CARBON / NITROGEN RATIO OPTIMIZATION AND PERIPHYTON DEVELOPMENT ON THE PRODUCTION AND SUSTAINABILITY OF *PENAEUS MONODON* (FABRICIUS) IN EXTENSIVE CULTURE SYSTEM

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By

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April - 2007

DECLARATION

I, **Johny T. Varghese**, do hereby declare that the thesis entitled **"CARBON / NITROGEN RATIO OPTIMIZATION AND PERIPHYTON DEVELOPMENT ON THE PRODUCTION AND SUSTAINABILITY OF *PENAEUS MONODON* (FABRICIUS) IN EXTENSIVE CULTURE SYSTEM"** is a genuine record of research work done by me under the supervision of **Prof. Dr. B. Madhusoodana Kurup**, Professor, School of Industrial Fisheries, Cochin University of Science and Technology 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.

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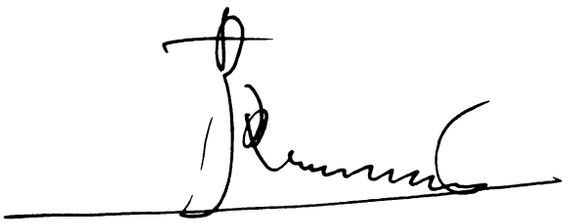
April - 2007

Johny T. Varghese

CERTIFICATE

This is to certify that the thesis entitled **"CARBON / NITROGEN RATIO OPTIMIZATION AND PERIPHYTON DEVELOPMENT ON THE PRODUCTION AND SUSTAINABILITY OF *PENAEUS MONODON* (FABRICIUS) IN EXTENSIVE CULTURE SYSTEM"** to be submitted by **Mr. Johny T. Varghese,** 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** of Cochin University of Science and Technology, under the faculty of **Marine Sciences.**



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JOHNY T. VARGHESE

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Chapter – 1

General Introduction

1. Background of the study

Aquaculture, the farming of aquatic animals and plants has turned out to be an important industry world wide. Aquaculture production systems used across the world differ widely depending on the species being cultured, the geographical location and socioeconomic context. Extensive, semi-intensive and intensive methods can be employed to produce shrimp and fish. Extensive methods have proved economically viable in the brackishwater area (Beveridge, 1987). Environmental issues have always been a point of debate in shrimp farm development. While the harvest from capture fisheries around the world has stagnated, aquaculture is viewed as a sound option to increase fish production and to play a vital role in providing food and nutritional security. However, the shrimp farming sector has been strongly opposed by environmental groups on many occasions not only in India but in many other countries around the globe. Legal interventions have been sought to curtail shrimp culture to preserve the coastal environment and the ecology. Though the polarization of opinion on the adverse impact of aquaculture in the nineties was very strong, there are signs of more tolerance lately to accommodate diverse views and opinions to allow development of shrimp farming in an environment friendly and sustainable manner.

In India, commercial shrimp farming started gaining roots during the mid-eighties only and during this period, this actually

attained peak in most of neighboring Asian countries especially China and Taiwan. Shrimp culture begun booming in early 1990's in India. However, the industry collapsed in 1995 - 96 due to disease outbreaks. Since then, shrimp aquaculture industry is facing severe criticism for the adoption of unsustainable culture practices, such as the discharge of pond water with high nutrients. Nitrogen plays a key role in aquaculture systems due to its dual functioning as a nutrient and toxicant. The excessive accumulation of toxic inorganic nitrogen in culture systems deteriorates the pond system by reducing the growth rate and survival rate of cultured organisms. Most of the coastal states in India are new to commercial scale shrimp farming. The lack of awareness on good farming practices and appropriate extension services have in fact led to a host of problems.

2. Sustainable development of shrimp farming – some issues for consideration

It is generally accepted that the days of maximum production oriented unsustainable shrimp farming practices are gone. Present day production has to take note of not only the markets but a host of technical issues as well as the concerns of the environment. The subject matter of sustainable shrimp farming is broad from farm level management practices to integration of shrimp farming into coastal area management, shrimp health management and policy,

socioeconomic and legal issues. The Aquaculture Authority permits stocking of post larvae up to 6 nos m^{-2} for farms within the CRZ and up to 10 nos m^{-2} outside the CRZ (Aquaculture Authority News, 2006). It is gratified to see that high percentage of farms are embracing low stocking densities in the country and enjoying a high success rate in doing so. The low stocking densities are working in terms of economics as well. Adoption of low stocking densities will be one of the key elements of sustainability in the years to come and needs to be promoted among the shrimp farmers.

The sustainability of the shrimp farming in Kerala is facing severe risks and crisis like degradation of estuarine ecosystem due to indiscriminate discharge of shrimp farm effluents loaded with high inorganic nitrogen, crop loss due to poor environmental conditions and recurrence of diseases due to the stress and strain the farmed shrimps are prone to. The shrimp farming sector has received criticism for excessive use of formulated feed containing high protein shrimp feed, of which around 50% is getting accumulated at the pond bottom as unconsumed (Avnimelech, 1999; Hari et al., 2004; Hari et al., 2006). The waste materials accumulated with the feces from the cultured stock, dead organism and organic fertilizers undergo decomposition. Thus, shrimps are exposed to toxic ammonia-N and nitrite-N, which are responsible for stress and strain in cultured shrimps. After oxygen, ammonia-N is the second most limiting factor

in the culture system for shrimp stocking density (Raveh and Avnimelech, 1979; Blackburn et al., 1988; Piedrahita, 1988; Krom and Neori, 1989; van Dam, 1990; Colt and Oriwicz, 1991; Hargreaves, 1998; Monroya et al., 1999). Furthermore, the discharge of inorganic nitrogen rich pond effluents increases pollution in the main water sources. Maintenance of good water quality with minimum water exchange and retention of nitrogenous nutrient input into harvestable products are thus emerging as most important requirements for sustainable shrimp farming.

3. Development of shrimp aquaculture in India

India, by virtue of having 8118 km long coastline, 2.02 million sq. km of Exclusive Economic Zone (EEZ) and extensive geographical stretch with varied terrain and climate, supports a wide diversity of inland and coastal wetland habitats. There are 3.9 million ha of estuaries and 3.5 million ha of brackishwater areas in the country. Out of this, 1.2 million ha of coastal area have been identified as suitable for brackishwater aquaculture and by the adoption of sustainable practices, it can yield optimum quantities of shrimp and other commercially valuable finfish and shell fish species.

The over exploitation of shrimp from natural sources and the ever increasing demand for shrimp and shrimp products in the world food market has resulted in a wide gap between the demand and

supply shrimp. This has necessitated the need for exploring new avenues for increasing shrimp production. The state-wise potential area and status of shrimp culture development in India is given in Table 1.1. The estimated brackishwater area suitable for undertaking shrimp cultivation in India is around 11.91 lakhs ha spread over 10 states and union territories viz. West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Pondicherry, Kerala, Karnataka, Goa, Maharashtra and Gujarat. Of this, only around 1.2 lakhs ha are now under shrimp farming and hence lot of scope exists for entrepreneurs to venture into this field. The marine products export from India has been rising over the years and the current export is worth about US $ 1478 million (FAO, 2005). The major markets for Indian shrimp are Japan, Western Europe and USA. Frozen shrimp is the largest export item in terms of value contributing 64% of the total export earnings followed by frozen cephalopod (15%), frozen fish (11%), dried fish (2%), etc. Today India stands amongst the major shrimp producing countries in the world with a growth rate of about 300.0 % over the last decade.

In India, shrimp farming has been traditionally practiced in the coastal states of West Bengal and Kerala. The traditional trap and hold farming system was characterized with low production levels of mixed species of finfishes and shell fishes. The importance of introducing scientific farming techniques will increase production and profitability from the traditional system.

Like any other agriculture / animal husbandry practice, shrimp culture has also been affected by health and disease problems. Initially, some bacterial diseases in localized shrimp farms with low mortality rates were noticed. However, viral diseases such as *Monodon baculo virus* and *white spot virus disease* syndrome were reported from shrimp farms in 1995 followed by a slump in Indian shrimp farming. Heavy stocking densities and poor farm management practices were attributed as major reasons for such disease out breaks in the country.

4. Role of carbohydrate addition in the shrimp culture system

The tiger prawn, *Penaeus monodon* (Fabricius) is the most extensively cultured crustacean in South-East Asian countries. This species is known to possess high growth rate and adapt to various culture systems. *Penaeus monodon* is considered as a candidate species for brackishwater culture. Protein is an essential nutrient for this culture organism and the major source of ammonium-N in culture farms is typically protein rich feed. Aquatic animals excrete ammonium-N, which may accumulate in the pond. Protein is an expensive feed component and high dependency on artificial feeding increases feed cost considerably. The expensive protein fraction should, therefore, be at optimal level. Furthermore, the protein

sparing effect of non-protein nutrients such as carbohydrate may be effectively utilized for reducing the feed cost. In highly aerated ponds, ammonium-N is oxidized by bacteria to nitrite-N and nitrate-N. Unlike carbondioxide, which is released to the air by diffusion or forced aeration, there is no effective mechanism to remove the nitrogenous metabolites out of the pond. Thus, intensification of aquaculture system is inherently associated with enrichment of the water with ammonium-N and other inorganic nitrogenous species. The management of such systems depends on developing methods to remove these compounds from culture pond.

Removal of excessive nitrogen from culture pond is commonly carried-out by frequent exchange and replacement of pond water. However this practice is constrained by the following reasons:

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

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

high surface area with immobilized nitrifying biomass enables a high nitrifying capacity in a controlled environment. One problem associated with biofiltration is the high cost involved and the need to treat and digest a large mass of feed residues.

An additional strategy that is getting more attention presently is the removal of ammonium from water through its assimilation into microbial proteins by addition of carbonaceous materials to the system. If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. A further important aspect of this process is the potential utilization of microbial proteins as a source of feed protein for fish or shrimp.

This, however, depends upon the ability of the animal to harvest such bacteria and to digest and utilize the microbial protein. One obvious constraint is the minimal size of particles that can be taken up by the fish and shrimp. Taghon (1982) reported that benthic invertebrates were able to take up microscopic glass bead when they were coated with proteins. This demonstrates that the chemical nature of particles may favor their harvesting by cultured organisms. The fact that relatively large microbial cell clusters are formed due to flocculation, alone or in combination with clay or feed particles (Harris and Mitchell, 1973; Avnimelech et al., 1982, 1984) and the resultant microbial protein additionally favors the growth of shrimp and fish.

Controlling inorganic nitrogen by manipulating carbon / nitrogen ratio is a potential method for aquaculture systems. This approach offers a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in culture ponds. Such a strategy can be practiced as an emergency response, ie., addition of a carbonaceous substrate in case of increased ammonium concentration. It is possible to add cheap sources of carbohydrates such as cassava meal and flour. However, additional pond aeration may be required to compensate the additional oxygen consumption. The conventional control measures include intensive exchange of pond water. Furthermore, it is not always practical to stop feeding to slow down TAN (Total ammonia nitrogen) build up. The proposed method enables to keep a high biomass and to bring-out a corrective means in case of failure of conventional control measures.

A more advanced approach is to adjust protein level in feed so as to avoid the build up of inorganic nitrogen in pond water. This approach was tested and proven successfully in intensive ponds that are continually mixed and aerated. The intensive culture of fish in these ponds is based on a system that is similar to biotechnological reactors (Avnimelech, 1998). The addition of carbohydrates was done as a part of recycling and increased utilization of protein through the utilization of microbial proteins. Production and utilization of microbial proteins (SCP, single-cell protein) have been studied

extensively during the last few decades (Tannenbaum and Wang, 1975). The major problem involved in economically sound utilization of SCP culture is harvesting, dehydration and packing of the material. In contrast, for in situ microbial protein culturing in the pond, all these expensive processing stages are not needed since harvesting is done by fish and shrimp, as part of the system.

Applicability of the same approach in earthen stagnant ponds is not trivial and has to be further studied in conventional fish and shrimp ponds. The addition of carbohydrates to feed may result in an accelerated sedimentation of organic matter to the pond bottom, where microbial biomass is not utilized by fish or shrimp and may increase the organic load in the pond.

The adjustments of the carbon / nitrogen ratio in feed as a means to control the pond water quality and sediment quality are studied. The objectives of this study are to evaluate the basic reactions and mechanisms affecting this process; to demonstrate its potential; to develop the quantitative means needed to adjust the carbon / nitrogen ratio and to control inorganic nitrogen accumulation in the farming system.

5. Periphyton based aquaculture

The term 'periphyton' refers to the microfloral community living attached to the surfaces of submerged objects in water (Wetzel, 1983).

This definition, however, does not include fungal, bacterial, protozoan and other attached animal components, which are included in the German word 'Aufwuchs'. Depending on the substrate types, periphyton communities are again subdivided as 'Epilithon' grown on rock, 'Epipelon' on mud or silt, 'Epipsammon' on sand and 'Epiphyton' on submerged macrophyte substrates. In microbiology, periphyton is often referred to as 'Biofilms' (Nielsen et al., 1997; Shankar et al., 1998; Shankar and Mohan, 2001). In aquaculture, the term periphyton has been used in a broader sense. Periphyton is defined as the entire complex of sessile aquatic biota attached to the substratum and includes associated detritus and microorganisms. Thus, the periphyton community comprises bacteria, fungi, protozoa, phytoplankton, zooplankton, benthic organisms and a range of other invertebrates and their larvae. Any material providing surface area, including coral reef, branches of different trees, higher aquatic plants, bamboo, PVC pipes etc., can be used for periphyton production.

The idea of periphyton based aquaculture is originally derived from the traditional method, (bush trap testing) locally called 'padal fishing' a unique fishing method used in the Ashtamudi estuary, Vambanad is of Kerala (South India). Locally available tree branches such as mango, mangroves and bamboo poles are placed in shallow open waters. These branches are known as padals which act as shrimp and fish aggregating devices. A large number of post larvae of

shrimp and fingerlings find shelter beneath the padals, foraging the peri and epiphyton developed from the submerged twigs and other structures used to construct them (Thomas et al., 2004), such as 'acadjas' of Cote Ivory Coast, West Africa (Welcomme, 1972) and the 'samarahs' of Cambodia (Shankar et al., 1998). Dense masses of tree branches or bamboo are established in lakes, lagoons or rivers the fish and shrimp are attracted by the provision of shelter from predators, suitable breeding habitats and the availability of natural food. These unique tools used in capture fisheries have recently been considered as models for novel periphyton based aquaculture systems.

There are two basic food sources for all organisms in extensive and semi-intensive ponds: primary productivity from algae and protein rich supplementary feed. Algae produce organic matter by using solar energy and carbondioxide through photosynthesis which can be further utilized, indirectly through secondary trophic levels (zooplankton, benthos, and invertebrates etc.) and directly, through grazing by fish and shrimp. Heterotrophic microorganisms are essential components of the food web in these two food source as thus decompose organic matter and release nutrients which can again be utilized by algae or consumed by the cultured organism (Colman and Edwards, 1987; Moriarty, 1997). However, a common assumption particularly in aquaculture is that the phytoplankton community is

most important in terms of energy fixation and fuelling of the food web. Research has shown that macrophytes and periphyton are significant dominant contributor to primary production (Moss, 1998).

6. Objectives, hypotheses and outline of the thesis

Controlling the inorganic nitrogen by manipulating carbon / nitrogen ratio is a method gaining importance in aquaculture systems. Nitrogen control is induced by feeding bacteria with carbohydrates and through the subsequent uptake of nitrogen from the water for the synthesis of microbial proteins. The relationship between addition of carbohydrates, reduction of ammonium and the production of microbial protein depends on the microbial conversion coefficient. The carbon / nitrogen ratio in the microbial biomass is related to the carbon contents of the added material. The addition of carbonaceous substrate was found to reduce inorganic nitrogen in shrimp culture ponds and the resultant microbial proteins are taken up by shrimps. Thus, part of the feed protein is replaced and feeding costs are reduced in culture systems.

The use of various locally available substrates for periphyton based aquaculture practices increases production and profitability (NFEP, 1997; Ramesh et al., 1999; Wahab et al., 1999a; Azim et al., 2001; Keshavanath et al., 2001a; Azim et al., 2002). However, these

techniques for extensive shrimp farming have not so far been evaluated. Moreover, an evaluation of artificial substrates together with carbohydrate source based farming system in reducing inorganic nitrogen production in culture systems has not yet been carried-out. Furthermore, variations in water and soil quality, periphyton production and shrimp production of the whole system have also not been determined so-far.

This thesis starts with a general introduction (present chapter), a brief review of the most relevant literature, results of various experiments and concludes with a summary (Chapter – 9). The chapters are organised conforming to the objectives of the present study. The major objectives of this thesis are, to improve the sustainability of shrimp farming by carbohydrate addition and periphyton substrate based shrimp production and to improve the nutrient utilisation in aquaculture systems.

The specific objectives of the present study can be outlined as:

1. To optimize the protein percentage in shrimp feeds by the control of carbon / nitrogen ratio.
2. To evaluate the effect of various mode of carbohydrate application and diet having various protein levels for the production and sustainability of *Penaeus monodon*.

3. To monitor the carbohydrate addition shrimp production relationship and culture sustainability for increasing the total revenue of the harvested shrimp and reducing the feed cost.
4. To examine the stocking density and carbohydrate addition relationship in the yield and sustainability of *Penaeus monodon.*
5. To asses the efficiency of various types of carbohydrates in the control of inorganic nitrogen and increasing production of *Penaeus monodon.*
6. To monitor the substrate based periphyton effect on water and soil quality parameters and to evaluate the quantitative production of additional excellent natural food source for the culture organism.
7. To optimize the fertilization rates in periphyton production in the absence of shrimp grazing pressure.
8. To asses the combined effects of periphyton and addition of carbohydrate in the production and sustainability of *Penaeus monodon.*
9. To reduce the water based inorganic nitrogen discharge in to environment this making shrimp farming more ecologically and environmentally sustainable.

Table 1.1

The state-wise potential area and status of shrimp culture development in India during 2005

Sr.	State	Estimated brackish water area (ha.)	Area under cultivation (ha.)
1	West Bengal	405,000	34,660
2	Orissa	31,600	11,000
3	Andhra Pradesh	150,000	50,000
4	Tamil Nadu	56,000	2,879
5	Pondicherry	800	37
6	Kerala	65,000	14,657
7	Karnataka	8,000	3,500
8	Goa	18,500	650
9	Maharashtra	80,000	716
10	Gujarat	376,000	884
		1,190,000	**118,983**

Source: Aquaculture Authority News, September 2006

Review of Literature

Review of Literature

As demand for fish, crustaceans and other aquatic organisms is increasing and capture fisheries reaches its maximum level of exploitation, food production through aquaculture attracts global attention. For the successful rearing of tiger prawn, development of a balanced artificial ration is essential (Banerjea, 1967). The optimum amounts of dietary protein and energy play key roles in the growth of shrimp in culture ponds (Tiemeier et al., 1965; Hastings, 1967). Studies in shrimp have indicated that those diets which are low in both protein and total energy resulted in reduced weight gain (Lee and Putnam, 1973; Garling and Wilson, 1976). High dietary protein essential for faster tissue growth and maintenance, is an expensive component of formulated diets and protein may be catabolised to meet the energy requirements of somatic growth (Capuzzo and Lameasten, 1979; Sedgwick, 1979). Higher dietary protein feed deteriorates the water and soil quality in shrimp grow-out ponds (Boyd, 1989). Furthermore, Avnimelech and Lacher (1979) and Boyd (1985) reported that total fed feeds only 50% of the feed is utilized by the cultured organism. The remaining 50% dietary protein feed is assumed to constitute feed waste, which is a major source of ammonium-N (Gaudy and Gaudy, 1980; Heaper, 1988). Colt and Armstrong (1981) reported that accumulation of toxic inorganic nitrogen species such as NH_4^+ and NO_2^- in water is one of the major

problems affecting the sustainability of shrimp farming. Ammonia-N is a highly toxic compound because it can easily cross most biological membranes and cause pH alterations which may reduce survival rates and impair various physiological mechanisms (Schmidt-Nielson, 1983; Campbell, 1991). The release of dissolved nutrients by a shrimp farm leads to an increase in their concentration in the receiving water body. This increase has been termed as 'hypernutrification' (Gowen and Bradbury, 1987). Increasing levels of total nitrogen, total inorganic nitrogen and total phosphorus in shrimp pond water poses serious environmental problem (Boyd, 1990).

The most widely documented process associated with commercial culture is the high accumulation of organic matter due to the deposition of solid waste from undigested feed (Brown et al., 1987; Gowen et al., 1991). The aquaculture industry is focusing on the development and refinement of water recycling technologies due to concerns related to the potential negative impacts of production on the environment (Klontz, 1979; Rosenthal, 1994). Typically, mechanical filtration removes particulate matter, while biological filtration removes dissolved wastes, including ammonia (Brune and Gunther, 1981; Kaiser and Wheaton, 1983; Losordo, 1991). However, it is not economically viable in a high surface area due to the higher cost. At the same time, good water quality is essential for ensuring survival and adequate growth rate (Boyd, 1990; Burford, 1997).

Aquatic animals excrete ammonium, which may accumulate in the pond bottom. A different approach is to estimate the amount of carbohydrate needed to be added in order to immobilize the ammonium excreted by the fish or shrimp in the culture system (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996). The manipulation of shrimp culture system for improved water quality and shrimp production requires a definite understanding of various physical, chemical and biological processes (Boyd, 1986). Reduction of dissolved inorganic nitrogen can be established in intensive, well aerated and circulated fish or shrimp ponds by the application of organic carbon sources (Avnimelech et al., 1989). The organic carbon rich substrates such as glucose, cassava and sorghum meal were used to control carbon / nitrogen ratio (Avnimelech et al., 1994). The control of inorganic nitrogen accumulation in pond is based upon carbon metabolism and nitrogen immobilizing microbial process. Bacteria and other microorganisms use carbohydrates (sugar, starch and cellulose) as a food, to generate energy and to produce proteins and new cells (Avnimelech, 1999). The resulting heterotrophic bacterial production (single cell protein) may be utilized as a food source of fish and shrimp (Beveridge et al., 1989; Rahmathulla and Beveridge, 1993; Burford et al., 2004a) and thus lowering the demand for supplemental feed protein (Avnimelech, 1999). Schroeder (1978) reported that carp can filter out particles

larger than 20 – 50 µm. According to Odum (1968) *Mugil cephalus* take up particles small as 10 µm. Interestingly Taghon (1982), found that benthic invertebrates were able to take up microscopic glass bead when they were coated with proteins.

Pond production systems in Southern Asian countries are becoming increasingly reliant on external resources (feed, fertilizers) to supplement or stimulate autochthonous food production in fish pond. Hickling (1962) and Heaper (1988) have demonstrated that fish production in fertilized ponds does not increase in direct proportion to increased fertilizer addition and that above a certain level, increasing fertilizer rates does not further increase fish yield. In most feed driven pond production systems, only about 15 – 30% of nutrient input is converted into harvestable products, the remainder being lost to the sediment, effluent water and the atmosphere (Acosta-Nassar et al., 1994; Gross et al., 2000). In nutrient rich environment, the substrate acted merely as a platform for periphyton (Moss, 1998). On the other hand, periphyton biomass in open water habitats strongly depended on substrates type (Blinn et al., 1980; Hansson, 1992; Vymazal and Richardson, 1995). The amount of periphyton biomass per surface area was found to be highly variable and was influenced by water depth (Konan-Brou and Guiral, 1994; Light and Beardall, 1998; Keshavanath et al., 2001a), nutrient availability (Elwood et al., 1981; Fairchild et al., 1985) grazing pressure (Hatcher and Larkum, 1983;

Hansson et al., 1987; Hay, 1991; Huchette et al., 2000) and seasonality, including environmental parameters (Hatcher and Larkum, 1983; Carpenter, 1986; Bothwell, 1988; Arfi et al., 1997; Ledger and Hildrew et al., 1998).

The term 'periphyton' is applied to the complex of sessile biota attached to submerged substrata such as stones and sticks and includes not only algae and invertebrates but also associated detritus and microorganisms. The assemblage of attached organisms on submerged surfaces, including associated non-attached fauna are referred to as periphyton (van Dam et al., 2002). The feasibility of periphyton based systems has been explored in brackishwater fish ponds in West Africa (Welcomme, 1972; Hem and Avit, 1994; Konan-brou and Guiral, 1994). Periphyton is a complex matrix of algae and heterotrophic microbes attached to submerged surfaces in streams and other shallow waters. It serves as an important food source for invertebrates and some fish (Apesteguia and Marta, 1979; Newman and McIntosh, 1989; Cattaneo et al., 1993). Periphyton based aquaculture systems offer the possibility of increasing both primary production and food availability for culture organism (Legenedre et al., 1989; Hem and Avit, 1994; Guiral et al., 1995; Wahab et al., 1999a). Greater abundance of net phytoplankton in ponds probably relates to higher nutrient input in those ponds (Boyd, 1989). Biochemical oxygen demand and ammonia-nitrogen were most closely related to

phytoplankton abundance and community variation in the grow-out ponds. Ammonia-nitrogen is an important algal nutrient (Boyd, 1989) and BOD is a measure of the organic material in ponds which is closely related to phytoplankton communities (Tookwinas and Songsangjinda, 1999). In shrimp ponds, ammonia concentration was not significantly affected by pond depth, although nitrite and nitrate were inversely related to pond depth (Carpenter et al., 1986). Presumably, reducing water depth in a pond with a high phytoplankton density will reduce light limitation of phytoplankton growth and thereby enhance nutrient uptake (Piedrahita, 1991). A periphyton mat consists of a solid matrix embedded with bacteria, algae, protozoa, fungi, zooplankton and small invertebrates (Kalpan et al., 1987; Bender and Phillips, 2004; Garcia-Meza et al., 2005). The selection of suitable species (Wahab et al., 1999a; Azim et al., 2001), selection of locally available substrates and the optimization of fertilizer dose are the major steps in periphyton based aquaculture. It is evident that periphytic algae need to be grazed constantly and kept at low biomass to maintain their high productivity (Hatcher, 1983; Hay, 1991; Huchette et al., 2000). Trials have demonstrated that fish production from ponds supplied with additional substrates for periphyton production is higher than that from substrate free controls (Legendre et al., 1989; Konan et al., 1991; Hem and Avit, 1994; Guiral et al., 1995; NFEP, 1997; Wahab et al., 1999a; Azim et al., 2001).

phytoplankton abundance and community variation in the grow-out ponds. Ammonia-nitrogen is an important algal nutrient (Boyd, 1989) and BOD is a measure of the organic material in ponds which is closely related to phytoplankton communities (Tookwinas and Songsangjinda, 1999). In shrimp ponds, ammonia concentration was not significantly affected by pond depth, although nitrite and nitrate were inversely related to pond depth (Carpenter et al., 1986). Presumably, reducing water depth in a pond with a high phytoplankton density will reduce light limitation of phytoplankton growth and thereby enhance nutrient uptake (Piedrahita, 1991). A periphyton mat consists of a solid matrix embedded with bacteria, algae, protozoa, fungi, zooplankton and small invertebrates (Kalpan et al., 1987; Bender and Phillips, 2004; Garcia-Meza et al., 2005). The selection of suitable species (Wahab et al., 1999a; Azim et al., 2001), selection of locally available substrates and the optimization of fertilizer dose are the major steps in periphyton based aquaculture. It is evident that periphytic algae need to be grazed constantly and kept at low biomass to maintain their high productivity (Hatcher, 1983; Hay, 1991; Huchette et al., 2000). Trials have demonstrated that fish production from ponds supplied with additional substrates for periphyton production is higher than that from substrate free controls (Legendre et al., 1989; Konan et al., 1991; Hem and Avit, 1994; Guiral et al., 1995; NFEP, 1997; Wahab et al., 1999a; Azim et al., 2001).

Laboratory based grazing trails also indicated that algal feeding species, such as Tilapias, can ingest more plant based food per unit time when presented as periphyton than as plankton (Dempster et al., 1993).

