), optimum water quality requirement for the grow-out of giant freshwater prawn are, water temperature 28-31^{0}C, pH 7-8.5 and dissolved oxygen 3-7 ppm. In the present study, these parameters are well within the limit. pH value was in the range of 6.48 - 6.59, which is very near to the prescribed optimum value. BFT not showed any influence on the temperature and pH values both in sediment and water. Dissolved oxygen was relatively higher in non-BFT tanks than BFT tanks. The higher

THB in the BFT tanks resulted in the utilization of oxygen for microbial metabolism and this may be attributed a reason for the low oxygen levels in BFT tanks. According to Asaduzzaman et al. (2009a), the oxygen budget in pond is affected by the autotrophic and heterotrophic processes. Heterotrophic bacteria (HB) constitute an important factor in terms of oxygen consumption, metabolic by-products they release after cellular lysis, for the competition they may have with autotrophic bacteria for oxygen and space (Nogueira et al., 2002). Ammonia concentration and feed protein have a direct proportional relation in aquaculture systems (Li and Lovell, 1992; Hari et al., 2004, 2006). Accumulation of dissolved nitrogen, especially ammonium, as a result of food addition and excretion of organisms reared at high density, are the main problems in intensive shrimp culture systems, affecting their food ingestion, growth and survival rates. Wang and Fast (1992), Tomasso (1994), Wasielsky et al. (1994), Ostrensky and Wasielsky (1995), and Cavalli et al. (1996), reported that ammonia-N and organic carbon increased in response to dietary protein concentration. Avnimelech (1999, 2009) with the help of a series of experiments proved that addition of carbohydrate reduce the need of dietary protein concentration.

The addition of carbohydrate source was useful to reduce the concentration of nitrogenous compounds both in water and sediment. This findings agree with Avnimelech and Mokandy (1988), Avnimelech et al. (1989), Avnimelech (1999), Hari et al. (2004, 2006), Varghese (2007), Asaduzzaman et al. (2008) and Kurup (2009). TAN concentration also decreasing as reported in the above studies. Shrimp exposure to high ammonium concentrations seems to reduce their resistance to diseases (Brock and Main, 1994). Zhu and Chen (1999) reported that autotrophic nitrifying bacteria remove ammonia at a sufficient rate to maintain water quality at a level adequate to prevent ammonia toxicity to the fish. Avnimelech (1999) reported significant reduction of TAN in the commercial-scale ponds of tilapia both in sediment and water column due to the addition of carbohydrate. In the present experiment, the toxic ammonia is converted into the less toxic nitrite and nitrate. The results of Mallasen et al. (2004) demonstrated that nitrate induces extremely low toxicity for giant river prawn larvae. The authors concluded that nitrate is not a limiting factor for giant river prawn larviculture. Tapioca powder and strong aeration may facilitated biofloculation (Crab et al., 2007; Schryver et al., 2008) and increase in the C/N ratio which resulted in high THB count, which immobilised TAN and helped in the synthesis of new bacterial cell (Hari et al., 2004; Asaduzzaman et al., 2008). Research showed that in crustacean culture systems, the toxic nitrogenous wastes effectively used by the phytoplankton and microbial activities (Shilo and Rimon, 1982; Diab and Shilo, 1988). Burford et al. (2004) strongly support the view that the addition of carbohydrate in culture system facilitated the increase of heterotrophic bacterial population during the culture time.

The concentrated bacterial population in the pond water or soil with carbon source is the goal of reducing inorganic nitrogen production in the shrimp and fish culture system (Boyd et al., 1984; Tucker and Lloyd, 1985; Chiayvareesajja and Boyd, 1993). For the better growth and production of giant freshwater prawn the desirable protein level in the feed is considered as 30-40% or excess of 40% (Balazs and Ross 1976; Millikin et al., 1980; Ashmore et al., 1985). In the conventional culture system feed with higher protein level 32 and 28 % supply during the initials culture period. Lower protein (24%) will provide only towards the end of the culture period. The above feeds having different protein levels were evaluated for the BFT experiments. Difference in the protein percentage of each experimental feed was four. The usage of higher dietary protein level (32% dietary protein feed) may result in the production of more inorganic nitrogen in the culture system. Like the protein content and the inorganic nitrogen compounds, the cost and protein content of the feed have a direct relation, ie; when the protein content of the feed increases the cost of the feed also increases. Since cost for the feed is major investment for aquaculture activity, the reduction of protein in the aquaculture feed even a single percentage will minimise the economic stress and dependency to fish meal for fish feed production.

Tapioca powder was used as biofloculating agent in this study. Bacteria utilized the added carbohydrate as food and synthesized microbial protein through the subsequent uptake of nitrogen from the system (Avnimelech et al., 1994). This conversion is an additional sink for ammonia and contributes to dissolved waste conversion (Schneider et al., 2006). The monthly chlorophyll values showed a fluctuating trend, the higher chlorophyll- *a* reading was reported from BFT applied tanks and this shows the presence of chlorophyll containing groups in the biofloc. The proliferation of bacterial population in aquaculture ponds results in a number of benefits (Boyd, 1995), such as reduction of blue-green algal population, inorganic nitrogen concentration, increasing dissolved oxygen and promotion of organic matter decomposition. Approach of biofloc is found as an effective environmental management method in the farming of giant freshwater prawn. The pollutant from the freshwater aquaculture, though very small when compared to the marine prawn culture system, its effects can be reduced by the application of bilfloc technology and by adopting this technique it is possible to culture prawns in small embankments with the production of less toxic effluent, without compromising the health of the animal. The reduction of feed protein from 32 to 24% by the application of biofloc technology is another valuable contribution to the aquaculture industry.

3.4.2 Comparison of the production of giant freshwater prawn in BFT applied and normal culture systems

Aeration, regular feeding and addition of suitable biofloculating agent, are the characteristics of a biofloc culture system, and these protocols are similar to the intensive aquaculture practices. In order to make the culture more profitable, the stocking density should be kept in high. In normal scampi culture system the stocking density varies 4 - 20/m^2 (New and Valenti, 2004). The expected production from such system is 500-5000 kg/year. Mancebo (1978) studied growth in tank-reared populations of the post-larval Malaysian prawns (*Macrobrachium rosenbergii*). The study was with animals that are stocked in cylindrical tanks at densities ranging from 33 to 115 prawns/m^2 (3.0 to 10.7 prawns/ft^2 grow-out cycles of 6 months). The results showed that populations of post-larvae (mean weight 0.016 to 0.060 g) grow to an approximate mean weight of 18 g within the 6-month cycle. The mortality ranges were 0.33%/day for the first 4 months and increased to 0.50%/day during the last 2 months of grow-out. In monoculture a stocking density of 4±1 PL/m^2 is desirable, depending on pond conditions. But in some countries the stocking density higher than 25PL/m^2 is practiced. Prawns grow fast under optimal conditions; optimal growth is 30-60 g within 6-8 months after stocking (Jayachandran, 2001). Perez (2000) studied the growth, survival, yield and size distribution of *M. rosenbergii.* Stocking density was 7 prawns/m^2 with a size of 1.3±0.2 g. The grow-out system was in earthen pond with an area of 1200 m^2 the stocking density used in the study was the same density used in a semi-intensive farming system (Sadek and El-Gayar, 1995). The study reported that survival of 36%, with the culture duration of 120 days. According to them, the low survival rate was due to bird predation. In this experiment, the stocking density was 15 prawns/m^2, the production ranged from 26.76-53.46 g/m^2/180days. The survival was 40-73% from non biofloc applied system and 64.5-86.9% from BFT applied system. From the present study it is clear that there is no compromise in the growth parameters of prawn grown in the BFT applied tanks fed with lower protein. Culture period, stocking density and survival of giant freshwater prawn under different culture system reported by various researchers are summarized in the Table: 4.8, while comparing this table, relatively higher stocking density and survival rate is encountered in the present study, especially in BFT applied culture systems.

Table 4.8 Culture period, stocking density and survival of giant freshwater prawn, *M. rosenbergii* in monoculture system reported by various researchers

SL No	Researcher	Type of culture system	Culture period (Days)	Stocking density	Survival (%)
1	Mancebo (1978)	Intensive	180	33-115/m^2	
2	Rouse and Stickney (1982)	Extensive	112		34
3	D'Abramo et al. (1989)		135		54-90
4	Malecha et al. (1981), New (1990), Yousuf-Haroon (1990), Valenti (1993)	Semi intensive	-	7-20/m^2	40-65
5	Daniels et al. (1995)		131		73-82
6	Tidwell et al. (1998)		106		58
7	Tidwell et al. (1999)		90		76
8	Tidwell et al. (2000)	Substrate based system	106	7.4/m^2	90
9	Perez (2000)	Semi intensive	120	7/m^2	36
10	Asaduzzaman et al. (2008)	C/N ratio optimized and periphyton based system	120	2/m^2	62.8-72.1
11	Present work	With and without biofloc technology applied	180	15/m^2	40-73 from non biofloc applied system and 64.5-86.9 from biofloc technology applied system

If carbon and nitrogen are well balanced in the bacterial substrate, ammonia in addition to organic nitrogenous waste will be converted into bacterial biomass (Henze et al., 1996). Studies revealed that biofloc is composed of aggregated, suspended particles formed in shrimp culture water and contain phytoplankton, bacteria, zooplankton and detritus material (Ju et al., 2008). By the application of BFT, the resulting heterotrophic bacterial production serves as the nutritious feed for the cultured animals. Since bacteria are single cell and composed of 50-60% of protein, the flocculated bacterial consortium may be utilized as a food source by the prawns. Studies show that Carp and Tilapia have the capacity to harvest bacterial floccules (Schroeder, 1978, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993). Prawns may consume the biofloc, which resulted in the relatively higher growth parameters in BFT applied tanks.

Rao (1967) reported that the gut of *M. rosenbergii* contain various items namely debris, sand, plant and animal tissues including filamentous algae. This shows the capacity of the organism to ingest the microalgae and other microbial material. John (1957) reported the specimen from paddy fields had paddy grains in their stomach. Feed with 32% protein is found to be optimum for the growth of the giant freshwater prawn (Hari, 2000). Mitra et al. (2005) suggested protein requirement percentage of 35-37, 28-30 and 38-40 for juvenile, adult and brood stock, respectively. In the present experiment, feed with three levels of protein with a difference of 4% were examined. Growth parameters were found to be more or less same in the tanks fed with high protein (32%) and the less protein feed with carbohydrate added treatments. The prawn yield parameters from the BFT applied ponds were found to be higher when compared to the normal culture systems. Mean prawn yield from the 32P, 24BFT and 28BFT showed statistically similar values, net prawn yield from the BFT tanks were higher and this shows that the BFT tanks may contain yet undiscovered 'growth factor' (Ju et al., 2008). All the growth parameters recorded from 32P and 24BFT found to be same. Survival rate is high in both treatments. Individual prawn weight or mean prawn weight and survival rate showed an inverse relationship. In the treatment 24P, survival rate was 40%, individual prawns with higher weights reported from this treatment (6.18 g). Food conversion ratio was also maximum in 24P. Feeding individual with lower protein (24%) from the postlarval stage onwards may affect the health and proper development of the organism, which may resulted in the competition and increased cannibalism among the organisms. Garson et al. (1986) made the same observation; according to the study, 25% dietary protein pelleted feed alone was not sufficient to cater the required supplemental nutrients to *Penaeus monodon* in intensive farming system. The lower survival in these tanks may support the growth of stronger individuals. Prawn harvested from these tanks was dominated by the presence of strong blue clawed individuals. Due to the lack of competition they attained maximum individual weight. This may be the reason for the high food conversion ratio from these tanks.

In shrimp culture systems phytoplankton and bacteria play a crucial role in the processing of nitrogenous waste (Shilo and Rimon, 1982). BFT was found to be very effective in the production of inorganic nitrogen at very low levels, besides showing better survival and higher shrimp yield. Studies of Thompson et al. (2002) concluded that biofilm effectively absorbs or transforms ammonium present in the water column has important implications for the health of *F. paulensis* juveniles, since this shrimp tolerates high amounts of nitrate (>15000 μM) and nitrite (>1000 μM) (Cavalli et al., 1996). High ammonium concentrations are lethal (Ostrensky and Wasielsky, 1995), or may seriously inhibit their food intake and growth (Wasielsky et al., 1994; Mirando, 1997). The chlorophyll- *a* content in the treatments showed significant variation. Highest mean values observed in BFT tanks. Chlorophyll- *a* containing organisms in biofloc (Ju et al., 2008), may facilitated the better growth of the animals and improved water quality. In the experiment of Thompson et al. (2002), the decline in ammonium concentrations was mainly related to an increase in chlorophyll-*a* in biofilms. According to them the ammonium was mainly absorbed by the microalgae that use this element to produce new biomass.

The prawns harvested from each culture system was weighed and categorised into four size groups viz, prawn having the weight more than 10 g, prawns weigh in between 6-10 g, weight comes under 1-5 g and below 1 g. When observing the treatment-wise production in 24P, the weight ranged from 3.2 g-18.8 g, in 28P it was 1g-11.3 g in 32P the lower weight was 1.9 g and maximum was 22.8 g while in biofloc systems the 24BFT produced animal with very low weight and the prawns obtained with higher weight was 15.4 g. Eventhough the low prawn weight was produced, from this system, maximum number of animals having above 10 g was also recorded from the same system. In 28BFT treatment, lower and higher weight was 3 g and 11.1g respectively. In higher protein fed BFT applied system (32BFT), the animal biomass ranged between 1.3 g-17.3 g. These observations are graphically represented in the Fig. 4.8

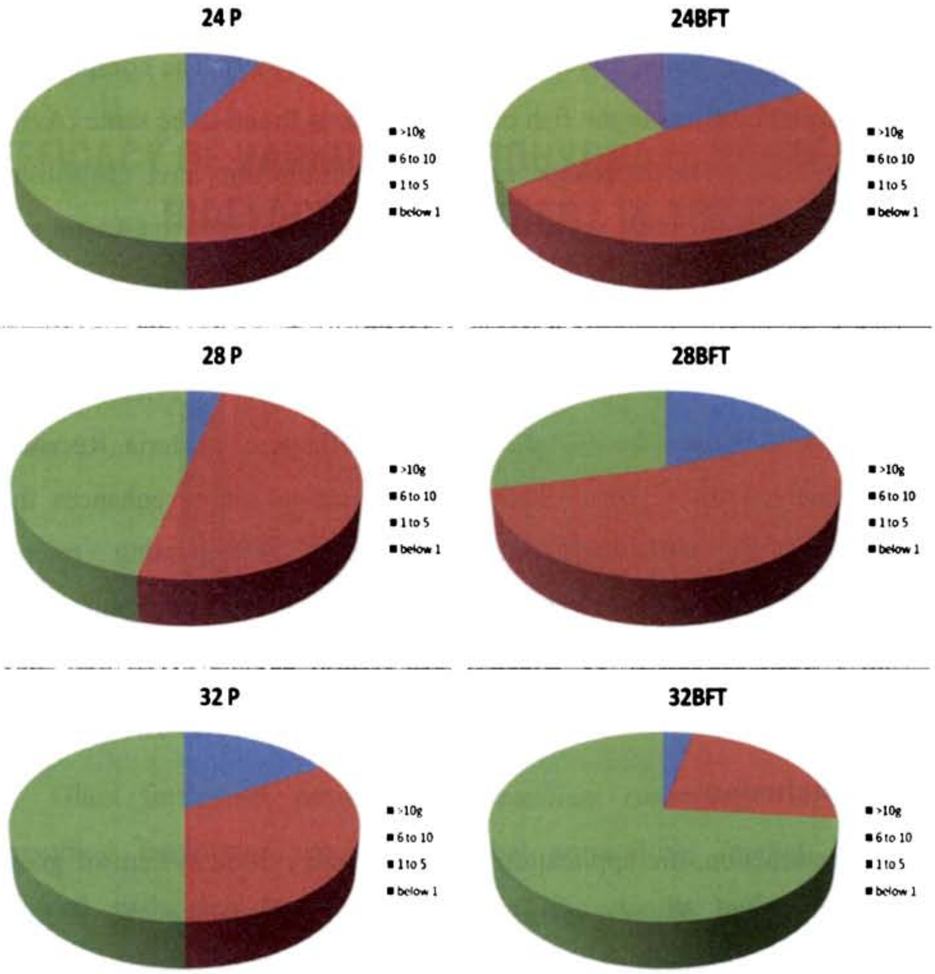

Fig. 4.8 Representation of various size-groups of prawns produced from with or without biofloc technology applied system fed with varying protein level

Biofilms have been considered as reservoirs of pathogenic bacteria, like *Vibrio harveyii*, which can affect shrimp cultures (Karunasagar and Otta, 1996). In this study organisms survived well and there were no symptoms of diseases by external examination of prawns. Studies of Thompson et al. (2002) recorded that the nitrogen uptake by a biofilm may help to reduce the occurrence of pathogenic bacteria, since these microorganisms normally occur in situations where nitrogenous compounds reach extremely high values (Brock and Main, 1994; Austin and Austin, 1999). The composition of biofloc and bioflims in the fish culture system is found to be same (Azim and Little, 2008). The microalgae associated with biofilms have the ablility to produce antibiotics that prevent pathogenic bacterial growth (Austin and Day, 1990; Alabi et al., 1999). Protozoa that inhabit biofilms could also control the abundance of pathogenic bacteria through grazing (Thompson et al., 1999). Thus, it is possible that, contrary to the expected effect, biofilm removal could increase the risk of developing pathogenic bacteria. Recently it was observed that excess addition of the carbon source enhances the development of polyhydroxyalkanoate (PHA) accumulating micro-organisms. The properties of PHAs are very similar to those of organic acids and have been proved to be effective bio-control agents, given that they beneficially affect the host's microbial balance in the gut.