Miller and Falace (2000) suggested two mechanisms for increasing fish production in artificial reef based system: (1) the additional shelter provided by the substrate allows more of the resource to flow into cultured organism biomass and (2) the new primary production and attached benthic secondary production fostered by the artificial substrate support a new food web, part of which will end up in cultured species biomass. Maximum periphyton biomass level is reported to be coinciding with the euphotic zone (Konan-Brou and Guiral, 1994; Keshavanath et al., 2001c). Wahab et al. (1999a) reported 53 genera of periphyton collected from scrap bamboo in fish ponds among which 12 genera were observed in verity. Huchette et al. (2000) identified 32 species of diatom as periphyton along with other micro and macro organisms from both animal and plant kingdoms growing on artificial substrates in Tilapia cages. The periphyton quantity varied substantially with substrate type, fertilization level, environment conditions and taxonomic composition (Paine and Vadas, 1969; Heaper, 1988; Makarevich et al., 1993; Napolitano et al., 1996; Ledger and Hildrew, 1998; Huchettu et al., 2000; Keshavanath et al., 2001a). The increased fertilization rate

amplified pond productivity. However, it results in drastic production of inorganic nitrogen in the culture system (Bormann et al., 1968; Vitsousek et al., 1979; Schimel and Firestone, 1989; Dail et al., 2001). Konan-Brou and Guiral (1994) and Keshavanath et al. (2001a) reported that maximum periphytic biomass levels coincided with photosynthetic compensation and the depth of culture pond. The biodegradable substrates viz. sugarcane bagasse, paddy straw, dried water hyacinth (*Eichornia crassipes*), kanchi, PVC pipes and bamboo poles were used in the culture system for the periphyton production but the highest periphyton growth occurred on bamboo poles (Ramesh et al., 1999; Umesh et al., 1999; Azim, et al., 2001; Keshavanath et al., 2001a; Azim et al., 2002; Joice et al., 2002; Mridula et al., 2003). Microbial communities containing algae, blue green algae, bacteria, protists, zooplankton and fungi embedded in an extracellular polysaccharide matrix develop on submerged surfaces. Within these communities, autotrophic or heterotrophic biomass dominates, depending on light, dissolved oxygen and nutrient availability (Hepher et al., 1989).

Worldwide aquaculture has been increasing rapidly in the last decade, approximately at an average rate of more than 10% per year (Muir, 1995; Tacon, 1997; Pedini and Shehadeh, 1997; World Bank, 1998; FAO, 2001), mainly due to the combined effects of increasing world population (Caddy and Griffiths, 1995), and the increasing

demand for aquaculture products in developed countries (Tacon, 1997; Lem and Shehadeh, 1997). Modified extensive production of juvenile shrimp is gaining increased attention worldwide as a potential means to improve aquaculture production via application as a transitional nursery system (i.e. between the hatchery and grow-out ponds). Stocking juvenile shrimp into grow-out ponds, as opposed to post larvae, is thought to improve production mainly by; increasing early survival rates in ponds, because juveniles are likely to be hardier and therefore more able to adapt to pond conditions (Samocha et al., 2002), and also to reduced grow-out duration (Samocha et al., 1993; Peterson and Griffith, 1999). Reduced growth and survival at higher densities are attributed to a number of factors like, a decrease in the availability of space and natural food sources (Maguire and Leedow, 1983; Peterson and Griffith, 1999); an increase in adverse shrimp behavior such as cannibalism (Abdussamad and Thampy, 1994); the degradation of water quality (Nga et al., 2005); and accumulation of undesirable sediment (Arnold et al., 2005, 2006). It is believed that added surface area created by the substrates enhance the colonization of epiphytic biota, which in turn provides a natural food supplement for the shrimp (Moss and Moss, 2004; Burford et al., 2004b). Shrimp was also cultured both with and without the addition of artificial substrates, on the surface of artificial substrates colonized with epiphytic biota, at each density to ascertain if *Penaeus monodon*

benefit from the addition of substrates during intensive production (Moss and Moss, 2004).

In shrimp culture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon, 1982; Diab and Shilo, 1988). The use of low protein feed led to a significant reduction in the feed based inorganic nitrogen accumulation in the pond (Li and Lovell, 1992). The addition of carbohydrate enhances the total heterotrophic bacterial population in the pond, which in turn results in further reduction of inorganic nitrogen. Middelburg and Nieuwenhuize (2000a), Benner (2002) and Bronk (2002) found that the presence of microbial community uptake the different nitrogenous substrates. Heterotrophic bacteria nitrogen uptake focused on dissolved inorganic nitrogen (DIN), especially ammonium-N (NH_4^+) and nitrate-N (NO_3^-) as an important nitrogen source (Antia et al., 1991; Middelburg and Nieuwenhuize, 2000b; Bronk, 2002; Zehr and Ward, 2002; Berman and Bronk, 2003). Thus, the low toxic inorganic nitrogen levels in the pond (Wahab et al., 2003) and utilization of microbial cells as feed act as favorable factors for the augmented shrimp production (Avnimelech, 1999; Burford et al., 2003, 2004b). The utilization of microbial protein depends on the ability of the target animal to harvest the bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). Extensive conditions and carbohydrate addition to the water column also

resulted in a significant increase in the THB count, together with observed lower TAN concentrations in water and sediment (Bronk, 2002). Carbohydrate addition also caused a significant reduction in NO_2^--N concentration in the water column, which can be attributed to low availability of TAN as substrate for nitrification (Avnimelech, 1999; Hari et al., 2004). Furthermore, lower TAN in the sediment positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). The addition of carbohydrate to intensively well-mixed production systems will reduce the TAN concentration through immobilization by bacterial biomass (Avnimelech and Mokady, 1988; Avnimelech et al., 1989; 1994; and Avnimelech, 1999). According to Avnimelech (1999) TAN concentrations were found low (2.0 mg l^{-1}) in carbohydrate added shrimp culture, when compared to the findings of Chen and Tu (1991) (6.5 mg l^{-1}) and Thakur and Lin (2003). Cotner et al. (2000) showed that water samples collected from Florida Bay having a TAN concentration of 7.4 – 17.1 µg l^{-1} enhanced microbial growth with glucose addition. Water exchange in ponds is limited or even null, leading to the accumulation of organic residue and to the development of dense heterotrophic microbial population (McIntosh, 2000; Avnimelech, 2003). In intensive aquaculture systems, inorganic nitrogen, including toxic ammonia and nitrite accumulate in the water (McIntosh, 2000). This problem is prevented through the addition of

carbonaceous substrates leading assimilation of the soluble inorganic nitrogen and its incorporation into microbial protein (Chamberlain et al., 2001; Tacon et al., 2002). The microbial protein, aggregated in microbial flocs serves as a rich source of amino acids and growth factors to fish and shrimp, leading to a significant recycling of protein and higher utilization of feed (Avnimelech et al., 1994; Chamberlain et al., 2001; Tacon et al., 2002). Recent research showed that carbohydrate addition in extensive shrimp ponds improved the nitrogen retention efficiency and had a positive effect on production (Hari et al., 2004).

The shrimp growth recorded in carbohydrate added culture conditions was not limited by any of the water quality parameters as they fell in the favorable limits for *Penaeus monodon* production (Chen et al., 1990; Hariati et al., 1996). The comparable net shrimp yield and FCR showed the possibility of reducing the dietary protein level in favor of addition of carbohydrate to the water column without any significant reduction in shrimp production (Hari et al., 2004). The reduction in TAN and NO_2^--N levels observed in carbohydrate added treatments could only be attributed to the increased THB population, which immobilized TAN for the synthesis of new bacterial cells (Hari et al., 2004). Burford et al. (2004b) suggested that 'flocculated particles' rich in bacteria and phytoplankton could contribute substantially to the nutrition of the *Litopenaeus vannamei* in intensive shrimp ponds.

Survival rates were similar in various experiments showed that water and sediment quality were favorable for *Penaeus monodon* cultivation (Hariati et al., 1996). Shrimp rely on natural foods even in fed ponds. Studies using stable isotope have shown that the natural biota can contribute to shrimp nutrition in less intensive systems (Parker and Anderson, 1989; Cam et al., 1991; Burford, 2000). Focken et al. (1998) found that 71% natural food in the stomachs of *Penaeus monodon* in semi-intensively managed fed pond. Four to ten percent of ^{15}N-enriched natural biota were retained by shrimp within 48 hours (Burford, 2000). O'Keefe (1998) suggested a reduced dietary protein level (25 – 30%) for *Penaeus monodon* in extensive type of shrimp culture systems against 30 – 40% and 40 – 50% in semi-intensive and intensive type of culture, respectively. Depending on the culture system, 16 - 21% of the total amount of nitrogen available in the system was retained in shrimp biomass. These values concur with 14% retention in semi intensive *Penaeus vannamei* ponds (Teichet-Coddington et al., 2000), 18% retention in semi-intensive Thai shrimps ponds (Briggs and Funge-Smith, 1994) and 21 – 22% retention in intensive *Penaeus monodon* shrimps ponds (Jackson et al., 2003). However, in closed intensive *Penaeus monodon* rearing systems, a 23 - 31% N recovery was recorded (Thakur and Lin, 2003). The nitrogen budget in the carbohydrate added shrimp culture system revealed that 16 - 21% of the total nitrogen input was retained in the

shrimp, 0.22 - 0.49% in the water, 67 - 71% in the sediment, and 2.1 - 2.7% was lost through water exchange (Hari et al., 2006). Extensive shrimp farming with low water exchange pollutes less surrounding surface waters than all other shrimp farming systems (Hari et al., 2006).

Chapter – 2

Optimization of protein percentage in the shrimp feed by the control of C / N ratio

1. Introduction

Tiger shrimp, *Penaeus monodon* (Fabricius) is the prime marine candidate species used for aquaculture in South East Asia. Asian countries are contributing to 91% of the world shrimp production (FAO, 2002). Of the total global shrimp production 90% comes from extensive and modified extensive types of farming (FAO, 2001). Aquaculture contributes significantly to the world food supply, providing around 30% of fisheries production (Anon, 1994; Shahidul et al., 2003). Since further resources are exploited beyond their sustainable limit by capture fisheries, aquaculture is expected to continue to have an important role in catering to the ever increasing demand for fish. However, aquaculture systems today have expanded large enough to have significant impact on environment, such as pond culture and cage culture systems produce a lot of wastewater by huge nutrient loading to the environment which might be a source of water pollution (Naylor et al., 1998, 2000). These circumstances have forced industry to use recirculating systems in aquaculture systems both in sea water as well as fresh water (Tanaka et al., 1994; Krom, 1989), which is very expensive and not profitable. The high concentration of ammonia produced by uneaten feeds and fish waste are toxic to the organism cultured in aquaculture systems (Roger and Klementon, 1985). Aquatic animals require high concentration of protein rich feed and the source of ammonium is typically high protein rich feed

supplied by the farmers. Major ecological impact of shrimp farming is the discharge of nutrient rich waters in to coastal waters that may cause severe damage to the ecosystem (Colt and Armstrong, 1981; Folke and Kautsky, 1992; Naylar et al., 1998; Shang et al., 1998). A reduction in environmental quality of estuary can have a negative effect on shrimp pond operations (Paul and van Veen, 1978; Smith, 1996, 1999). Discharging water from ponds is a common management practice to ensure adequate water quality for shrimp growth. The discharge of high load of nutrients and suspended solids bring about adverse effects on the receiving water like enhancing ammonia-N content (Naylor et al., 1998; Smith et al., 1999). Therefore, efficient method for removal of ammonia-N and increase in yield from aquaculture system are needed for the development of sustainable shrimp farming techniques.

Poor shrimp survival and yield in extensive shrimp culture system have been attributed to several factors, including low quality of shrimp seed, poor environmental conditions and management (Sinh, 1994; de Graaf and Xuan, 1998; Johnston et al., 1999; Johnston et al., 2000a, b). Better environmental conditions are essential for the survival and adequate growth in shrimp (Boyd, 1976; Burford, 1997). Low primary productivity and rapid rate of benthic decomposition have already been suggested as possible factors limiting shrimp production (Alongi et al., 1999a, b). One of the

potential measures to improve sustainability in shrimp production system is the addition of organic carbon rich substrate (glucose, cassava, sorghum meal or cellulose) to control the carbon / nitrogen ratio (C / N ratio) (Avnimelech, 1999). Reducing water exchange to 0% in carbohydrate added treatments have no significant effect on shrimp growth, survival or production (Hopkins et al., 1993; Hopkins, 1994; Hopkins et al., 1995a, b; Martinez-Cordova et al., 1995; 1996). In USA, the regulation of pond discharge has led to the efforts for minimizing water exchange (Tucker, 1985; Wang, 1990; Hopkins et al., 1993). Reduction of dissolved inorganic nitrogen can be established in extensive system, well aerated and circulated fish or shrimp ponds by the application of organic carbon sources (Avnimelech et al., 1989; Avnimelech, 1999). The manipulation in the C / N ratio may result in a shift from an autotrophic to a heterotrophic system (Avnimelech, 1999; Browdy et al., 2001). The heterotrophic bacterial population utilizes the inorganic nitrogen to synthesize bacterial protein and new cells (single cell protein) and it may be utilized as a food source by the culture organism (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993) especially shrimp (Burford et al., 2004a), thus minimizing the demand for supplementary feed protein (Avnimelech, 1999). Taghon (1982) found that benthic invertebrates were able to take up microscopic glass beads when they are coated with proteins.

The effect of addition of organic carbon source with constant mixing and aeration on the reduction of inorganic nitrogen in aquaculture ponds of extensive, intensive or super intensive types is reported by Avnimelech (1999). An investigation on the effect of addition of organic carbon source and optimization of protein percentage in the shrimp feed in extensive stagnant shrimp ponds by water quality and shrimp production is warranted. The suspended solids distributed in the water column are believed to contribute to the success of this technique. Sine the bulk of shrimp is still grown in extensively managed stagnant water ponds, even a small impact of carbohydrate addition on production and sustainability will have a major effect on global shrimp production.

The objectives of the present study are:

1. To determine the suitable dietary protein level for the addition of carbohydrate.
2. To assess the level of enhancement of heterotrophic bacterial population due to the addition of carbohydrate in extensively managed shrimp ponds.
3. To evaluate whether the addition of carbohydrate is useful in improving the ecological sustainability in shrimp farms.
4. To examine whether the addition of carbohydrate helps in increasing shrimp production.

2. Materials and methods

Experiment design

Tank allocation for each treatment was done following completely randomized design and triplicate tanks were maintained for each treatment. The experiment had a 2 x 2 factorial design with two levels of dietary protein (25% and 40%) with and without carbohydrate source addition directly to the water column. The treatments without carbohydrate addition are abbreviated as P25 and P40, while the treatments with carbohydrate addition are abbreviated as P25 + CH and P40 + CH.

Experimental setup

The experiment was carried out in 1200 liter fiber reinforced plastic (FRP) tanks having an effective bottom area of 1.86 m^2. All the tanks were provided with uniform sediment layer (6 cm thick) brought from an extensive shrimp culture pond (Pokkali shrimp farm). The culture tanks were filled with 26 ppt saline water from Cochin estuary, which was pumped into a concrete tank and kept for 1 week for conditioning. The water level was maintained at 50 cm without water exchange during the 60 days experiment. Post larvae of *Penaeus mondon* (PL 20) purchased from a commercial hatchery were nursed for 30 days in FRP tanks at a stocking density of 250 m^{-2}. After 30 days, uniform sized (0.357 ± 0.01 g) juveniles were stocked at

a density of 6 juveniles m^{-2} in the culture tank. To stimulate phytoplankton bloom, culture tanks were fertilized with urea and super phosphate at a rate of 4 and 1 g m^{-2} $week^{-1}$ during the first three weeks of the experiment. 25% and 40% crude protein diets were prepared for shrimp diet (Higashimaru Feeds India Limited, Kuthyathodu, S.India). Locally purchased tapioca flour was used as carbohydrate source. Shrimps were fed with experimental feed at 15% of initial weight and adjusted gradually to 6% at the end of the culture. The pelleted shrimp feed distributed evenly over the tank's surface, twice daily at 08.00 and 18.00 hours. Pre-weighed carbohydrate was mixed with tank water in a beaker and applied to the water column uniformly followed by first feeding during the day. Shrimps were harvested by draining the tanks; individual length, weight and survival were recorded.

The quantity of carbohydrate (CH) added in the experiment

Carbohydrate addition was calculated following Eq. (1) (Avnimelech, 1999), and assuming that the added carbohydrate contains minimum 50% carbon, the CH addition needed (ΔCH) to reduce the total ammonia nitrogen concentration by 1 g N m^{-3} is 20 g m^{-3}.

$\Delta CH = \Delta N / 0.05$ (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):

ΔN = Quantity of feed x %N in feed x %N excretion (2)

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

ΔCH = Quantity of feed x %N in feed x %N excretion / 0.05 (3)

According to Eq. (3), 390 gm tapioca flour is required for each kg of 25% dietary protein and 620 gm tapioca flour for each kg of 40% dietary protein.

Water and sediment quality monitoring

Water quality parameters viz, temperature (mercury thermometer), salinity (hand refractometer), water pH (pH pen) and secchi disk reading were measured directly from the tank and dissolved oxygen (Winkler method, APHA, 1995) in site at 09.00 AM on a daily basis. Water samples were collected using horizontal water sampler from three locations of each tank and pooled together. Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. The water samples were filtered through

GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrate-N (cadmium reduction), nitrite-N and total ammonia nitrogen (TAN) (Phenol hypochlorite method) (Grasshoff et al., 1983). Biological oxygen demand (5 days BOD) of water samples was estimated following APHA (1995). Monthly chlorophyll-a in non-filtered water column samples was analyzed following standard methods (APHA, 1995). Sediment samples were collected from six locations using PVC pipes (2 cm diameter). Sediment pH was measured by pH pen. 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 (Mudroch et al., 1996) also estimated. The net protein value (NPV) of shrimp was measured by Jean guillaume et al. (2001). Monthly observation of total heterotrophic bacteria (THB) count in the water and sediment was also estimated by the standard procedures (APHA, 1995) and expressed as colony forming unit (cfu).

Shrimp production was measured by:

The growth of shrimp was estimated with the help of formula

$$\text{Growth} = \frac{\text{Final measurement} - \text{Initial measurement}}{\text{Initial measurement}} \times 100$$

The specific growth rate (SGR) was calculated by following the formula

$$SGR = \frac{(\log \text{final weight} - \log \text{initial weight})}{\text{Days of experiment}} \times 100$$

Feed conversion ratio (FCR) was calculated following the formula

$$FCR = \frac{\text{Feed consumed (Dry weight)}}{\text{Live weight gain (Wet weight)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Live weight gain}}{\text{Protein consumed in dry weight}}$$

Food conversion efficiency (FCE)

$$= \frac{\text{Total live weight gained by fish} \times 100}{\text{Total dry weight of feed offered}}$$

$$\text{Average daily weight gain (ADG)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Time interval}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of shrimp} \times 100}{\text{Initial number of shrimp}}$$

Statistical analysis

All the non-repeatedly measured variables (shrimp growth, yield, SGR, FCR, FCE, PER, ADG, survival rate and net protein value of shrimp) were analyzed by One-Way ANOVA Tukey HSD software using SPSS 11.5. Daily, biweekly and monthly water and sediment parameters were compared by using Two-Factor ANOVA without replication was performed using Microsoft Excel 2000 XP. Significant treatment effects were separated by calculating the least significant difference at 5% level.

3. Results

Water and sediment quality parameters

The mean values of daily water quality parameters such as temperature, water pH, dissolved oxygen, salinity and secchi disk readings are shown in Table 2.1. No significant difference ($P>0.05$) was observed among the treatments in temperature (28.12 – 28.13 ^{o}C), water pH (7.84 – 7.88) and dissolved oxygen (5.99 – 6.12 mg l^{-1}). However, secchi disk reading showed significant variation ($P<0.05$) among treatments, with higher value in treatment P25 and P40 + CH (50.97 and 51.37 cm), while it was lower in treatment P25 + CH and P40 (45.90 and 49.00 cm). The dietary protein level and carbohydrate addition had no significant effect ($P>0.05$) on the alkalinity, BOD and

soil pH (Table 2.2), the values being in the range 51.00 – 52.43 mg $CaCO_3$ l^{-1}, 3.50 – 4.45 mg l^{-1} and 7.80 – 7.85 respectively.

The mean values of biweekly water and sediment treatment wise TAN, nitrite-N, nitrate-N and THB results were summarized in Table 2.2. The treatment with carbohydrate addition showed significant reduction ($P<0.05$) in inorganic nitrogen production in water and sediment. The treatment P40 showed significantly ($P<0.05$) higher water TAN (9.02 µg l^{-1}), nitrite-N (3.59 µg l^{-1}) and nitrate-N (7.47 µg l^{-1}) concentration while it was lowest in the treatment P25 + CH (3.04, 0.96 and 1.95 µg l^{-1}). The results of ANOVA showed that dietary protein level in the diet have significant effect ($P<0.05$) in the production of inorganic nitrogen concentration in sediment. Higher THB population in water and sediment were observed in carbohydrate added treatments (P25 + CH and P40 + CH) (Table 2.2). However, chlorophyll-a and organic carbon showed no significant differences ($P>0.05$) between the treatments.

The effect of carbohydrate addition and dietary protein levels on water and soil quality of treatments during the culture period is shown in Fig. 2.1 and in Fig. 2.2 respectively. The results revealed that addition of carbohydrate to water column was effective in reducing ($P<0.05$) the TAN and nitrite-N levels during the rearing period. Among the sampling periods treatment P40 showed highest water TAN (23.65 µg l^{-1}), nitrite-N (11.41 µg l^{-1}) in sampling period

four and in nitrate-N (21.64 μg l^{-1}) concentration was observed during the sampling period five (Fig. 2.1). In sediment highest TAN concentration was observed in treatment P40 (53.24 μg l^{-1}) followed by treatment P25 (52.31 μg l^{-1}) during the sampling period five. The THB population during the culture period in water ranged from 47.33 – 132.67 x 10^5 cfu ml^{-1} while in sediment it ranged from 9.39 – 25.66 x 10^7 cfu ml^{-1} (Fig. 2.1 & 2.2). During the culture period treatment P40 + CH showed higher THB concentration followed by treatment P25 + CH. 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. 2.1 & 2.2).

Shrimp yield parameters

Details of shrimp harvested from experimental ponds with and without carbohydrate source addition are shown in Table 2.3. Significantly higher individual shrimp weight was recorded in the treatment P40 + CH, P40, and P25 + CH (2.40 - 3.02 g) than in P25 treatment (1.35 g). Higher shrimp yield was recorded in P25 + CH (14.2 g m^{-2}) when compared to P25 (7.4 g m^{-2}). The treatments with carbohydrate added (P40 + CH and P25 + CH) showed significantly ($P<0.05$) higher SGR value than without carbohydrate added

treatment (P40 and P25). The carbohydrate addition have significant effect ($P<0.05$) on shrimp SGR than the dietary protein level. Lower FCR values (3.33) were recorded in carbohydrate added treatment (P25 + CH) than P25 (6.18) and it was not significantly different ($P>0.05$) from P40 (3.28) and P40 + CH (2.68). The dietary protein level with the addition of carbohydrate had a significant effect ($P<0.05$) on the FCR values among the treatments. The one-way ANOVA results showed that the ADG values of P25 + CH, P40 and P40 + CH (0.03 – 0.04 g m^{-2}) were significantly different ($P<0.05$) from the treatment P25 (0.01 g m^{-2}). PER (1.17) and net protein value (NPV) (27.17%) results also showed that treatment P25 + CH utilized the maximum protein for yield. Survival of the shrimp did not vary significantly ($P>0.05$) among the treatments (68 – 74%).

4. Discussion

The nature of water quality is an important tool in aquaculture pond management, because results of such analysis are indicative on the suitability of water for aquaculture production or the concentrations of some of the parameters are suboptimal (Boyd et al., 1994). According to Boyd (1976) the optimum ranges for extensive shrimp culture variables are water temperature 26 - 32 ^{o}C, pH 7.5 - 8.5, alkalinity 50 - 90 mg $CaCo_3$ l^{-1} and dissolved oxygen 4.5 - 8.0 mg

l^{-1}. In the present study, the water temperature, water pH, alkalinity and dissolved oxygen were found to be in optimal ranges during the culture periods, while the secchi disk reading showed significant variations. Chei (1992) recommends the optimum water secchi disk value at 40 – 50 cm. Dewan et al. (1991) and Ahmed (1993) observed an inverse relationship between secchi value and chlorophyll-a in ponds. In the present study, chlorophyll-a concentration showed significant variations. Binch et al. (1997) found that alkalinity, water and bottom soil pH are significantly correlated with shrimp yield. The results of the present study revealed that the temperature, dissolved oxygen, alkalinity, water and bottom soil pH variables were in the optimal ranges and there is no significant variation among treatments during the culture.

Addition of carbohydrate to the pond water column resulted in significant reduction in the concentration of TAN both in water and sediment and this finding concurs with that of Avnimelech and Mokady (1988), Avnimelech et al. (1989), (1994) and 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 study, carbohydrate added treatments (P25 + CH and P40 + CH) showed significant increase of total heterotrophic bacteria population during the culture period. Burford et al. (2004a) strongly support the view that the addition of

carbohydrate in culture system facilitated the increase of heterotrophic bacterial population during the culture time. In the shrimp culture system, the toxic nitrogenous wastes were effectively used by the phytoplankton and microbial activities (Shilo and Rimon, 1982; Diab and Shilo, 1988). However, the water TAN concentrations in treatment P40 (9.02 ± 9.30 μg l^{-1}) was higher when compared to treatment P40 + CH (3.73 ± 4.30 μg l^{-1}). Low TAN concentrations were recorded in the present study due to the addition of carbohydrate, on the other hand, higher THB were observed in water and sediment in these treatments. 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; Queiroz and Boyd, 1998). The dissolved inorganic nitrogen limitation can be established in the culture system, fish or shrimp pond by adding a carbon-rich several other substrate like glucose and cassava meal cellulose powder (Avnimelech and Mokady, 1988; Avnimelech et al., 1989; Avnimelech et al., 1994; Avnimelech, 1999) and molasses (Burford et al., 2004b). In the present study, tapioca flour was used as a carbohydrate source for the microbial consumption by increasing the bacterial population. Bacteria utilized the added carbohydrate as food and synthesized microbial protein through the subsequent uptake of nitrogen from the system (Avnimelech et al., 1994).

Subsequent reduction of inorganic nitrogen and enhancement of THB in the carbohydrate added treatment strongly agrees with the above statement. The resulting heterotrophic bacterial production (single-cell protein) may be utilized as a food source by Carp and Tilapia (Schroeder, 1978; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993). Avnimelech et al. (1989) demonstrated a practical technique to recycle excess N into fish or shrimp. 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.

Wang et al. (1992) reported that ammonia-N and organic carbon were increased in response to dietary protein concentration. The results of the present study showed that dietary level had a significant effect on the concentration of toxic inorganic nitrogen species and in organic carbon. The rate of dietary protein in pelleted feed was particularly promoting the level of organic carbon in the system (Rubright et al., 1981; Garson et al., 1986; Anderson et al., 1987; Hernandez-Liamas et al., 1993). The addition of carbohydrate in to extensive culture system is a potential means to reduce the concentration of inorganic nitrogen (Avnimelech and Mokady, 1988; Avnimelech et al., 1989, 1994; Avnimelech, 1999; Browdy et al., 2001). The control of inorganic nitrogen was made possible by the

utilization of the inorganic-N to synthesize bacterial protein and new cells (Avnimelech, 1999). Avnimelech et al. (1992, 1994) proved that addition of carbohydrate reduced the need of dietary protein concentration. In the present study 25% dietary protein feed and 40% dietary protein feed were used for comparing dietary protein. The usage of higher dietary protein level (40% dietary protein feed) was resulted in the production of more inorganic nitrogen concentration in the culture system. Conversely, while applying the appropriate level of dietary protein level (25% dietary protein feed) with addition of carbohydrate was found effective the production of inorganic nitrogen production at very low levels, besides showing better survival and higher shrimp yield. On the other hand, according to Garson et al. (1986) the 25% dietary protein pelleted feed alone was not sufficient to cater the required supplemental nutrients to *Penaeus monodon* in intensive farming system.

No significant difference was observed in the survival among the treatments and it can be attributed to the ideal water and soil quality conditions prevalent in the culture systems (Hernandez-Llamas et al., 1995). Among various treatments, the net shrimp yield was significantly higher in carbohydrate added treatments. Burford et al. (2004b) suggested that 'flocculated particles' rich in bacterial and phytoplankton could contribute substantially to the nutrition of the *Litopenaeus vannamei* in intensive shrimp ponds. Natural food from

the shrimp pond contributed 75.09% of *Penaeus subtilis* stomach contents while the formulated feed contributed only 15.16% in semi-intensive culture system (Nunes et al., 1997). The utilization of microbial protein depends on the ability of target animal to harvest bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). The net shrimp yield with low protein diet (25%) together with addition of carbohydrate was comparable with the treatment having 40% dietary protein and this finding would manifest that *Penaeus monodon* can well utilize additional protein, which may be derived from the bacterial biomass, as a consequence to the addition of carbohydrate. The carbohydrate addition was beneficial in the extensive shrimp culture practices by increasing the production, reduced feed cost and the reduced inorganic nitrogen production in water and soil of the culture system. These results are highly useful in making the shrimp farming more ecologically sustainable and economically viable.

In conclusion, addition of carbohydrate to the extensive shrimp culture system reduced the demand for dietary protein level from 40% to 25% without compromising the shrimp production. The direct addition of carbohydrate to water column was useful in increasing the total heterotrophic bacterial population and resulted in augmenting shrimp production. The levels of inorganic nitrogen species in water column were lower due to the subsequent uptake by bacteria.

Profitability of the shrimp farming operation can be improved by the addition of carbohydrate to ponds by reducing the feed cost and fetching higher revenue from harvested shrimp. Furthermore, the utilization of microbial protein by the shrimp was also useful in increasing the net protein value (NPV) in shrimp. This type of culture will definitely improve the sustainability of shrimp farming under extensive and modified extensive methods of farming systems due to conversion of more N inputs of the pond in to harvestable products.

Table 2.1

Daily water quality parameters of indoor tanks stocked with *Penaeus monodon*

Variable	Treatments (mean ± SD)			
	P25	**P25 + CH**	**P40**	**P40 + CH**
Temperature (^{o}C)	28.12 ± 0.82^{a}	28.13 ± 0.79^{a}	28.12 ± 0.80^{a}	28.13 ± 0.81^{a}
Water P^{H}	7.88 ± 0.21^{a}	7.87 ± 0.19^{a}	7.85 ± 0.20^{a}	7.84 ± 0.26^{a}
DO (mg l^{-1})	6.10 ± 1.09^{a}	6.11 ± 1.12^{a}	6.12 ± 1.15^{a}	5.99 ± 1.16^{a}
Salinity (ppt)	26.34 ± 0.86^{a}	26.72 ± 1.04^{b}	26.75 ± 1.05^{b}	26.83 ± 1.13^{b}
Secchi disk reading (cm)	50.97 ± 3.02^{a}	45.90 ± 8.52^{b}	49.00 ± 4.38^{b}	51.37 ± 2.66^{a}

Results from ANOVA Two-factor without replication

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

Table 2.2

Effect of carbohydrate addition and dietary protein level on the water and sediment quality in indoor

	Treatments (mean ± SD)			
	P25	**P25 + CH**	**P40**	**P40 + CH**
Water quality variable				
BOD (mg l^{-1})	3.97 ± 2.30^{a}	4.07 ± 2.10^{a}	3.50 ± 2.00^{a}	4.45 ± 1.80^{a}
Alkalinity (mg $CaC0_3$ l^{-1})	51.87 ± 16.80^{a}	51.70 ± 16.40^{a}	51.00 ± 16.90^{a}	52.43 ± 16.90^{a}
TAN (ug l^{-1})	4.68 ± 5.00^{a}	3.04 ± 3.90^{a}	9.02 ± 9.30^{b}	3.73 ± 4.30^{a}
Nitrite-N (ug l^{-1})	2.18 ± 1.90^{ab}	0.96 ± 0.80^{b}	3.59 ± 4.30^{a}	1.45 ± 0.80^{b}
Nitrate-N (ug l^{-1})	4.97 ± 7.10^{ab}	1.95 ± 1.50^{b}	7.47 ± 11.20^{a}	3.58 ± 4.30^{b}
Chlorophyll-a (ug l^{-1})	25.67 ± 16.10^{a}	25.4 ± 11.40^{a}	28.43 ± 14.50^{a}	24.61 ± 18.80^{a}
THB (10^{5}cfu ml^{-1})	72.00 ± 33.10^{a}	92.00 ± 52.80^{a}	55.33 ± 32.40^{a}	104.67 ± 34.50^{a}
Sediment quality variable				
Soil pH	7.85 ± 0.23^{a}	7.84 ± 0.21^{a}	7.82 ± 0.23^{a}	7.80 ± 0.21^{a}
TAN (ug l^{-1})	36.21 ± 16.20^{a}	32.92 ± 14.90^{b}	36.23 ± 16.70^{a}	31.14 ± 14.20^{b}
Nitrite-N (ug l^{-1})	0.05 ± 0.02^{a}	0.05 ± 0.02^{a}	0.04 ± 0.02^{a}	0.05 ± 0.02^{a}
Nitrate-N (ug l^{-1})	3.29 ± 1.80^{a}	2.72 ± 1.50^{a}	2.69 ± 1.40^{a}	2.76 ± 1.40^{a}
Organic carbon (ug l^{-1})	13.62 ± 3.40^{a}	16.14 ± 3.40^{a}	14.52 ± 3.80^{a}	14.63 ± 2.80^{a}
THB ($10^{'}$cfu ml^{-1})	13.10 ± 8.70^{a}	19.43 ± 7.10^{a}	10.84 ± 3.40^{a}	17.53 ± 12.20^{a}

Results from ANOVA Two-factor without replication

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

Table 2.3

Effect of carbohydrate addition and protein levels on weight, shrimp yield, SGR, FCR, and survival of *Penaeus monodon* in Indoor trials

Variable	Treatments (Mean ± SD)			
	P25	**P25 + CH**	**P40**	**P40 + CH**
Individual shrimp weight gain (g)	1.35 ± 0.32^{b}	2.40 ± 0.10^{a}	2.46 ± 0.32^{a}	3.02 ± 0.44^{a}
Net shrimp yield (g m^{-2})	7.4 ± 0.31^{c}	14.2 ± 0.84^{b}	15.0 ± 1.64^{ab}	18.4 ± 2.52^{a}
Specific growth rate (SGR)	2.18 ± 0.43^{b}	3.17 ± 0.07^{a}	3.20 ± 0.21^{a}	3.54 ± 0.23^{a}
Feed conversion ratio (FCR)	6.18 ± 1.71^{a}	3.33 ± 0.14^{b}	3.28 ± 0.40^{b}	2.68 ± 0.37^{b}
Protein efficiency ratio (PER)	0.66 ± 0.15^{b}	1.17 ± 0.04^{a}	0.75 ± 0.09^{b}	0.92 ± 0.13^{ab}
Average daily weight gain (ADG)	0.01 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}
Net protein value (%)	16.67 ± 0.50^{c}	27.17 ± 1.00^{a}	17.50 ± 0.95^{c}	22.73 ± 1.69^{b}
Survival rate (%)	68.2 ± 19.02^{a}	70.7 ± 36.20^{a}	74.4 ± 15.04^{a}	73.9 ± 13.91^{a}

Results from Tukey One-way ANOVA

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

Fig. 2.1

The effect of carbohydrate addition and dietary protein levels on the water quality parameters

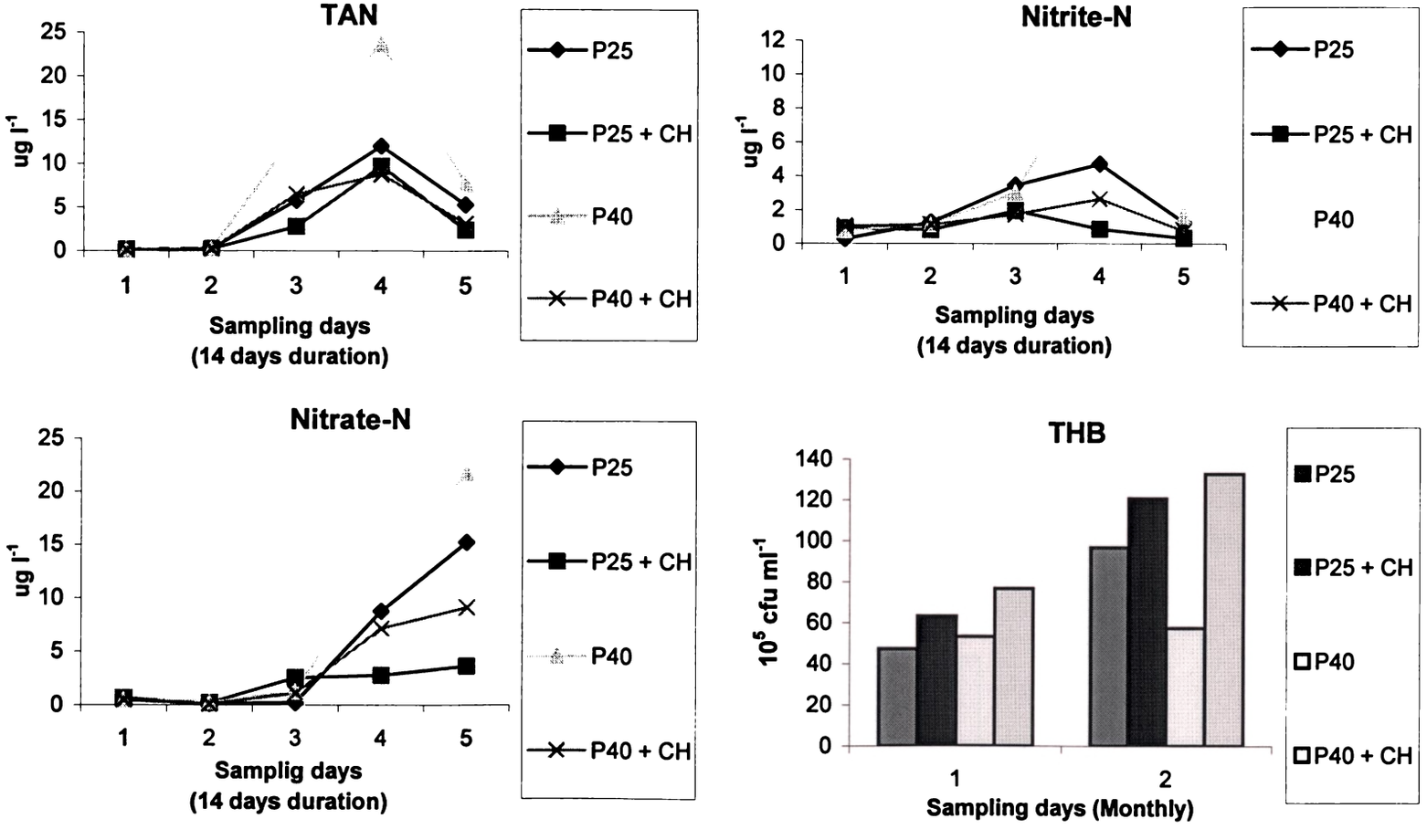
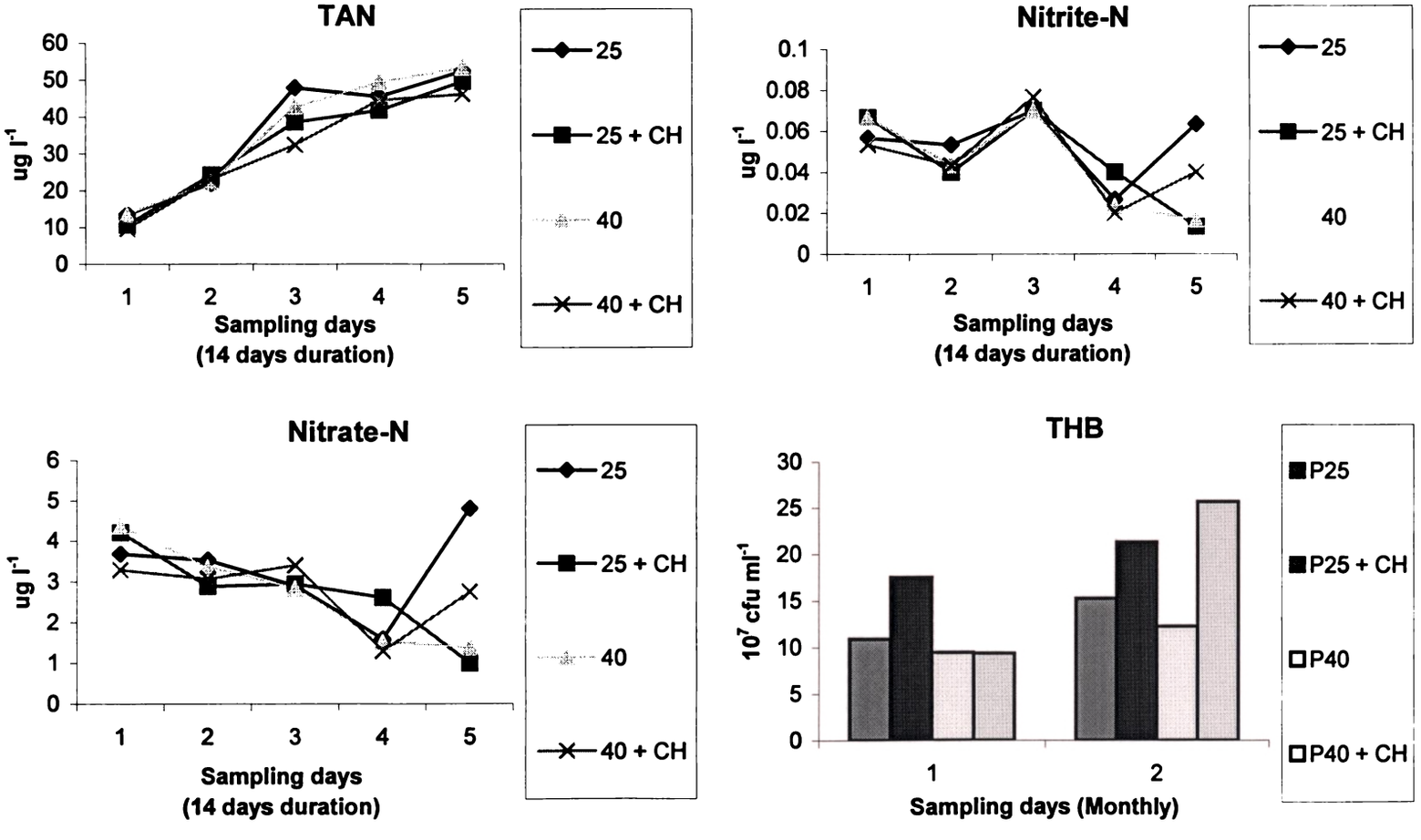


Fig. 2.2

The effect of carbohydrate addition and dietary protein levels on the sediment qauality parameters



Chapter – 3

Effects of various modes of carbohydrate application and diets having various protein levels in the production and sustainability of *Penaeus monodon* (Fabricius)

1. Introduction

Penaeus monodon, the tiger prawn is the most extensively cultured crustacean in South-East Asia (FAO, 2000). Owing to its high growth rate (CIFRI, 1985), ability for adaptation to various culture systems (Alava and Lim, 1983) and good response to compounded feed (Lee, 1971; Forster, 1972; Aquacop, 1976; Dall, 1992), *Penaeus monodon* is proved an ideal species for mass cultivation in estuarine and brackish water ecosystem. Shrimp farming has been a rapidly growing industry in many tropical and subtropical regions due to its high export potential. However, the increasing importance of shrimp aquaculture may influence the productivity of shrimp farms (Anon, 1994). Consecutive farming practices increase the toxic inorganic nitrogen concentration, which may cause stress to culture organisms affecting adversely to their growth (Dall et al., 1990). Coastal brackishwater shrimp farms are known to exert substantial impact on surrounding environment. Therefore, shrimp farming industries have been under increasing pressure to reduce environmental impacts and improve sustainability (Wu et al., 1994). Physical and chemical properties of water in shrimp farms are useful indicators of farm management practices (Dierberg and Kiattisimkul, 1996) and, at the same time, properties of effluent water are important to understand the impact of farm effluents on the receiving environment (Chua et al., 1989). Environmental

manipulation to boost culture production requires a basic understanding of the physical, chemical and biological processes occurring in the culture system. The addition of carbohydrate source helps to increase the microbial population and these microorganisms utilize the inorganic nitrogen present in the culture system to produce microbial protein. Feed present in the culture system has also been found to influence water quality and shrimp health (Jory, 1995; Burford and Williams, 2001). The shrimps cultured in extensive culture system utilize the organic matter, bacteria, phytoplankton, and zooplankton which results in high shrimp yield (Bombeo-Tuburan et al., 1993; Allan et al., 1995). Reduction in eutrophication results from optimizing pond water quality. This has been achieved largely by trial and error approach of new designs and management based on indoor observations. However, there is increasing need to understand the natural processes taking in place pond systems which would be helpful in reducing discharge loads and improve water quality (Tacon, 1996; Burford et al., 2001; Jory et al., 2001).

Shrimp farming industry is facing increasing pressure to lower its environmental impact (Naylor et al., 1998). A major concern is the discharge of nutrients from shrimp farms into coastal waters, while largely contributes to increased algal bloom, oxygen depletion and reduced biodiversity. Most of the nutrients discharged from intensive shrimp farms originate from formulated protein rich feeds (Funge-

Smith and Briggs, 1998). Experimental studies indicated that feed inputs during the production cycle account for the bulk of nitrogen inputs to many shrimp ponds (Briggs and Funge-Smith, 1994; Jackson et al., 2003). Nitrogen (N) plays a key role in the dynamics of aquaculture systems due to its dual role, in various forms, as a nutrient and toxicant. Dynamics of nitrogen in intensive aquaculture ponds have been studied by Hargreaves (1997), Lorenzen et al. (1997) and Lefebvre et al. (2001).

Therefore, efforts to improve feeding strategies must focus on both optimizing production and minimizing waste. One of the potential management measures to improve the sustainability in shrimp production systems is the addition of organic carbon rich substrate (glucose, cassava, sorghum meal or cellulose) to control the carbon / nitrogen ratio (C / N ratio) (Avnimelech, 1999). Reduction of dissolved inorganic nitrogen can be achieved in intensive, well aerated and circulated fish or shrimp ponds by the application of organic carbon sources (Avnimelech et al., 1989; Avnimelech, 1999). The resulting bacterial production (single cell protein) may be utilized as a food source by carp or tilapia (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993) and shrimp (Burford et al., 2003; Hari et al., 2004). Further improving the technique of carbohydrate addition in extensive production systems can have a major impact on the sustainability of shrimp farming, especially when considering that

the vast majority of shrimp production in Asia is still harvested from extensively managed ponds.

Manipulations of shrimp culture systems to improve water quality and to enhance production require a thorough understanding of the physical, chemical and biological processes taking place (Boyd, 1986). The objectives of present study are,

(1) To examine the effectiveness of various modes of carbohydrate application in the production and sustainability of shrimp farming.

(2) To find out whether the addition of carbohydrate can lower the protein content of the applied feed.

(3) To monitor which mode of carbohydrate application increases the heterotrophic bacterial population.

(4) To find out whether the application is useful in augmenting the shrimp production and

(5) To quantify the extent of reduction in toxic inorganic nitrogen levels in the pond by the various modes of application.

2. Materials and methods

Experiment design

The experiment tanks were allocated following complete randomized design with 25% and 40% dietary protein level with or without carbohydrate addition. 40% dietary protein feed was

pulverized and repelletised with the addition of required quantity of carbohydrate source at 620 g per kg of feed, which resulted a 25% protein diet with carbohydrate application through the diet were conducted for the comparison. A 25% and 40% protein diet were further abbreviated as P25 and P40. The treatments that received carbohydrate addition to the water column are further referred to as P25 + CH and P40 + CH. The dietary protein feed repelletised with carbohydrate was abbreviated as CHD.

Experimental setup

The experiment was carried out in 6 m^3 concrete tanks having an effective bottom area of 6 m^2. All the tanks were provided with a uniform 7 cm thick sediment layer taken from an extensive shrimp culture pond. Lime was added initially at 3 kg $tank^{-1}$. Culture tanks were filled with 22 ppt saline water from the Cochin estuary. Water level in the culture tanks was maintained at 100 cm during the whole culture period. Overflow pipes facilitated a runoff of rainwater with minimum mixing with tank water. To stimulate phytoplankton development, culture tanks were fertilized with urea and super phosphate at the rate of 4 and 1 g m^{-2} $week^{-1}$ during the first six weeks of culture. Cattle dung was also added to the tanks at 5, 2, 3 and 2 kg $tank^{-1}$ at the onset of the 2^{nd}, 3^{rd}, 5^{th} and 7^{th} week of culture, respectively. Twenty days old post larvae (PL 20) of *Penaeus monodon*

purchased from a commercial hatchery were stocked in the tanks at a density of 7 PL m^{-2}. Commercial shrimp feeds containing 25% and 40% crude protein were used during the experiment (Higashimaru Feeds India Limited, Kuthyathodu, S.India). Tapioca flour was used as carbohydrate source. The quantity of carbohydrate was calculated following Avnimelech (1999); i.e. 390 g and 620 g tapioca flour per kg of the 25% and 40% diet fed, respectively.

Treatments were executed in triplicate and were assigned randomly to the 15 tanks used in this study. Daily feeding rates of shrimps were 15% body weight at the start of the experiment, and declined gradually to 3% at the end of the culture period. Feed was distributed evenly over the tank's surface, twice daily at 08.00 and 18.00 hours. The pre-weighed tapioca flour was mixed in a beaker with tank water and uniformly distributed over the tank's surface directly after the feed application at 8.00 AM. Shrimps were harvested by draining the tanks 120 days after stocking. Individual length, weight and survival were recorded.

Water and sediment quality parameters

Temperature (mercury thermometer), salinity (hand refracto meter), water pH (pH pen) and transparency (Secchi disc) were measured directly from the tank and dissolved oxygen (Winkler method, APHA, 1995) daily in situ at 09.00 AM. Sediment and water

samples were collected on biweekly basis between 09.00 and 10.00 hours. Water samples were collected using a horizontal water sampler from three locations of each tank and pooled together before analysis. Composite water column samples were filtered through a GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrate-N (cadmium reduction), nitrite-N and total ammonia nitrogen (TAN) (phenol hypochlorite method) (Grasshoff et al., 1983). The biological oxygen demand (5 day BOD) of the water samples were estimated following APHA (1995). Monthly chlorophyll-a in non-filtered water column samples was analyzed following standard methods (APHA, 1995). Sediment samples were collected from six locations using 2 cm diameter PVC pipes. Sediment pH was measured by pH pen. The organic carbon in the sediment was determined following El Wakeel and Riley (1957). Exchangeable TAN, nitrite-N and nitrate-N were measured according to Mudroch et al. (1996). The net protein value of shrimp was measured by Jean guillaume et al. (2001). Monthly observation of total heterotrophic bacteria (THB) count in the water and sediment was estimated following the standard procedures (APHA, 1995) and expressed as colony forming units (cfu).

Statistical analysis

Daily, biweekly and monthly water and sediment parameters were compared using Two-Factor ANOVA without replication using

Microsoft Excel 2000 XP. All the non-repeatedly measured variables (shrimp growth, yield, SGR, FCR, FCE, PER, ADG, survival rate and net protein value of shrimp) were analyzed by One-Way ANOVA Tukey HSD software using SPSS 11.5. Significant treatment effects were separated by calculating the least significant difference at 5% level.

3. Results

Water quality parameters

The daily water quality parameters viz. water pH, dissolved oxygen (DO) and secchi disk reading were not shown any significant variation ($P>0.05$) in treatments (Table 3.1). Treatments wise significant variations ($P<0.05$) were observed in the parameters such as alkalinity, salinity, biological oxygen demand (BOD) and temperature. Table 3.1 shows the significant variation of salinity which can be attributed to the temperature variation. The addition of carbohydrate to water column significantly reduced ($P<0.05$) the total ammonia nitrogen (TAN) (Fig. 3.1a), in treatment P25 + CH (5.91 $\mu g\ l^{-1}$) when compared to the addition of carbohydrate to the diet (7.04 $\mu g\ l^{-1}$) and the treatment P25 showed the concentration 9.79 $\mu g\ l^{-1}$. The details of TAN concentrations are shown in Table 3.2. The addition of carbohydrate reduced the nitrite-N concentration and the lowest value was recorded in the treatment P25 + CH (Fig. 3.1b). The nitrate-N

concentration of the treatments fluctuated between 3.13 – 3.61 μg l^{-1}, however, no significant variations (P>0.05) were observed (Table 3.2). The total heterotrophic bacterial population (THB) in the carbohydrate added water column treatments showed significant increase (P<0.05) when compared to other treatments (Fig. 3.1d). The highest THB population was observed in P25 + CH (37.0 X 10^5 cfu ml^{-1}) and in treatment CHD, 23.5 X 10^5 cfu ml^{-1} which were not statistically significant (P>0.05). Treatment wise THB values are given in Table 3.2. Carbohydrate with out addition, addition to either water column or diet did not had any significant effect (P>0.05) on chlorophyll-a (Table 3.2).

During the culture period the highest TAN production was recorded at sampling period 3 (Fig. 3.1a) while the maximum value in nitrite-N was observed in the sampling period 4 (Fig. 3.1b) whereas nitrate-N was highest at sampling period 5 (Fig. 3.1c). The temporal variation of inorganic nitrogen production decreased in carbohydrate added water column treatments. THB population showed increase during the culture period. There is no variation in chlorophyll-a during the culture period.

Sediment quality parameters

No significant variation (P>0.05) was observed among treatments in soil pH and the values ranged between 8.24 – 8.33. The

sediment TAN levels in the experimental tanks with carbohydrate addition in water column showed significant difference ($P<0.05$) (Table 3.2). The lowest mean TAN concentration was observed in the treatment P25 + CH (17.84 μg l^{-1}). During the culture period variation ($P<0.05$) was observed and the highest TAN concentration was recorded in treatment P40 at sampling period 9 (38.92 μg l^{-1}) (Fig. 3.2a). The sediment nitrite-N and nitrate-N levels in the treatment were not significantly ($P>0.05$) affected by carbohydrate addition (Table 3.2). During the culture period nitrite-N and nitrate-N concentrations showed increase (Fig. 3.2b and c). There was no treatment wise effect on organic carbon level in the sediment ($P>0.05$) (Table 3.2). During the culture period increase was observed in organic carbon which was in the range 6.09 – 17.98 μg l^{-1} (Fig. 3.2d). The addition of carbohydrate to water column had significantly influenced the THB population both treatment wise and did not show any significant variation ($P>0.05$) in treatment CHD (Table 3.2) (Fig. 3.2e). High THB population was recorded in treatment P25 + CH (74.56 X 10^7 cfu ml^{-1}) in contrast to lowest in treatment P40 (41.54 X 10^7 cfu ml^{-1}).

Growth, yield and survival of shrimp

Results of shrimp yield, individual weight, SGR, FCR, PER, FCE, ADG, net protein value and survival rate are presented in Table

3.3. Individual shrimp weight and shrimp yield were significantly higher ($P<0.05$) in carbohydrate added treatment. The higher shrimp yield was recorded in P40 + CH (154 g m^{-2}) and it was not different from ($P>0.05$) treatment P25 + CH (148 g m^{-2}) and P40 (138 g m^{-2}). Significantly higher ($P<0.05$) SGR values were observed in low protein diet with addition of carbohydrate (P25 + CH) than high protein diet (P40) without carbohydrate addition. SGR was more influenced by the carbohydrate addition ($P<0.05$) than the dietary protein level ($P>0.05$) however, there is no interaction with higher dietary protein. Significantly lower ($P<0.05$) FCR values showed in carbohydrate added treatments (P25 + CH) than P25 and was comparable to P40 and P40 + CH. The results of the present study showed that addition of carbohydrate, dietary protein level and their interaction had a significant effect on the PER values. The shrimp survival ranged from 77 - 83% and the dietary protein level or the addition of carbohydrate had no significant effect ($P>0.05$).