4.5 Conclusion

In conclusion, the application of BFT in the culture system of giant freshwater prawn, *M. rosenbergii*, was useful in reducing the protein percentage from 32 to 24, without affecting the yield. So, farmers can adopt farming with 24% protein with BFT application. Toxic metabolite compounds like ammonia is shown to be get reduced by the bacterial metabolism. The reduced protein percentage in the feed is compensated by the consumption of flocculated microbial protein by the animal in effect, that results in conversion of more N inputs of the pond into harvestable products. Use of feed with lesser protein percentage will reduce the production cost. Biofloc technology is one of the futuristic technology for increasing the ecological and environmental sustainability of prawn farming.

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Chapter 5

EFFICACY OF VARIOUS CARBOHYDRATE SOURCES AS BIOFLOCULATING AGENT IN THE GROW-OUT SYSTEM OF GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII*

Contents

5.1 Introduction

Giant freshwater prawn, *Macrobrachium rosenbergii* is a highly demanding freshwater species in global aquaculture market. India is endowed with rich freshwater resource like ponds, tanks, lakes and reservoirs, which are ideal for the production of freshwater prawns. Scampi fetch much higher price than finfishes like carps, catfishes, etc. and the production would therefore help to increase the income of the rural fish farmers and improve their economic status. The operation of intensive aquaculture of freshwater prawn demands high investment and technical expertise, which are not affordable by resource-poor farmers (Asaduzzman et al., 2010b). Efforts are needed to intensify aquaculture by using the resources derived from other agricultural systems and manipulating natural food thereby maximizing overall nutrient retention (Azim and Little, 2006).

Biofloc technology is a novel technology for increasing the production and reducing the harmful chemicals from the aquaculture systems (Avnimelech, 2003, 2010). This technique is also referred as the built in bioreactors (Kurup, 2010b). BFT is successfully employed both in shellfish and finfish culture systems (Milstein et al., 2001; Burford et al., 2003, 2004; Avnimelech, 2005; Wasielsky et al., 2006; Serfling, 2006). The factors favoring the production of biofloc are mixing intensity, dissolved oxygen, organic loading rate, temperature, pH and organic carbon source (Schryver et al., 2008). The organic carbon can be supplied either as additional organic carbon source like glucose, acetate, glycerol etc. or by changing the feed composition by increasing its organic carbon content (Avnimelech, 1999).

Shrimp growth depends on the nutritional quality of dietary protein. Feed represents about 60% of the production cost in the extensive, semi-intensive and intensive farms. Therefore, attention has been paid towards reducing feed cost by way of use of less expensive and highly nutritive ingredients or by better consumption and assimilation of feeds by the animals (Varghese, 2007). Since protein is an expensive component of the fish diet, optimization of protein level in diet is necessary (Gümus and Ikiz, 2009). Optimal protein requirement level can be achieved by the addition of non-protein sources such as cheap carbohydrates (Varghese, 2007; Hari et al., 2004, 2006; Saritha, 2009). The results of several studies have pointed out the importance of using less expensive energy sources such as lipids and carbohydrate in order to save protein (Gallego et al., 1994; Okoye et al., 2001). Carbohydrates and lipids are cheaper energy sources compared to proteins. Optimal level of protein and the protein-sparing effect of non-protein nutrients such as lipids or carbohydrates may be effective in reducing feed costs (Gumus and Ikiz, 2009). Carbohydrate is proven to be a suitable energy source for carnivorous, omnivorous or herbivorous fishes (Zaid and Sogbesan, 2010). The protein sparing effect of lipids has been shown to be effective in several fish species (De-Silva and Anderson, 1998; Sargent and Tacon, 1999). Grains and grain products are the main carbohydrate sources in the diets of cultivated fish (Tacon, 1993), an attempt at fulfilling the energy requirement of fish through the use of roots and tubers could probably ameliorate the stiff competition with cereals and grains (Zaid and Sogbesan, 2010). Roots and tubers which could probably improve the feeds, water stability and nutrients retention, increase efficiency of digestibility and reduce cost of fish feed production (Falayi et al., 2003, 2004).

The carbon sources play a pivotal role in the biofloc formation, composition and its nutritive values (Hollender et al., 2002; Oehmen et al., 2004). The bioflocs production depends on the quality of added substrate and its C:N ratio (Avnimelech, 2007). Different carbohydrate sources like glucose, cassava meal, cellulose powder, molasses, tapioca flour, starch and wheat flour have been employed by various workers to enhance the bacterial production in extensive as well as intensive aquaculture systems (Avnimelech and Mokady, 1988; Avnimelech et al., 1994; Avnimelech, 1999, 2007; Buford et al., 2004; Hari et al., 2004, 2006; Varghese, 2007; Azim and Little, 2008, Asaduzzaman et al., 2008, 2009a, 2009b, 2010a, 2010b). However the organic carbon source is reported to determine, to a large degree, 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 (Hollender et al., 2002; Oehmen et al., 2004). The carbohydrate source should be economically viable, easily and locally available, non-toxic and compatible with the culture system and reared animals. For example, the glycerol which is a by-product in the bio-fuel industry is utilising as a biofloculating agent in some part of the world (Schryver et al., 2008).

On the basis of these assumptions, the present experiment is framed to study the effect of five locally available carbohydrate sources as biofloculating agent for controlling toxic metabolites in the semi-intensive culture system of giant freshwater prawn and its effect on prawn production.

5.2 Materials and methods

5.2.1 Tank allocation

Indoor experiments were conducted in FRP tanks with 1200L capacity and with an effective bottom area of 1.86 m^2, five triplicate treatments were maintained in the prawn hatchery complex of School of Industrial Fisheries, CUSAT. Post-larvae 20 stage of *M rosenbergii* purchased from local hatchery used for the experiment. Prawns were stocked at a rate of 250/m^2 for one week and they were fed with crumble, sinking starter feed having a crude protein percentage of 32 (*Grow best* scampi feeds). All tanks were provided with sand collected from the upper streams of Vembanad lake system which is well known for the natural habitat of *M. rosenbergii.* Tanks were filled with municipal water with a depth of 60 cm. All tanks were facilitated with 2 air stone-hoses type of diffuser system which is fitted to 5 HP blower. Aeration was provided 24 hours throughout the experiment for better biofloculation. Tanks were kept one week for dechlorination. Urea and super phosphate were added as fertilizers at a dosage of 4 and 1 g/m^2 during the first three weeks (Varghese, 2007). After one week all tanks were stocked with prawns at a rate of 15/m^2 (New, 2002). Before stocking initial weight of the organism (0.159±0.1g), initial water and sediment parameters were recorded. Commercial pelletized sinking prawn feed with a dietary protein level 24 was selected as experimental feed (From the results of Chapter 4). Feed was in pellet form and for initial feeding it was repelletized into smaller size.

5.2.2 Preparation of carbohydrate source and feeding

Five easily and locally available carbohydrate sources Viz. tapioca flour (*Manihot esculaneta*), yam flour (*Amorphophallus sp*), wheat flour (*Triticum aestivum*), rice flour (*Oryza sativa*) and potato flour (*Solanaum tuberosum*) were selected as carbohydrate sources for biofloclation. Rice flour and wheat flour were purchased from the local market in powdered form which was meant for the culinary purpose. While tapioca, yam and potato were purchased from vegetable market. Raw tubers were purchased, peeled and washed thoroughly, made into small pieces and soaked in water overnight. Next morning water drained and the pieces were kept in oven at 60^{0}C till it dried completely. After that slices were powdered in a mixer grinder, sieved through 35 µm sieves and powder stored in air-tight container (Saritha, 2009). By processing 1 kg of raw tuber, 500 g of corresponding powder was obtained.

Prawns were fed with experimental feed at 15% of initial weight (1-60 days) and adjusted gradually to 6% at the end of the culture (60-120 days). The daily feeding ration for each treatment was calculated and adjusted by estimating the monthly sampled mean biomass. The ration was divided and distributed twice daily with similar portions between 0900 and 1000 h in the morning and between 1700 and 1800 h in the evening. The

C:N ratio of the treatments was calculated using the formula of Avnimelech (2000) and it was found to be 13.4 for all the treatments. The quantity of carbohydrate added was calculated following the theory of Avnimelech (1999) and Hari et al. (2004, 2006) as explained in Chapter 4 (Section 4.2.2). Pre-weighed carbohydrate source was mixed in a glass beaker with the water collected from the corresponding culture tanks; 37.2 g for 100 g of feed was added in the tanks and poured directly to the water column after first feeding (Avnimelech, 1999). The culture tanks treated with tapioca flour, yam flour, wheat flour, rice flour, potato flour were represented as T, Y, W, R and P, respectively (Fig. 5.1). All the systems were maintained for 120 days without any water exchange. Water loss due to evaporation was compensated by the addition of dechlorinated water as per requirement.

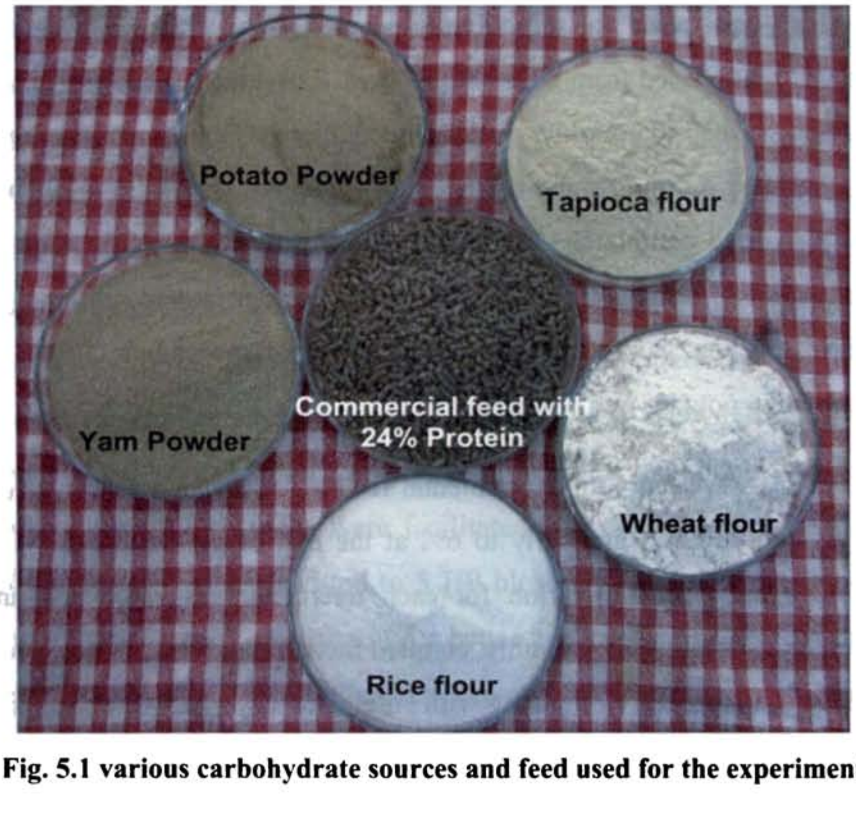

Fig. 5.1 various carbohydrate sources and feed used for the experiment

5.2.3 Prawn yield parameters

Harvesting was done by hand picking after complete draining of the culture tanks. Individual length and weight were recorded. Individual prawn weight gain, net prawn yield (gm/m^2), mean weight gain, net prawn yield, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), average daily weight gain (ADG) and survival rate, were calculated as described in Chapter 4 (Section.4.2.4).

5.2.4 Assessment of water and sediment quality parameter

Water quality parameters, temperature and pH were measured insitu at 0900 h on daily basis. Water samples were collected using a horizontal water sampler from three locations of each tank and pooled together. Sediment samples were collected from three locations using PVC pipes. Both water and sediment samples were transported to the laboratory within two hours after collection and analyzed. Sediment and water samples were collected on biweekly basis . Composite water column samples were filtered through GF/C Whatman glass filter paper and the filtrates were analyzed for nitrate-nitrogen (NO_3-N) (resorcinol method) nitrite-nitrogen (NO_2-N), total ammonia nitrogen (TAN) (phenol hypochlorite method). Chlorophyll-*a* in non-filtered water column samples were estimated following standard methods (APHA, 1995), dissolved oxygen (APHA, 1995) and biological oxygen demand (5 days BOD) was estimated following APHA, (1995). The organic carbon in the sediment was determined flowing El wakeel and Riley (1957) exchangeable TAN, nitrite-nitrogen, nitrate-nitrogen in the sediment were measured (Mudroch, et al., 1996). Nitrate estimate was done by resorcinol method. Total heterotrophic bacteria (THB count) in the water and sediments were estimated following the standard procedure (APHA, 1995) and expressed as colony forming units (cfu)

5.2.5 Statistical analysis

All non-repeatedly measured variables (prawn growth, yield, FCR, SGR and PER, survival) were analyzed by one-way ANOVA Tukey HSD programme using SPSS 17 software. If a main effect was significant, the ANOVA was followed by Tukey's test at $p<0.05$ level of significance. Water and sediment quality parameters were analyzed with two-way ANOVA.

5.3 Results

The average values recorded for the various physiochemical parameters like temperature, pH and dissolved oxygen are given in Table 5.1. These parameters were well within the optimum rage (New and Singholka, 1985) for the rearing of *M. rosenbergii* and were not found to be affected by the addition of different sources of carbohydrate. Water and sediment quality parameters, such as temperature, pH, dissolved oxygen, BOD and organic carbon were in the range of 27.7 - 27.8^{0}C, 6.38 - 6.54 and 6.50 - 6.57, 7.1 - 7.4 mg/l, 3.68 - 3.89 mg/l and 15.53 -16.42 mg/g respectively. Organic carbon and dissolved oxygen showed significant difference among treatments. Among the various treatments, the TAN in the water column has no significant difference but the sediment TAN is lower in tapioca added system (0.72 mg/l) and maximum (0.95 mg/l) was in the system where wheat was used as carbohydrate source. Nitrite values of both water and sediment have not showed any significant difference among treatments. But nitrate values in water showed significantly lower values in treatment (2.14 mg/l) where potato flour was added.

Concentrations of TAN, nitrate and nitrite recorded from water and sediment showed flcutuating trends.When comparing the week-wise values, significantly lower TAN concentration in water was observed at the end of the culture period, especially in the 7th biweek. Higher values were observed in the initial biweeks (2nd 3rd and 4th). Whereas nitarite value showed a reverse trend when compared to TAN. Significantly higher values were observed in the final biweeks however all other biweeks not showed any difference. Nitrate value showed a trend similer to that of TAN. Lower TAN values was in 7th biweek whereas higher was in 2nd,4th, and 5th biweeks. TAN values in sediment was siginificantly higher during the middle of the experimental period (5th biweek), while lower values were observed in 1st and 2nd biweek. Significantly lower nitrate values were observed in the sediment during the 1st , 2nd and 3rd biweeks and it was low in 7th biweek. Nitrate concentation was significatly higher during 7th and 8th biweeks and it was lower during 5th biweek.

Monthly parameters from various treatments are presented in Table 5.4. The bacterial count also did not exhibit any difference among treatments. ANOVA results showed significant variation in the bacterial count in month-wise data. Lower numbers of colonies were observed in first month (21.52x10^{3} cfu) while it was higher during the last (fourth) month (150.16 x10^{3} cfu).