4. Discussion

One of the major water quality problems in the culture system is the accumulation of toxic inorganic nitrogenous species at the pond bottom. Avnimelech (1999) reported that the addition of carbohydrate in to intensive aquaculture is a potential means to reduce the concentration of inorganic nitrogen. Among inorganic nitrogen

species, TAN concentration is the most dangerous toxic component in the system, the toxic TAN concentration was low (5.91 - 14.69 μg l^{-1}) in the present study when compared to 6.5 mg l^{-1} reported by Chen et al. (1990) and Thakur and Lin (2003) (198 - 519.1 μg l^{-1}) in *Penaeus monodon* rearing systems. Ammonia-N and nitrite-N are two compounds that can be toxic to shrimp (Chien, 1992). The results of the present study showed that the dietary protein levels had significant role in the production of toxic inorganic nitrogen concentration in the shrimp culture systems as higher TAN and nitrite-N levels were recorded in treatments fed with high percentage of protein diets. The concentration of toxic inorganic nitrogen especially ammonia-nitrogen was higher in the culture system fed with total protein feed (Das et al., 1995) which concurs with the present results. Glencross et al. (2001) reported that the lower protein level in the diet was lower the ammonia concentration in the culture system. The present study proved that addition of carbohydrate directly to the water column was much more useful in significantly reducing ($P<0.05$) the inorganic nitrogen production both in water and soil (Table 3.2). Similar effect of inorganic nitrogen reduction has been reported by Avnimelech and Mokady (1988), Avnimelech (1999), Burford et al. (2003), Hari et al. (2004). Martin et al. (1998) showed that high protein diet and feces could increase the concentration of particle and dissolved matter in the sediment. Decomposition of

bottom waste results in the formation of TAN, nitrite-N and nitrate-N (Blackburn et al., 1988; Garnier and Barillier, 1991). TAN is directly excreted by the shrimp (Burford and Williams, 2001; Burford and Longmore, 2001). The results of the present study showed that TAN was increased in response to dietary protein concentration and total protein fed. Rinj (1996) stated that accumulation of inorganic nitrogen, particularly ammonia-N, in the sediment is common in pellet fed ponds.

In the present study no significant difference could be noted in organic carbon in ponds with or without carbohydrate addition. However, the organic carbon showed a gradual increase during the progressive of culture (Fig. 3.2d). The organic wastes produced in the culture system are finally converted into organic carbon and that will be deposited in the pond bottom (Abdel-Rahman et al., 1979; Pascual et al., 1983). The present experiment results revealed that the carbohydrate application through diet did not have any significant effect in sustainability and shrimp yield. Toxic inorganic nitrogen control induced by feed (carbohydrate source) availability of bacteria and its increase (Avnimelech et al., 1992). The treatment CHD result showed that there is no significant variation in the THB population when compared with carbohydrate added to the water column treatment.

In shrimp aquaculture ponds, the variation of BOD during the culture period is mainly due to the phyto-zooplankton growth which is stimulated by the addition of fertilizers and wastage of product that supplied as feed (Francis et al., 2003). The present study resulted BOD showed significant variation between the treatments. However, chlorophill-a did not show treatment wise variation in the study. Phytoplankton numbers and primary production rates were high in conventional shrimp and fish ponds (Yusoff and McNabb, 1989; Knud-Hansen et al., 1991; Burford, 1997). The addition of carbohydrate to water column was useful in the significant increase of the THB population during the culture period, which might have utilized the inorganic nitrogen species. It has been reported that deleterious nitrogenous wastes were effectively used by the phytoplankton and microbial activity in shrimp culture system (Shilo and Rimon, 1982; Diab and Shilo, 1988). During the initial sampling period chlorophyll-a did not show significant variation (Table 3.2) and the steady increase in chlorophyll-a concentration after the eight week showed the utilization of inorganic nitrogen from water. Thus, it appeared that the increase of THB population was useful in reducing toxic inorganic nitrogen concentration in carbohydrate added to the water column treatments. According to Avnimelech (1999) the inorganic nitrogen can be controlled by its utilization to synthesize bacterial protein and new cells. Furthermore, the utilization of

microbial protein depends on the ability of target animal to harvest bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). The juvenile and adult shrimp eat a wide variety of micro-invertebrates and phyto-zooplankton material (Dall, 1992; Smith et al., 1992). The diet also changes seasonally, depending on prey availability (Wassenberg, 1990). The biochemical composition of the major prey items of shrimp were determined by Dall et al. (1991, 1992). They found the natural diet to be high in protein (70 to 80%). Low TAN concentration in shrimp farms can be limitation of phytoplankton growth (Burford and Gilbert, 1999). The higher yield in the pond where carbohydrate added to water column of the present study revealed that *Penaeus monodon* is capable of utilizing the additional protein, which might have derived from the bacterial biomass, produced by the addition of carbohydrate.

The individual shrimp weight and net shrimp yield were significantly higher in carbohydrate added to the water column treatments. In the present study, the similar net shrimp yields were observed in the treatment P25 + CH and P40. It has been reported that toxic nitrogen concentration was reduced while the heterotrophic production increased in carbohydrate added ponds (Avnimelech, 1999; Burford et al., 2003; Burford et al., 2004b). Favorable conditions, better food and environment will helps to increase the yield and survival of shrimp (Robertson and Phillips, 1995; Paez-

Osuna et al., 1997). However, the present results revealed that carbohydrate addition to pond did not have any significant effect on the survival of shrimp. Similar survival rates in all treatments showed that water and sediment quality were favorable for *Penaeus monodon* cultivation (Hariati et al., 1996). Table 3.3 showed the addition of carbohydrate to the water column with 25% dietary protein feed produced higher SGR, FCR, PER, ADG and net protein value when compared to CHD, P25 and P40. The treatment P40 + CH is equal or higher but it is not significantly different. Austreng et al. (1977) observed that the carbohydrate application results tended to produce more protein and less fat in culture species bodies. The higher shrimp yield in the carbohydrate added treatments in the present study showed that *Penaeus monodon* can well utilize the additional bacterial protein as a result of carbohydrate addition.

In conclusion, manipulation C / N ratio by the addition of carbohydrate in extensive shrimp culture system improved the water quality in ponds by reducing toxic inorganic nitrogen levels. The mode of carbohydrate application through diet had no significant effect in reduction of toxic inorganic nitrogen production and increasing the shrimp yield. The demand for dietary protein level can be significantly reduced in favor of carbohydrate addition to the water column without compromising the shrimp production. The added carbohydrate to the water column facilitated increased heterotrophic bacterial growth

which in turn augmented shrimp production. . The protein level in the diet was reduced from 40% to 25% without compromising shrimp production. The culture minimized the water discharge due to the minimum production of inorganic nitrogen. Carbohydrate addition in combination with reduction of the dietary protein level improved the sustainability of shrimp farming in extensive shrimp culture systems.

Table 3.1

Daily water quality parameters in the outdoor tanks stocked with *Penaeus monodon*

Variable	Treatments (mean ± SD)				
	CHD	**P25**	**P25 + CH**	**P40**	**P40 + CH**
Temperature (^{o}C)	29.49 ± 1.28^{a}	29.41 ± 1.79^{a}	29.19 ± 1.50^{ab}	28.84 ± 1.74^{b}	29.06 ± 1.53^{b}
Water P^{H}	8.12 ± 0.53^{a}	8.19 ± 0.57^{a}	8.02 ± 0.43^{a}	8.03 ± 0.46^{a}	8.01 ± 0.48^{a}
DO (mg l^{-1})	6.66 ± 2.62^{a}	6.43 ± 3.37^{a}	6.35 ± 2.25^{a}	6.11 ± 1.95^{a}	6.74 ± 2.43^{a}
Salinity (ppt)	21.96 ± 2.41^{b}	21.41 ± 2.74^{b}	22.85 ± 2.51^{a}	22.15 ± 2.41^{ab}	22.04 ± 2.24^{ab}
Secchi disk reading (cm)	59.11 ± 27.04^{a}	61.33 ± 26.11^{a}	66.26 ± 24.19^{a}	60.48 ± 23.72^{a}	60.93 ± 22.58^{a}

Results from ANOVA Two-factor without replication

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

Table 3.2

Effect of carbohydrate addition and dietary protein level on the water and sediment quality in outdoor

	Treatments (mean ± SD)				
	CHD	**P25**	**P25 + CH**	**P40**	**P40 + CH**
Water quality variable					
BOD ($mg\ l^{-1}$)	3.93 ± 1.98^{ac}	3.31 ± 1.86^{ab}	2.96 ± 1.85^{b}	3.70 ± 2.05^{bc}	4.30 ± 2.05^{c}
Alkalinity ($mg\ CaC0_3\ l^{-1}$)	80.31 ± 29.54^{a}	79.37 ± 34.88^{b}	84.64 ± 33.09^{ab}	68.21 ± 28.14^{c}	84.32 ± 30.93^{ab}
TAN ($ug\ l^{-1}$)	7.04 ± 5.99^{ab}	9.79 ± 6.91^{a}	5.91 ± 4.98^{b}	14.69 ± 15.84^{c}	8.61 ± 10.60^{ab}
Nitrite-N ($ug\ l^{-1}$)	1.40 ± 2.07^{b}	2.03 ± 2.96^{a}	0.81 ± 1.12^{c}	3.38 ± 5.01^{d}	1.99 ± 3.15^{a}
Nitrate-N ($ug\ l^{-1}$)	3.38 ± 1.67^{a}	3.14 ± 1.23^{a}	3.13 ± 1.42^{a}	3.61 ± 1.99^{a}	3.29 ± 1.75^{a}
Chlorophyll-a ($ug\ l^{-1}$)	28.97 ± 37.74^{a}	30.33 ± 31.24^{a}	45.03 ± 56.86^{a}	37.35 ± 26.08^{a}	60.85 ± 33.24^{a}
THB ($10^5 cfu\ ml^{-1}$)	23.5 ± 6.74^{cd}	20.8 ± 6.95^{d}	37.0 ± 14.26^{ab}	29.7 ± 9.62^{bc}	31.9 ± 11.62^{ab}
Sediment quality variable					
Soil pH	8.33 ± 0.34^{a}	8.25 ± 0.21^{a}	8.29 ± 0.18^{a}	8.24 ± 0.22^{a}	8.26 ± 0.16^{a}
TAN ($ug\ l^{-1}$)	20.70 ± 8.10^{a}	20.16 ± 8.20^{a}	17.84 ± 6.67^{d}	25.94 ± 9.97^{c}	22.0 ± 8.87^{b}
Nitrite-N ($ug\ l^{-1}$)	0.02 ± 0.02^{a}	0.03 ± 0.02^{a}	0.03 ± 0.02^{a}	0.03 ± 0.03^{a}	0.03 ± 0.02^{a}
Nitrate-N ($ug\ l^{-1}$)	0.14 ± 0.15^{a}	0.11 ± 0.09^{a}	0.12 ± 0.10^{a}	0.1 ± 0.08^{a}	0.12 ± 1.00^{a}
Organic carbon ($ug\ l^{-1}$)	11.12 ± 4.03^{a}	10.39 ± 3.92^{a}	12.13 ± 3.84^{a}	11.01 ± 3.61^{a}	11.52 ± 3.68^{a}
THB ($10'cfu\ ml^{-1}$)	50.61 ± 41.59^{o}	45.22 ± 29.01^{o}	74.56 ± 50.71^{a}	41.54 ± 23.36^{o}	73.80 ± 44.53^{a}

Results from ANOVA Two-factor without replication

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

Table 3.3

Effect of carbohydrate addition and protein levels on weight, shrimp yield, SGR, FCR, and survival of *Penaeus monodon* in Outdoor trial

Variable	Treatments (Mean ± SD)				
	CHD	**P25**	**P25 + CH**	**P40**	**P40 + CH**
Individual shrimp weight gain (g)	21.80 ± 1.18^{c}	21.11 ± 0.76^{c}	26.21 ± 0.45^{ab}	23.74 ± 0.29^{bc}	26.94 ± 1.70^{a}
Net shrimp yield (g m^{-2})	124.89 ± 10.24^{bc}	113.88 ± 10.72^{c}	148.44 ± 5.26^{a}	138.49 ± 7.52^{ab}	154.05 ± 7.93^{a}
Specific growth rate (SGR)	6.52 ± 0.05^{b}	6.49 ± 0.03^{b}	6.71 ± 0.01^{a}	6.61 ± 0.05^{ab}	6.73 ± 0.06^{a}
Feed conversion ratio (FCR)	1.40 ± 0.12^{ab}	1.54 ± 0.14^{a}	1.18 ± 0.04^{bc}	1.26 ± 0.06^{bc}	1.13 ± 0.05^{c}
Protein efficiency ratio (PER)	2.86 ± 0.23^{o}	2.56 ± 0.24^{o}	3.34 ± 0.11^{a}	1.94 ± 0.1^{c}	2.16 ± 0.1^{ao}
Feed conversion efficiency (%)	71.61 ± 5.87^{bc}	65.29 ± 6.15^{c}	85.10 ± 3.02^{a}	79.40 ± 4.31^{ab}	88.32 ± 4.55^{a}
Average daily weight gain (ADG)	0.17 ± 0.01^{c}	0.17 ± 0.01^{c}	0.21 ± 0.00^{ab}	0.19 ± 0.01^{bc}	0.22 ± 0.01^{a}
Net protein value (%)	23.60 ± 1.05^{cd}	21.43 ± 1.10^{d}	27.70 ± 1.25^{b}	25.50 ± 0.90^{bc}	28.50 ± 1.10^{a}
Survival rate (%)	81.75 ± 2.75^{a}	76.98 ± 4.96^{a}	80.95 ± 4.12^{a}	83.33 ± 0.01^{a}	81.75 ± 2.75^{a}

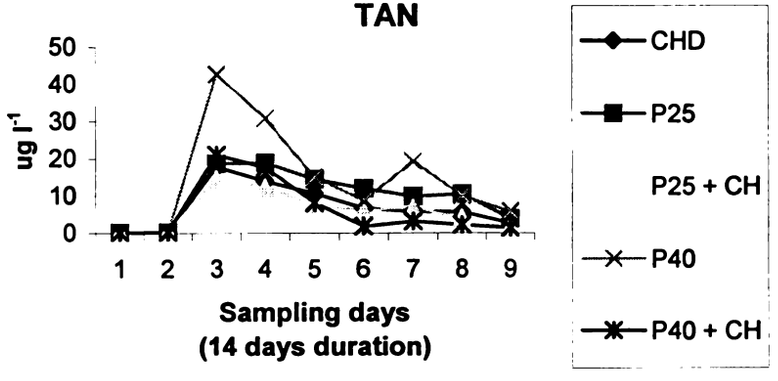
Results from Tukey One-way ANOVA

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

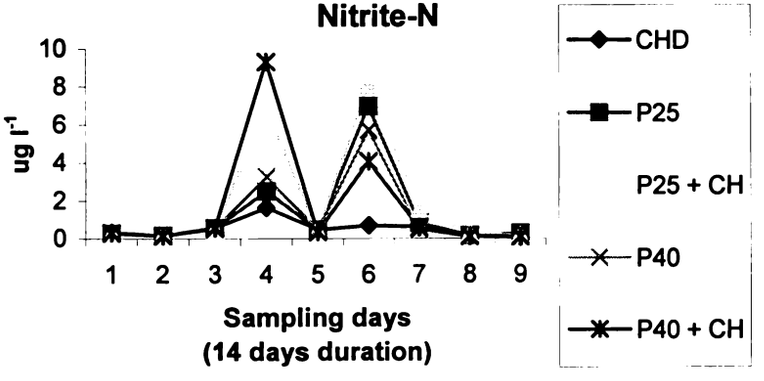
Fig. 3.1

The effect of carbohydrate addition and dietary protein levels on the water quality parameters

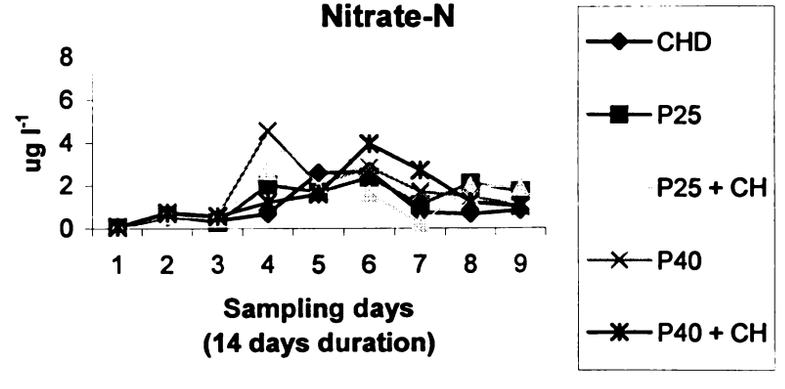
(a)



(b)



(c)



(d)

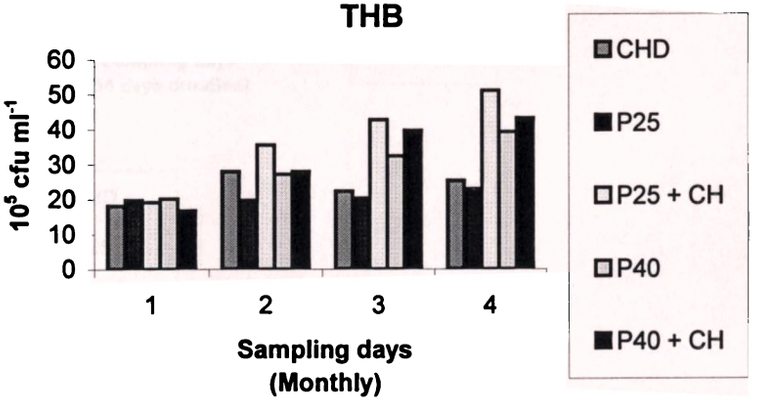
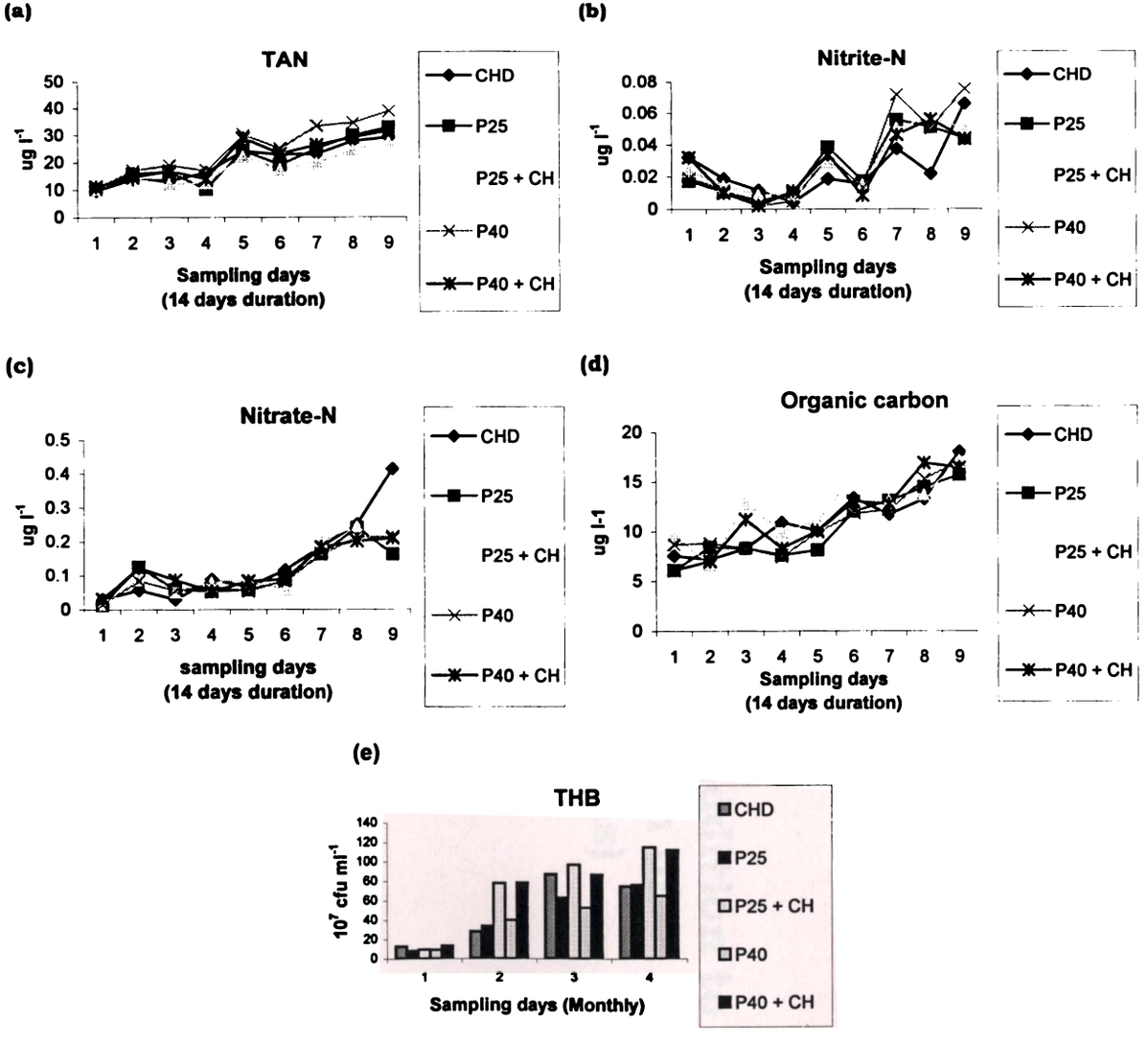


Fig. 3.2

The effect of carbohydrate addition and dietary protein levels on the sediment quality parameters



Chapter – 4

On-farm trials on carbohydrate addition to the extensive farming system of *Penaeus monodon* (Fabricius)

1. Introduction

Over the past three decades, shrimp farming sector had undergone spectacular changes due to the intervention of innovative technologies which were very much useful for enhancing guaranteed returns and profit and therefore a renewed interest was seen among the shrimp farmers of the country. The potential of aquaculture to meet the challenges of food security and to generate employment and foreign exchange earning are clearly demonstrated during the rapid expansion of this sector with a higher annual growth rate when compared to that of livestock. If aquaculture is to develop and provide for current and future needs in a sustainable manner, development of ecologically sustainable farming systems are the essential pre-requisites. *Penaeus monodon* is the prime candidate and extensively cultured shrimp and therefore, development of suitable ecofriendly culture method is essential for its sustainable production (Shi-Yen and Chun-Yang, 1992). Recently aquaculture industry is paying much attention on the development and refinement of a culture systems which can alleviate negative impacts of water and soil nitrogenous toxicants developed from the inputs used in the ponds (Klontz, 1979; Rosenthal, 1994). In most feed-driven ponds, less than 30% of nutrients inputs are converted into harvestable products, the remainder being lost to sediments, effluent water and atmosphere (Acosta-Nassar et al., 1994; Beveridge et al., 1994; Olah et al., 1994).

Pond production systems are also reliant on the environment at large to disperse and assimilate wastes (Beveridge and Phillips, 1993). Increasing the efficiency of resource use and improving productivity in general at the on-farm level and promoting responsible aquaculture are some of the essential steps to be followed in this direction.

Shrimp aquaculture is one of the fastest growing economic activities in coastal areas of the Asia-Pacific region where more than 85% of world's farmed shrimp is produced (FAO, 2002). Shrimp farming in Asia-Pacific region has been expanding since the early seventies and reached an industrial scale followed by increasing demand for shrimp in the export market. Despite rapid explosion of shrimp farming in Asia-Pacific region for the last two decades, water and sediment qualities and their implications in shrimp production as well as the impacts of farm effluents on the receiving ecosystem remains poorly understood. Coastal brackishwater shrimp farms are claimed to exert substantial impacts on the surrounding environment. Therefore, shrimp farming industries have been under increasing pressure to reduce environmental impacts and improve sustainability (Wu et al., 1994). Physical and chemical properties of water in shrimp farms are useful indicators of the farm environment (Dierberg and Kiattisimkul, 1996) and at the same time, properties of effluent waters are important to understand the impacts of farm effluents on the receiving environments (Chua et al., 1989). Although treatment of

farm effluents and reuse for shrimp offers a potential tool for reducing environmental impacts (Chin and Ong, 1994), shrimp farms in developing countries usually lack these systems. Environmental manipulation to boost aquaculture production requires a basic understanding of the physical, chemical and biological processes occurring in the systems. To understand the chemical processes and to establish information on the fate of the added nutrient particularly nitrogen is essential (Daniels and Boyd, 1989).

Most of the Asian countries including India and South American countries experienced serious decline, even collapse in cultured shrimp production. The production losses are principally due to poor rearing environment, management practices and outbreak of diseases (Primavera, 1998) and these issues have also raised questions relating to its sustainability in purely practical terms. The intensification of aquaculture activities has brought about severe degradation of land resources, deterioration of water and soil qualities, salination and land use pattern, thus threatening the long-term sustainability of aquaculture (Phillips et al., 1993, Primavera, 1998). Loading of ponds and the surrounding surface waters with prawn farming effluents has also been linked to outbreaks of diseases and production loss (Joseph et al., 2001). In recent years, there is a serious concern about potential environmental impacts of shrimp farming and the necessity of managing shrimp farms based on

environmental interactions. Though a wealth of information is available on the water and sediment characteristics of intensive and semi intensive shrimp ponds (Jackson et al., 2003; Martin et al., 1998; Tookwinas and Songsangjinda, 1999; Hopkins et al., 1993), similar information on feed driven extensive shrimp grow outs are rare. Wahab et al. (2003) reported that though most of the water quality parameters are within acceptable levels in the extensive shrimp ponds of Bangladesh, sub lethal levels of ammonia can be present and create stressing conditions. Bashar and Amin (2003) reported that there exists marked variation in the water quality of pond, discharge points and adjacent river of the extensive and semi intensive shrimp farms of Bangladesh. The ecology and shrimp production in the extensive shrimp ponds of Mekong delta Vietnam were subjected to series of studies, notably by Alongi et al. (1999 a & b) who reported that high suspended solids and nutrient concentrations, low and highly variable pH and dissolved oxygen concentrations, and variations in salinity due to intense rainfall limit shrimp production. Inadequate management practices and environmental deterioration (Binch et al., 1997) were also attributed as reasons for the low production of these ponds. The available information on Pokkali shrimp culture are mostly on quantification of annual production of shrimp during when these fields were used for trapping and holding of shrimps (Menon, 1954; George, 1974; Kurup

et al., 1992). Nair et al. (1988) made an attempt to correlate the environmental parameters with the shrimp catch in the Pokkali fields under trap and grow system and reported that the conditions for growth are good only for those fields located proximal to estuarine mouth.

The objective of this study is to evaluate the on-farm production on two feeds, one divided with high protein diet (Pelleted diet with about 40% of protein normally used as supplementary feed in the farming practices) and another with low protein diet (25%) with the addition of carbohydrate by conducting trials in extensive farming system.

The objectives of the present study are:

(1) To develop a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in the pokkali shrimp farms of Kerala.

(2) To effectively control inorganic nitrogenous species in the effluent discharge from the Pokkali fields and reciprocally converting the N inputs of the pond in to the maximum harvestable product.

(3) To develop a technology by which dietary protein level 40%applied in the shrimp farms to 25% without compromising shrimp production.

(4) To reduce the feed cost in the shrimp farming and ensure higher profit from the harvested shrimp.

(5) To improve the environmental sustainability of extensive shrimp farming system.

2. Materials and method

Experiment design

The experiments were designed with two different dietary protein levels viz. 40% dietary protein (P40) and 25% dietary protein feed with carbohydrate addition treatments (P25 + CH) for comparison of shrimp yield and environmental conditions in the Pokkali fields of Kerala where *Penaeus monodon* cultured. Eight earthen Pokkali ponds, each having 250 m^2 bottom area (shrimp–rice alternate farms) of Cochin, Kerala were selected for the on-farm trials. The ponds were prepared following the usual pre-stocking procedures of an extensive shrimp farm, which includes draining up of the ponds after rice cultivation, cleaning the aquatic weeds and remaining of the rice crop, strengthening of the dike and periphery. Lime was applied at 2000 kg ha^{-1}. Elimination of the weed and predatory fishes were done by the application of tea seed cake at 60 kg ha^{-1}. Saline water used to fill the ponds was filtered through nylon net screens to avoid the entry of fish eggs and larvae. Water depth was maintained at 1 meter throughout the experimental period. Initially, cattle dung was applied at 1000 kg

ha^{-1} $crop^{-1}$. However, urea and single super phosphate were added to water column at 80 and 20 kg ha^{-1}, respectively on a weekly basis for first two months to initiate algal bloom in ponds. Lime was added to the pond at 10 kg $pond^{-1}$ $week^{-1}$ to maintain the pH and alkalinity. PL 20 of *Penaeus monodon* (0.016 ± 0.01 g) purchased from the commercial hatchery was stocked @ 6 PL m^{-2}.

Four randomly allocated ponds were used for each treatment. Commercial 25% and 40% crude protein shrimp feeds were used for the experiment (Higashimaru Feeds India Limited, Kuthyathodu, S.India). Shrimps were fed with 20% of their initial body weight, and gradually the feeding rate declined to 4% at the end of the culture period. Tapioca flour was pre-weighed and applied uniformly over the pond surface on a daily basis. At 390 g tapioca flour for each kilogram of 25% dietary protein feed were used. Shrimps were harvested on 105th day of culture, total shrimp yield from each ponds were estimated, and sub samples were weighed and counted for calculating the average weight and shrimp survival.

Water and sediment quality parameters

Water quality parameters, temperature (mercury thermometer), dissolved oxygen; Winkler method (APHA, 1995), salinity (hand refracto meter), water pH (pH-Scan-Eutech instruments, Singapore)

and transparency (Secchi disc) were measured in situ at 09.00 AM on daily basis. Water samples were collected using a horizontal water sampler from three locations of each pond and pooled together. Sediment samples were collected from three locations using PVC pipes (4 cm diameter). Both water and sediment samples were transported to the laboratory with in two hours after collection in iceboxes and analysed. Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. Composite water column samples were filtered through a GF/C Whatman glass fiber filter 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 was performed using standard method (APHA, 1995). Biological oxygen demand was measured (5 day) APHA (1995). Sediment pH was measured by pH pen. The organic carbon in the sediment was determined following El Wakeel and Riley (1957). Exchangeable total ammonia nitrogen (TAN), nitrite-N, nitrate-N in the sediment were measured (Mudroch et al., 1996). The net protein value of shrimp was measured by Jean guillaume et al. (2001).Total heterotrophic bacteria (THB) count in the water and sediment was estimated following the standard procedures (APHA, 1995) and expressed as colony forming units (cfu).

Statistical analysis

All the non-repeatedly measured variables (shrimp growth, yield, SGR, FCR, FCE, PER, ADG, survival rate and net protein value of shrimp) were analyzed by ANOVA: Single-Factor software using Microsoft Excel 2000 XP. Daily, biweekly and monthly water and sediment parameters were compared by using Two-Factor ANOVA without replication was performed using Microsoft Excel 2000 XP. Significant treatment effects were separated by calculating the least significant difference at 5% level.

3. Results

Water quality parameters

The results of the daily and biweekly water quality parameters from different treatments such as temperature, water pH, dissolved oxygen (DO), biological oxygen demand (BOD), salinity, secchi disk reading and alkalinity results are presented in Table 4.1 and 4.2 respectively. Temperature (32.6 – 32.7 ^{0}C), DO (5.8 mg l^{-1}), BOD (4.4 mg l^{-1}), salinity (14.8 – 14.9 ppt) and alkalinity (8.6 – 8.8 mg $CaCo_3$ l^{-1}) did not vary significantly ($P>0.05$) between the treatments. However, significant variations ($P<0.05$) were observed in water pH and secchi disk reading, the mean pH values for treatment P40 and P25+CH

were respectively 7.6 and7.7 while the secchi disc reading of the former treatments were 60.9 and 59.4 cm respectively.

The total ammonia nitrogen (TAN) concentrations was low in treatment P25 + CH when compared to P40 which was significantly different ($P<0.05$). Total heterotrophic bacterial (THB) population showed significant variation ($P<0.05$) in carbohydrate added treatments. Highest THB was recorded in P25 + CH (57.04×10^5 cfu ml^{-1}) (Fig. 4.1d). Nitrite-N, nitrate-N and Chlorophill-a did not show any significant variation ($P>0.05$) (Table 4.2) between the treatments.

The inorganic nitrogen concentration of nitrite-N, nitrate-N and TAN showed a decreasing tendency with the advancement of culture period (Fig. 4.1a, b, c). Whereas THB and chlorophyll-a concentrations showed an increasing trend during the culture period. The values of THB and chlorophyll ranged from 40.59 – 57.04×10^5 cfu ml^{-1} and 17.97 – 18.89 µg l^{-1} respectively (Table 4.2).

Sediment quality parameters

TAN and nitrate-N showed significant variation ($P<0.05$) in the treatment and lower values were observed in the treatment P25 + CH (Table 4.2). The THB population also showed significant increase ($P<0.05$) in P25 + CH where the mean value was 53.8×10^7 cfu ml^{-1} (Fig. 4.2d). The soil parameters such as pH, nitrite-N, nitrate-N and organic carbon did not show any significant difference ($P>0.05$) among

the treatments. The results of the sediment quality parameters are presented in Table 4.2.

The addition of carbohydrate significantly reduced ($P<0.05$) the TAN concentrations from 26.6 (P40) to 25.7 $\mu g\ l^{-1}$ in treatment P25 + CH. TAN and nitrite-N in the sediment showed temporal variation due to the accumulation (Fig. 4.2a, b). The nitrate-N concentration did not vary during the experiment and the level was in the range 0.03 – 0.16 $\mu g\ l^{-1}$ (Fig. 4.2c).

Shrimp yield and survival

The shrimp harvest details such as individual shrimp weight, net shrimp yield, FCR, SGR and survival rate are given in Table 4.3. In the treatment P25 + CH the individual shrimp weight at harvest was higher than that of treatment P40. In P25 + CH where the low protein diet with addition of carbohydrate to the water column was added to the water column was resulted in the production of 64.4 gm $^{-2}$ while in the treatment P40, the application of 40% diet has resulted only the shrimp production of 44.8 m^{-2}. The shrimp production between these two treatments was also found significantly different ($P<0.05$). The higher SGR was also recorded in treatment P25 + CH which was significantly different ($P<0.05$) from the treatment P40. Significant difference ($P<0.05$) was also observed in FCR value, which was lowest in treatment P25 + CH than to P40. The variables of PER,

FCE, ADG and NPV were higher in the treatment P25 + CH and it was significantly different ($P<0.05$) from that of treatment P40. The survival of shrimp was not affected ($P>0.05$) by the application of tapioca powder in treatment P25 + CH and it ranged between 35.5 – 42.2%.

Cost and economic analysis of on-farm trial

The results of the cost and economic analysis of on-farm trial details are given in Table 4.4. The total variable cost is much higher in treatment P40 (IRS. 97, 420.0 /-) when compared with the treatment P25 + CH (IRS. 73, 302.0 /-). In P40 and P25 + CH treatments total applied quantity of dietary protein shrimp feed is 1320 kg. The cost of 1 kg of 40% dietary protein feed is IRS. 46.0 /- while the same in the case of 1 kg of 25% dietary protein feed is IRS. 26.0 /-. The total tapioca powder applied along with 25% dietary shrimp feed is 130 kg which was purchased at the rate of IRS. 10.0 /- kg. The cost of feed and carbohydrate source for P25 + CH was lower than P40 due to the higher cost of high protein shrimp feed. The harvested shrimp from P25 + CH and P40 belonged to 40 and 50 counts, respectively. The total revenue from the harvested shrimp (ha^{-1}) was 54% higher in treatment P25 + CH than in P40 due to the combined effect of better yield and higher price of shrimp commensurate with their marketable size. A 40% reduction of feed cost was recorded in the P25 + CH

treatment when compared to treatment P40. The net profit from the P25 + CH was IRS. 1,14,292.0 /- which was much higher than P40 (IRS. 20,422.0 /-). Furthermore, the benefit cost ratio was significantly higher in treatment P25 + CH (1.4) than in treatment P40 (0.2) (Table 4.4).

4. Discussion

The results of the present study revealed that the growth of *Penaeus monodon* was far better in carbohydrate added culture system when compared to high protein fed treatment. Catacutan (1991) and Rosas et al. (2000) also reported similar findings with carbohydrate application. Pond dynamics is governed by complex interaction between water column and sediment components (Funge-Smith and Briggs, 1998). In the present study, the treatment with carbohydrate addition showed significant reduction in TAN both in sediment and water column. These results are in full conformity with the findings of Avnimelech and Mokady (1988), Avnimelech et al. (1989, 1994) and Avnimelech (1999). A critical DO concentration of 2.5 mg l^{-1} is required for normal growth of *Penaeus monodon* (Allan and Maguire, 1993). In the present study, the mean concentration of DO level was 5.8 mg l^{-1} which is well above the critical limits and there was no significant difference could be discernible between the treatments. In extensive culture systems, dissolved oxygen

concentrations at early morning were in the range 3 and 5 mg l^{-1} which is considered favourable for growth in *Penaeus monodon* without causing stress (Hariati et al., 1996). The optimal conditions for farming of *Penaeus monodon* recommended by NACA (1994) are as follows: dissolved oxygen, 3.8 – 6.0 mg l^{-1}; water pH, 7.5 – 8; salinity, 10 - 20 ppt; and ammonia, <0.1 mg N l^{-1}. In the present study, these parameters were with in the prescribed limits of NAGA (Table 4.1 and 4.2).

In the present study TAN level in the water column was 1.40 – 4.50 µg l^{-1} which is very low compared to 25 – 50 µg l^{-1} as reported Briggs and Funge-Smith (1994), Phillips (1994) and Jackson et al. (2003). In P25 + CH, the TAN concentration in the water and sediment significantly decreased during the progression of culture period. TAN is produced by microbial degradation of waste feed and by shrimp excretion (Burford and Williams, 2001; Burford and Longmore, 2001); TAN is rapidly utilised by the phytoplankton community (Burford and Glibert, 1999). In the present study, addition of carbohydrate to the water column was found useful in reducing the TAN concentrations and concurrently, higher THB population was observed both in water and sediment. According to McGoogan and Galtin (1998), Rudacille and Kohler (1998)and Conquest et al. (1998), the addition of properly adjusted carbonaceous material have the ability to potentially eliminate ammonium from the system and increase the bacterial

population. Mahamood (1986) reported usage of natural isotope abundance to identify the food source of post larval and juvenile *Penaeus monodon* in aquaculture ponds. Chen and Chen (1992) and Rothlisberg (1998) observed that *Penaeus monodon* can eat plankton directly over the grow-out period. In the present study, the contribution of phytoplankton to natural food did not vary significantly among the treatments as evidenced by the chlorophyll-a concentrations. However, significant increase was observed in the concentration of chlorophyll-a during the culture period. It can be attributed to the concentration of organic wastes in the pond bottom which might have enhanced the rate of primary production and chlorophyll-a as reported by Hopkins et al. (1993, 1995a, b).

In sediment, significantly higher TAN and nitrate-N concentrations were observed in the higher protein diet fed (P40) treatment. Waste accumulated in the sediment (unconsumed feed pellets, faeces, etc.) are degraded and ammonia-N, nitrite-N and nitrate-N are formed (Blackburn et al., 1988; Garnier and Barillier, 1991). Furthermore, ammonia is released in the water through the excretion of the shrimp (Wajsbrot et al., 1989). The maximum sediment TAN level reported during the present study is 47.69 $\mu g\ l^{-1}$ (P40). Chien (1989), Morales et al. (1991) and Rinj (1996) reported that the most common problem in feed fed ponds is the accumulation of organic matter and of inorganic nitrogen, particularly ammonia-N

ranging from 50.0 – 250 μg l^{-1} in the sediment. Due to the addition of carbohydrate to the water column, significant increase in organic carbon accumulation might have taken place which might have derived from the dead and decayed section, metabolic wastes from cultures organisms and faeces wastages, etc. (Boyd, 1992; Martin et al., 1998).

Nair et al. (1988) reported invariably low DO values in the Pokkali fields, conversely, in the present study, values the DO value was not subjected to extreme fluctuations. The water pH ranged from 7.6.to 7.7 and this in comparison with the optimal levels of 7.5 - 8.0 as reported by NACA (1994) indicate that the pH levels are ideal for shrimp farming. Pokkali field are characterised by acid sulphate soils and therefore the pH will be comparatively low (Nair et al., 1988) Joseph et al. (2001) reported the BOD levels in Kandeleru creek-water (Andhra Pradesh, India) as 14.6 mg l^{-1}, where ponds were stocked with shrimps @ 20 - 28 m^{-2} and this in comparison with BOD levels in the present study is farther. TAN levels in the extensive shrimp farms of Bangladesh were in the range 0.5 - 2.6 mg l-1 (Wahab et al., 2003) and this is far higher observed in the present study. Nair et al. (1988) reported ammonia concentrations from seasonal Pokkali fields in the range 0.5 to 2.5 mg l^{-1} during the summer months and this is on a higher side when compared to the values recorded from carbohydrate added treatments. The lower ammonia levels recorded now can be due

to the application of carbohydrate in these ponds. It can be asserted that pH, DO, water TAN and BOD in the present study are below the levels as prescribed by the Govt. of India at discharge points in creeks or estuary which are used as sources and disposal points for shrimp farming (Aquaculture Authority of India, 2001). Morales et al. (1991) reported that the concentrations of (NH_4 - NH_3)-N ranging from 23.4 to 236 mg kg^{-1} wet sediment, from shrimp ponds in Spain and for (NO_2 - NO_3)-N, concentration could range from 6.4 to 16.2 mg kg^{-1}. These values (ammonia and nitrite) are far above those observed in the present study. Pond bottom conditions are reported to be very crucial for shrimp growth and its well-being since it lives most of the time near the bottom burrows in to the soil and ingest bottom soil (Boyd, 1989; Chien, 1989, Avnimelech and Ritvo, 2003). The highest levels of TAN and NO_2 in sediments recorded in the carbohydrate added treatment was 25.7 µ moles l^{-1} and therefore it can reasonably be inferred that the floor conditions of CH added ponds are highly favourable for the shrimp for its dwelling and is not critical in providing sensitivity to diseases.

The addition of carbohydrate to the culture system favours microbial development as it provides a food source and it facilitates subsequent uptake of nitrogen from the culture system, by the synthesis of microbial proteins (Avnimelech et al., 1992). The present study proved that the addition of carbohydrate source significantly

increases THB population in the water and sediment in the Pokkali fields. The utilisation of microbial protein depends on the ability of target animal to harvest bacteria and its ability to digest and utilise the microbial protein (Avnimelech, 1999). The higher yield in the carbohydrate added treatment showed that *Penaeus monodon* can well utilise the additional protein derived from the harvested bacterial biomass as result of carbohydrate addition in the farms. It would thus appear that the principal of microbial protein synthesis by the addition of carbohydrate source and its consumption by the shrimp is equally effective at par with the indoor and out door experiments carried out in the earlier part of this thesis. The net shrimp yield, FCR and net protein value were higher in the treatment fed with 25% protein diet together with carbohydrate addition to the water column. Similarly, better feed and protein conversion coefficients were recorded in tilapia, fed with low protein and carbohydrate source (Avnimelech and Mokady, 1988; Avnimelech et al., 1989). The on-farm results showed that the dietary protein level had a significant effect on the level of toxic inorganic nitrogen species. Chua et al. (1989), Robertson and Philips (1995) and Paez-Osuna et al. (1997) reported that there exist a direct correlation between the increase of toxic inorganic nitrogen level both in water and sediments of shrimp grow-outs commensurate with the dietary protein concentration in the total protein feed applied to the system. Governments of several developed

countries have already been adopting policies to reduce the pollution of aquatic environments from shrimp farm effluents, thus stressing the importance of studies on the production of waste product from aquaculture farms (Gonzalez-Vila et al., 1996; Twarowska et al., 1996; Easter et al., 1996). Based on the results of the present study, it can be asserted that the addition of carbohydrate to the water column is useful in significantly reducing the toxic inorganic nitrogen species in the culture system and thus immensely useful in maximising the harvestable product from the nitrogen applied in the shrimp farms and reciprocally reducing the wastage of nitrogen through farm effluent. The higher growth rate and yield of culture organism can be attributed to the favourable toxic inorganic nitrogen level (Shilo and Rimon, 1982; Diab and Shilo, 1986) and increases the heterotrophic bacterial population in the carbohydrate added ponds (Burford et al., 2003; Burford et al., 2004b). Protein conversion efficiency of the shrimp in carbohydrate added ponds were higher, revealing that the input feed protein along with the microbial protein were effectively converted into shrimp biomass. Moriarty (1976) proved that increasing bacterial population in the culture system could be an important consideration in the growth and yield of penaeid shrimp. This may suggest that shrimp may derive some direct nutritional benefit from bacteria. The presence of higher level of bacterial population and relatively lower level of inorganic nitrogen in the

carbohydrate added treatment would manifest that the tapioca flour is a good source of organic carbon as it was well utilised by the heterotrophic bacterial population. Several other carbohydrate sources like glucose and cassava meal cellulose powder (Avnimelech and Mokady, 1988; Avnimelech et al., 1989; Avnimelech et al., 1994; Avnimelech, 1999), molasses (Burford et al., 2004a) were used in the fish and shrimp ponds to reduce the inorganic nitrogen production.

The economic analysis of on-farm trials was carried out and the results showed that the combination of low protein diet together with carbohydrate addition substantially reduced the feed cost in contrast to the high protein feed driven pond. The selection of Tapioca flour as the carbohydrate source was a much more practical approach, basically of three reasons such as (1) due to its low coast (IRS. 10 kg^{-1}) (2) easy availability (3) low protein content. Carbohydrate application promoted shrimp growth with out special sparing effect and nitrogen excretion was lowered (Gauquelin, 1996). The combined effect of high shrimp yield and higher market prize for the shrimp produced from carbohydrate added source increases the revenue by 54% and the net profit by 400% when compared to the high protein diet. Hari et al. (2004) proved that the carbohydrate addition with low protein feed was useful in the substantial reduction of feed cost and improving the net shrimp yield.

In conclusion, the innovative approach of feeding low protein shrimp diet with addition of carbohydrate in the extensive shrimp farms was found practicable approach for improving the ecological sustainability and increasing production and profitability.

Table 4.1

Daily water quality parameters in the on-farm ponds stocked with *Penaeus monodon*

Variable	Treatments (mean ± SD)	
	P 40	**P25 + CH**
Temperature (°C)	32.7 ± 1.0[a]	32.6 ± 1.8[a]
Water P^H	7.6 ± 4.2[b]	7.7 ± 4.6[a]
DO (mg l^{-1})	5.8 ± 2.3[a]	5.8 ± 1.3[a]
Salinity (ppt)	14.8 ± 1.3[a]	14.9 ± 1.3[a]
Secchi disk reading (cm)	60.9 ± 7.7[a]	59.4 ± 5.3[b]

Results from ANOVA Two-factor without replication

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

Table 4.2

Effect of carbohydrate addition and dietary protein level on the water and sediment quality in on-farm

	Treatments (mean ± SD)	
	P40	**P25 + CH**
Water quality variable		
BOD (mg l^{-1})	4.4 ± 2.0[a]	4.4 ± 1.6[a]
Alkalinity (mg $CaC0_3$ l^{-1})	8.8 ± 1.8[a]	8.6 ± 2.3[a]
TAN (ug l^{-1})	3.4 ± 1.8[a]	1.4 ± 1.3[b]
Nitrite-N (ug l^{-1})	0.16 ± 0.08[a]	0.14 ± 0.05[a]
Nitrate-N (ug l^{-1})	0.95 ± 0.65[a]	0.79 ± 0.61[a]
Chlorophyll-a (ug l^{-1})	17.97 ± 15.7[a]	18.89 ± 26.0[a]
THB (10^5cfu ml^{-1})	40.59 ± 18.53[b]	57.04 ± 20.7[a]
Sediment quality variable		
Soil pH	6.3 ± 0.1[a]	6.3 ± 0.1[a]
TAN (ug l^{-1})	26.6 ± 11.1[a]	25.7 ± 8.9[b]
Nitrite-N (ug l^{-1})	0.08 ± 0.06[a]	0.08 ± 0.05[a]
Nitrate-N (ug l^{-1})	0.14 ± 0.16[a]	0.07 ± 0.07[b]
Organic carbon (ug l^{-1})	19.6 ± 4.6[a]	20.3 ± 5.6[a]
THB ($10'$cfu ml^{-1})	41.5 ± 11.9[b]	53.8 ± 17.3[a]

Results from ANOVA Two-factor without replication

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

Table 4.3

Effect of carbohydrate addition and protein levels on weight, shrimp yield, SGR, FCR, and survival of *Penaeus monodon* in On-farm trial

Variable	Treatments (Mean ± SD)	
	P40	**P25 + CH**
Individual shrimp weight gain (g)	21.0 ± 0.8^{b}	25.7 ± 1.7^{a}
Net shrimp yield (g m^{-2})	44.8 ± 7.8^{b}	64.4 ± 12.2^{a}
Specific growth rate (SGR)	7.6 ± 0.0^{b}	7.8 ± 0.0^{a}
Feed conversion ratio (FCR)	2.2 ± 0.0^{b}	1.9 ± 0.1^{b}
Protein efficiency ratio (PER)	1.01 ± 0.03^{b}	2.00 ± 0.11^{a}
Feed conversion efficiency (%)	43.9 ± 1.7^{b}	53.6 ± 3.5^{a}
Average daily weight gain (ADG)	0.22 ± 0.0^{b}	0.27 ± 0.0^{a}
Net protein value (%)	19.78 ± 3.4^{b}	45.15 ± 7.5^{a}
Survival rate (%)	35.5 ± 7.0^{a}	42.2 ± 10.5^{a}

Results from ANOVA single-factor

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

Table 4.4

Cost (IRS.) and economic analysis of *Penaeus monodon* on-farm trial (per hectare)

Particulars	Quantity	Rate (IRS.)	Treatments	
			P40	P25 + CH
Variable cost				
Pond preparation	20 man days	150.0	3000.0	3000.0
Eradication	60 kg tea seed cake	30.0	1800.0	1800.0
Labour charge for eradication	4 man days	150.0	600.0	600.0
Lime	3200 kg	1.2	3840.0	3840.0
Cattle dung	1000 kg	0.5	500.0	500.0
Urea	320 kg	1.5	480.0	480.0
Super phosphate	80 kg	3.0	240.0	240.0
Shrimp seed	60,000 nos.	0.3	15000.0	15000.0
Shrimp feed (40% protein diet)	1320 kg	46.0	60720.0	0.0
Shrimp feed (25% protein diet)	1320 kg	26.0	0.0	34320.0
Tapioca flour	130 kg	10.00	0.0	1300.0
Salary of farm assistant	3 months	2500.00	7500.0	7500.0
Power cost	200 units	1.50	300.0	300.0
Harvest cost (IRS. kg^{-1})		5.00	2240.0	3222.0
Fuel cost	44 l	27.30	1200.0	1200.0
Total variable cost			97,420.0	73,302.0
Fixed costs				
Interest (5.83%)			5,680.0	4,274.0
Depreciation (1.94%)			1,890.0	1,422.0
Total fixed costs			7570.0	5696.0
Production				
Total shrimp yield (kg ha^{-1})			447.9[b]	644.3[a]
Price of shrimp (IRS. kg^{-1})			280.0	300.0
Economic analysis				
Total production costs			1,0,4990.0	78,998.0
Gross return (IRS.)			1,25,412.0[b]	1,93,290.0[a]
Net profit (IRS.)			20,422.0[b]	1,14,292.0[a]
Benefit / cost ratio			**0.2[b]**	**1.4[a]**

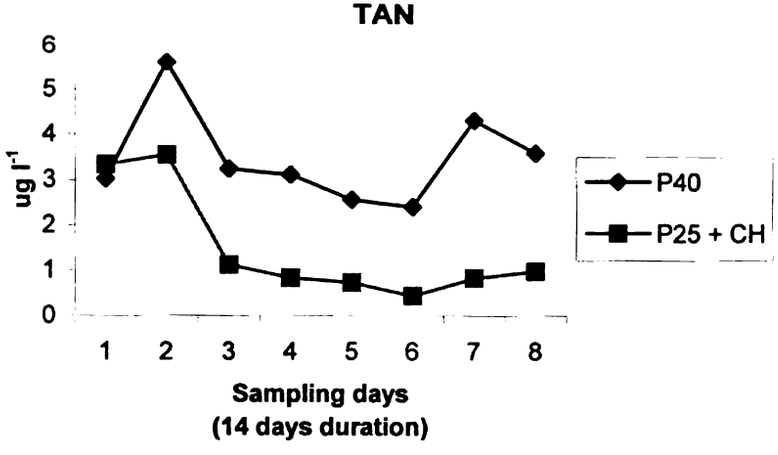
Results from ANOVA single-factor

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

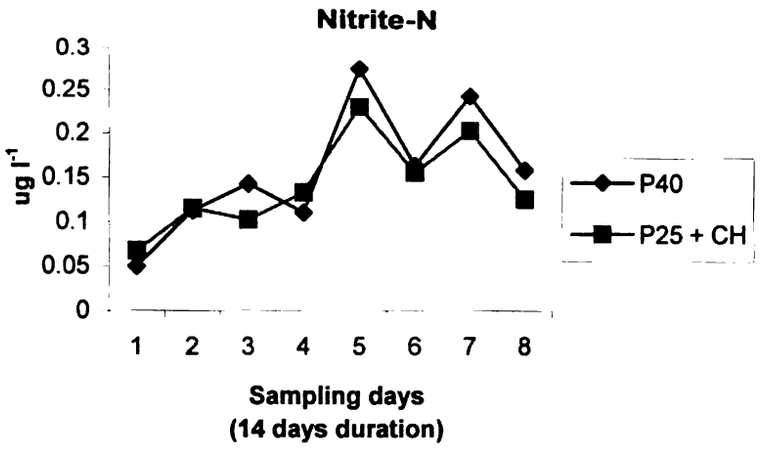
Fig. 4.1

The effect of carbohydrate addition and dietary protein levels on the water quality parameters

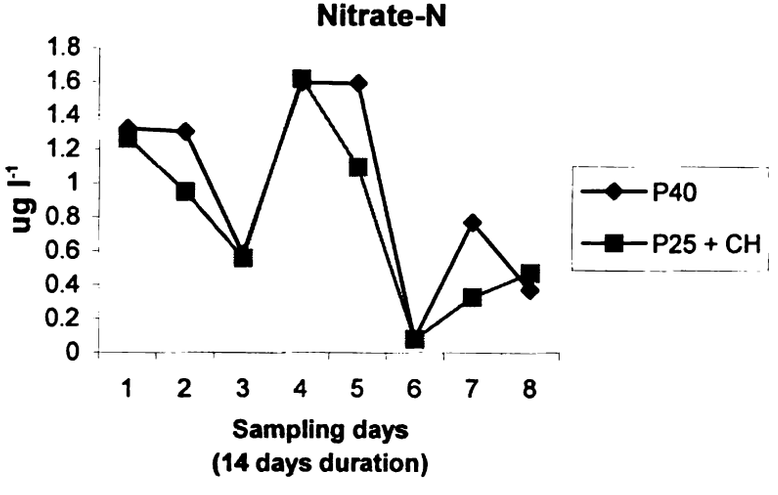
(a)



(b)



(c)



(d)

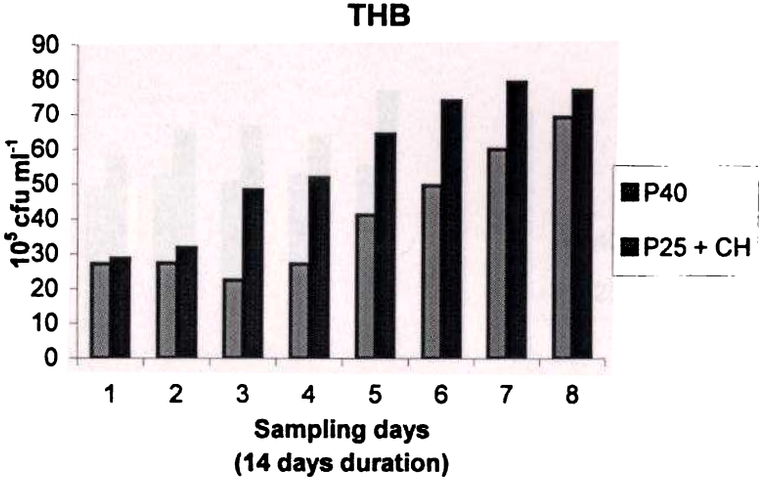
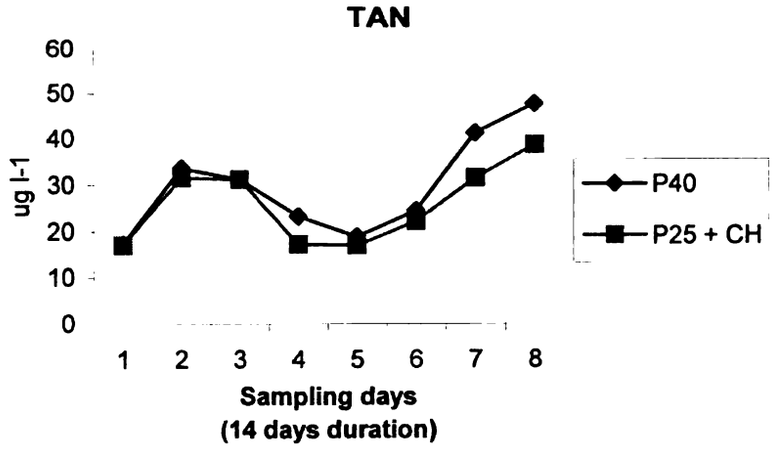