Table 5.1 Daily water and sediment parameters in the tanks treated with various carbohydrate sources as biofloculating agents

Treatments	**T**	**Y**	**W**	**R**	**P**
Temperature (^{0}C)	27.7±.25^{a}	27.7±.35^{a}	27.7±.38^{a}	27.7±.38^{a}	27.8±.35^{a}
pH (water)	6.54±.26^{a}	6.44±.34^{a}	6.50±.32^{a}	6.38±.33^{a}	6.47±.32^{a}
pH (sediment)	6.50±.35^{a}	6.57±.26^{a}	6.57±.26^{a}	6.52±.21^{a}	6.56±.31^{a}

Results from Tukey One-way ANOVA

Treatments with mean values in same row with different superscripts differ significantly ($p<0.05$)

Table 5.2 Biweekly water quality parameters in the tanks treated with various carbohydrate sources as biofloculating agents

Treatments	T	Y	W	R	P
DO (mg/l)	7.18±.39^{ab}	7.10±.34^{a}	7.30±.37^{ab}	7.18±.33^{ab}	7.40±.39^{b}
BOD (mg/l)	3.89±.93^{a}	3.68±1.03^{a}	3.82±1.45^{a}	3.89±1.29^{a}	3.70±1.03^{a}
Nitrite (mg/l)	0.59±0.49^{a}	0.65±0.54^{a}	1.05±0.87^{a}	0.91±0.71^{a}	0.77±0.57^{a}
Nitrate (mg/l)	3.28±1.31^{b}	3.33±1.89^{b}	3.57±1.75^{b}	4.30±1.95^{b}	2.14±1.29^{a}
TAN (mg/l)	0.092±0.2^{a}	0.098±0.05^{a}	0.084±0.03^{a}	0.087±0.04^{a}	0.099±0.06^{a}

Results from Tukey Two-way ANOVA

Treatments with mean values in same row with different superscripts differ significantly ($p<0.05$)

Table: 5.3 Biweekly sediment quality parameters in the tanks treated with various carbohydrate sources as biofloculating agents

Treatments	T	Y	W	R	P
OC (µg/g)	16.42±1.68^{b}	15.83±1.29^{ab}	15.89±1.40^{ab}	16.29±1.29^{b}	15.53±1.20^{a}
Nitrite (mg/l)	1.50±0.99^{a}	2.06±1.43^{a}	2.043±1.44^{a}	1.76±1.37^{a}	1.81±1.59^{a}
Nitrate (mg/l)	3.68±1.98^{a}	4.63±1.75^{a}	3.80±1.60^{a}	4.46±2.04^{a}	4.12±0.97^{a}
TAN (mg/l)	0.72±0.31^{a}	0.90±0.23^{ab}	0.95±0.26^{b}	0.84±0.31^{ab}	0.85±0.26^{ab}

Results from Tukey Two-way ANOVA

Treatments with mean values in same row with different superscripts differ significantly ($p<0.05$)

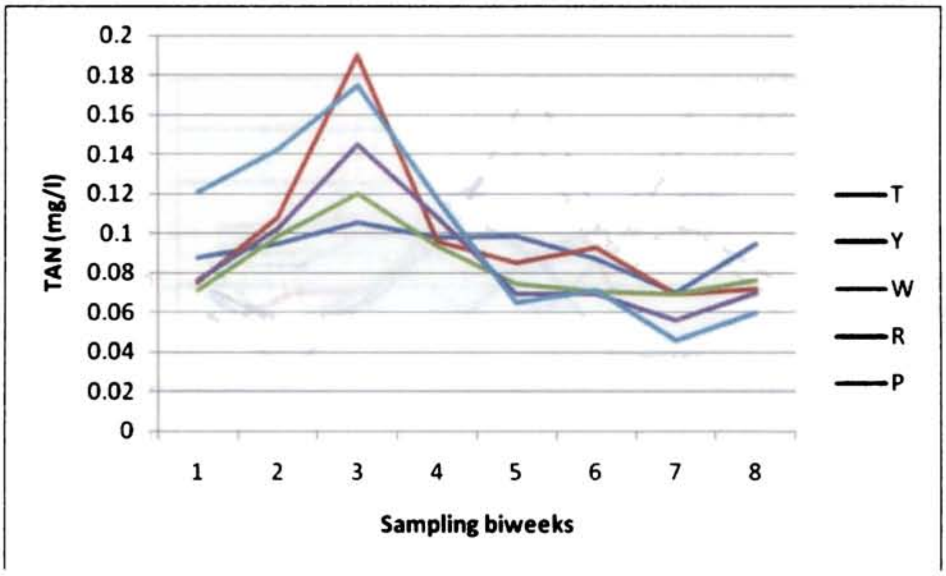

Fig. 5.2 Effect of various carbohydrate sources on the water TAN, in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

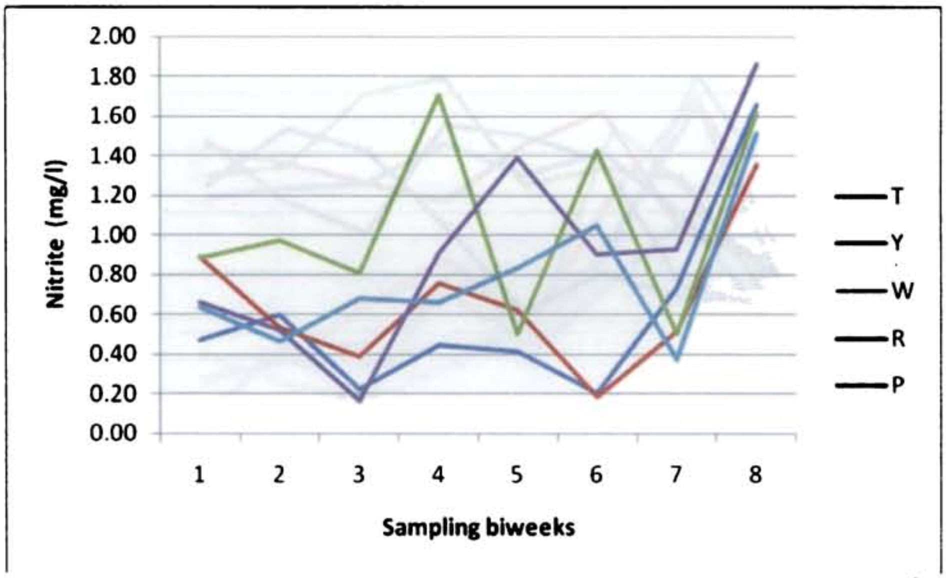

Fig. 5.3 Effect of various carbohydrate sources on the water nitrite in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

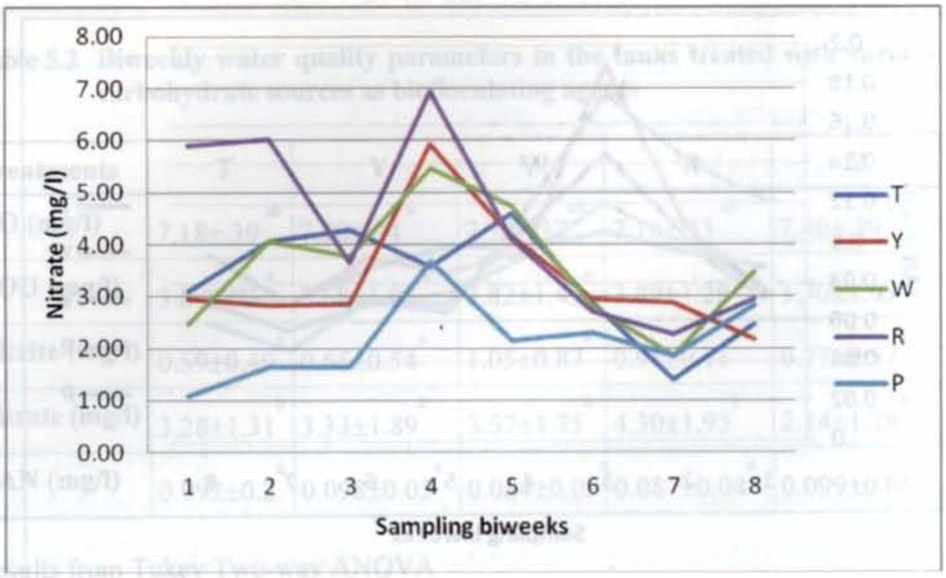

Fig. 5.4 Effect of various carbohydrate sources on the water nitrate in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

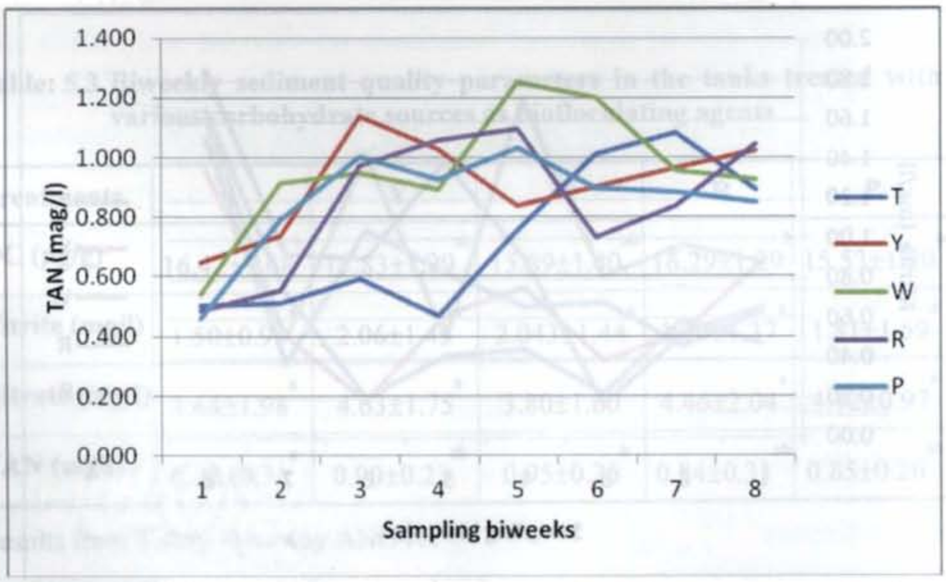

Fig 5.5 Effect of various carbohydrate sources on the sediment TAN in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

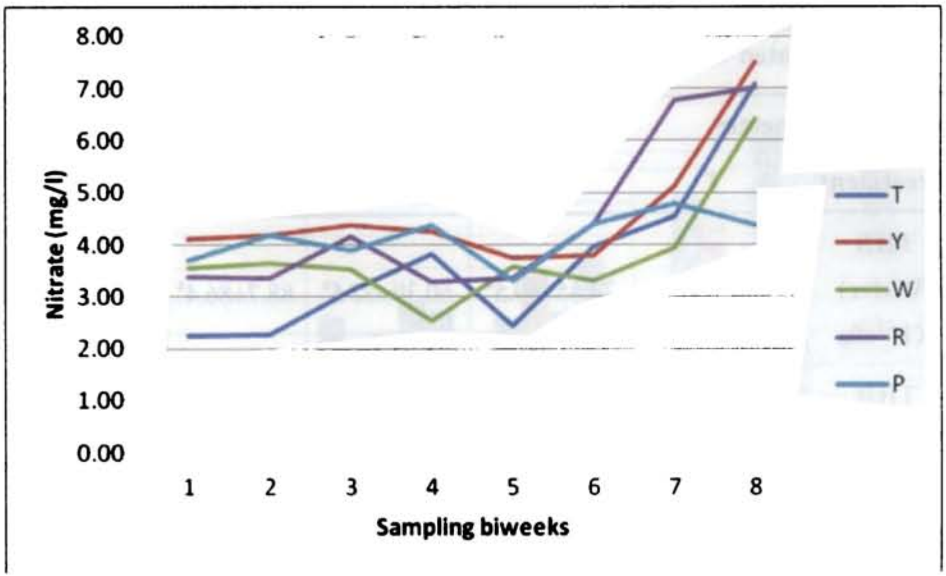

Fig. 5.6 Effect of various carbohydrate sources on the sediment nitrate in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

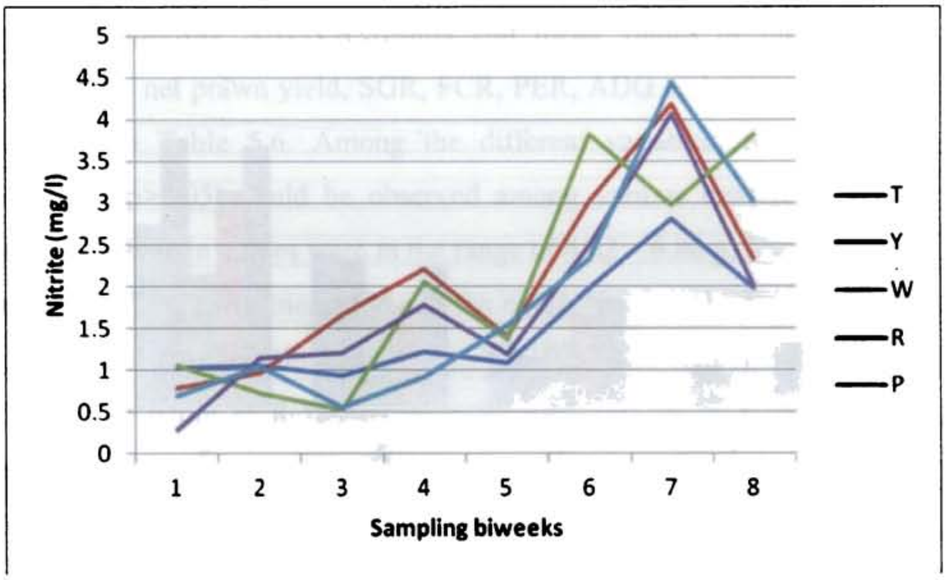

Fig. 5.7 Effect of various carbohydrate sources on the sediment nitrite in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Table 5.4 Monthly water and sediment quality parameters in the tanks treated with various carbohydrate sources as biofloculating agents

Monthly parameters					
Treatment	**T**	**W**	**Y**	**R**	**P**
THB (Water) X10^{3}cfu	99.9±61^a	74.45±69.5^a	60.39±52.5^a	88.7±86.4^a	72.9±58.5^a
THB (Sediment) X10^3 cfu	154.0±110.7^a	141.3±121.8^a	112.4±69.3^a	203.0±166^a	140.7±83.3^a
Chlorophyll a (mg/l)	26.5±7.4^a	27.2±7.7^a	26.4±6.6^a	29.5±9.4^a	27.5±7.7^a

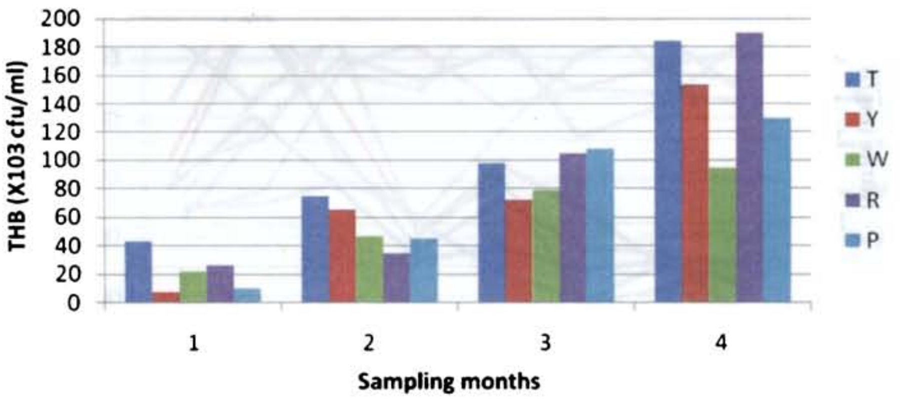

Fig. 5.8 Effect of various carbohydrate sources on the Total Heterotrophic bacterial count (water) in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

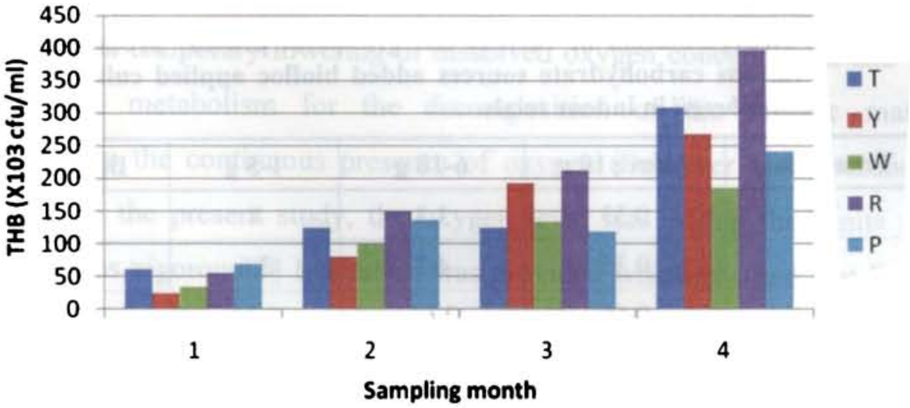

Fig. 5.9 Effect of various carbohydrate sources on the Total Heterotrophic bacterial count (sediment) in indoor tanks stocked with giant freshwater prawns, *M. rosenbergii*

Growth parameters

The one-way ANOVA results and mean values of mean prawn weight gain, net prawn yield, SGR, FCR, PER, ADG and survival rate are presented in Table 5.6. Among the different variables, no significant variations (p>0.05) could be observed among various treatments. Mean prawn weight gain values were in the range of 4.62 – 6.80 g. The net prawn yields of different treatments were in the range 40.11 – 52.72 g/m^2 and the highest value was recorded in treatment P. The SGR values ranged from 0.93 – 1.20 while FCR ranged between 0.91 – 1.25. FCR values were almost equal in all treatments. The protein efficiency ratio varied from 3.39 – 4.57 whereas average daily weight gain was in the range 0.026 – 0.044 g. Survival rate of the prawns did not vary (62.82 – 81.23%) among the different treatments. The size-groups of prawns harvested from various systems were also recorded. The organisms were mainly classified into 4 groups, below 1 g, 1-5 g, 6-10 g and above 10 g, majority of the animal harvested comes in between 1-5 g (Table 5.5).