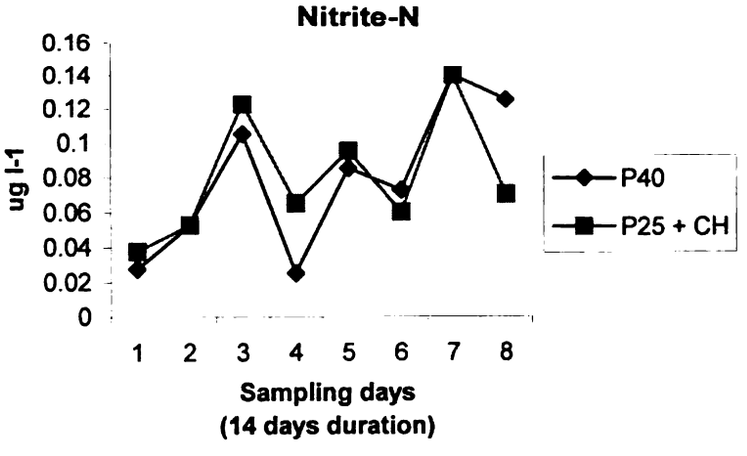
Fig. 4.2

The effect of carbohydrate addition and dietary protein levels on the sediment qauality parameters

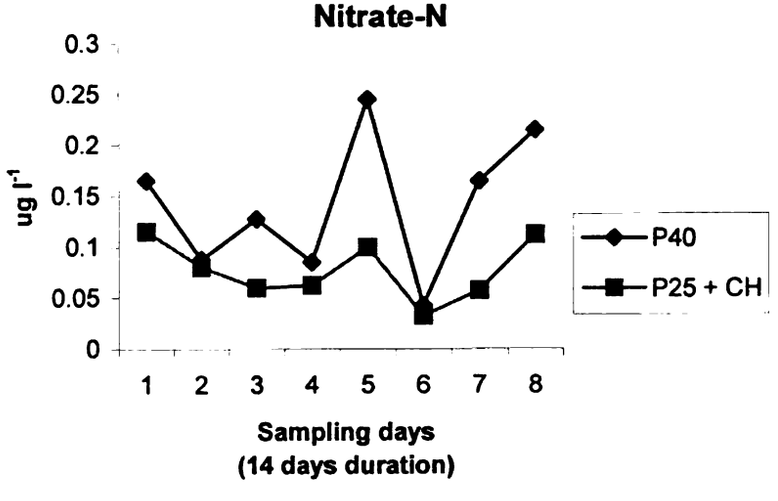
(a)



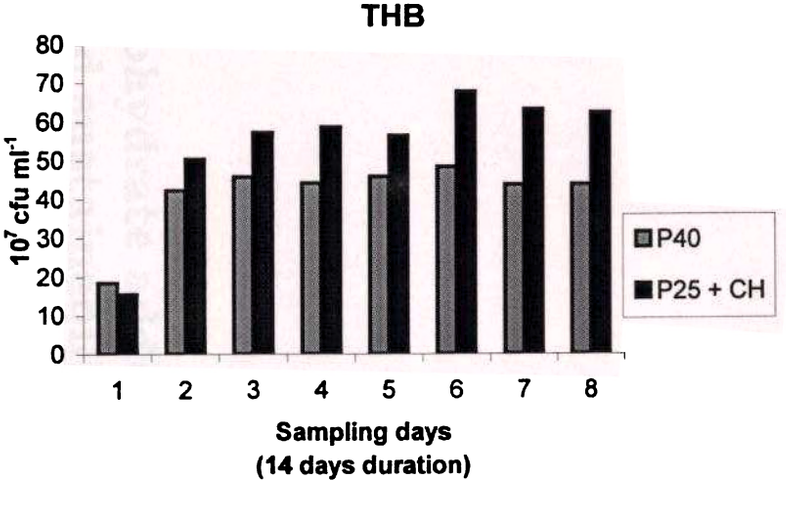
(b)



(c)



(d)



Chapter – 5

Stocking density and carbohydrate addition relationship in the yield and sustainability of *Penaeus monodon* (Fabricius)

1. Introduction

The production of brackishwater shrimps showed a strong increase in the 70's and 80's. In 1990, the world production reached some 750,000 MT (FAO, 1992). However, many problems arose in most of the producing countries (Thailand, Philippines, Indonesia, Equator and in India). In general, the decrease in productivity of aquaculture sites was imputed to over-intensification total surface of ponds on the same site, stocking densities, number of rearing cycles per year leading to the deterioration of the surrounding environment (Folke and Kautsky, 1992) and of pond water and pond sediment quality (Lin, 1989; Chen, 1992; Phillips et al., 1993). Stress reduced shrimps resistance to pathogenic diseases, resulted in mass mortality (Liao, 1990; Chua, 1993). Development of aquaculture activities at a particular site cannot be carried out only by considering planned facilities and the quality of the water on the site at its origin (Briggs, 1993). There is a strong relationship between the quality of the water in the pond and that in the water in the surrounding environment. Degradation of surrounding water quality will be faster as the quantities of wastes discharged entering in the environment increase, and as the site is confined (Martin et al., 1993), that is to say as the volume and flow of the receiving body are decreased.

An inverse relationship exists between Food Conversion Ratio FCR and waste production. FCR is related to parameters dealing with

nutrition adjustment of the daily food ration to size and biomass, and to parameters such as stocking density. Many works show an inverse relationship between the growth of shrimp and stocking density (Ray and Chien, 1992; Lee et al., 1986; Daniels et al., 1995; Sandifer et al., 1987).

Wastes generated by aquaculture activities, mainly faeces and unconsummated feed pellets, first settle in the sediment. As a consequence organic wastes and metabolites of degraded organic matter accumulate in the sediment of ponds (Boyd, 1992; Fast and Boyd, 1992; Hopkins et al., 1994). Part of the waste is flushed out of the ponds, immediately or later after the organic matter has been degraded.

Many authors have studied waste formation and pond management (Wang, 1990; Hopkins et al., 1993, 1994), including water and sediment in shrimps ponds (Hunter et al., 1987; Chen et al., 1990; Boyd, 1992; Ray and Chien, 1992; Funge-Smith, 1993; Tunvilai et al., 1993a, b; Boyd et al., 1994). Nevertheless, studies on the dynamic and quantification of nutrient flows in marine shrimp ponds are few. One report is that of Briggs and Funge-Smith (1994) on nitrogen and phosphorus budgets in commercially operated marine shrimp ponds in south-east Asia (stocking density ranging from 5 to 50 shrimp m^{-2}). However, the new tendency is to rear shrimps with stocking densities lower than 10 to 25 shrimp m^{-2}.

The high profitability and generation of foreign exchange have provided the major driving force in global expansion of shrimp culture, attracting both national governments and international development agencies. The key factor in the growth of Asian shrimp industry has been private sector initiative including the environment of multinational corporations (Chamberlain, 1991; Bailey and Skladany, 1991). Nutrient content of the feed will influence growth, survival and the amount of metabolic and excreted waste products entering the system. A substantial consequence of artificial feeding is the amount of waste produced through pellet fragmentation, leaching loss, residual feed and undigested material have been reported. Feeding strategies have also been found to influence water quality and shrimp health (Jory, 1995; Burford and Williams, 2001). A major source of ammonium is the typically protein rich feed because of the energy production pathway and the oxidation and catabolism of proteins (Heaper, 1988). Shrimp industry is characterized by extensive and intensive production in relatively small farms which stock at high densities and input of formulated feeds, intensive aeration, and relatively low rate of water exchange (Kongkeo, 1997). The extensive shrimp culture systems are characterized by low rate of water exchange and long retention time and therefore, the processes taking place within the pond will have a major bearing on water quality. The stress induced by poor water quality which may result in

reduced growth rates, weakened resistance to disease or direct mortality problems with water quality are often linked to culture intensity (Wang and Fast, 1992). Extensive and Intensive farming is characterized by rapidly increasing level of inputs added to the ponds, which are subjected to recycling. Pond process may be expected to respond to these increasing inputs, resulting in changes in many water quality parameters.

Effluents from aquaculture ponds typically are enriched in suspended organic solids, nitrogen and phosphorus. This may contribute significantly to elevated nutrient loadings in coastal environments (Chua et al., 1989; Dierberg and Kiattisimkul, 1996; Paez-Osuna et al., 1997; Paez-Osuna et al., 1998). Available information indicated that in shrimp pond most of these materials originate from supplementary feeds (Briggs and Funge-Smith, 1994; Robertson and Phillips, 1995; Paez-Osuna et al., 1997). Water exchange is still a common management practice in shrimp pond operation because of the deleterious effects of effluents from shrimp ponds on water quality of coastal zone (Ziemann et al., 1992; Twilley et al., 1993; Dierberg and Kiattisimkul, 1996; Bardach, 1997). The cumulative impact of pond effluent on the environmental quality of estuaries is proportional to the discharge volume and nutrient concentration (Twilley, 1989; Csavas, 1994). A reduction in environmental quality of the estuary can also have a negative effect on

shrimp pond operation (Smith, 1996). Several methods have been proposed to ameliorate the impact of shrimp pond effluents on the water quality of adjacent estuaries including, improved pond designs (Dierberg and Kiattisimkul, 1996; Sandifer and Hopkins, 1996), reducing the inorganic production in the pond and minimizing the water exchange rates (Hopkins et al., 1993; Martinez-Cordova et al., 1995).

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. The objectives of this study are:

(1) To find out the effectiveness of C / N ratio in the control of inorganic nitrogen accumulation during culture period in shrimp farms stocked with different stocking density.

(2) To evaluate various stoking densities with 25% dietary protein feed with and without carbohydrate addition.

(3) To examine the shrimp growth and yield in each stocking density.

2. Materials and methods

Experiment design

The experiment tanks allocation for each treatment followed a complete randomized design with 25% shrimp dietary protein feed with or without carbohydrate addition directly to the water column. The juvenile *Penaeus monodon* were stocked at a density of 3, 7 and 12 m^{-2}. The treatments without carbohydrate addition are abbreviated as 3, 7 and 12, while the treatments with carbohydrate addition as 3 + CH, 7 + CH and 12 + CH.

Experimental setup

The experiment was carried out in 6 m^3 concrete tanks having an effective bottom area of 6 m^2. All the experimental tanks were

provided with a uniform sediment layer (7 cm thick) collected from the pokkali shrimp farm. Lime was added initially at 3 kg $tank^{-1}$ and cattle dung @ 5 kg $tank^{-1}$. Culture tanks were filled with 25 ppt saline water from Cochin estuary by pumping after conditioning for one week. For stimulating phytoplankton bloom, the tanks were fertilized with cattle dung at 5, 2, 3, and 2 kg $tank^{-1}$ at 2^{nd}, 3^{rd}, 5^{th}, and 7^{th} week respectively. Urea and super phosphate were also added at the rate of 4 and 1 g $week^{-1}$ for first six week. Twenty days old post larvae (PL 20) (0.015 ± 0.01 g) of *Penaeus monodon* purchased from a commercial hatchery were stocked in the tank at a density of 3, 7, 12 juveniles m^{-2}. Commercial shrimp feed containing 25% crude protein (Higashimaru Feeds India Limited, Kuthyathodu, India) was used for the experiment. Carbohydrate source for addition to water column was purchased from local market. Tapioca flour was used as carbohydrate source, the quantity being calculated following Avnimelech (1999); *i.e.* 390 g tapioca flour for each kg of 25% dietary protein, respectively.

Shrimps were fed with experimental feed at 15% of initial weight and adjusted gradually to 3% body weight at the end of culture. Feed was distributed evenly over the tank's surface, twice daily at 8.00 and 18.00 hours. Pre-weighed carbohydrate source was mixed with tank water and applied to the water column uniformly followed by the feeding during the morning. Shrimps were harvested after draining

the tanks on 100th day of culture; individual length, weight and survival were recorded.

Water and sediment quality parameters

Daily water quality parameters such as temperature (mercury thermometer), salinity (hand refractometer), secchi disk (transparency) and pH (pH pen) were measured directly from the tank and dissolved oxygen (Winkler method, APHA, 1995) daily in situ at 09.00 AM. 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 (2 cm diameter). Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. The water samples were filtered through GF/C Whatman glass fiber filter 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 was analyzed following standard methods (APHA, 1995). Biological oxygen demand (5 days) 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). Total heterotrophic bacteria (THB) count in the water and sediment was

also estimated by the standard procedures (APHA, 1995) and expressed as colony forming unit (cfu).

Statistical analysis

Statistical analysis of daily, biweekly water and sediment quality parameters were done by ANOVA: Two-Factor without Replication performed using Microsoft Excel 2000. For non-repeatedly measured variables (individual growth rate, net shrimp yield, SGR, FCR, FCE, PER, ADG, Survival rate) were analyzed by One-Way ANOVA, Tukey HSD software using SPSS 11.5. Significant treatment effect was separated by calculating the least significant difference at 5% level.

3. Results

3.1. Stocking density 3 m^{-2}

Daily water quality parameters *viz.*, temperature, water pH, dissolved oxygen, salinity, secchi disk reading and biweekly alkalinity with 3 and 3 + CH additions are shown in Table 5.1 & 5.2. No significant difference could be seen between these parameters ($P>0.05$). Biological oxygen demand showed significant difference ($P<0.05$). The total ammonia nitrogen (TAN), nitrite-N, nitrate-N, chlorophyll-a, total heterotrophic bacteria (THB) of the water and Soil total ammonia nitrogen, nitrite-N, nitrate-N, total heterotrophic

bacteria and organic carbon are summarized in Table 5.2. In water, nitrite-N and THB in water showed significant difference ($P<0.05$) (Fig. 5.1). The ANOVA showed that nitrate-N showed significant difference ($P<0.05$) (Fig. 5.1).

Among the sediment parameters, soil pH, Organic carbon and nitrite-N (Fig. 5.2) showed no significant difference ($P>0.05$) (Table 5.2). Conversely, TAN, nitrate-N and THB showed variation during the sampling period (Fig. 5.2).

Treatment 3 with 25% dietary protein with addition of carbohydrate source, water and soil TAN, nitrite-N and nitrate-N were found low when compared to treatment without addition of carbohydrate. THB concentration showed slight increase in carbohydrate added treatment when compared to treatment without carbohydrate addition (Tables 5.2).

The average individual shrimp weight recorded in 3 + CH was 30.8 ± 1.5 g and this was very high when compared with 26.7 ± 1.9 g recorded in treatment without carbohydrate addition. SGR, FCR, PER, FCE and ADG were higher in 3 + CH than 3. The one-way ANOVA result showed significant difference ($P<0.05$). The carbohydrate added treatment evinced high shrimp yield. Net shrimp yield was also high in 3 + CH treatments but statistically it was not significant (Table 5.3).

3.2. Stocking density 7 m^{-2}

Temperature, pH, dissolved oxygen, salinity and secchi disk reading, ANOVA results of various water quality parameters in 7 and 7 + CH are shown in Table 5.1. No significant difference (P>0.05) was seen.

Among the treatments, biweekly water TAN, nitrite-N, nitrate-N and THB showed significant difference (P<0.05) (Fig. 5.1). The sediment nitrite-N (Fig. 5.2), organic carbon and soil pH showed no significant difference (P>0.05). Sediment TAN, nitrate-N and THB showed significant difference (P<0.05) (Fig. 5.2). The mean values with standard deviation are shown in Table 5.2.

The individual shrimp average weight in 7 + CH was higher than that of 7. The SGR and FCR were also higher in carbohydrate added treatment. The addition of carbohydrate to the water column with 25% protein diet had a significant effect (P<0.05). No statistical difference in shrimp survival was observed (P>0.05) between the treatments (Table 5.3).

3.3. Stocking density 12 m^{-2}

Temperature, pH, dissolved oxygen, salinity, secchi disk reading and alkalinity did not vary between the treatments (Table 5.1 & 5.2). No significant difference was found between these parameters (P>0.05).

The mean values with standard deviation of water TAN, nitrite-N, nitrate-N, THB (Fig. 5.1) and chlorophyll-a are presented in Table 5.2 and the values showed significant difference ($P<0.05$). Addition of carbohydrate may increase the level of water and soil THB population ($P<0.05$) (Fig. 5.1 & 5.2). The mean values with standard deviation of sediment TAN, nitrite-N, nitrate-N, organic carbon, bulk density and THB are summarized in Table 5.2. Significant variation was found in the sediment TAN, and nitrate-N ($P<0.05$). However, no significant difference ($P>0.05$) was observed in sediment nitrite-N and organic carbon. Conversely, sediment THB showed significant difference ($P<0.05$).

The individual shrimp average weight showed a significant difference ($P<0.05$). SGR and FCR were higher in carbohydrate added tank when compared to tank without carbohydrate addition ($P<0.05$). No significant difference ($P>0.05$) was observed in the survival rate (Table 5.3).

3.4. Comparison of different levels of stocking density without addition of carbohydrate

In treatment where no addition of carbohydrate was done, there was no significant difference ($P>0.05$) in respect of temperature, water pH, dissolved oxygen, salinity and secchi disk (Table 5.1).

However, water TAN, nitrite-N, nitrate-N, THB, (Fig. 5.1) chlorophyll-a and the sediment TAN, nitrate-N and THB (Fig. 5.2) were showed significant variation ($P<0.05$) (Table 5.2). Conversely, sediment pH, nitrite-N and organic carbon showed no significant difference ($P>0.05$) between different stocking density.

The net shrimp yield was found higher in stocking density 12 followed by 7 and 3. The individual shrimp average weight was highest in 3 followed by 7 and 12, the difference is statistically significant ($P<0.05$). The SGR and FCR were higher in 3 followed 7 and least at 12 which were also significantly different ($P<0.05$). Survival rate of shrimp did not vary among the treatments ($P>0.05$). Higher survival rate was recorded at 3 followed 7 and it was least at 12 (Table 5.3).

3.5. Comparison of different levels of stocking density in treatment having addition of carbohydrate

In the treatment of 3 + CH, 7 + CH, 12 + CH addition, no significant difference ($P>0.05$) was observed in respect of temperature, water pH, sediment pH, dissolved oxygen, salinity and secchi disk reading (Table 5.1 & 5.2). However, significant difference was observed ($P<0.05$) in water quality parameters such as TAN, nitrite-N, nitrate-N, THB, (Fig. 5.1) BOD, alkalinity and chlorophyll-a (Table 5.2).

Significant difference was also observed in respect of sediment TAN, nitrate-N and THB (Fig. 5.2). However, no significant difference ($P>0.05$) was found in nitrite-N and organic carbon.

The individual average shrimp weight at harvest was highest ($P<0.05$) in 3 + CH followed by 7 + CH while it was least in 12 + CH. The addition of carbohydrate to the water column had significant effect ($P<0.05$) on shrimp yield. The higher SGR was observed in the treatment with 3 + CH followed by 7 + CH and 12 + CH which was significantly different ($P<0.05$). FCR value was highest in 3 + CH followed by 7 + CH and was lowest in 12 + CH. Among the three, no significant difference ($P>0.05$) was observed in survival rate. The survival rate was very high in 3 + CH treatments while it was low in 12 + CH (Table 5.3).

4. Discussion

Controlling the inorganic nitrogen by manipulating the carbon / nitrogen ratio is one of the potential control methods suitable for aquaculture systems. The daily water quality parameters temperature, water pH, dissolved oxygen salinity and secchi disk reading were with in the favorable limit during the culture period. Banerjea (1967) and Boyd (1974) reported that the above 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 6.6 which did not show any variation between treatments. The bottom soil pH is an important variable in pond aquaculture and values between 6.5 – 7.5 normally considered as acceptable (Jackson, 1958; McLean, 1982; Boyd and Tucker, 1992; Hendershot et al., 1993 and Bloom, 1999). It has also reported that a significant reduction in TAN in both sediment and water column was recorded in treatments with carbohydrate addition (Avnimelech, 1999; Avnimelech et al., 1989; 1994). In the present study, TAN concentrations of water and sediment showed a cumulative increase in control tanks with stocking density of 3, 7, 12 when compared with treatments 3 + CH, 7 + CH and 12 + CH. The low TAN concentration seen in carbohydrate added treatments irrespective of stocking density is indicative of the effect of carbohydrate addition in reducing the concentration of inorganic nitrogen. This approach seems to be a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in the pond. Avnimelech and Mokady (1988) reported that the addition of carbohydrate is an effective method to reduce concentrations of inorganic nitrogen in intensive aquaculture practices and the results of the present study strongly corroborate with this findings. The results of present study revealed there was an increase in the level of toxic inorganic nitrogen commensurate with the increase of stocking density. Ghosh and Mohanty (1981), Brady (1990), Phillips et al. (1993), Ayub et al. (1993) and Csavas (1994) reported that the toxic

inorganic nitrogen concentration in culture systems increases by the rate of feeding, unconsumed feed and the rate of excretion and the present findings show full agreement with the above.

In the present study, the significant increase was noticed in total heterotrophic bacterial (THB) count in both water and soil of all carbohydrate added treatments and it can be attributed to the addition of carbohydrate. Aikyama et al. (1989) reported that carbohydrate source (glucose and starch) application to the culture system provided better environment for multiplying bacterial population. The reduction of TAN and corresponding increase of THB observed in the present study can be attributed to the utilization of TAN from water and sediment as reported by Avnimelech and Mokady (1988), Avnimelech et al. (1989, 1994) and Avnimelech (1999). The heterotrophic bacteria may utilize TAN, DO (dissolved oxygen) and convert them to microbial protein. Microorganisms present in shrimp culture systems can take up significant amounts of NH_4^+, and consumed oxygen from the water column (Pomeroy et al., 1965). It may also be noted that the significant increase of THB might have resulted in significant decrease of BOD in carbohydrate added treatment. Moriarty (1996, 1997 and 1998) recommended that augmented microbial population can increase BOD consumption. According to the available reports (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993 and Avnimelech, 1999) the

heterotrophic bacteria produced single cell protein might be utilized as a food source by carp and tilapia whereby lowering the demand for supplemental feed protein In the present study, higher shrimp yield, FCR and FCE were recorded in the carbohydrate added treatments when compared to the control. Utilization of microbial protein depends on the ability of the target animal to harvest (Avnimelech, 1999). Several studies on the diet of *Penaeus* species in estuaries and coastal waters indicated that shrimps feed mostly on small phyto-zoobenthos (Tiews et al., 1976; Marte, 1980; 1982; Smith et al., 1992). It has also reported that *Penaeus monodon* is able to select microbial food (Apud et al., 1981 and El-Hag, 1984).

The survival rate of shrimp was similar among treatments, which showed that carbohydrate addition to ponds does not have any significant effect in survival. According to Chen and Kou, (1992) the similar survival rate encountered in the present study is indication of providing optimal condition for cultured organism. It would appear that addition of carbohydrate to the pond did not make the habitat unhospitable to *Penaeus monodon* as the water quality parameters were well with in favorable limits (Chen et al., 1990; Hariati et al., 1996). However, the survival rate was lower in 12 and 12 + CH when compared to other treatments and this can be attributed to the effect of high stocking density. High mortality of 40 - 50% has been reported in dense culture systems (8 kg m^{-2}) (Avnimelech and Lacher, 1979;

Boyd, 1985 and Muthuwani and Lin, 1996) due to the ammonium excreted by the fish or shrimp in the pond.

In the present study, net shrimp yield was significantly high in carbohydrate added treatments. Furthermore, the individual shrimp weight, SGR, FCR, FCE, PER, and average daily weight attained also showed higher values in carbohydrate added treatments. The studies conducted with dietary feed containing constant protein level revealed that the presence of microbial protein may influence the PER and FCE in *Penaeus monodon* (Abdel-Rahman et al., 1979; Hajra et al., 1988 and Teshima and Kanazawa, 1986). The higher growth rate and shrimp yield observed in carbohydrate added treatment can be attributed to the low inorganic nitrogen levels and better-feed utilization as reported by Wahab et al. (2003). Similarly lower TAN in sediment might have positively influenced the food intake and health of the shrimps as opined by Avnimelech and Ritvo (2003). Maximum individual shrimp weight observed in treatment 3 + CH might be due to low stocking density. According to Biddle et al. (1977) and Bautista (1986), low stocking density in the culture may facilitate maximum area for foraging and therefore, individual shrimp growth will be relatively higher.

In conclusion, the manipulation of C / N ratio by carbohydrate addition is possible in stocking densities up to 12 m^{-2} and the possibility of application of this technology in higher stocking

densities need to be investigated. Based on the results of the present study, it can be recommended that C / N ratio can be manipulated in the semi intensive farming system of *Penaeus monodon* for the economic and ecological sustainability.