Table 5.5 Representation of various size-groups of prawns produced from various carbohydrate sources added biofloc applied culture of *M. rosenbergii* in indoor trials

Treatment	Above 10 g	6-10 g	1-5 g	Below 1 g
T	0.33	3.3	4	1.3
Y	0.33	4.3	3.6	0.33
W	0.66	2.66	5.33	0
R	0.33	4.66	2.66	2
P	0.33	3.6	6	1

Table 5.6 Effect of various carbohydrate sources on weight, prawn yield, SGR, FCR, and survival of *M. rosenbergii* in indoor trials

Variable	T	Y	W	R	P
Mean prawn weight gain	4.62±0.23^{a}	6.80±3.32^{a}	5.26±2^{a}	5.34±1.95^{a}	5.73±2.52^{a}
Net prawn yield (g/m^{2})	40.64±14.6^{a}	45.82±22.3^{a}	40.11±17.2^{a}	43.69±18.85^{a}	52.72±16.91^{a}
SGR	1.08±0.08^{a}	1.20±0.28^{a}	1.10±0.23^{a}	0.93±0.06^{a}	1.10±0.02^{a}
FCR (Excluding biofloc)	1.20±0.07^{a}	1.14±0.28^{a}	1.25±0.20^{a}	1.15±0.19^{a}	0.91±0.07^{a}
PER	3.47±0.20^{a}	3.79±0.90^{a}	3.39 ±0.60^{a}	3.66±0.56^{a}	4.57±0.34^{a}
ADG	0.026±0.00^{a}	0.044±0.03^{a}	0.031±0.02^{a}	0.031±0.02^{a}	0.033±0.02^{a}
Survival rate	72.66±1.09^{a}	62.82±30.46^{a}	67.56±22.45^{a}	72.00±25.28^{a}	81.23±21.94^{a}

Results from Tukey One-way ANOVA

Treatments with mean values in same row with different superscripts differ significantly ($p<0.05$)

5.4 Discussion

The addition of carbonaceous substrate to the water column may resulted in temporary lowering of dissolved oxygen concentration and the microbial metabolism for the decomposition of the organic matter necessitate the continuous presence of oxygen (Schryver and Verstraete, 2009). In the present study, the oxygen level was within the limits and continuous vigorous 24 h aeration was provided which ensured that DO is not a limiting factor. Water quality parameters showed that they are good for the culture of giant freshwater prawn. This revealed that BFT is positively affecting the system by improving the water quality (Boyd and Zimmerman, 2000). High heterotrophic bacterial counts observed due to addition of carbohydrate in all treatments are found to be accomplished by a reduction of biological oxygen demand (BOD) in various treatments. Bratvold and Browdy (1998, 2001) reported that total bacterial counts and oxygen consumption rate were comparable in zero water exchange shrimp ponds. Culture system with low water exchange during intensive production of crustacean shellfishes has been achieved with closed recirculation system (Reid and Arnold 1992; Samocha et al., 2002; Mishra et al., 2008). However, such systems have high capital and operating costs (Ebeling and Timmons, 2007). A potentially cheaper alternative systems is the zero water exchange biofloc system, to which results in the formation of flocculated particles (microbial flocs) rich in bacteria and phytoplankton can be developed (Mc Intosh, 2000a, 2000b; Bufford et al., 2004; Wasilesky et al., 2006). The basic principle of biofloc technology is to reduce the toxic components from the culture system, the TAN, nitrate, nitrite values showed no significance difference, among the treatments, but it is comparatively lower with conventional culture systems (Avnimelech and Lacher, 1979; Avnimelech and Mokady, 1988; Avnimelech, 1998, 2000, 2006). The limitation of dissolved inorganic nitrogen can be maintained in fish or shrimp pond by adding carbon-rich substrates like glucose, cassava meal, cellulose powder, molasses etc. (Avnimelech and Mokady, 1988; Avnimelech et al., 1984, 1986, 1989, 1994; Avnimelech, 1999; Burford et al., 2004). The addition of carbonaceous substances will improve the water quality and productivity of ponds.

The effect of dietary carbohydrate on fish growth seems to depend on the source, dietary concentration and digestibility, the level of dietary intake, rearing conditions and fish species (Hilton and Atkinson, 1982; Kim and Kaushik, 1992; Krogdahl et al., 2005). The protein-sparing effect of different sources and levels of carbohydrates have been debated upon (Hilton and Atkinson, 1982; Wilson, 1994; Stone, 2003). All the carbohydrate sources applied to water column of various treatments were found effective in biofloculation by the significant increase in the total heterotrophic counts and this finding fully concurs with Burford et al. (2003). Locally available flour, molasses and starch were the common biofloculant used in this type of culture system (Avnimelech, 1999; Burford et al., 2004; Hari et al., 2004, 2006; Varghese, 2007; Sairtha, 2009).

In India, especially in state of Kerala, since the underground tubers like tapioca, yam, etc. consumed as the major food stuff, these sources were easily and cheaply available in local markets on demand. The five carbohydrate sources selected were tested in BFT applied grow-out of *P. monodon* (Varghese, 2007) and in the larviculture of *M. rosenbergii* (Saritha, 2009). Yen and Chun-Yang (1992) compared three carbohydrate sources, viz. glucose, dextrin and corn starch in favour of substituting the dietary protein. Avnimelech (1999) reported that with the addition of sugar (glucose) and cassava meal as carbonaceous substrate, there was a significant reduction in the accumulation of TAN, nitrite-N and nitrate-N concentration in tilapia farms. Megahed (2010) conducted on-farm trial to evaluate the effects of feeding on pellets with different protein levels in the presence and absence of the bioflocs on water quality. survival and growth of the green tiger shrimp (*P. semisulcatus*) in intensive types of shrimp culture systems. Wheat flour was the biofloulating agent used for that study. Cotner et al. (2000) reported that glucose addition to water reduced TAN concentration from 17.1-7.4 µg l^{-1} due to the enhancement of microbial growth. Asaduzzaman et al. (2008, 2009a, 2009b) used tapioca powder as the carbohydrate source for biofloculation. The study was carried out in Bangladesh. Later it was found that the availability of tapioca powder in Bangladesh was irregular and it has poor acceptance by farmers due to its higher price. Asaduzzaman et al. (2009b) recommended that identification of an alternative cheap on-farm carbohydrate source, which could potentially be produced within the farmer's traditional agricultural systems, is essential for economic sustainability of biofloc technology. On the basis of the series of assumptions, Asaduzzman et al. (2010b) compared the efficacy of tapioca starch and maize flour (*Zea mays*) as biofloculating agent. The similar inorganic N-species concentrations and other water quality parameters in ponds supplied with both maize flour and tapioca starch showed the possibility of using low-cost maize flour as cheap carbohydrate source for maintaining good water quality in C:N ratio optimised system. Results of pond ecological and growth data revealed that maize flour can be a good source of organic carbon to maintain a high C: N ratio in C/N controlled periphyton-based freshwater prawn ponds. In the present experiment, the maize flour was not evaluated because in

Kerala, it is not a prime cultivated grain and its local availability is also not common.

The effect of various types of carbohydrates such as starch, dextrin, glucose and sucrose on the growth and feed efficiency of the prawn were compared by Deshimaru and Yone (1978) and they concluded that sucrose is a suitable source of dietary carbohydrate for the prawn, whereas starch, dextrin and specially glucose are less desirable. Wilson (1994) showed that cooked starch and dextrin are utilized more efficiently than simple sugars by most fish. Bergot (1979) fed 120 or 300 g/kg of glucose or starch to rainbow trout and found that 300 g glucose was optimal. Tian et al. (2010) demonstrated that grass carp grows better when fed a glucose than starch diet. No consistent results about different complexities of carbohydrates utilization among fish species of different food habits have been achieved so far. Hung et al. (1989) found that white sturgeon utilized glucose and maltose more efficiently than fructose, sucrose, lactose, dextrin or starch. Inappropriate feeding practices in aquaculture may lead to feed wastage and insufficient feed being provided, resulting in higher production costs (Mihelakakis et al., 2002) and contamination of the aquatic environment (Ng et al., 2000). Efficient feeding strategy provides better growth and production (Cho et al., 2003; Eroldogan et al., 2004).

Usually in grow-out of giant freshwater prawn the commercial feed protein ranged from 22% to 38.5% (Crab et al., 2009). Kurup and Prajith (2010) optimized the protein percentage in giant freshwater prawn grow-out system as 24 with the application of biofloc technology, where tapioca powder was the bioflocating agent. Devi (2009) studied biofloc production in *Penaeus monodon* culture system under varying pH levels. Author also used tapioca powder as carbohydrate source. Crab et al. (2009) conducted

15 day experiment to evaluate the effect three carbohydrate sources; acetate, glycerol and glucose on the nutritional values of floc as a feed for post larvae of giant freshwater prawn. When compared to the above sources, carbohydrate sources chosen for the present experiment is locally and easily available and cheep also. When acetate, glycerol, glycerol+ *bacillus* and glucose were selected as bioflocuating agent in prawn culture system, authors reported a survival rate of 25±7%, 60±0%, 70±0% and 75±7% respectively. In this study, the survival rate was better when compared to this, where in the maximum and minimum survival rates observed were 81.52±21% and 62.82±30%. Crab et al. (2009) evaluated the efficiency of carbohydrate source for 15 days, whereas the present experiment was with the duration of 120 days. Better survival may be due to the lowering of toxic metabolites as a result of biofloculation or BFT may make it possible to increase growth yield and survival level at low water replacement rates with a potential addition of natural food resource. Kurup and Saritha (2010) and Saritha (2009) applied biofloc technology in the larviculture of giant freshwater prawn. Higher survival and good water quality parameters were recorded in the study. Saritha (2009) evaluated the efficiency of five carbohydrate sources. Carbohydrate sources opted is same as in the present experiment. All the carbohydrate sources applied to water column in various treatments were found to be effective for biofloculation which was manifested by the significant increase in the Total heterotrohic bacterial count and this finding fully concur with Burford et al. (2003) and Varghese (2007).

In general, the result of the study revealed that the various carbohydrate sources scanned in this experiment have the capacity to reduce the organic and inorganic nitrogen species developed as the result of animal metabolism and the selected five carbohydrate source were equally effective in controlling the toxic compounds and there is no significant effect on production. So, it is advisable to choose any of the above carbohydrate sources for the biofloculation process in the culture of giant freshwater prawn for making the practice ecologically and economically sustainable. Shi-Yen and Chun-Yang (1992) compared three carbohydrate sources, viz. glucose, dextrin and corn starch in favour of substituting the dietary protein. Varghese (2007) and Saritha (2009) observed no difference among the various carbohydrates sources in keeping the levels of TAN and NO_2^- -N under control. The recent studies have shown that glycerol-grown bioflocs have good nutritional properties and that they can be used as an additional feed source for giant freshwater prawn postlarvae (Crab et al., 2010a).

The glycerol used in the biofloc culture of *Artemia franciscana* showed a negative effect on the survival of artemia nauplii (Crab et al., 2010b). The criteria to select carbonaceous substrates should be its bio-availability, ability to disperse in water and its cost. A readily bio-degradable substrate is preferable in very intensive systems. The substrate should be soluble or given in fine powdered form, so as to slow its sedimentation rate and to keep it suspended in the water as much as possible. Finally, one should select substrates that are not costly. Carbonaceous substrates such as molasses, cassava meal, wheat or other flour have been successfully used by many researchers. It is possible to add carbonaceous substrates as an emergency measure in cases of an increase in inorganic nitrogen levels (e.g. after a period of cloudy days). An addition of 20-25 g carbonaceous substrate is needed to immobilize 1 g of inorganic nitrogen. A detailed discussion of the quantitative effects of C/N ratios is given by Avnimelech (1999). According to Hajra et al. (1988) the high survival rates of shrimp are mainly due to the favourable limit of environmental conditions for the organism. Once the carbon source is added to the culture water, it will be metabolised very quickly by the resident biofloc community. A solution to overcome the toxicity problem is partitioned addition of lower levels of the carbon source to the culture pond instead of one single addition (Crab et al., 2010a).

Prawn harvest details revealed that none of the parameters showed significant difference among treatments. Varghese (2007) carried out similar studies in extensive culture system of *Penaeus monodon* with the same carbohydrates sources and the results were similar. The survival rates of prawns were also similar among the treatments which indicate that all carbohydrate sources not have any adverse effect in destroying the shrimp habitat. In the present study, the net prawn yield and FCR were comparable in all treatments and it may be inferred that the level of interaction between the low dietary protein (24%) and different types of carbohydrate sources were similar. Furthermore, the lower TAN level in sediment might have influenced positively the food intake and health of the prawns (Avnimelech et al., 1995; Avnimelech, 1999; Hari et al., 2004, 2006; Varghese, 2007).

5.5 Conclusion

Biofloculation by the addition of different carbohydrate sources in the present experiment would indicate that carbohydrate added to the system facilitated the immobilization of inorganic nitrogen and from the results it is clear that carbohydrate sources can be utilized as a possible means to reduce the concentration of toxic metaboliotes from the culture tanks. In conclusion, the five locally available carbohydrate sources such as potato flour (P), yam flour (Y), rice flour (R), wheat flour (W) and tapioca flour (T) are equally effective and useful for the biofloculation process in the culture system of giant freshwater, *M. rosebergii,* and the scanned carbohydrate sources have the ability to controlling the inorganic nitrogen production in shrimp ponds by adjusting C:N ratio and they work well with the feed having a reduced protein percentage. While selecting the carbohydrate source in BFT ponds, it should be cheap, locally available and do not cause any harm to the cultured animal. More research is required to be finding out the efficiency of utilizing other cheap carbohydrate sources like sugarcane waste, molasses, coco, yam tuber and other agricultural wastes. Standardization is required for the use of liquid carbohydrate substrates such as sugarcane juice. The composition and nutritional value of the floc formed in the different carbohydrate sources used in aquaculture systems also need further investigation.

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Chapter 6

EFFECT OF ADDITION OF TWO MAJOR CARPS IN VARIOUS PERCENTAGES TO THE BFT APPLIED MONOCULTURE SYSTEM OF GIANT FRESHWATER PRAWN

Contents

6.1 Introduction

Aquaculture accounts for about one–third of total fishery production and more than two-third of inland fish production in India (Sharma and Leung, 2000). Indian aquaculture is dominated by freshwater fishes, including Indian major carps, Chinese carps and giant freshwater prawn. These are mainly produced in ponds, often integrated with crops and livestock (Schneider et al., 2000). Freshwater prawns can be cultured in the existing fish ponds along with major carps and can be integrated into rice farming to increase the income of rice cultivation. The most important aspects of polyculture are the ability to increase the productivity through more efficient utilization of natural food available (Hepher and Pruginin, 1981). In India, polyculture of the freshwater prawn, *M. rosenbergii*, and Indian major carps is a lucrative economic activity, and normally prawns are stocked with species like rohu, catla, silver carp and grass carp (Ayyappan, 2006). Carps have a number of advantages over other fish species. First, the carps can use feeds with moderate protein and fishmeal content. Secondly, they can be reared in ecologically efficient and environmentally benign polyculture systems that make optimum use of the natural productivity of the ponds and water bodies in which they are stocked. Thirdly, because of a huge and growing consumer base, traditions and relatively low prices, carps have good markets in Asian countries (Uddin et al., 1994; FAO, 1997). Polyculture of freshwater prawns with Indian major carps together with silver and grass carps enhances total production (Parameswaran et al., 1992) and have no adverse effect on the survival, growth or yield of other species (Lilyestrom et al., 1987). Finally, carp culture has lower production costs, fewer input requirements, fewer environmental problems and a smaller risk of disease outbreaks compared to shrimp culture (ADB/NACA, 1996).

In the present work, *Labeo rohita* and *Catla catla* were selected as the species for polyculture with freshwater prawns due to a variety of reasons. Rohu and catla are important commercial aquaculture fish species. In addition, rohu was chosen as the planktivorous fish species because it can utilise food from the whole water column (Das and Moitra, 1956) as well as having high consumer preference and market value in south Asia, especially in India and Bangladesh (Dey et al., 2005).

Prawn yield is influenced only by prawn stocking rate and not by the species of fish used (carps and tilapia) or by different stocking rates or different feeding and manuring regimes. However, the growth of carp and tilapia polycultured with prawns appear to be strongly affected by the number tilapia stocked and the feeding/manuring strategy (New and Valenti, 2000). Prawns utilize the bottom of the pond effectively in monoculture. The inclusion of freshwater prawns in a polyculture system almost always has synergistic beneficial effects, which include: more stable dissolved oxygen levels; reduction of predators; coprophagy (consumption of fish faeces by prawns), which increases the efficiency of feed: higher pond productivity (all species); and the potential to increase total value of the crop by the inclusion of a high-value species (FAO, 2002).

In highly aerated ponds, ammonium is oxidized by bacteria to nitrite and nitrate species. 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. Thus, intensification of aquaculture system is inherently associated with enrichment of the water with respect to ammonium and other organic nitrogenous species. The management of such system depends on developing methods to remove these compounds from the pond. 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. 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 protein as a source of feed protein for shrimp or fish. Utilization of microbial protein depends upon the ability of the animal to harvest the bacteria and its ability to digest and utilize the microbial protein and C/N ratio can be manipulated in the semi-intensive farming system of crustaceans for the economic and ecological sustainability (Varghese, 2007).

Against this background, main objectives of the present study are

1) To find out the efficiency of addition of two Indian major carps in various percentages to the biofloc technology applied prawn culture system

2) To find out its effect in water and sediment quality

3) To select the suitable stocking density and species for the BFT applied polyculture system

6.2 Material and methods

6.2.1 Experimental design

The experiment was carried out in concrete tanks having an effective bottom area of 6 m^2 (3×2×1 m). All the tanks were provided with a uniform 7 cm deep loamy sediment layer taken from an extensive shrimp culture pond. The soil was made of 20.7% clay, 29.8% silt and 49.5% sand. All tanks were completely independent and fully exposed to natural sunlight. Experiments were maintained in triplicate following complete randomized design. Tanks were drained and cleaned, sediment layer ploughed manually and exposed to sunlight for one week (Fig.6.1). One foot water was filled in all tanks and lime was added initially at 3 kg $tank^{-1}$. Municipal water was filled in all the tanks. Cattle dung, urea and super phosphate were used as fertilizers. Fertilization was done with cattle dung at the rate of 5 kg $tank^{-1}$, urea and super phosphate, 4 and 1 g m^2/ week (Varghese, 2007). All tanks were provided with 4 air-stone hose type of diffuser system 50 cm above the sediment layer for 24 hours throughout the experiment for increasing biofloculation, which was fitted to a 5 hp blower. Tanks were kept one week for dechlorination and plankton growth. Water level in the culture tanks was maintained at 1 m throughout the culture period. Tanks were facilitated with overflow pipes which maintained the water level as one metre which was covered with fine meshed nets prevented the escaping of prawns and fishes. Post larvae of *M. rosenbergii* were purchased from the Kerala Government Regional shrimp hatchery, Azheekode, Kerala, India. Prawns were stocked at the rate of 90 PL/tank

($15PL/m^2$). Two Indian major carp fingerlings catla (*Catla catla*) and rohu (*Labeo rohita*) were purchased from a private hatchery. Prawns and fishes were having weight and length of 0.158± 0.012 g and 1.36 cm, 9.8±2.25 g and 9±2.5 cm, respectively. Catla and rohu were stocked at various percentages. A combined stocking density of 20 fishes/tank was maintained. Details of prawn and fish stocking densities and experimental codes are given in Table: 6.1. Feed with a crude protein percentage of 24% was used for both prawns and fish. Prawns were fed with experimental feed at 15% of initial weight (1-60 days) and adjusted gradually to 6% at the end of the culture (61-120 days). Fishes were fed at 5% of the initial weight (1-60 days) and adjusted gradually to 3% at the end of the culture period (61-120 days).

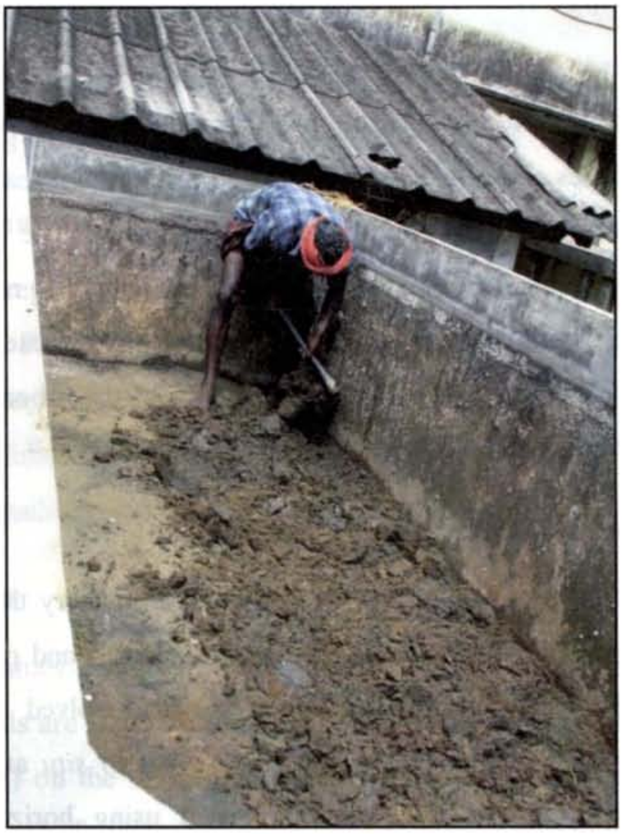

Fig. 6.1 Preparation of tanks before stocking

Table 6.1 Experimental designs and stocking density of fishes in the BFT applied monoculture system of giant freshwater prawn, *M. rosenbergii*

Sl No.	Treatments Stocking density in 6 m^2 tanks Prawns	 Rohu	 Catla	Experimental code
1	90	20	0	100R
2	90	15	5	75R+25C
3	90	10	10	50R+50C
4	90	5	15	75C+25R
5	90	0	20	100C

6.2.2 Harvesting

At the end of the experiment (120th day), tanks were drained and prawns and fishes were collected by hand picking (Fig. 6.13-6.15). Total length of the fishes and prawns was measured using a dial reading caliper from the tip of the rostrum to the tip of the telson. The weight was measured by weighing the animals from each treatment, after removing the water from their body with tissue paper. Specific growth rate, net prawn yield, and individual prawn yield were recorded (As described in Chapter 3) during the sampling days.

6.2.3 Water and sediment quality monitoring

Water quality parameters such as temperature (mercury thermometer), salinity (hand refractometer), Secchi's disk (transparency) and pH (pH pen) were measured directly from the culture tank and dissolved oxygen was measured following Winkler method (APHA, 1995) *in situ* at 09.00 AM daily. Biweekly water samples were collected using horizontal water sampler from three locations of each tank and pooled together. Sediment samples were collected from six locations using PVC pipes (2cm diameter). Sediment and water samples were collected on biweekly basis between 0900 and 1000 hours. The water samples were filtered through GF/C Whatman filter paper and the filtrate was analyzed for nitrate-N (cadmium reduction), nitrite-N and total ammonia nitrogen (TAN) (phenol hypochlorite method) (Grasshoff et al., 1983). Chlorophyll- *a* in non-filtered water column samples were analyzed following standard methods (APHA, 1995). Biological oxygen demand (5day BOD) of water samples was estimated following APHA (1995). The organic carbon in the sediment was determined following El Wakeel and Riley (1957). Exchangeable TAN, nitrite-N and nitrate-N in the sediment were also measured (Mudroch et al., 1996). Monthly total heterotrophic bacteria (THB) count in the water and sediment was also estimated following standard procedures (APHA, 1995) and expressed as colony forming units (cfu).

6.2.4 Data analysis

All non-repeatedly measured variables (prawn and fish growth, yield, FCR,SGR and PER, survival) were analyzed by one-way ANOVA Tukey HSD programme using SPSS 17 software. If a main effect was significant, the ANOVA was followed by Tukey's test at $P<0.05$ level of significance. Water and sediment quality parameters were analyzed with two-way ANOVA.

6.3 Results

Mean values of water and sediment quality parameters of different treatment ponds are shown in Table 6.2- 6.5. Treatments had no significant effect ($P>0.05$) on the daily parameters such as water temperature, water and sediment pH. Temperature was 27-28^{0}C among treatments; water pH ranged from 7.7-8, while sediment pH values were comparatively low with a range of 6.5-6.6. The *Secch's* disk reading values showed increasing trend with the increasing stocking density of catla. Average value of 62.2 cm was recorded from treatment 100C tank, the lower value 28.8 cm was from the treatment 100R. The visibility was increasing with the increase in the stocking density of catla. Dissolved oxygen concentration was 6.7-6.8 mg/l, BOD values raged 3.7-3.9 mg/l. Water and sediment TAN, nitrate and nitrite did not show any significant difference among treatments, except the nitrite values recorded from the sediment. THB values were significantly differs among treatments. Both in the water and sediment, higher bacterial colonies were observed in the catla dominated treatments and lower values were recorded from the rohu dominated systems. THB was found to be increasing when the number of catla stocking increased in the tanks. Chlorophyll-*a* values were also differed among treatments and significantly lower chlorophyll values were recorded from 50C+50R tanks and higher was from 100R tanks followed by 75R+25C.

The growth parameters showed a fluctuating trend. Mean prawn weight gain, specific growth rate, average daily weight gain and survival rates among the treatment were significantly different. Higher mean prawn weight (27 g) was recorded from 100C, SGR and ADG were also higher in this treatment with a values of 1.78 and 0.22 g, respectively. Higher and lower survival was obtained from 25R+75C and 100C with a percentage of 82 and 18, respectively. Catla yield showed that net fish yield and FCR were significantly differing among treatment. The net fish yield from this treatment was 1.2 kg. Lower FCR was from 75R+25C and 100C. Except survival, rohu yield did not demonstrate any difference among the tanks. Higher survival was from the treatment 25R+75C (80%).

Table 6.2 Daily water and sediment quality parameters recorded from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

Treatment	100R	75R+25C	50C+50R	75C+25R	100C
Temperature (C^{0})	27.9±0.5^{a}	28±0.4^{a}	28±0.4^{a}	28±0.4^{a}	28±0.4^{a}
pH water	8±0.9^{a}	7.7±0.7^{a}	8±0.8^{a}	7.8±0.8^{a}	7.7±0.7^{a}
pH sediment	6.5±0.2^{a}	6.6±0.2^{a}	6.6±0.2^{a}	6.5±0.3^{a}	6.5±0.1^{a}

Table 6.3 Biweekly water quality parameters recorded from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

Treatment	100R	75R+25C	50C+50R	75C+25R	100C
Visibility (cm)	28.8±16.8^{a}	49.4±22.4^{b}	50.6±13.3^{b}	54.8±16.9^{b}	62.2±13.5^{b}
DO (mg/l)	6.7±0.51^{a}	6.7±0.45^{a}	6.9±0.37^{a}	6.9±0.49^{a}	6.7±0.52^{a}
BOD (mg/l)	3.9±0.57^{a}	3.7±0.64^{a}	3.7±0.58^{a}	3.8±0.41^{a}	3.7±0.66^{a}
Nitrite (mg/l)	0.95±0.76^{a}	1.17±0.90^{a}	1.47±1.05^{a}	1.38±1.05^{a}	1.14±0.97^{a}
Nitrate (mg/l)	3.14±1.35^{a}	3.02±1.40^{a}	3.50±1.87^{a}	3.91± 2.42^{a}	3.32 ±1.55^{a}
Ammonia (mg/l)	0.95±1.07^{a}	0.76±0.98^{a}	0.53±0.49^{a}	0.72±0.86^{a}	0.67±0.56^{a}

Table 6.4 Biweekly sediment quality parameters recorded from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

Treatment	100R	75R+25C	50C+50R	75C+25R	100C
OC (mg/l)	20.7±0.59^{a}	20.7±0.57^{a}	20.8±0.55^{a}	20.7±0.50^{a}	20.8±0.50^{a}
Nitrite (mg/l)	1.51±0.96^{b}	1.30±0.93^{ab}	1.11±0.78^{ab}	0.99±0.88^{ab}	0.71±0.57^{a}
Nitrate (mg/l)	2.76±1.34^{a}	2.93±1.80^{a}	3.18±1.66^{a}	3.55±1.59^{a}	3.18±1.62^{a}
Ammonia (mg/l)	3.19±1.04^{a}	3.41±0.99^{a}	3.42±1.09^{a}	3.03±1.06^{a}	3.52±0.86^{a}

Table 6.5 Monthly water and sediment quality parameters recorded from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

Treatment	100R	75R+25C	50C+50R	75C+25R	100C
THB(Water) X10^{3} cfu	60.8±37.1^{a}	83±55^{ab}	76.9±71^{ab}	80.4±70.3^{ab}	121.8±100^{b}
THB(Sediment) X10^{3} cfu	152.17±72^{a}	174.2±97^{ab}	192.6±102^{ab}	194.6±86^{ab}	249.5±162^{b}
Chlorophyll a (µg/l)	77.2±21.2^{c}	66.7±17^{bc}	47.1±12^{a}	59.6±17^{ab}	54.5±20^{ab}

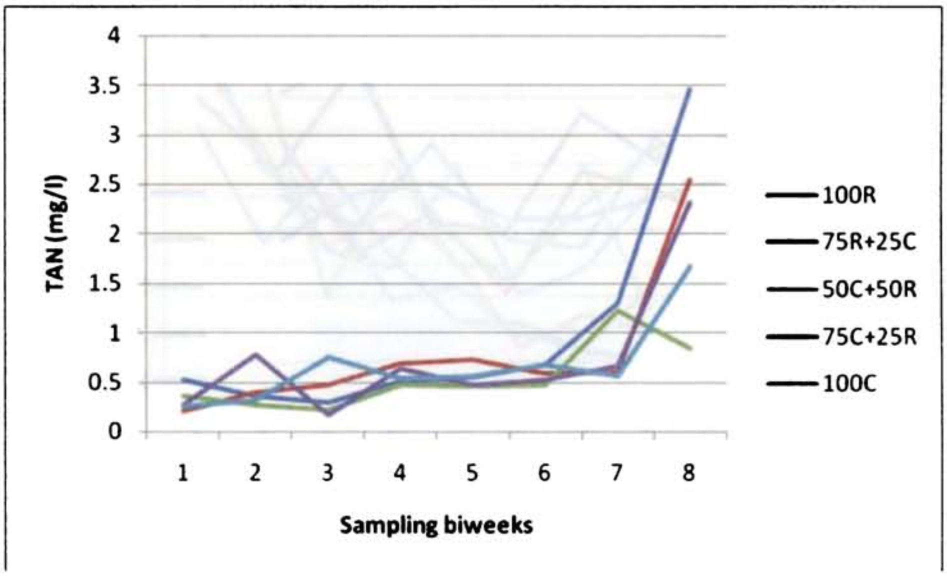

Fig. 6.2 Variation of total ammonium nitrogen (TAN) in water from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

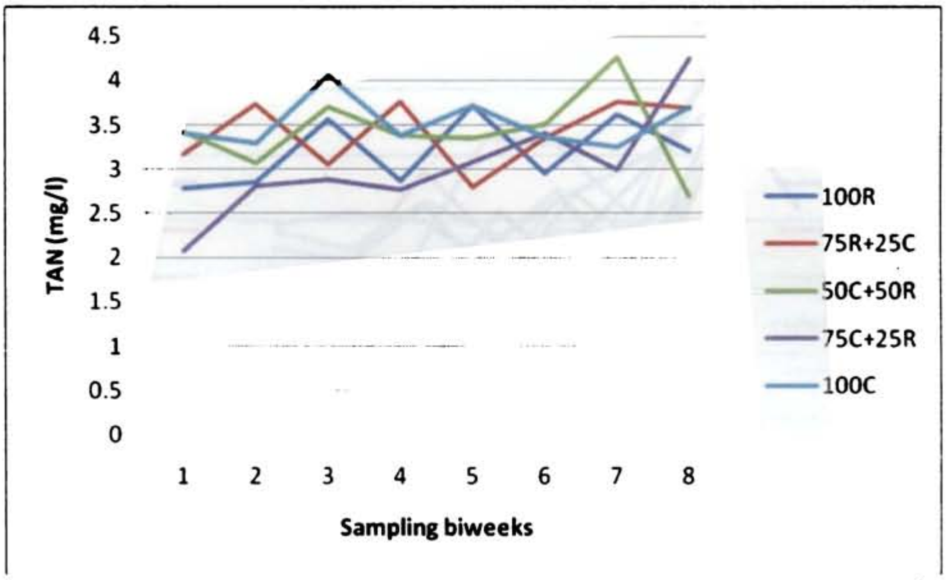

Fig. 6.3 Variation of total ammonium nitrogen (TAN) in bottom soil from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

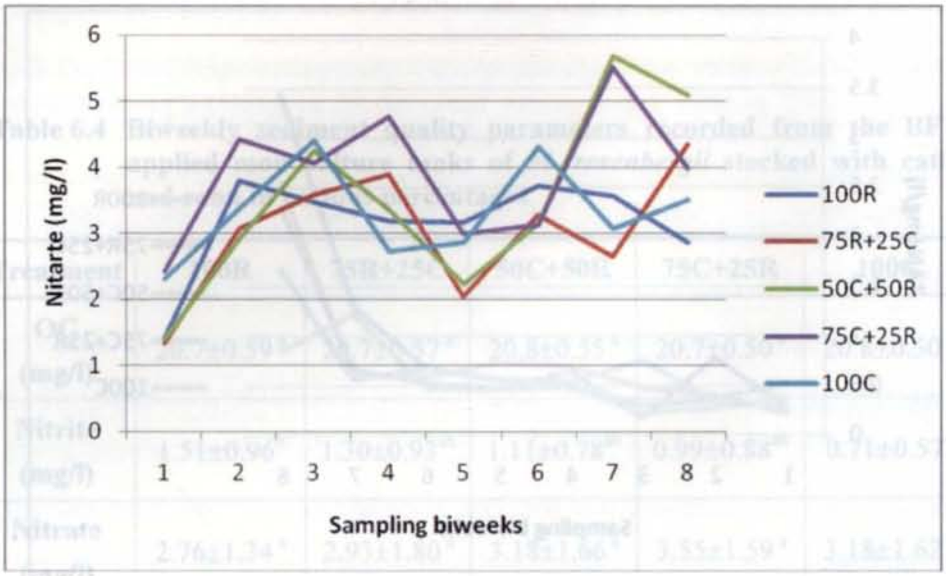

Fig. 6.4 **Variation of Nitrate in water from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages**