Table 5.1

Daily water quality parameters in the varying stocking density tanks stocked with *Penaeus monodon*

Variable	Treatments (mean ± SD)					
	3	**3 + CH**	**7**	**7 + CH**	**12**	**12 + CH**
Temperature (^{o}C)	30.6 ± 0.4^{a}	30.2 ± 0.3^{a}	29.6 ± 0.8^{a}	29.6 ± 0.5^{a}	30.1 ± 0.9^{a}	29.6 ± 0.5^{a}
Water P^{H}	8.3 ± 0.03^{a}	8.3 ± 0.03^{a}	8.3 ± 0.03^{a}	8.3 ± 0.01^{a}	8.3 ± 0.03^{a}	8.3 ± 0.03^{a}
DO (mg l^{-1})	5.8 ± 0.2^{a}	5.8 ± 0.4^{a}	5.7 ± 0.4^{a}	5.8 ± 0.4^{a}	5.7 ± 0.3^{a}	5.8 ± 0.6^{a}
Salinity (ppt)	24 ± 0.6^{a}	24 ± 0.6^{a}	24 ± 0.6^{a}	24 ± 0.5^{a}	24 ± 0.7^{a}	24 ± 0.6^{a}
Secchi disk reading (cm)	58.4 ± 24.7^{a}	62.4 ± 23.4^{a}	63.9 ± 23.6^{a}	58.4 ± 21.9^{a}	61.6 ± 22.1^{a}	59.8 ± 21.9^{a}

Results from ANOVA Two-factor without replication

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

Table 5.2

Effect of carbohydrate addition on the water and sediment quality in varying stocking density trial with *Penaeus monodon*

	Treatments (mean ± SD)					
	3	**3 + CH**	**7**	**7 + CH**	**12**	**12 + CH**
Water quality variable						
BOD (mg l^{-1})	3.6 ± 0.2^{a}	3.3 ± 0.2^{b}	3.6 ± 0.1^{a}	3.3 ± 0.3^{b}	3.4 ± 0.2^{c}	3.3 ± 0.3^{b}
Alkalinity (mg $CaC0_3$ l^{-1})	56.5 ± 5.8^{bc}	49.2 ± 6.3^{c}	68.0 ± 4.3^{a}	57.4 ± 7.2^{c}	70.8 ± 2.0^{a}	64.3 ± 11.6^{ab}
TAN (ug l^{-1})	3.7 ± 2.4^{b}	2.6 ± 1.5^{c}	4.0 ± 2.5^{a}	3.4 ± 2.0^{d}	4.2 ± 2.6^{a}	3.6 ± 2.1^{bd}
Nitrite-N (ug l^{-1})	3.97 ± 1.5^{c}	4.05 ± 1.6^{ab}	4.01 ± 1.6^{bc}	4.08 ± 1.6^{a}	4.08 ± 1.6^{a}	4.16 ± 1.6^{a}
Nitrate-N (ug l^{-1})	1.15 ± 0.39^{a}	1.08 ± 0.35^{d}	1.30 ± 0.47^{b}	1.16 ± 0.39^{a}	1.36 ± 0.46^{c}	1.29 ± 0.44^{b}
THB (10^{5}cfu ml^{-1})	49.2 ± 4.1^{c}	60.4 ± 11.1^{a}	57.9 ± 5.8^{a}	66.6 ± 12.4^{b}	62.3 ± 9.3^{ab}	75.8 ± 16.9^{d}
Chlorophyll-a (ug l^{-1})	27.5 ± 6.1^{ab}	23.6 ± 2.0^{b}	27.1 ± 3.8^{ab}	35.2 ± 4.7^{c}	27.0 ± 3.3^{ab}	29.4 ± 3.9^{a}
Sediment quality variable						
Soil P^{H}	6.6 ± 0.2^{a}	6.6 ± 0.2^{a}	6.6 ± 0.3^{a}	6.6 ± 0.3^{a}	6.6 ± 0.3^{a}	6.6 ± 0.2^{a}
TAN (ug l^{-1})	20.3 ± 15.4^{b}	13.2 ± 8.8^{c}	23.1 ± 17.0^{ab}	12.4 ± 8.7^{bc}	24.2 ± 17.7^{a}	15.0 ± 9.5^{c}
Nitrite-N (ug l^{-1})	0.048 ± 0.0^{a}	0.046 ± 0.0^{a}	0.047 ± 0.0^{a}	0.047 ± 0.0^{a}	0.051 ± 0.0^{a}	0.051 ± 0.0^{a}
Nitrate-N (ug l^{-1})	2.5 ± 0.5^{ad}	2.2 ± 0.6^{bd}	2.5 ± 0.5^{ac}	2.0 ± 0.4^{b}	2.4 ± 0.5^{cd}	2.2 ± 0.4^{d}
Organic carbon (ug l^{-1})	12.74 ± 2.0^{a}	12.78 ± 2.8^{a}	12.34 ± 1.6^{a}	13.39 ± 3.3^{a}	13.50 ± 2.3^{a}	14.1 ± 3.5^{a}
THB ($10'$cfu ml^{-1})	80.0 ± 9.0^{a}	94.4 ± 24.0^{ab}	85.9 ± 16.4^{ac}	95.7 ± 23.0^{b}	88.1 ± 19.9^{bc}	100.5 ± 26.0^{a}

Results from ANOVA Two-factor without replication

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

Table 5.3

Effect of carbohydrate addition in varying levels of stocking density on weight, shrimp yield, SGR, FCR, and survival of *Penaeus monodon*

Variable	Treatments (Mean ± SD)					
	3	**3 + CH**	**7**	**7 + CH**	**12**	**12 + CH**
Individual shrimp weight gain (g)	26.7 ± 1.9^{c}	30.8 ± 1.5^{a}	25.4 ± 1.5^{d}	28.1 ± 1.5^{b}	19.6 ± 2.1^{f}	22.1 ± 1.0^{e}
Net shrimp yield (g m^{-2})	75.0 ± 4.7^{d}	87.5 ± 4.4^{d}	166.6 ± 9.8^{c}	186.3 ± 9.8^{c}	225.0 ± 9.0^{b}	257.1 ± 8.7^{a}
Specific growth rate (SGR)	7.4 ± 0.003^{c}	7.6 ± 0.003^{a}	7.4 ± 0.001^{d}	7.5 ± 0.005^{b}	7.1 ± 0.002^{f}	7.2 ± 0.000^{e}
Feed conversion ratio (FCR)	1.2 ± 0.09^{c}	1.09 ± 0.04^{d}	1.6 ± 0.3^{b}	1.2 ± 0.09^{cd}	2.1 ± 0.03^{a}	1.9 ± 0.07^{a}
Protein efficiency ratio (PER)	1.8 ± 0.14^{o}	2.2 ± 0.08^{a}	1.5 ± 0.31^{c}	1.9 ± 0.13^{o}	1.1 ± 0.01^{c}	1.2 ± 0.04^{ac}
Feed conversion efficiency (%)	77.2 ± 5.8^{b}	91.2 ± 3.6^{a}	63.1 ± 12.8^{c}	78.9 ± 5.6^{b}	46.4 ± 0.7^{d}	50.9 ± 1.8^{d}
Average daily weight gain (ADG)	0.26 ± 0.0008^{c}	$0.30 \pm .0.0011^{a}$	0.25 ± 0.0002^{d}	0.28 ± 0.0003^{b}	0.19 ± 0.0005^{f}	0.22 ± 0.0001^{e}
Survival rate (%)	72.2 ± 5.5^{a}	74.0 ± 3.2^{a}	61.9 ± 12.5^{a}	69.8 ± 4.9^{a}	59.2 ± 0.8^{a}	57.4 ± 2.1^{a}

Results from Tukey One-way ANOVA

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

Fig. 5.1

The effect of carbohydrate addition and varying stocking density on the water quality parameters

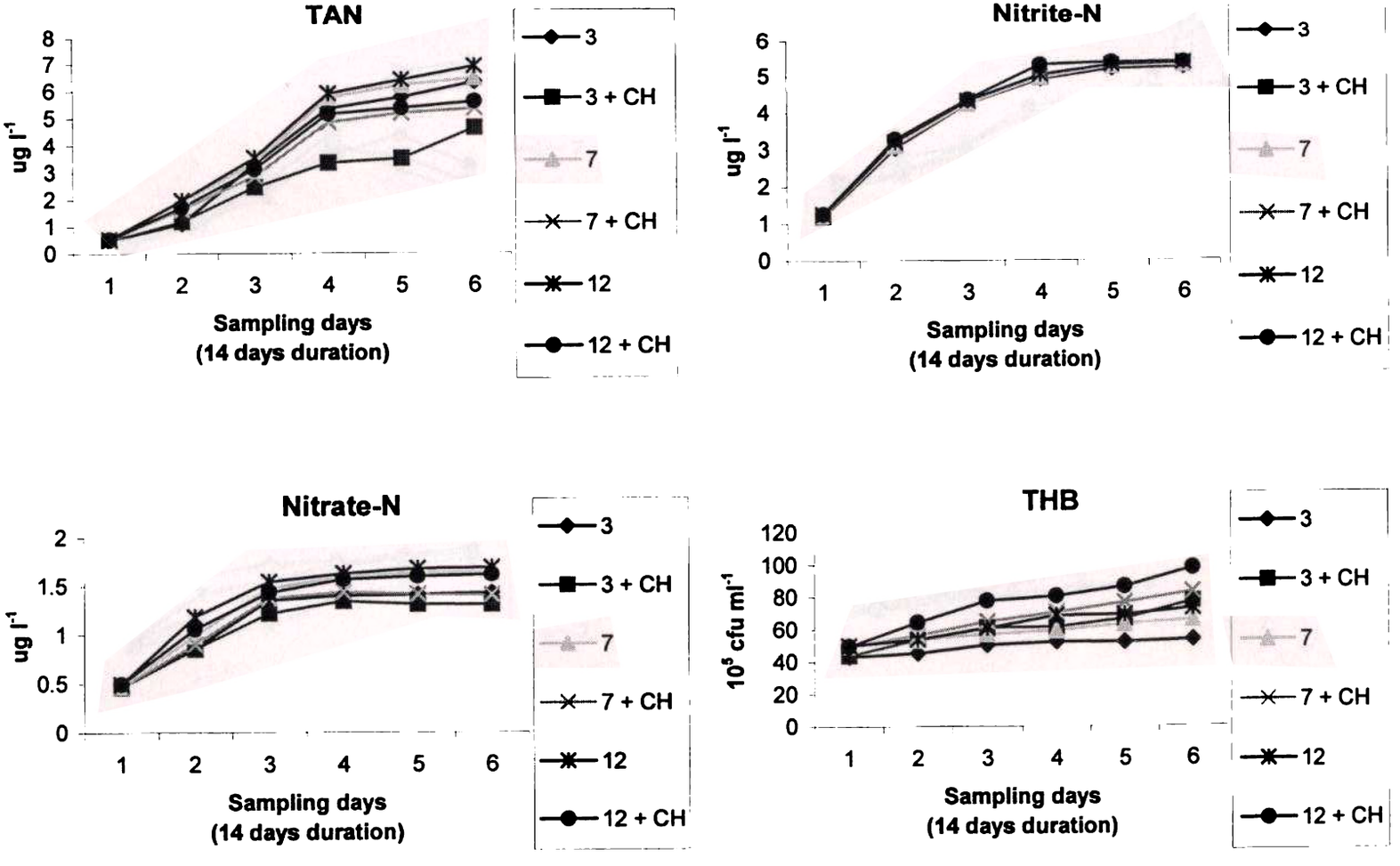
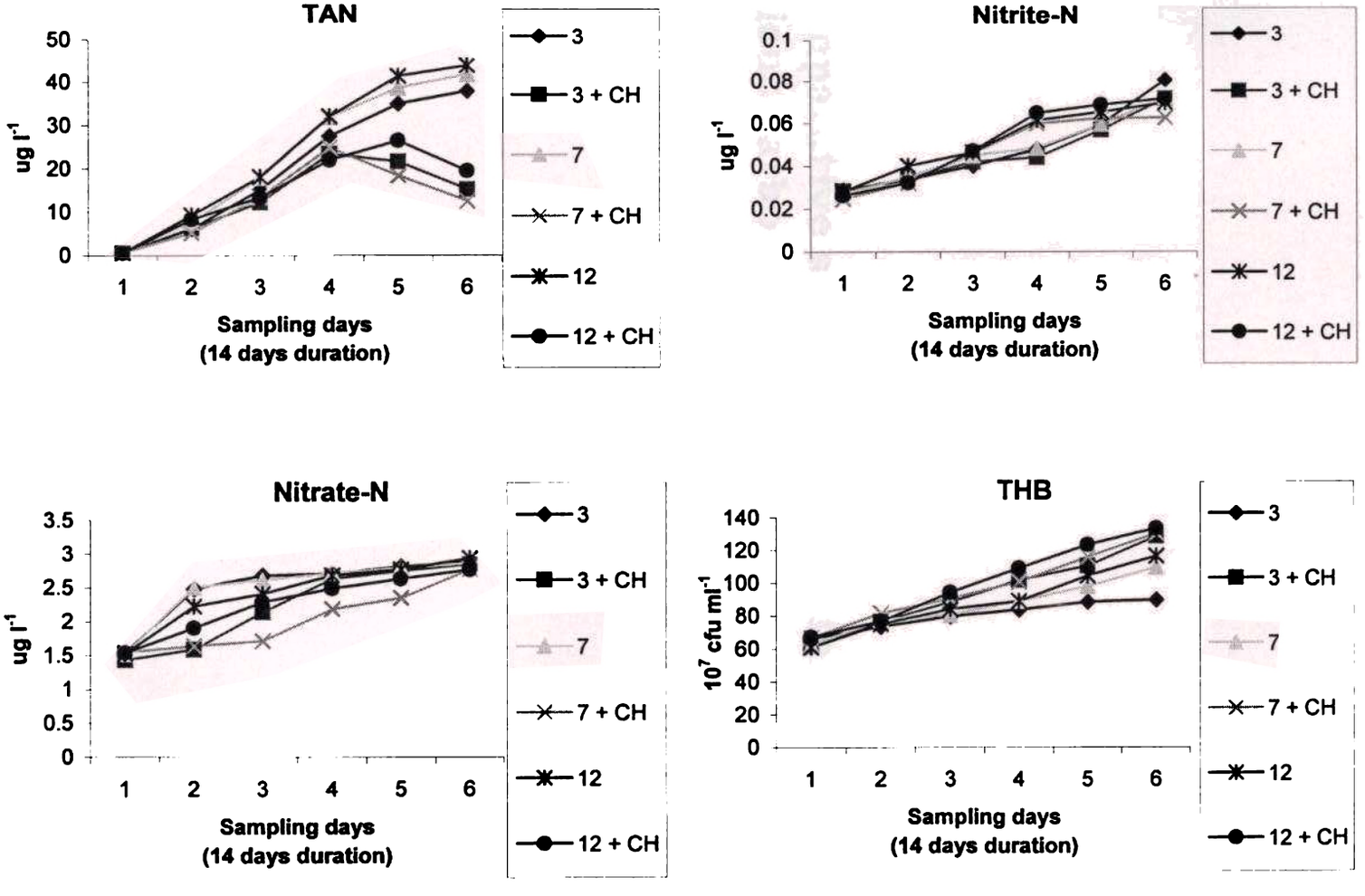


Fig. 5.2

The effect of carbohydrate addition and varying stocking density on the sediment qauality parameters



Chapter – 6

Comparative study on the performance of various carbohydrate sources in the control of inorganic nitrogen concentration in the grow-out of *Penaeus monodon* (Fabricius)

1. Introduction

The tiger prawn, *Penaeus monodon* offers a higher potential as one of the prime candidature species for large-scale commercial culture in India. Shrimp farming in ponds is one of the major aquaculture activities in the tropical and subtropical countries contributing 26.1% of the global shrimp production (Tacon, 2003). Low inputs of feed, fertilizers and low stocking densities in extensive and modified extensive types of culture contribute to the bulk of global shrimp production (90%) (FAO, 2001). Shrimp (*Penaeus monodon*) production in Taiwan was declined since 1988 due to pathogenic and non-pathogenic factors discharged into open waters (Liao and Chen, 1996). Late in 1994, massive shrimp mortality associated with diseases were increasingly reported from southern Thailand, India and Malaysia (Wongteerasupaya et al., 1996). The pathogenic organisms found in shrimp ponds were discharged with the wastewater, flowed to the sea, were transported to other areas and then pumped into other shrimp farms. In order to reduce the transformation of disease effects to reduce pond discharge as little as possible, have been undertaken during the culture period (Bashour and Al-Jaloud, 1984; Wang, 1990). However, absence of water circulation in culture pond may cause acute water and soil acute toxicity to cultured organisms causing severe stress and leading low survival rate (Twilley, 1989; Millamena, 1990; Hopkines et al., 1994).

Further more, the waste generated during culture, mainly feces and unconsumed feed, settle on the bottom. Mineralization of accumulated organic matter under anaerobic conditions leads to the formation of toxic metabolites like ammonia-N and nitrate-N, spoiling the living environment of the shrimp. Aquatic animals including shrimp excrete nitrogen waste in the form of ammonium, which may accumulate in the pond. The discharge of nutrient rich effluents from culture ponds to coastal waters is becoming a major environmental concern with the expansion of shrimp culture operation in many parts of world (van Dam, 1990; Fast and Boyd, 1992; Hopkins et. al., 1994; Avnimelech and Ritvo, 2003).

Protein is the main expensive component in diet and in artificial feeds when shrimps are cultured intensively. Dietary protein has been reported as the most essential nutrient for the growth of shrimps (Andrews et. al., 1972; Balazs et. al., 1973; Forster and Beard, 1973; Venkataramiah et. al., 1975; Alava and Lim, 1983). High protein diet is mainly used for the faster growth rate and higher survival in shrimp culture. However, optimal protein requirement level can be reduced by the addition of non-protein source such as cheap carbohydrate sources. 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. Shrimp growth depends on the nutritional quality of dietary protein. The protein content of feed mainly depends expensive ingredients like fish meal, shrimp meal etc. At the same time, carbohydrate ingredients are the last expensive dietary energy sources. The effectiveness of various kinds of carbohydrate sources has been studied in different penaeid species since 1969. The application of carbohydrate source can reduce the amount of protein utilization and following this principle Shiau and Peng (1992) had evaluated the effectiveness of glucose, dextrin and starch as carbohydrate sources in the farming of *Penaeus monodon*. McGoogan and Galtin, 1998; Rudacille and Kohler, 1998; Conquest et al., 1998 reported that the adjustment of C / N ratio in the feed can effectively control the pond water quality.

The present study was undertaken with a view to evaluate effectiveness of five locally available carbohydrate sources such as Potato flour (P), Yam flour (Y), Rice flour (R), Wheat flour (W) and Tapioca flour (T) in controlling the inorganic nitrogen production by adjusting C/N ratio.

2. Materials and Methods

Study area

The experiment was carried out in 15 reinforced rectangular reinforced concrete tanks concrete tanks ,each having 6 m^3 having an

effective bottom area of 6 m^2, with a depth 1 m. The experiment tank was provided with a uniform sediment layer (7 cm thick) collected from the extensive shrimp culture pond of Cochin. All tanks were completely independent and fully exposed to natural sunlight.

Experimental design

Five various carbohydrate sources were selected for the evaluation of their performance in controlling the inorganic nitrogen production by adjusting C/N ratio. The tank allocation for each treatment was done completely randomized and triplicate tanks were maintained for each treatment. Commercial shrimp feed containing 25% crude protein (Higashimaru Feeds India Limited, Kuthyathodu, S.India) was used for the experiment. Carbohydrate source were purchased from local market. Potato flour (P), Yam flour (Y), Rice flour (R), Wheat flour (W) and Tapioca flour (T) were sieved through 35 µ and used as carbohydrate source. The quantity of carbohydrate added was calculated following the theory of Avnimelech (1999); i.e. 390 g of each carbohydrate source for each kg of 25% dietary protein, respectively. Shrimps were fed with experimental feed at 15% of initial weight and adjusted gradually to 3% body weight at the end of the culture. Feed was distributed evenly over the tank's surface, twice daily at 8.00 and 18.00 hours. Pre weighed carbohydrate source was mixed with tank water and applied to the water column uniformly

followed by the feeding in the morning. Shrimps were harvested after draining the tanks on 105th day of culture, their individual length, weight and survival were recorded.

Tank preparation and stocking

Before beginning the experiment, tanks were drained and cleaned. Lime was added initially at 3 kg tank^{-1} and cattle dung @ 5 kg tank^{-1}. Culture tanks were filled with 24 ppt saline water drawn from the Cochin estuary, stored in concrete storage tank and kept for one week for settlement. The tanks were fertilized with cattle dung @ 5, 2, 3, and 2 kg tank^{-1} at 2nd, 3rd, 5th, and 7th week respectively. Urea and super phosphate were also added @ 4 and 1 gm week^{-1} for first six weeks.

Twenty days old post larvae (PL 20) (0.015 ± 0.01 g m^{-2}) of *Penaeus monodon* purchased from a commercial hatchery were stocked in the tank at a density of 7 juveniles m^{-2}.

Water and sediment quality monitoring

Water quality parameters such as temperature (mercury thermometer), salinity (hand refractometer), pH (pH pen) and secchi disk (transparency) were measured directly from the tank on a daily basis and dissolved oxygen following Winkler method (APHA, 1995) *in situ* at 09.00 AM. 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 (2 cm diameter). Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. The water sample was filtered through GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrate-N (cadmium reduction), nitrite-N and TAN (total ammonia nitrogen) (Phenol hypochloriate method) (Grasshoff et al., 1983). Biological oxygen demand (BOD) (5 days) 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 measured following Mudroch et al., 1996) Monthly chlorophyll-a in non-filtered water column samples was analyzed following standard methods (APHA, 1995). Total heterotrophic bacteria (THB) count in the water and sediment were also estimated by the standard procedures (APHA, 1995) and expressed as colony forming unit (cfu).

Harvesting

At the end of the experiment tanks were drained and shrimps were collected. Total length of the shrimp 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.

Data analysis

Statistical analysis of daily, biweekly and monthly water and sediment quality parameters were analyzed using Two-Factor ANOVA without replication was performed using Microsoft Excel 2000. For individual growth rate, net shrimp yield, SGR, FCR, FCE, PER, ADG, Survival rate One-Way ANOVA Tukey HSD software using SPSS 11.5. Significant treatment effects were separated by calculating the least significant difference at 5% level.

3. Results

Water quality parameters

The water quality parameters such as temperature, water pH, dissolved oxygen, salinity, alkalinity of the different treatments were in the range 28.29 – 28.52 °C, 8.26 – 8.39, 5.30 – 5.52 mg l^{-1}, 24.79 – 24.88 ppt and 55.22 – 58.55 mg $CaCo_3$ l^{-1} respectively. There was no significant differences ($P>0.05$) in any of the above water quality parameters among the different treatments (Table 6.1 and 6.2). The secchi disk readings ranged from 57.79 – 60.33 cm (Table 6.1). Potato, yam and rice flour added treatments showed significant difference ($P<0.05$) in secchi disk reading when compared to wheat and tapioca flour added treatments ($P<0.05$).

Among the various treatments, potato flour and yam flour added treatments showed significant difference ($P<0.05$) from that of rice flour, wheat flour and tapioca flour carbohydrate added treatment in respect of BOD. The BOD values ranged from 2.98 – 4.35 mg l^{-1} (Table 6.2). The nitrite-N ranged from 0.97 – 1.29 µg l^{-1}, and while comparing the variation among the treatments, potato added treatment showed significantly difference ($P<0.05$) from yam, rice and tapioca flour added while and there was no difference between potato and wheat ($P>0.05$) . In the case of Nitrate-N, it ranged from 2.97 – 3.22 µg l^{-1} and among various treatments Potato showed significant difference ($P<0.05$) from that of rice and wheat. THB values ranged from 69.04 – 86.21 x 10^5cfu ml^{-1} and the rice and tapioca treatments showed significant difference from other treatments. Chlorophyll-a values ranged from 27.83 – 38.76 µg l^{-1}, and among the treatments, the yam, rice and wheat flour added treatments showed significant difference ($P<0.05$) from tapioca. The results of the water quality parameters recorded from various treatments at biweekly intervals are shown in Table 6.2.

The temporal variation of water quality parameters depicted in Fig. 6.1. The TAN concentration over the sampling periods peaked at 56th day (Period 5) and the highest concentration recorded was 14.39 µg l^{-1} (W). The results of the two-way ANOVA showed that, besides carbohydrate addition, the dietary protein level had a significant effect

in the inorganic nitrogen level ($P<0.05$) in water. The highest nitrite-N value observed during the culture period 2.72 µg l^{-1} (W) is on 28th day (Period 3). Where as the same in respect of nitrate-N concentration was registered on 42nd day 4.87 µg l^{-1} (W) (Period 4). The THB counts showed a gradual increase in all treatments and highest THB count was recorded in treatment rice (145 x 10^5 cfu ml^{-1}).

Sediment quality parameters

The results of the soil analysis of different treatments (Table 6.2) revealed that addition of different carbohydrate sources did not have any significant effect ($P>0.05$) in soil pH during the culture period and this parameter showed a variation from 6.57 – 6.63. The rice flour and tapioca flour added treatment showed significantly lower ($P<0.05$) TAN concentration when compared to potato and yam. The TAN was in the range from 14.71 – 17.18 µg l^{-1} in various treatments. The nitrite-N varied from 0.41 – 0.59 µg l^{-1}, and among the treatments significant difference ($P<0.05$) was observed in yam, rice, wheat and tapioca when compared with potato. Nitrate-N was in the range 1.84 – 2.11 µg l^{-1} potato, wheat and tapioca showed significant variation from yam. Notably, organic carbon in Potato flour added treatment showed significant difference ($P<0.05$) from yam and rice. The yam, wheat and tapioca flour added treatments showed significantly different from rice. The organic carbon varied between

11.62 - 12.50 μg l^{-1} and the Potato, yam and rice flour added treatment showed significant difference ($P<0.05$) with wheat and tapioca. In the case of soil THB population, it ranged from 44.63 - 60.83 x 10^7cfu ml^{-1}. Tapioca showed significant variation ($P<0.05$) with potato, yam and rice flour.

In the case of soil TAN (Fig. 6.2) higher concentration ($P<0.05$) was observed on 56th day (Period 5) with 28.82 μg l^{-1} in treatment T. Thenceforth the soil TAN concentrations showed lowering and at the end of the culture, it was plummeted to 9.46 μg l^{-1} in treatment W. In nitrite-N the highest value was observed on 28th day (Period 3) with 0.97 μg l^{-1} in treatment T and thereafter a significant reduction was noticed ($P<0.05$). The nitrate-N values varied from 1.35 - 1.86 μg l^{-1} in the treatments, the highest nitrate-N value was observed at the 42nd day (Period 4) of culture. Thereafter (Period 4) a significant reduction was observed in nitrate-N concentration. The THB also showed gradual increase during the progression of culture period, which ranged from 8.07 - 124.00 x 10^7cfu ml^{-1}.

Shrimp harvest production details

The One-Way ANOVA results and mean values of individual shrimp weight, net shrimp yield, SGR, FCR, PER, FCE, ADG and survival rate are presented in Table 6.3. Among the different variables there is no significant variations ($P>0.05$) could be observed among

various treatments. Individual shrimp weight gain values were in the range of 25.70 – 26.95 g. The net shrimp yields of different treatments were in the range 141.37 – 147.01 g m^{-2} and the highest value was recorded in treatment P (P>0.05). The SGR values ranged from 6.75 – 6.76 while FCR ranged between 1.19 – 1.24. FCR of P (1.19), W (1.20) was lower (P>0.05) than T (1.24). The protein efficiency ratio varied from 3.18 – 3.30 while feed conversion efficiency ranged from 81.09 - 84.33% whereas average daily weight gain was in the range 0.214 – 0.216 g. Survival rate of the shrimp did not vary (78.57 – 80.95%) among the different treatments.

4. Discussion

In the present study, the efficiency of five cheap carbohydrate sources were compared in terms of controlling the inorganic nitrogen production by adjusting C/N ratio in shrimp culture. Shi-Yen and Chun-Yang (1992) compared three carbohydrate sources, viz. glucose, dextrin and corn starch in favor of substituting in the dietary protein. In the present study, there was no significant differences in the water and soil quality parameters such as in temperature, water pH, salinity, soil pH and dissolved oxygen. Water pH and soil pH are important variable in pond aquaculture, a neutral condition (pH 7) is ideal but values from 6.5 - 8.5 are normally considered acceptable (Banerjea, 1967; Boyd, 1974; Munsiri et al., 1995; Boyd, 1995).

Hariati et al. (1996) found that the dissolved oxygen concentrations between 3.0 – 5.0 mg l^{-1} were considered favorable for growth of *Penaeus monodon* without causing stress. In transparency the secchi disk readings showed significant difference among various treatments and it can be attributed to variation of nutrients during the culture and this finding is complementary to that of Hari et al. (2004), Smith et al. (2002) and Kuo-Feng Tseng et al. (1998).

All the carbohydrate sources applied to water column of various treatments were found effective in optimizing the carbon / nitrogen ratio which is manifested by the significant increase in the total heterotrophic counts and this finding fully concur with Burford et al. (2003). Furthermore, the high heterotrophic bacteria counts observed due to addition of carbohydrate in all treatments is found to be accomplished by a reduction of biological oxygen demand (BOD) in various treatments. Bratvold and Browdy (1998 and 2001) reported that total bacterial counts and oxygen consumption rate were comparable in zero water exchange shrimp ponds. High concentration of two nitrogen species Ammonia-N and nitrite-N are toxic to shrimp (Chien, 1992).

In the present study, TAN concentration levels in the water column ranged from 0.43 – 14.00 μg l^{-1} and this much lower when compared to Al-Zeid et al. (1988) who reported a concentration of 750 - 3250 μg l^{-1} TAN in the commercial shrimp farms. It appears that the

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addition of all types of carbohydrate was useful in the control of TAN. As shrimp are carnivorous omnivorous organisms and are mainly bottom living organism (New, 1987), the sediment inorganic nitrogen production also affect in the survival and growth of the organism. The sediment TAN showed a significant reduction after the 56th day of culture and the sediment nitrite-N and nitrate-N values were also showed similar reduction. Conversely, a significant increase of sediment THB could be observed during the culture period. 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. With the addition of different carbohydrate source, there was significant increase in organic carbon was also observed. The carbon content may deposited at the bottom after the utilization of carbohydrate source assuming that added carbohydrate contain 50% carbon (Avnimelech and Lacher, 1979; Boyd, 1985; Muthwani and Lin, 1996). The inorganic nitrogen accumulation in the culture system is by metabolism and nitrogen immobilizing microbial processes. Bacteria and other microorganisms use carbohydrate sources as food, to generate energy and grow i.e., to produce proteins and new cells (Avnimelech, 1999). The increase registered in THB population (17.33 - 133.27 x 10^5cfu ml^{-1}) in treatments applied with various carbohydrate sources in the present

study would indicate that carbohydrate is accompanied by the immobilization of inorganic nitrogen. It appears that all the carbohydrate sources can be utilized as a potential means to reduce the concentration of inorganic nitrogen from the culture system, which concurs with the findings of Avnimelech (1998), Avnimelech and Lacher (1979) and Avnimelech and Mokady (1988).