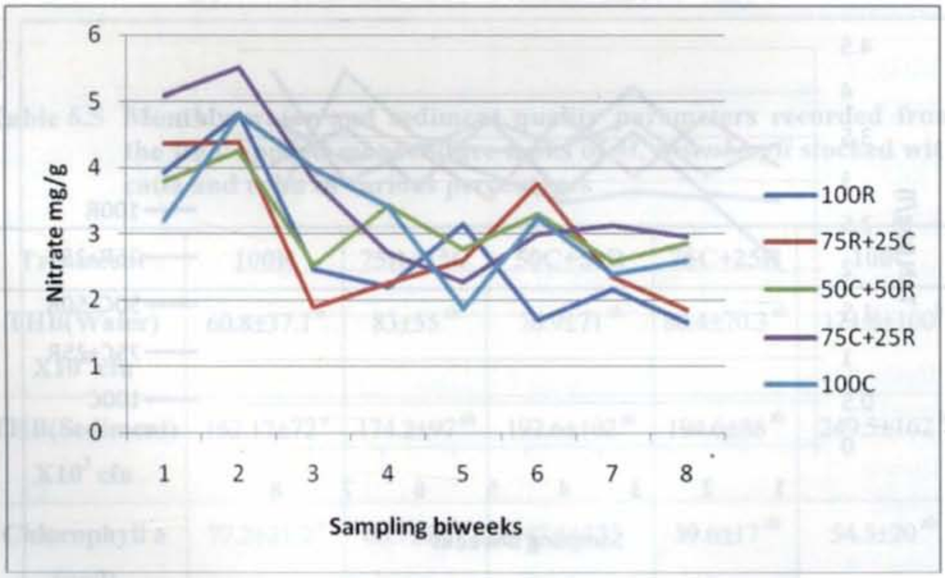

Fig: 6.5 **Variation of Nitrate in sediment from the BFT applied monoculture tanks of M. rosenbergii stocked with catla and rohu in various percentages**

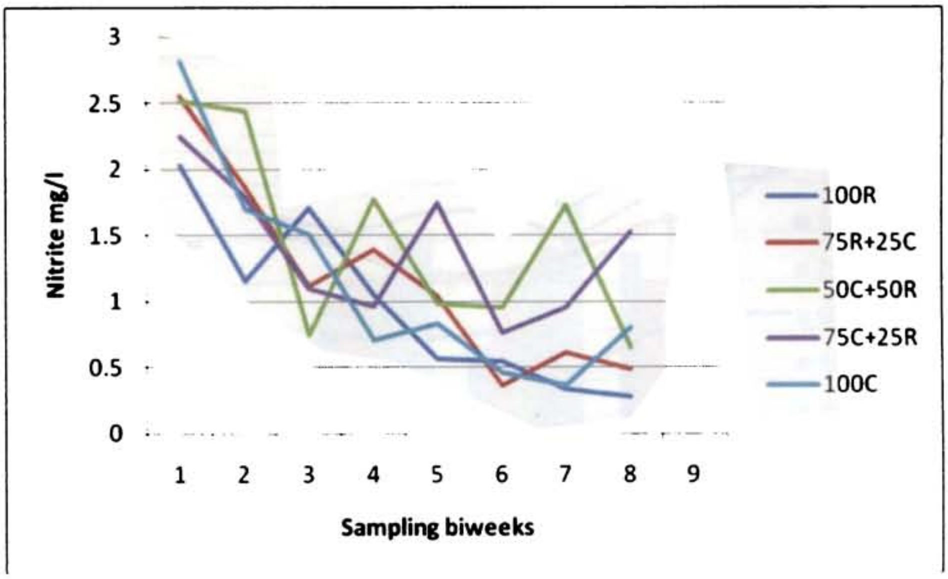

Fig. 6.6 **Variation of Nitrite in water from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages**

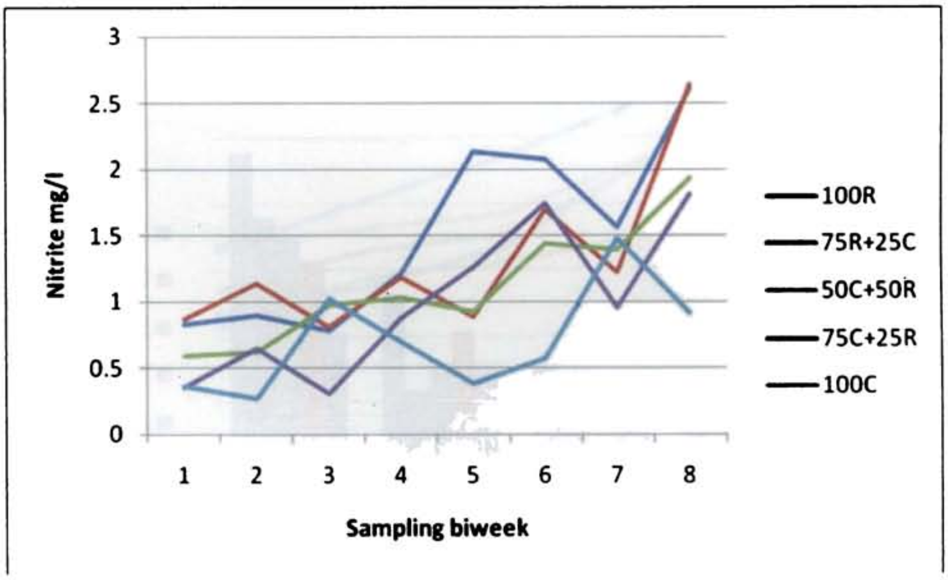

Fig. 6.7 **Variation of Nitrite in bottom soil from the BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages**

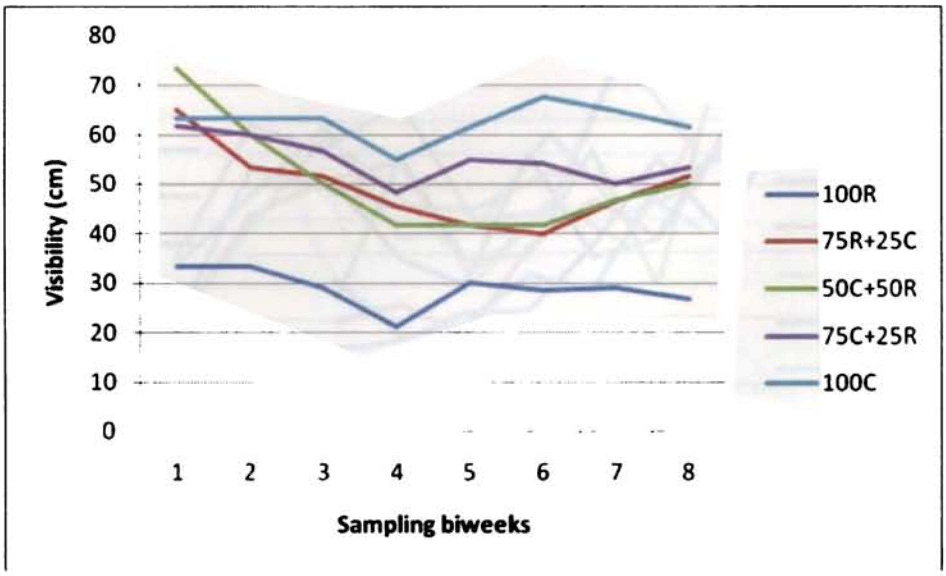

Fig. 6.8 **Biweekly variation in the *Secchi* disk depth recorded from BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages**

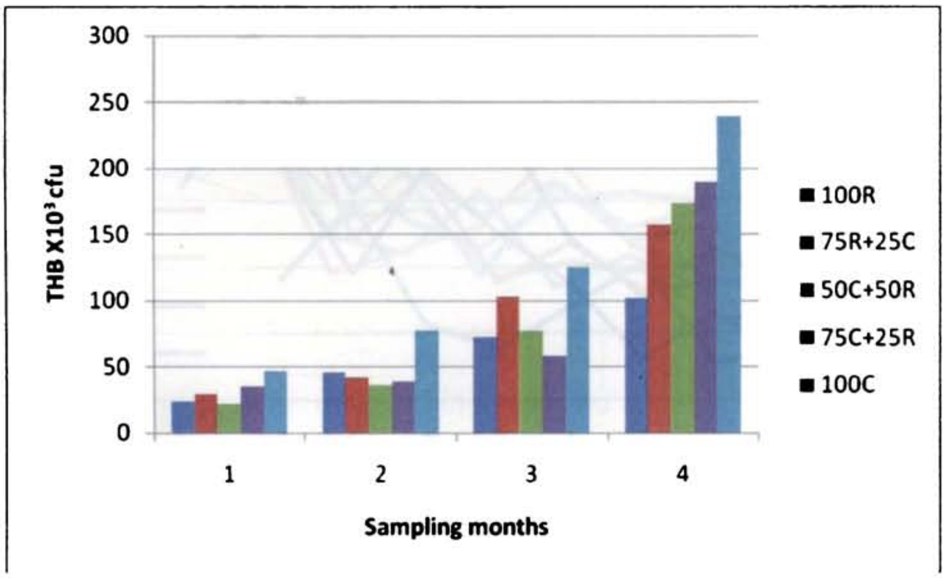

Fig. 6.9 **Monthly variations of Total heterotrophic bacteria in the water column of BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages**

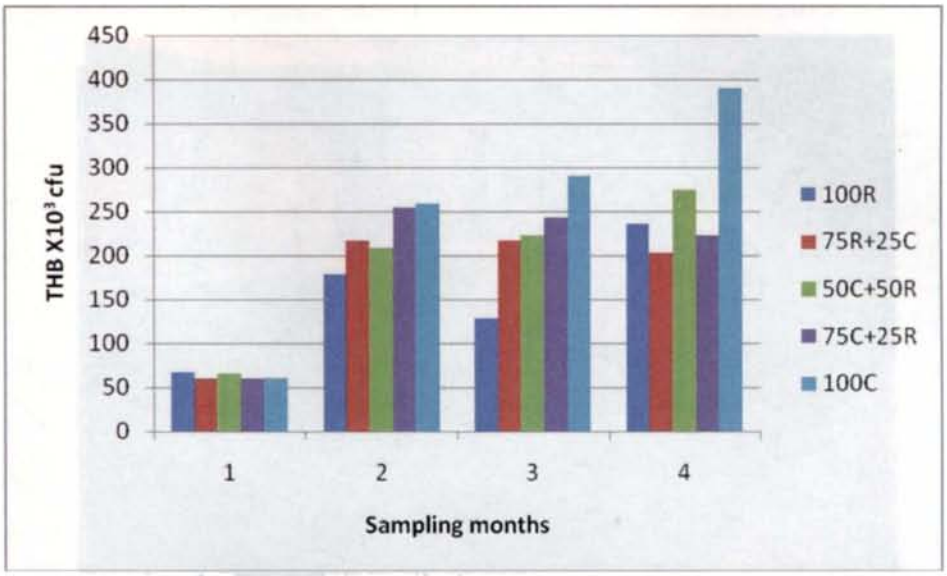

Fig: 6.10 Monthly variations of Total heterotrophic bacteria in the sediment of BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

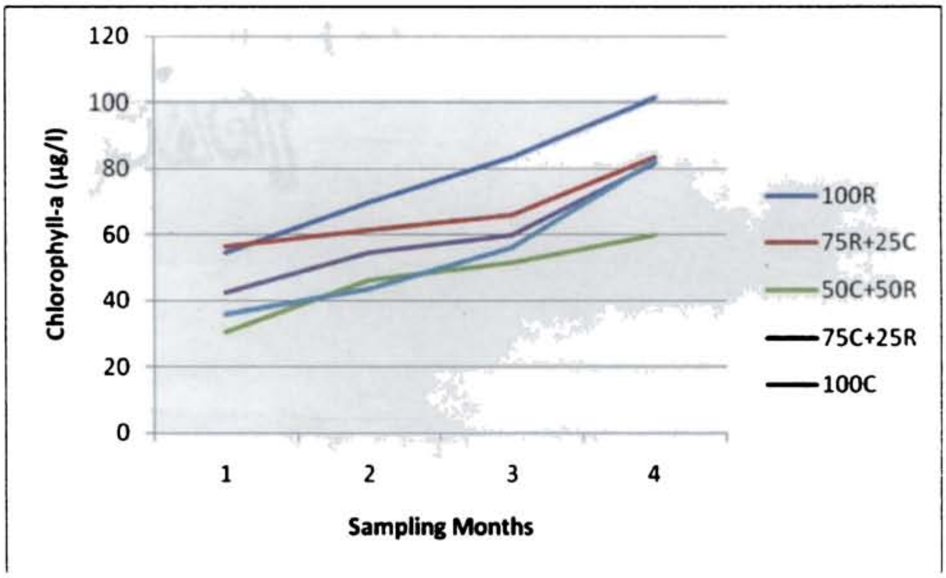

Fig. 6.11 Monthly variation of Chlorophyll-a in BFT applied monoculture tanks of *M. rosenbergii* stocked with catla and rohu in various percentages

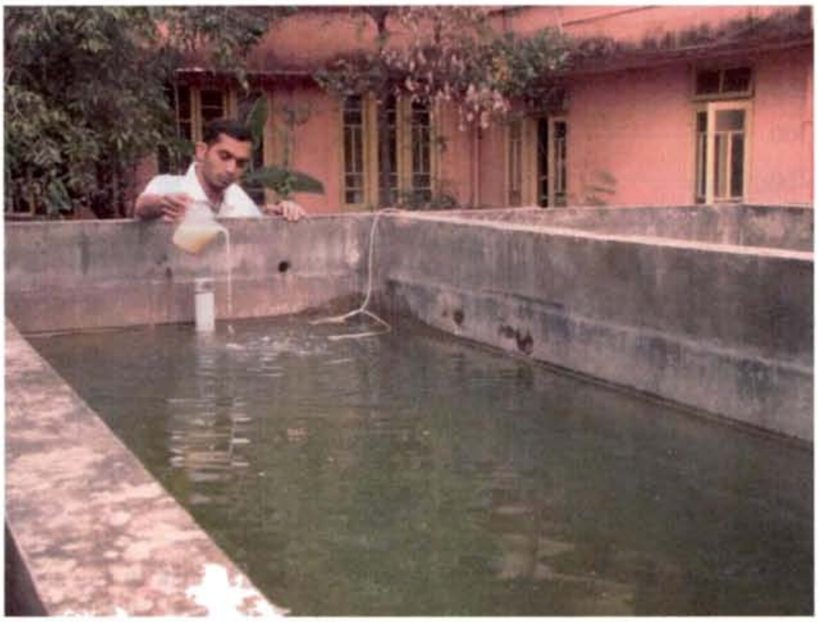

Fig. 6.12 Addition of carbohydrate source to the water column

Fig. 6.13 Prawns harvested from experimental tanks

Fig: 6.14 Rohu harvested from experimental tanks

Fig. 6.15 Catla harvested from experimental tanks

Table 6.6 Comparison of means (and SD) of yield parameters of prawn and fishes added in various percentage to the BFT applied monoculture system of giant freshwater prawn, *M. rosenbergii* by ANOVA.

Variable	Treatment				
Prawn	**100R**	**75R+25C**	**50R+50C**	**25R+75C**	**100C**
Mean prawn weight gain (g)	5.91±0.90^{a}	6.21±0.38^{a}	8±1.67^{ab}	10.26±1.70^{ab}	27.01±16.6^{c}
Net prawn yield (g/tank)	264.38±53.15^{a}	245±314^{a}	410.4±35.3^{a}	517.3±93.5^{a}	609±236^{a}
FCR	1.03±0.18^{a}	4.73±5.71^{a}	0.65±0.05^{a}	0.52±0.10^{a}	0.50±0.25^{a}
SGR	1.30±0.05^{a}	1.32±0.02^{a}	1.41±0.07^{ab}	1.5±0.06^{ab}	1.78±0.31^{c}
PER	4.12±0.82^{a}	3.81±4.9^{a}	6.3±0.55^{a}	8±1.45^{a}	9.4±3.69^{a}
ADG (g)	0.04±0.00^{a}	0.05±0.00^{a}	0.06±0.01^{ab}	0.08±0.01^{ab}	0.22±0.13^{c}
Survival rate (%)	56.7±9.7^{abc}	35±24.9^{ab}	67.36±10.2^{bc}	82.6±17^{c}	18.1±1.73^{a}
Catla	**100R**	**75R+25C**	**50R+50C**	**25R+75C**	**100C**
Mean fish weight gain (g)	***	98.86±18.9^{a}	56±1^{a}	75.4±7.1^{a}	174.6±110^{a}
Net fish yield (g/tank)	***	247±56.8^{a}	182±5^{a}	359±127^{ab}	1275±771^{b}
FCR	***	0.86±0.11^{a}	2.58±0.07^{b}	2.11±0.67^{b}	0.84±0.46^{a}
SGR	***	0.49±0.06^{a}	0.29±0.00^{a}	0.39±0.03^{a}	0.63±0.30^{a}
PER	***	4.3±1.0^{a}	1.5±0.05^{a}	2±0.73^{a}	5.6±3.4^{a}
ADG (g)	***	0.82±0.15^{a}	0.46±0.00^{a}	0.52±0.05^{a}	1.45±0.92^{a}
Survival rate (%)	***	60±0.00^{a}	50±0.00^{a}	40±11.5^{a}	47±11.5^{a}
Rohu	**100R**	**75R+25C**	**50R+50C**	**25R+75C**	**100C**
Mean fish weight gain (g)	137.8±35^{a}	114.9±64.5^{a}	106.9±24.3^{a}	116.8±77.3^{a}	***
Net fish yield (g/tank)	934±465^{a}	559.6±249.8^{a}	478.6±125.8^{a}	414.3±169.2^{a}	***
FCR	1.21±0.65^{a}	1.43±0.60^{a}	1.02±0.26^{a}	0.63±0.23^{a}	***
SGR	0.94±0.09^{a}	0.84±0.20^{a}	0.85±0.08^{a}	0.84±0.22^{a}	***
PER	14.5±7.2^{a}	8.7±3.8^{a}	7.4±1.9^{a}	6.4±2.6^{a}	***
ADG (g)	1.14±0.29^{a}	0.95±0.53^{a}	0.89±0.20^{a}	0.97±0.64^{a}	***
Survival rate (%)	38.3±14.4^{a}	39.9±6.6^{a}	50±10^{ab}	80±20^{b}	***

6.4 Discussion

M. rosenbergii, being a benthophagic omnivore, is an excellent candidate for polyculture. Culture of prawn with fish also improves the ecological balance of the pond water, preventing the formation of massive algal blooms (Cohen et al., 1983). Ling (1969) recommended polyculture of *M. rosenbergii* with non-carnivorous freshwater fish such as carps and tilapia. Polyculture of freshwater prawns is recommended by duly considering a series of factors like species compatibility, interaction with environment, stocking size and essentially the targeted market and its economics. The production cost of prawn may be lowered by the correct choice of fish species and their stocking rates (New and Valenti, 2000). Fish that 'filter' pond water are quite efficient in this respect. Zimmermann and Rodrigues (1998) reported that 74% of research papers published on the polyculture of freshwater prawns was the combination with 'filtering' fishes (plankton feeders). The remaining 26% papers referred to the polyculture of *M. rosenbergii* with a wide variety of other species: other freshwater prawns (Martinez et al., 1985; Rajyalakshmi and Maheswardu, 1986; Durairaj and Umamaherswari, 1991), freshwater crawfish (D'Abramo and Daniels, 1992), mullets (Cohen, 1989; Hulata et al., 1990), catfish (Cohen, 1985; Avault, 1986; D'Abramo et al., 1986; Heinen et al., 1987; Lamon, 1988), marine shrimp (in rotation) (Srinisavan et al., 1997) and aquatic plants (Das et al., 1991; Roy et al., 1991).

Present experiment was an attempt to incorporate major carps in the biofloc technology applied monoculture system of giant freshwater prawn. Studies reported that the several characteristics of water quality were influenced by polyculture and they found that these effects were always positive, in that main factor relating to this work is that the polyuculture method decreases the need to exchange water in ponds. Water temperature was initially within the optimal range for the polyculture of giant freshwater prawn with Indian major carps. The Secchi disk depth reading was directly propotional with increasing catla numbers due to lower abundance of phytoplankton, indicating that catla grazed more on developed flocs. Results of periphyton based experiment of Azim et al. (2001) reported the same observation. Dewan et al. (1991) and Ahmed (1993). reported an inverse relationship between Secchi depth value and chlorophyll *a* in aquaculture ponds. Secchi disc reading and the cholorophyll values are the indication of plankton development in the pond. Plankton has a profound effect on water quality in several ways (Boyd, 1982; Smith and Piedrahita, 1988). Phytoplankton are the major source of dissolved oxygen in fish ponds and indirectly a source of detritus upon which most bacterial respiration is based, the major sink for oxygen (Boyd, 1973). Phytoplankton is the major source of productivity in aquatic ecosystems, having a direct relationship with fish production (Talling, 1965). New and Valenti (2000) reported that presence of carp and tilapia results in improvement in water quality. According to Hepher and Pruginin (1981), the beneficial effect of polyculture is due to dissolved oxygen stability, reduction of predators and coprophagy. In the present study, dissolved oxygen concentrations were generally suitable for fish culture throughout the experimental period, no significant difference was observed in the case of dissolved oxygen concentrations in any of the treatments and this may be attributed to the addition of aeration to the culture system. Water and sediment quality parameters not showed any difference among treatment except values of sediment nitrite. Nitrite concentration in sediment showed significantly higher values in 100R tanks. Varghese (2007) reported that nitrogenous compound concentrations of water and sediment cumulatively increase with increase in stocking density. But in the present experiment, there is no significant variation in the concentration of TAN and nitrate among treatments. The biofloc formed in all the treatments may reduce the concentration of nitrogenous compound and it can be attributed to bacterial metabolism. Microorganisms present in shrimp culture systems can take up significant amounts of ammonia, and consumed oxygen from the water column (Pomeroy et al., 1965).

Banerjea (1967) and Boyd (1974) reported that if daily variables were in the acceptable level, it will help survival and growth of the culture species. In the present study, the soil pH was found to be 6.5-6.6 which did not show any variation between treatments which concurs with Varghese (2007). The bottom soil pH is an important variable in pond aquaculture and values between 6.5 – 7.5 normally considered as acceptable (Jackson, 1958; Mc Lean, 1982; Boyd and Tucker, 1992; Hendershot et al., 1993; Bloom, 1999).

Feeding ration was calculated separately for prawns and fishes, animals fed with low protein commercial feed (24%). Initially, prawns and fishes were fed with 15 and 5% of their body weight respectively. Later, it was reduced to 6 and 3% respectively. Feed used was sinking type. It ensured the availability to the prawns. Ahmed et al. (1996) conducted stocking density experiment with freshwater prawn ($0.6/m^2$), silver carp, catla, rohu and mrigal (fish stocking ratio 3.5:1.5:3.0:2.0). Where 32% crude protein feed was used for feeding.

In a polyculture setting, prawn and carp can utilize different food niches efficiently. Polyculture of prawn without bottom feeder species allows the prawns to obtain their sufficient share of the pelleted feed, as a certain proportion of the feed will sink to the bottom. In addition, it allows the prawn to graze on bacterial films on the bottom substrate and ultimately results in better growth performance of prawn (Hossain and Islam, 2006). In the present study, the significant high values in total heterotrophic bacterial (THB) count in both water and soil were recorded from 100C tanks. In 100R, significantly lower bacterial count was recorded and this indicates that the capacity of rohu to harvest the bacterial flocs. Many workers reported that the heterotrophic bacterial protein might be utilized as a food source by carp and tilapia whereby lowering the demand for supplemental feed protein (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993; Avnimelech, 1999). The properly adjusted and added carbohydrate was found useful in reducing the inorganic nitrogen concentration in the culture pond by the well utilization of heterotrophic bacterial population (Avnimelech, 1999). In the previous studies conducted in this connection, several other carbohydrate sources like glucose and cassava meal cellulose powder (Avnimelech and Mokady, 1988; Avnimelech et al., 1989, 1994; Avnimelech, 1999), molasses (Burford et al., 2004) and tapioca (Hari et al., 2004, 2006) were used in fish and shrimp ponds to reduce the inorganic nitrogen concentration for increasing the yield and getting high survival rate. Aikyama et al. (1989) reported that carbohydrate source (glucose and starch) application to the culture system provided better environment for multiplying bacterial population. In the present study, tapioca flour was used as carbohydrate source for the direct application to the culture system (Fig. 6.12). Tapioca powder is found to be an ideal source of carbon as it is cheaply available in Kerala.

The stocking density of prawns and fishes in the polyculture system varies from region to region and farm to farm. Prawns may be predators depending on the size of the fishes (Roberts and Kuris, 1990). In this experiment, relatively larger fishes were stocked with prawn. Roberts and Kuris (1990) recommended the stocking of fishes with prawns having the weight of 0.25 to 0.50 g. In the present experiment, prawns and fishes were having weight of 0.158±0.012 g and 9.8±2.25 g, respectively, and stocking density was 90 prawns/tank (15/m^2) and 20 fishes/tank (3.3/m^2). Mires (1987) successfully stocked *M. rosenbergii* juveniles at 5-7.5/m^2 in polyculture with Nile tilapia (*Oreochromis niloticus*) fingerlings stocked at 6000 to 7000/ha. Based on the result of Chapter 3, the stocking density of prawn was selected as 15/m^2. Higher stocking density is the characteristics of biofloc based aquaculture. Stocking density of prawn selected in this study is relatively higher when compared to other studies (Jose et al., 1992; Islam et al., 1999). But nearly similar stocking density was adopted by Hossain and Islam (2006). The authors conducted polyculture of prawns with carps at a stocking density of 10000-12500/ha. In the present study the production of prawns ranged from 40.8-101.5 kg/ha with a culture period of 120 days. Islam et al. (1999) reported a production of 172 kg/ha/year, where *M. rosenbergii* was stocked at 15000/ha with silver carp, catla, rohu and mrigal (1.75:0.75:1.50:1.0) at a stocking density of 5000/ha in each pond. The production of prawn in the present study was higher than that of Haque et al. (2003), who used prawn stocking densities of 3750, 5000, 6250, 7500, 8750, 10000/ha and catla, rohu and mrigal (1:1:1) were stocked at a density of 11250/ha and led to a production of 120.9-216.1kg/ha, while the production of carp was 2248.8-2359.2 kg/ha

Polyculture increases the yield of fish over monoculture at similar stocking densities by reducing inter-specific resource competition, and probably by mutual enrichment of diet through synergy (Hossain and Islam, 2006). Prameswaran et al. (1992) showed that the polyculture of the freshwater prawns with Indian major carp (rohu) together with exotic carp enhanced total production. Lilyestrom et al. (1987) reported that polyculture appeared to have no adverse effect on the survival, growth or yield of either species. Cannibalism amongst prawn does not seem to be exacerbated by presence of other fishes or crustaceans (New and Valenti, 2000). Martino and Wilson (1986) reported that the culture system with *M. rosenbergii*, Mosambica tilapia and the American crayfish, the animal interaction were inter-specific and that the cannibalism, which was detected among crustaceans was not influenced by the presence of tilapia. In the present experiment, prawn survival was less from 100% catla added tanks (18.1±1.73%), the fishes being surface feeder, occupied the top of water column so the prawn-fish interaction may be negligible or nil. In the rohu added tanks, relatively good survival was obtained. Even though survival was low in 100% catla tanks, mean prawn weight was 3.7±1 times greater than other treatments. Catla, being a surface feeder it may occupied in the upper ecological niche of the culture pond and the prawns may in the situation like that of monoculture system, this may triggered the cannibalism among prawns and the individuals survived might have utilised applied artificial feed and biofloc effectively, this may be the reason for lower FCR values.

The prawns showed better FCR values, except in the rohu dominated system. Rohu may consume both artificial feed and biofloc developed in the ponds to the maximum level. Studies revealed that the feed consumption by the prawns in the polyculture system is low when compared to the monoculture system (New and Valenti, 2000). Lilyestrom et al. (1987) showed that when polycultured with channel catfish (*Ictalurus punctatus*) fed formulated rations, freshwater prawns made less use of the artificial feed than the catfish. In the present experiment FCR of prawns ranged from 0.50-4.7, as mentioned earlier the higher FCR was from the 100% rohu and 75% rohu added tanks. This confirmed that in catla dominated system, there is no overlapping of the feeding niche, so the good FCR is by the effective utilisation of biofloc developed in the middle and bottom of the water column by the prawns. New (1988) reported that for *M. rosenbergii*, an FCR of 1.8-3.0 may be acceptable for dry diets, while moist diets made on the farm containing waste prawn heads, trash fish, etc. would have an FCR of 3.0-5.0. Jena et al. (2002) reported FCR values of 1.47-1.69 for carps in various stocking densities; in the present study, the FCR values ranged 0.63-2.58 and this is lower than those of earlier studies (Das et al. 1975, 1977; Sinha and Saha, 1980).

Evaluation of production performance in carp polyculture with different stocking densities and species combinations was studied by Jena et al. (2002). The results showed that the biomass contribution of the treatments stocked with Indian major carps was maximum by catla, followed by mrigal and rohu in treatments stocked at two different densities. Working with three Indian major carp species, Das et al. (1977, 1980) recorded similar results, with maximum contributions of catla followed mrigal and rohu under two different experimental trial.

6.5 Conclusion

Stocking finfishes in biofloc based monoculture system of freshwater prawns has the potential of increasing total yield. Prawns having higher market value than finfishes, it also ensure economic sustainability. Final results showed that prawn yield and survival was better in catla dominated tanks. It is recommended to incorporate 25% rohu and 75% catla in the biofloc based culture system of giant freshwater prawns. The results of the study also recommend to stock relatively larger catla for biofloc based culture. Fish production was also higher in the 100% catla tank. When adding catla in higher percentages, it should ensure the availability of hiding objects in the culture ponds in order to reduce the chance of cannibalism. Rohu and catla equally have the ability to harvest the biofloc, catla consumes the planktonic contributes in the floc whereas rohu grazes on the bacterial protein.

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Chapter 7

SUMMARY AND CONCLUSION

Aquaculture, rearing plants and animals under controlled conditions is growing with an annual growth rate of 8.3% in the period 1970–2008 (FAO, 2010). This trend of growth is essential for the supply of protein-rich food for ever increasing world population. But growth and development of aquaculture should be in sustainable manner, preferably without jeopardizing the aquatic environment. Among the aquaculture species, crustaceans have good demand and economic value both in domestic and international markets. Giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), is known as *Attukonchu* or *Kuttanadan konchu* in *Malayalam* and it is an indigenous crustacean species in the whole of the Indo-Pacific region. It is the only freshwater crustacean having very high economic value. Giant freshwater prawn based aquaculture industry has gained importance in many Asian countries, which contributed to over 98% of global freshwater prawn production. During the culture of freshwater prawn, two issues of environmental concern requires careful attention, viz, the escape of prawns from grow-out pond and the discharge of polluted effluent into natural waters. Among the two, the second one is considered as the serious issue because the release of nutrient-rich water to the natural environment results in eutrophication and besides the challenges of biosecurity. Nearly 70% of the protein-rich feed applied in the prawn culture system is getting accumulated in the pond bottom which leads to the development of toxic inorganic species (NH_4^+ and NO_2^-) in the pond sediment. Only 20- 40% of the nitrogen is incorporated to prawn tissue, while the remaining is lost to the pond and ultimately to the environment. As such, no effective mechanism is available to remove the accumulated nitrogenous metabolites from the pond bottom. There are several ways to eliminate ammonia from the aquaculture systems, such as exchange and replace water, use of bioflitration system or establishing a recirculatory aquaculture system (RAS), reduce or stop feeding, flush the pond with fresh water, reduce the stocking density, aerate the pond in emergencies, reduce the pH level, etc. But these methods are expensive and labourious and economically not feasible or can cause harm to the cultured animals.

An additional strategy that is recently gaining attention is the removal of ammonium from the water through its assimilation into microbial protein by addition of carbonaceous material to the system (Avnimelech, 1999). This novel technology is referred as biofloc technology (BFT). Biofloculation by the addition of properly adjusted carbohydrates could potentially eliminate the problem of inorganic nitrogen accumulation. An added advantage of this technology is the potential utilization of microbial protein as a source of protein for fish and shrimp. Biofloc technologies can provide a major contribution towards meeting these goals while producing high quality, safe, attractive and socially acceptable products. Biofloc technology facilitates intensive culture, while reducing investment and maintenance costs and incorporating the potential to recycle feed. Land based prawn culture utilize land and freshwater. On a global scale, water resources are becoming scarce and expensive. About 41% of the world population today lives in the water stressed river basins. In 2050, 70% of the world population will face water shortage. In this regard world aqua farmers recognized the importance of zero water exchange systems. BFT is based upon the principle of zero or minimal water exchange to maximize biosecurity while minimizing external environmental effects.

In the present study, the application of BFT in the nursery rearing and farming of giant freshwater prawn, *M. rosenbergii,* is attempted. The result of the study is organised into eight chapters. In the first chapter, the subject is adequately introduced. Various types of aquaculture practices followed, development and status of Indian aquaculture, present status of freshwater prawn culture, BFT and its use for the sustainable aquaculture systems, theory of BFT based aquaculture practices, hypothesis, objective and outline of the thesis are described. An extensive review of literature on studies carried out so far on biofloc based aquaculture are given in chapter 2.

The third chapter deals with the application of BFT in the primary nursery phase of freshwater prawn. Several workers suggested the need for an intermediate nursery phase in the culture system of freshwater prawn for the successful production. Thirty day experiment was conducted to study the effect of BFT on the water quality, and animal welfare under the various stocking densities.

Result revealed that an intermediate nursery phase is essential in the aquaculture of giant freshwater prawn, *M. rosenbergii.* BFT application in the primary nursery system found to be useful in enhancing the animal growth parameters and water quality. Higher survival, animal health and quality were observed in 200 and 200BFT. Even though higher production is obtained from the lower stocking densities, when considering the economic aspects of the culture, it is better to select the next stocking density (600) with biofloc application. The results of this study recommend a stocking density of 6PL/L in the biofloc applied primary nursery rearing of giant freshwater prawn.

Application of BFT in the grow-out of freshwater prawn and its effect on the water quality and growth parameters were studied and the results are given in fourth chapter. Feeds having three protein levels were evaluated. Six treatments with triplicates were maintained and prawns fed with the protein percentage 32, 28 and 24 with and without biofloc application were the treatment selected. All tanks were maintained for 180 days without water exchange. Data on water quality, sediment quality and prawn yield parameters were analyzed by ANOVA. The results showed that the application of BFT in the culture system of giant freshwater prawn, *M. rosenbergii*, reduced the protein percentage from 32 to 24 without affecting the yield. So farmers can adopt the biofloc technology with 24% crude protein in the farming of giant freshwater prawn. Toxic metabolite compounds like ammonia found to get reduced by the bacterial metabolism. The reduced protein percentage in the feed is compensated by the consumption of flocculated microbial protein by the animal which results in conversion of more nitrogen inputs of the pond in to harvestable products. Use of lesser protein will reduce the production cost. Biofloc technology is the futuristic technology for improving the ecological and environmental sustainability of prawn farming.

The efficiency of various type of carbohydrate in the control of inorganic nitrogen and increasing production of *M. rosenbergii* have been evaluated and the results are presented in chapter 5. The experiments were carried out in 1.2 m^2 tanks having sandy bottom. Feed with a dietary protein of 24% with addition of each carbohydrate sources such as tapioca flour, wheat flour, rice flour, potato flour and yam flour were compared.

Stocking density at the rate of 15 post larvae (PL 20) of *M. rosenbergii* /m^2 was used in the experiment. In this study, the results undoubtedly proved that the addition of each cheap carbohydrate source has the efficiency to reduce the total ammonia nitrogen (TAN) and nitrite-N production in the water and soil. Water TAN showed no significant difference among treatments. Biofloculation by the addition of different carbohydrate sources in the present experiment would indicate that carbohydrate added to the system facilitate the immobilization of inorganic nitrogen and from the results it is clear that carbohydrate sources can be utilized as a possible means to reduce the concentration of toxic metabolites in the culture environment. In conclusion, the five locally available carbohydrate sources such as potato flour (P), yam flour (Y), rice flour (R), wheat flour (W) and tapioca flour (T) are equally effective and useful for the biofloculation process in the culture system of *M. rosebergii* and the screened carbohydrate sources have the ability in controlling the inorganic nitrogen production in shrimp ponds by adjusting C:N ratio and these sources work well with the feed having a reduced protein percentage. While selecting the carbohydrate source in BFT ponds, it should be cheap, locally available and should not cause any harm to the cultured animal.

In the sixth chapter, efficiency of addition of two Indian major carps in various percentages to the biofloc technology applied prawn culture system on the water and sediment quality were investigated. This was done to understand optimizing the stocking density and species suitable for the BFT applied polyculture system. Prawn stocking density was fixed as 90 per tank (15/m^2) and two Indian major carps catla and rohu were added to the prawn tanks at various percentages. Water, sediment and growth parameters were evaluated. Growth parameters were analyzed using one-way ANOVA whereas water and sediment quality were compared with two- way ANOVA.

The study concluded that stocking finfishes in biofloc-based monoculture system of freshwater prawns has the potential of increasing total yield. Prawns having a higher commercial value than finfishes besides ensuring economic sustainability. Results showed that prawn yield and survival was better in catla dominated tanks. Based on the results of the study, it is recommended to incorporate 25% rohu and 75% catla in the biofloc-based culture system of giant freshwater prawns. The results of the present study also recommend to stock relatively larger catla for biofloc-based culture system. Fish production was also higher in the 100% catla tank. When catla was added in higher percentages it should ensured that the hiding objects in the culture ponds shall be used in order to reduce the chance of cannibalism among prawns. rohu and catla equally have the ability to harvest the biofloc, catla consumes the planktonic contributes in the floc whereas rohu grazed on the bacterial consortium suspended in the water column.

In Chapter 8, recommendations and future research perspectives in the field of biofloc based aquaculture is presented.

The study concluded that the major advantages of the application of BFT in aquaculture systems are

- 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 prawns
- The level of protein utilization is doubled in microbial reuse system
- The dense heterotrophic microbial biomass decreases the chance of outbreak of microbial diseases and finally
- The technology enables high yield in environmentally and economically sustainable system

In essence, biofloc technology could be useful in improving the sustainability of fish/shellfish farming in both extensive and modified extensive culture systems. There exists great scope for further improvement of this management strategy, not only by optimising the quantity of carbohydrate addition at various intensities of culture, but also by comparing the potential of other carbohydrate sources. Additional research is required with respect to feed composition, planning and feeding rate determination.

In one word, "Biofloc aquaculture is a key for the development of sustainable aquaculture".

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Chapter 8

RECOMMENDATIONS AND FUTURE RESEARCH PERSPECTIVES

India is endowed with vast growth potential for expanding aquaculture production for several reasons. Firstly, catering to the escalating demand for food fish can only met from aquaculture since the production from capture fisheries is almost got stagnated. Secondly, there exist great potential for increasing productivity by transferring appropriate technology at farm level. Development of new technologies and genetic improvement in fish stocks are also required for improving fish productivity. Thirdly, the substantial area shall be brought with the purview of aquaculture. For example, the country has about 2.2 million ha of freshwater ponds/tanks, of which only 0.325 million ha have been utilized for aquaculture. Similarly, of the 0.9 million ha of brackishwater areas suitable for aquaculture, only 70 700 ha have been developed (Kumar, 1996). The country also possesses other vast areas suitable for aquaculture, such as low-lying wetlands, canals, lakes, reservoirs and rivers. Fourthly, the country is endowed with rich fish germplasm resources, which need to be evaluated for culture potential. So far, only a few species have brought into culture basket. Farming of other species are yet to be realized. However all aquaculture practice should be done in a sustainable manner. Unsustainable aquaculture development will only generate short and medium-term profits for multinational cooperation's at the expense of long-term ecological imbalance and social instability. Practicing ecological aquaculture, organic aquaculture, polyculture, integrated aquaculture, mollusc farming, use of closed or less discharge aquaculture systems, etc are some of the way to sustainable aquaculture. Biofloc technology is one among such innovative system. Development alternative systems are needed to ensure that in the future aquaculture can contribute to the growing need for seafood products.

Biofloc technology being developed in the past few years there should be a need for familiarising and popularising this technology among the farmers of the world especially in the major aquaculture producers like China and India. Nowadays the lion part of the biofloc based research is carrying out mainly in the European countries. More focused research and technological innovations for the application based researches have to be developed. India being ranked in the list of major aquaculture producers in the world, biofloc based aquaculture and allied subjects have tremendous research potential and practical application. The present study embarks upon the scope of BFT based aquaculture in India and recommends the following points which need further research and consideration.

1. Biofloc technology can be applied for the major cultivable herbivorous and plankton feeding species like Indian major carps, coldwater fishes (mahseer), brackishwater species (mullets, mugil), etc.
2. Research need to be carried out to investigate the efficiency of this technique in carnivorous finfish culture system using an intermediate fish species which have the capacity to harvest the bioflocs (eg: sea bass-tilapia combined culture)

3. Previous research showed that BFT is more effective in the culture systems of tilapia. In Kerala (India), the culture of cichlid species *Etroplus suratensis* have a great scope and potential, since tilapia and *Etroplus* showed close resemblance in there feeding and ecological characteristics, the application BFT in *Etroplus* culture has to be tested
4. Standardisation of methodology, techniques and equipments for the pond construction, management, pre-stocking, post-stocking and harvest technologies for biofloc based aquaculture is needed.
5. Research must be concentrated on for developing some effective less energy utilising aeration devices which may suitable for biofloc-based aquaculture. The idea of construction of farms near the area having high wind action, and utilization of wind energy for aeration has to be explored; this will reduce the production cost.
6. Combination of BFT with other innovative techniques such as periphyton based aquaculture, fish driven re-suspension systems with various species are another topic of research.
7. Combining aquaponics, ornamental fish culture and BFT is an effective method for promoting horticulture, aquaculture and floriculture in the rural areas of India especially in the Northeastern states and cold climate regions.
8. Feed-based research like direct harvesting and feeding of biofloc, developing and commercialising bioflocs as feed

ingredients, incorporation of biofloc in the feed, its digestibility by animal etc.

9. Biotechnological applications like screening microbes and small invertebrates in the biofloc for the production of antimicrobial products, probiotics etc.
10. Study on the Characterisation of polyhydroxyalkanoate (PHA) and polyhydroxybutoate (PHB) in the BFT culture system and its response to cultured animals need to be investigated.
11. Complete microbial characterisation and identification of Invertebrate biofloc contributors of freshwater and brackishwater biofloc based systems.
12. Taxonomic variation of floc with respect to water and sediment parameters, carbohydrate sources, physical, chemical factors etc.
13. Production of effective artificial biofloc stock in laboratory using various microbial combinations.

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Awards

ASIAN FISHERIES SOCIETY
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YOUNG SCIENTIST AWARD, 2011

Presented to ***Mr. Prajith K.K.*** *for his paper entitled* **"Application of Biofloc Technology (BFT) for the sustainable aquaculture of Giant freshwater prawn, *Macrobrachium rosenbergii*: Effect on water quality and production"** *on the occasion of the Ninth Indian Fisheries Forum, Chennai.*

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THIRD PLACE

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EFFICACY OF VARIOUS CARBOHYDRATE SOURCES AS BIOFLOCULATING AGENT IN THE CULTURE SYSTEM OF GIANT PRAWN *Macrobrachium rosenbergii* (DE MAN): EFFECT ON WATER QUALITY AND PRODUCTION

presented by

Mr. Mark Oliver, Student Director
World Aquaculture Society, Asian-Pacific Chapter

Publications

- **Prajith, K. K.** and Madusoodana Kurup, B. 2011. Application of Biofloc technology (BFT) for the sustainable aquaculture of Giant freshwater prawn, Macrobrachium rosenbergii: effect on water quality and production, p.88. In: Gopalakrishnan, A. *et al.* (Eds.), *Renaissance in Fisheries: Outlook and Strategies - Book of Abstracts,* 9th Indian Fisheries Forum, Central Marine Fisheries Research Institute, Kochi and Asian Fisheries Society, Indian Branch, 19-23 December 2011, Chennai, India, 381 pp.
- **Prajith, K. K.** 2011. Biofloc technology (BFT) – The futuristic technology for the sustainable aquaculture, p.125. In: Gopalakrishnan, A. *et al.* (Eds.), *Renaissance in Fisheries: Outlook and Strategies - Book of Abstracts,* 9th Indian Fisheries Forum, Central Marine Fisheries Research Institute, Kochi and Asian Fisheries Society, Indian Branch, 19-23 December 2011, Chennai, India, 381 pp.
- **K.K Prajith** and B. Madhusoodana Kurup, efficacy of various carbohydrate sources as biofloculating agent in the culture system of Giant prawn, *Macrobrachium rosenbergii* (De man): Effect on water quality and Production, Asia Pacific Aquaculture Conference, January, 2011 Cochin, India
- B.Madhusoodana Kurup and **K K Prajith** - Application of biofloc technology in the culture system of *Macrobrachium rosenbergii*, in World Aquaculture Society, San Diego, California from March 1 - 5, 2010

Article published in popular journals

- Biofloc technology for fish culture (in Malayalam) - *Karshakasree* Magazine publishing by Malayalamanorama, December 2011 Edition, Page No 60-61

മീൻവളർത്തൽ

മത്സ്യക്കൃഷിക്ക് ബയോഫ്ലോക്ക് രീതി

കെ.കെ. പ്രജിത്ത്

മീൻവളർത്തൽ

ച്ചതാണ്.

ഇവയ്ക്ക് 32 ശതമാനം മാംസ്യം അടങ്ങിയ 100 ഗ്രാം തീറ്റ നൽകുമ്പോൾ 49 ഗ്രാം അന്നജസ്രോതസ് ചേർക്കണം. മത്സ്യക്കൃഷിയിൽ രാവിലെയും വൈകിട്ടുമായി രണ്ടുനേരമാണ് തീറ്റ നൽകാറ്. പ്രഭാത തീറ്റ നൽകിയ ഉടനെ നിശ്ചിത അളവിൽ അന്നജസ്രോതസ് ഒരു കപ്പിലോ ബക്കറ്റിലോ കുളത്തിൽനിന്നെടുത്ത വെള്ളത്തിൽ കലക്കി കുളത്തിലേക്ക് ഒഴിക്കാം. കൃഷി തീരുന്നതുവരെ ദിവസവും ഇങ്ങനെ ചെയ്യണം.

ഇന്ത്യയിൽ

നമ്മുടെ രാജ്യത്ത് ഈ സാങ്കേതികവിദ്യ പ്രചാരംനേടി വരുന്നതേയുള്ളൂ. ആദ്യമായി ബയോഫ്ളോക്ക് സാങ്കേതികവിദ്യ അടിസ്ഥാനമാക്കിയ കൃഷി കൊച്ചി ശാസ്ത്രസാങ്കേതിക സർവകലാശാലയിലെ സ്കൂൾ ഓഫ് ഇൻഡസ്ട്രിയൽ ഫിഷറീസ് വിഭാഗത്തിന്റെ നേതൃത്വത്തിലാണ് നടന്നത്. 40% മാംസ്യം അടങ്ങിയ തീറ്റയാണ് ഒരു പരീക്ഷണത്തിൽ നൽകിയത്. രണ്ടാമത്തെ പരീക്ഷണത്തിൽ ബയോഫ്ളോക്ക് രീതിയിൽ 25% മാംസ്യവും അതിനൊപ്പം ചെലവു കുറഞ്ഞ അന്നജവും നൽകി. രണ്ടു പരീക്ഷണങ്ങളിൽനിന്നും കിട്ടിയ മീനിന്റെ അളവ് ഒന്നു തന്നെയായിരുന്നു. അതായത് മരച്ചീനിപ്പൊടിയുടെ നേരിയ പ്രയോഗം മൂലം കാരച്ചെമ്മീന്റെ തീറ്റയിലെ മാംസ്യത്തിന്റെ അളവ് 40 ശതമാനത്തിൽനിന്നും 25% ആയി കുറയ്ക്കാൻ സാധിച്ചു. ഇതുമൂലം ചെമ്മീൻ കൃഷിയിൽ ഒരു കിലോ തീറ്റയുടെ ഉപയോഗത്തിൽ 25 രൂപ ലാഭമാണുണ്ടാക്കാൻ കഴിഞ്ഞത്. ഇതേ രീതിയിൽ ആറ്റുകൊഞ്ചിന്റെ കൃഷിയിലും 24% മാംസ്യം അടങ്ങിയ തീറ്റ ഉപയോഗിച്ച് ബയോഫ്ളോക്ക് രീതി പരീക്ഷിക്കുകയുണ്ടായി. അതിലും മികച്ച വിളവാണ് കിട്ടിയത്. ഈ സാങ്കേതികവിദ്യ ആറ്റുകൊഞ്ചിന്റെയും കാരച്ചെമ്മീന്റെയും ഹാച്ചറിയിലും നഴ്സറിയിലും വിജയകരമാണെന്ന് തെളിഞ്ഞിട്ടുണ്ട്.

വിളവെടുത്ത ആറ്റുകൊഞ്ച്

ബയോഫ്ളോക്ക് രീതിയിൽ ബാക്ടീരിയകളുടെ എണ്ണം വളരെയധികം വർധിപ്പിക്കുകയാണ് ചെയ്യുന്നതെന്നു നാം കണ്ടു കഴിഞ്ഞു. അപ്പോൾ സ്വാഭാവികമായും ഒരു സംശയം ഉണ്ടാകാം. മീനുകൾക്ക് ബാക്ടീരിയ മൂലം രോഗങ്ങൾ ഉണ്ടാകില്ലേ. എന്നാൽ ഈ കൃഷിരീതിയിൽ രോഗസാധ്യത വളരെ കുറവാണെന്ന് ഗവേഷണങ്ങളും അനുഭവങ്ങളും സാക്ഷ്യപ്പെടുത്തുന്നു. കാരണം, കൂടിയ തോതിൽ അന്നജം കുളത്തിലേക്ക് ചേർക്കുമ്പോൾ സൂക്ഷ്മജീവികൾ പോഷണപരമായി അസന്തുലിതാവസ്ഥയിലെത്തുന്നു. ഈ വിപരീതസാഹചര്യം മറികടക്കാൻ അവ ശരീരത്തിനുള്ളിൽ ചില പ്രത്യേക രാസവസ്തുക്കൾ ഉൽപാദിപ്പിക്കുന്നു. ബാക്ടീരിയകളെ ഭക്ഷിക്കുന്ന മീനുകളുടെ ഉള്ളിൽ ഇത്തരം രാസവസ്തുക്കൾ എത്തുമ്പോൾ അവയുടെ രോഗപ്രതിരോധശേഷിയാണ് വർധിക്കുന്നത്.

വാണിജ്യാടിസ്ഥാനത്തിലുള്ള വിപണനത്തിനായി സംസ്കരിച്ച ബയോഫ്ളോക്ക്

ബയോഫ്ളോക്ക് രീതിയിൽ കൃഷി നടത്തുമ്പോൾ ചില കാര്യങ്ങൾ പ്രത്യേകം ശ്രദ്ധിക്കേണ്ടതാണ്.

- നിർദേശിക്കപ്പെടുന്ന ശാസ്ത്രീയ പരിപാലനരീതി പിന്തുടരുക.
- വായുസങ്കലനത്തിനായി എയ്റേറ്ററുകളുടെ എണ്ണം, സ്ഥാനം എന്നിവ കൃത്യമായി ക്രമീകരിക്കുക.
- സ്ഥിരമായ വായുസങ്കലനം ഉറപ്പാക്കുക.
- അമിതമായി ഉൽപാദിപ്പിക്കപ്പെടുന്ന ഫ്ളോക്കുകൾ നീക്കം ചെയ്യുകയും വേണം.

വിലാസം: റിസർച്ച് സ്കോളർ,
അക്വാകൾച്ചർ ലബോറട്ടറി,
സ്കൂൾ ഓഫ് ഇൻഡസ്ട്രിയൽ
ഫിഷറീസ്, കൊച്ചി ശാസ്ത്രസാങ്കേ
തിക സർവകലാശാല,
കൊച്ചി–682 016. ഫോൺ: 94477 26227

ബയോഫ്ളോക്ക് രീതി പിന്തുടരുന്ന ഇന്തോനേഷ്യയിലെ ചെമ്മീൻപാടം.

● ഡിസംബർ 2011 ● കർഷകശ്രീ 61

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