The water and sediment characteristics of five treatments where five different sources of carbohydrate were applied revealed that all of them are effective in bringing down the water and sediment inorganic nitrogen production due to their capability in enhancing the water and soil total heterotrophic bacterial population. However, nitrite-N value in water was slightly higher in potato (1.29 $\mu g\ l^{-1}$) when compared to other treatments, while it was lowest in tapioca and rice. Conversely, TAN and Nitrite-N in sediment showed higher values in rice and tapioca. Cotner et al. (2000) reported that glucose addition to water reduced TAN concentration from 17.1 - 7.4 $\mu g\ l^{-1}$ due to the enhancement of microbial growth. The results of the present study show that the various carbohydrate sources studied are equally effective for the control of carbon / nitrogen ratio.

The survival rates of shrimp were also similar among the treatments which indicate that all carbohydrate sources did not have any adverse effect in destroying the shrimp habitat. According to Ansuman Hajra et al., (1988) the high survival rates of shrimp are

mainly due to the favorable limit of environmental conditions for the organism. Removal of accumulated inorganic nitrogen from water and sediment increased shrimp yield from 1.0 to 6.2 ton ha^{-1} $year^{-1}$ and the survival from 10% to 60% (Lemonnier and Brizard, 2001). In the present study, the net shrimp yield and FCR were comparable in all treatments and it maybe inferred that the level of interaction between the low dietary protein (25%) 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 shrimp (Avnimelech et al., 1995; Avnimelech, 1999; Hari et al., 2004). 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 in controlling the inorganic nitrogen production in shrimp ponds by adjusting C/N ratio and reducing the level of protein in the shrimp feed.

Table 6.1
Daily water quality parameters of various carbohydrate added outdoor tanks stocked with *Penaeus monodon*

Variable	Treatments (mean ± SD)				
	P	**Y**	**R**	**W**	**T**
Temperature (^{o}C)	28.41 ± 1.31^{a}	28.49 ± 1.26^{a}	28.52 ± 1.27^{a}	28.29 ± 1.08^{a}	28.52 ± 1.24^{a}
Water P^{H}	8.27 ± 0.23^{a}	8.37 ± 0.26^{a}	8.28 ± 0.29^{a}	8.39 ± 0.23^{a}	8.26 ± 0.17^{a}
DO (mg l^{-1})	5.52 ± 0.32^{a}	5.52 ± 0.35^{a}	5.46 ± 0.41^{a}	5.30 ± 0.47^{a}	5.42 ± 0.43^{a}
Salinity (ppt)	24.88 ± 1.12^{a}	24.83 ± 1.09^{a}	24.79 ± 1.22^{a}	24.83 ± 1.09^{a}	24.88 ± 1.08^{a}
Secchi disk reading (cm)	60.17 ± 3.40^{a}	60.33 ± 3.37^{a}	59.88 ± 3.07^{a}	58.54 ± 2.90^{b}	57.79 ± 2.95^{b}

Results from ANOVA Two-factor without replication

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

Table 6.2

Effect of various carbohydrate addition on the water and sediment quality in outdoor

	Treatments (mean ± SD)				
	P	**Y**	**R**	**W**	**T**
Water quality variable					
BOD (mg l^{-1})	4.25 ± 0.32^{a}	4.35 ± 0.45^{a}	3.07 ± 1.18^{b}	2.98 ± 0.96^{b}	3.29 ± 1.05^{b}
Alkalinity (mg $CaC0_3$ l^{-1})	55.22 ± 17.94^{a}	55.99 ± 16.97^{a}	55.33 ± 13.72^{a}	56.19 ± 14.41^{a}	58.55 ± 14.57^{a}
TAN (ug l^{-1})	7.08 ± 4.29^{a}	6.96 ± 4.92^{a}	7.67 ± 4.85^{a}	7.67 ± 4.92^{a}	7.27 ± 4.67^{a}
Nitrite-N (ug l^{-1})	1.29 ± 0.80^{a}	1.14 ± 0.76^{b}	0.97 ± 0.63^{b}	1.22 ± 0.81^{ab}	1.03 ± 0.69^{b}
Nitrate-N (ug l^{-1})	2.97 ± 0.95^{a}	3.08 ± 1.00^{ao}	3.22 ± 0.99^{o}	3.18 ± 1.01^{o}	3.09 ± 0.85^{ao}
THB (10^{5}cfu ml^{-1})	72.29 ± 44.22^{b}	69.04 ± 36.88^{b}	86.21 ± 48.41^{a}	85.21 ± 45.26^{a}	73.33 ± 45.76^{b}
Chlorophyll -a (ug l^{-1})	34.33 ± 11.39^{a}	35.61 ± 18.24^{a}	38.76 ± 4.71^{a}	36.98 ± 6.34^{a}	27.83 ± 2.92^{b}
Sediment quality variable					
Soil pH	6.63 ± 0.15^{a}	6.59 ± 0.15^{a}	6.60 ± 0.15^{a}	6.57 ± 0.17^{a}	6.63 ± 0.12^{a}
TAN (ug l^{-1})	15.00 ± 6.52^{ab}	15.96 ± 7.42^{a}	17.18 ± 7.33^{c}	14.71 ± 6.31^{b}	16.69 ± 8.10^{d}
Nitrite-N (ug l^{-1})	0.41 ± 0.37^{a}	0.54 ± 0.30^{b}	0.59 ± 0.29^{b}	0.54 ± 0.30^{b}	0.55 ± 0.29^{b}
Nitrate-N (ug l^{-1})	2.11 ± 0.49^{a}	1.84 ± 0.39^{b}	1.96 ± 0.47^{ab}	2.08 ± 0.47^{a}	2.05 ± 0.42^{a}
Organic carbon (ug l^{-1})	12.50 ± 4.18^{a}	12.09 ± 4.19^{o}	11.62 ± 4.22^{c}	12.38 ± 4.96^{ao}	12.13 ± 4.20^{ao}
THB ($10'$cfu ml^{-1})	46.58 ± 35.59^{a}	44.63 ± 30.77^{a}	50.60 ± 36.54^{ao}	56.04 ± 43.97^{oc}	60.83 ± 39.39^{c}

Results from ANOVA Two-factor without replication

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

Table 6.3

Effect of various carbohydrate addition on weight, shrimp yield, SGR, FCR, and survival of *Penaeus monodon* in outdoor trial

Variable	Treatments (Mean ± SD)				
	P	**Y**	**R**	**W**	**T**
Individual shrimp weight gain (g)	25.95 ± 0.28[a]	25.84 ± 0.48[a]	25.82 ± 0.44[a]	26.95 ± 0.41[a]	25.70 ± 0.26[a]
Net shrimp yield (g m^{-2})	147.01 ± 7.86[a]	143.66 ± 11.51[a]	142.06 ± 6.13[a]	145.72 ± 13.08[a]	141.37 ± 7.81[a]
Specific growth rate (SGR)	6.76 ± 0.01[a]	6.75 ± 0.02[a]	6.75 ± 0.02[a]	6.76 ± 0.02[a]	6.75 ± 0.01[a]
Feed conversion ratio (FCR)	1.19 ± 0.06[a]	1.22 ± 0.10[a]	1.23 ± 0.05[a]	1.20 ± 0.11[a]	1.24 ± 0.07[a]
Protein efficiency ratio (PER)	3.30 ± 0.18[a]	3.23 ± 0.26[a]	3.20 ± 0.14[a]	3.28 ± 0.30[a]	3.18 ± 0.18[a]
Feed conversion efficiency (%)	84.33 ± 4.51[a]	82.40 ± 6.60[a]	81.49 ± 3.51[a]	83.58 ± 7.50[a]	81.09 ± 4.48[a]
Average daily weight gain (ADG)	0.216 ± 0.002[a]	0.215 ± 0.003[a]	0.215 ± 0.003[a]	0.216 ± 0.003[a]	0.214 ± 0.002[a]
Survival rate (%)	80.95 ± 4.76[a]	79.37 ± 4.96[a]	78.57 ± 2.38[a]	80.16 ± 5.99[a]	78.57 ± 4.12[a]

Results from Tukey One-way ANOVA

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

Fig. 6.1

The effect of various carbohydrate addition on the water quality parameters

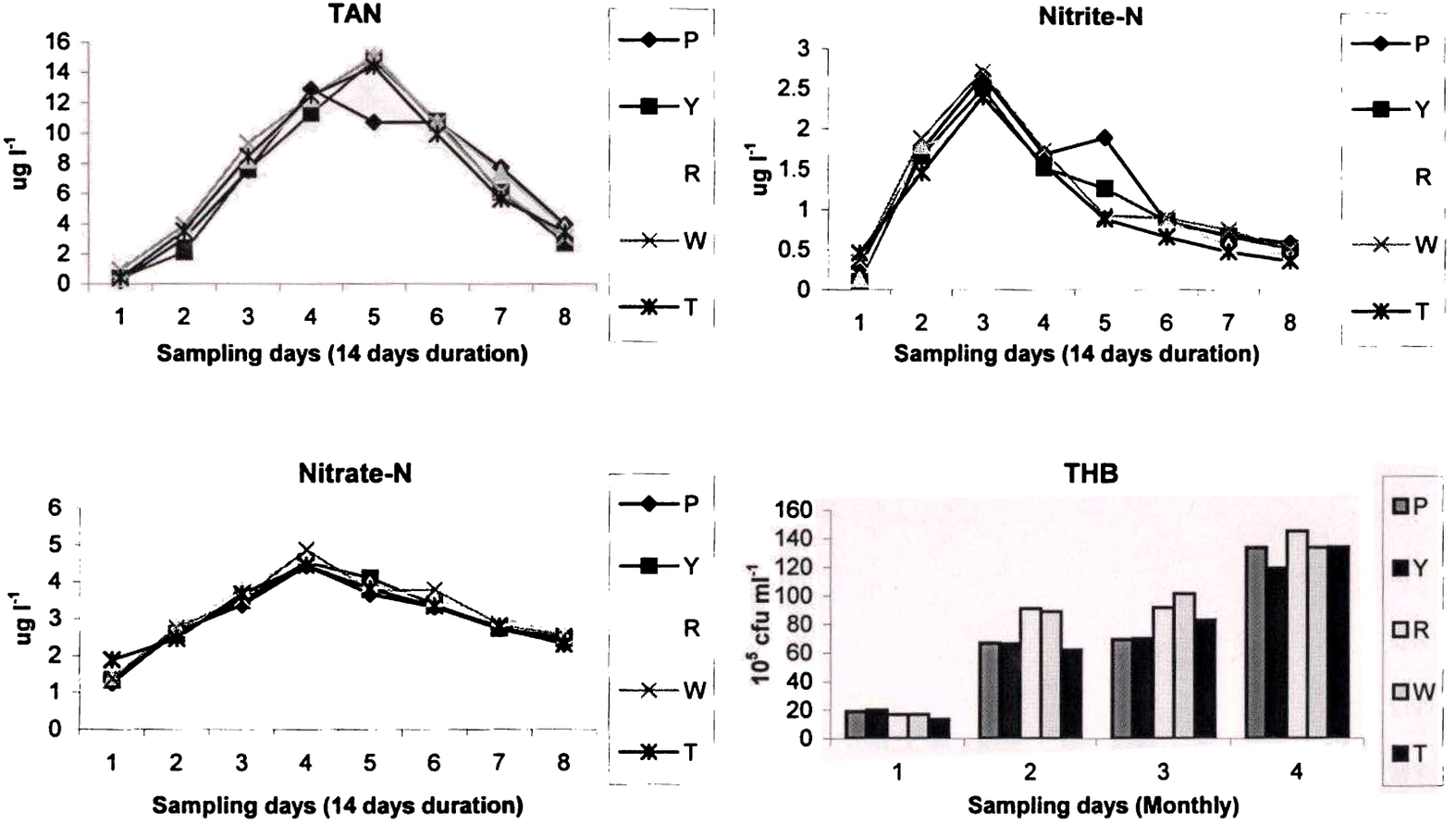
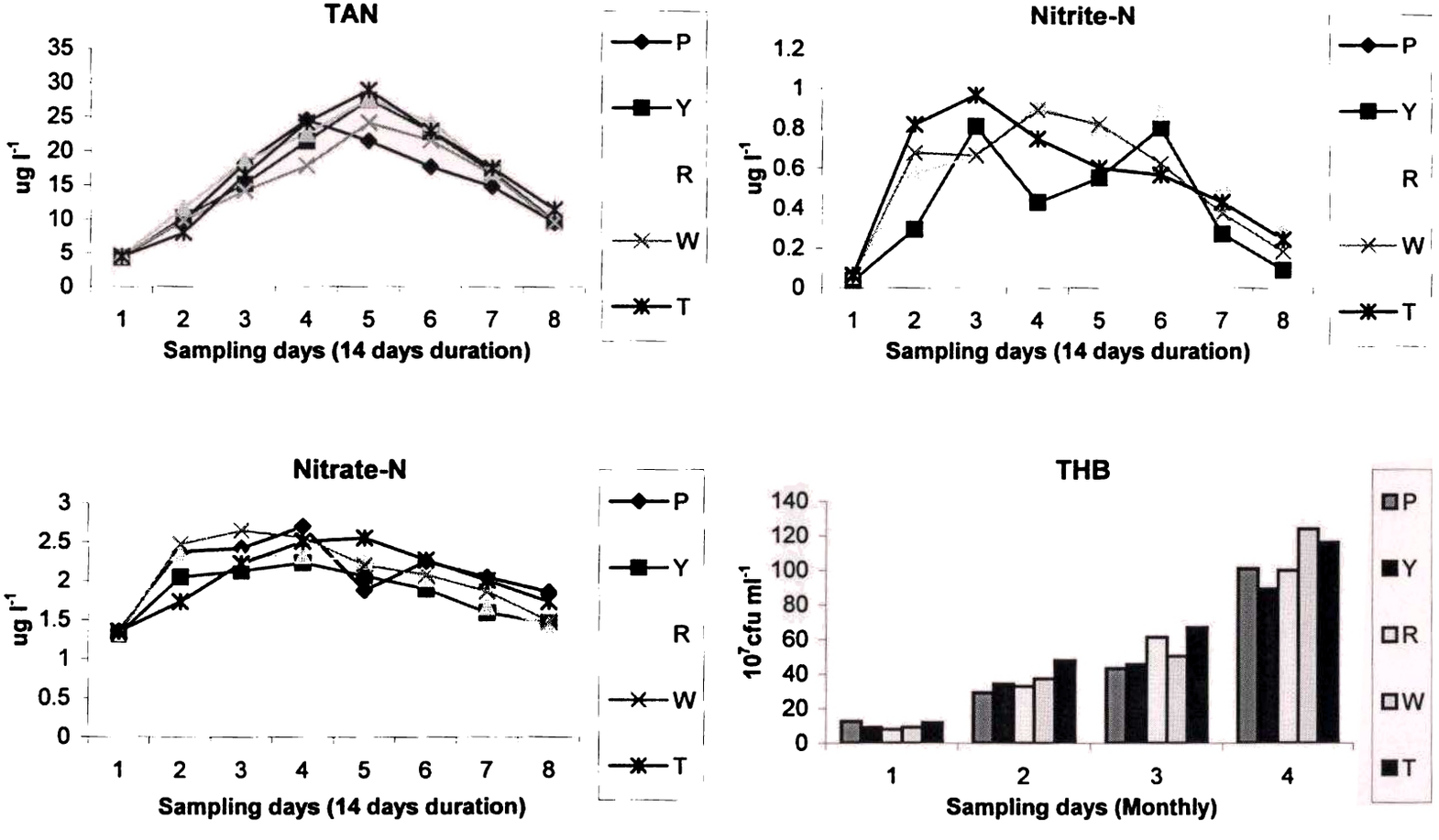


Fig. 6.2

The effect of various carbohydrate addition on the sediment qauality parameters



Chapter – 7

Periphyton production in two substrates at different levels of fertilization rate

1. Introduction

Demand for crustaceans and other aquatic organisms increases, while natural fisheries fastly approaching the optimal level of exploitation and therefore, aquaculture is the only solution for enhancement of production. The community of microscopic algae that grow attached to a variety of submerged substrata is an essential component of lotic ecosystems. This community, called periphyton, is responsible for most of the primary production (McIntire, 1973; Apesteguia and Marta, 1979) and constitutes the food source for several invertebrates (Cattaneo et al., 1993). It also plays a major role in the metabolic conversion and partial removal of biodegradable material in ponds and rivers (Lau and Liu, 1993). Microbial food webs are integral part for all aquaculture ponds and have a direct impact on productivity (Moriarty, 1997). The term periphyton refers to the microbial communities living on submerged surfaces, including bottom sediments, submerged plants and solid natural or man made underwater structure. The term 'periphyton' is applied to the complex of sessile biota attached to submerged substrata such as stones and sticks, and includes algae and invertebrates but also associated detritus and microorganisms. South Asia contributes to about 90% of the world's aquaculture production, the bulk of which is from ponds and rice fields (FAO, 2000). Growth in production is possible by increased reliance on external resources like fertilizers and feed.

Because of the higher cost of external inputs there is a diminishing interest among the farmers to continue with the farming practices (O'Riordan, 1992). Periphyton based aquaculture through the use of artificial substrates in the shrimp farms, can improve the efficiency of conversion of nutrients into harvestable products. The idea of periphyton based aquaculture is originally derived from traditional methods, such as the 'padal fishing' a unique fishing method in the Ashtamudi estuary of Kerala (South India). Locally available tree branches such as mango and mangroves are kept submerged in shallow open water which act as shrimp and fish aggregating devices. A large number of post larvae of shrimps and fish fingerlings find shelter beneath the padals, foraging the peri and epiphyton developed from the submerged twigs and other structures used to construct them (Thomas et al., 2004). Sustainable use of natural resources to enhance the production of low input aquaculture system can help to increase the income and food security of people in rural areas (NACA, 2000). Periphyton based aquaculture can be one of the essential means of increasing shrimp production. The feasibility of periphyton based systems has been explored in brackishwater fish ponds in West Africa (Welcomme, 1972; Hem and Avit, 1994; Konan-Brou and Guiral, 1994) and was found to enhance primary production and food availability and increase shrimp production. Trials have demonstrated that the aquaculture production with additional substrates for

periphyton production is higher than that from substrate free controls (Legendre et al., 1989; Konan et al., 1991; Hem and Avit, 1994; Guiral et al., 1995; NFEP, 1997; Wahab et al., 1999b; Azim et al., 2002). The external resources such as feed and fertilizers supplement or stimulate autochthonous food production in the grow-out system for shrimp growth. In most feed driven ponds, only less than 30% of nutrients inputs are converted into harvestable products, the remaining being lost to the sediment, effluent water and the atmosphere (Acostra-Nassar et al., 1994; Beveridge et al., 1994; Olah et al., 1994). Culture systems are also reliant on the environment at large to disperse and assimilate waste (Beveridge and Phillips, 1993).

The development of viable low cost technologies and their applications to current farming practices would help in enhancing aquaculture production. By providing suitable substrates, heterotrophic food production can be increased which will support the shrimp production. Substrates provide the site for epiphytic microbial production which can be eaten by shrimp. The shrimp harvested microorganisms directly in significant quantities, either from microbial biofilm on detritus or from naturally occurring flocks in water column (Schroeder, 1978). There is high variability in periphyton communities and abiotic factors like light, temperature, nutrient availability or type of substrate. The organisms colonizing substrates include microalgae, micro, meio and mesofauna, fungi and

bacteria. Most of them are small organisms with short life cycle of days or weeks, making the communities highly dynamic and responsive to environmental changes (Vermaat and Hootsmans, 1994). Experiments with artificial substrates have shown that periphyton can increase the production of pond harvestable product compared to systems without substrates (Pardue, 1973; Hem and Avit, 1994; Wahab et al., 1999a, b; Azim et al., 2002; Keshavanath and Wahab, 2001, Keshavanath et al., 2001a, b).

This chapter presents the results of the experiments conducted to evaluate the effectiveness of two locally available artificial substrates such as Bamboo and Kanchi in the production of periphyton under different types of fertilization application in varied levels. The objectives of this study are shown below:

1. To assess whether the addition of Bamboo and Kanchi as artificial substrates in the grow-outs make any changes in the optimal water and soil quality requirements of the shrimp.
2. To find out the optimum dosage of fertilizer consortium applied in the grow-out for maximum production periphyton.
3. To find out the variation in the suspended and attached algal growth in different substrata used for the study.

2. Materials and methods

Experimental design

The experiment was carried out in concrete tanks having an effective bottom area of $6m^2$. The experimental tanks were filled with a uniform sediment layer (7 cm thick) collected from the pokkali shrimp farm. Lime was added initially at 3 kg $tank^{-1}$. Bamboo and kanchi (*Bambusa sp.*) were used as substrate for periphyton growth plus while the treatment without substrate was used as control. Experiments were maintained in triplicate following complete randomized design. Cattle dung, urea and super phosphate were used as fertilizers in this study. In treatment 1C cattle dung, urea and super phosphate were applied at the rate of 1500,100 and 50 kg ha^{-1} respectively with out substrate. Whereas the above fertilizers with the given dosages with Bamboo substrate is the treatment 1 B while the above fertilizer dose with substrate kanchi is considered as treatment 1 K. Second fertilizer dose of cattle dung 3000 kg ha^{-1}, urea 150 kg ha^{-1} and super phosphate 100 kg ha^{-1} with substrate bamboo stands for treatment 2 B while second fertilizer dose with substrate kanchi is the treatment 2 K. In the third fertilizer dose, cattle dung 4500 kg ha^{-1}, urea 200 kg ha^{-1} and super phosphate 150 kg ha^{-1} respectively with substrate bamboo represents treatment 2.5 B while the above fertilizer dosages with substrate kanchi is the treatment 2.5 K. The bamboo poles (mean length - 2.0 m the effective water area in 1.5 m

and mean diameter – 5.5 cm) were vertically planted the pond at a density of 9 poles m^{-2} while kanchi were planted horizontally at a density of 34 poles m^{-2} (mean length – 2.0 m and mean diameter – 1.5 cm). Culture tanks were filled with 22 ppt saline water pumped from the Cochin estuary which was conditioned for a period of one week. The tanks were drained on the 75th day of experiment.

Water and sediment quality parameters

Water quality parameters such as temperature (mercury thermometer), salinity (hand refractometer), transparency (secchi disk) and pH (pH pen) were measured directly from the tank while dissolved oxygen was measured following Winkler method (APHA, 1995) at 09.00 AM on a daily basis. 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 (2 cm diameter). Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. The water samples were filtered through GF/C Whatman glass fiber filter 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 (5 day 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). Total Kjeldahl nitrogen in the periphyton was estimated (Mudroch et al., 1996). Total heterotrophic bacteria (THB) count in the water and sediment was estimated following standard procedures (APHA, 1995) and expressed as colony forming unit (cfu).

Determination of periphyton biomass

From each tank three poles were selected randomly and 2 x 2 cm^2 samples of periphyton were taken from different depths per poles. The areas were carefully scraped with the scalpel blade to remove the periphyton. After the sampling the poles were replaced to the original place. The materials collected were pre weighed and dried at 105^{0}C until constant weight and kept in dessiccator. The samples are then transferred to a muffle furnace and ashed at 450^{0}C for 6 hours and weighed. The dry matter, acid free dry matter and ash content were determined by weight following APHA (1995).

The pheophytin-a concentrations were determined by the following standard method APHA (1995). After the removal, the material was immediately transferred to tube containing 10 ml 90% acetone, sealed and transferred to the refrigerator for storing

overnight. Samples were homogenized for 30 sec with a tissue grinder and centrifuged for 10 min at 3000 rpm. The supernatant was transferred to the cuvettes, acidified by addition of three drops of 0.1 *N* HCl and absorption measured for the pheophytin-a.

Study of taxonomic composition of periphyton and plankton

The periphyton samples were taken from randomly selected poles from an area of 2 x 2 cm^2, each of different depth per pole and pooled together. The samples were collected on biweekly basis after the substrate installation. Pooled samples were preserved in 5% buffered formalin and after the vigorous shaking, a 1 ml of sub sample was transferred to a Sedgewick-Rafter cell (S-R cell), the number of colonies were counted on 10 randomly selected field of chamber under a binocular microscope (Azim et al., 2001). The periphyton sample densities were calculated by the formula.

$$N = (P \times C \times 100)/S$$

Where N = number of periphyton cells per cm^2 surface area; P = number of periphytic units counted in ten fields; C = volume of final concentrate of the sample (ml); S = area of scraped surface cm^2.

For the taxonomic identification of plankton, the samples were collected by passing 5 liter of water taken from the four locations of each tank and filtered through a 45 µ mesh size plankton net. The

concentrated sample was then transferred to a 100 ml measuring cylinder and made up to 100 ml with distilled water. Then the samples were preserved with 5% formalin solution. The 1 ml of plankton sub samples were estimated by using Sedgewick-Rafter cell (S-R cell) under the microscope. The plankton densities were calculated by the formula:

$$N = (P \times C \times 100)/L$$

Where N = the number of plankton cells per liter of original water; P = the number of plankton counted in the ten fields; C = the volume of the final concentration of the sample (ml); L = the volume (liters) of the tank water sample.

Taxa were identified to genus level using keys of Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976), Bellinger (1992) and Sreekumar (1996).

Statistical analysis

Statistical analysis of daily, biweekly and monthly (THB) water, sediment quality parameters and periphyton biomass (dry matter, pheophytin-a, ash content, protein, nitrogen (%) and ash free dry matter) were done by ANOVA: Two-factor without replication performed using Microsoft Excel 2000. The periphyton and plankton taxonomic data were analyzed by SPSS 11.5 One-way Tukey-HSD

test. Significant treatment effect was separated by calculating the least significant difference at 5% level.

3. Results

Water and sediment quality parameters

The water quality parameters in treatment with or with out substrates and fertilization effect were: temperature (30.17 – 30.83 °C), dissolved oxygen (6.69 – 6.81 mg l^{-1}) and salinity (20.89 – 21.00 ppt) and there was no significant difference ($P>0.05$) among the treatments. On the other hand, water pH (8.36 – 8.52), secchi disk reading (56.67 – 62.39 cm) and soil pH (8.29 – 8.42) have shown significant difference ($P<0.05$). The water quality parameters with mean with standard deviation are given in Table 7.1 and 7.2. The highest water pH was recorded in 2 K treatment in contrast to lowest in 2.5 B. Highest secchi disk reading was observed in 2.5 B while it was lowest in 1 K. In the case of soil pH the highest value was observed in 2.5 K where as it was lowest in 2 B treatment (Table 7.2).

Among the water quality parameters, biological oxygen demand (3.01 – 3.38 mg l^{-1}), alkalinity (43.44 – 53.50 mg $CaCO_3$ l^{-1}), total ammonia nitrogen (TAN) (4.81 – 6.41 µg l^{-1}), nitrite-N (0.20 – 0.34 µg l^{-1}) and total heterotrophic bacteria showed statistically significant difference among the treatments ($P<0.05$) (Table 7.2). Among the various water quality parameters, nitrate-N (0.63 – 0.76 µg l^{-1}) and

chlorophyll-a (39.61 – 55.94 μg cm^{-2}) showed significant difference ($P<0.05$) between the treatments (Table 7.2). Among the sediment quality parameters, total ammonia nitrogen (TAN) (14.36 – 30.51 μg l^{-1}), nitrite-N (0.08 – 0.14 μg l^{-1}), nitrate-N (0.30 – 0.43 μg l^{-1}), organic carbon (11.07 – 18.03 μ l^{-1}) and total heterotrophic bacteria (89.33 – 144.22 x 10^7 cfu ml^{-1}) showed significant difference ($P<0.05$) (Table 7.2). In treatment where higher levels of fertilization was applied (2.5 B and K), the water and soil total ammonia nitrogen and soil organic carbon showed high values which was statistically significant ($P<0.05$). Highest total ammonia nitrogen in water was observed in 2.5 K treatment (6.41 μg l^{-1}) which was found significantly different ($P>0.05$) from 2.5 B treatment (6.13 μg l^{-1}). In respect of soil, treatment 2.5 K showed highest total ammonia nitrogen followed by 2.5 B (Table 7.2).

During the culture period, higher total ammonia nitrogen concentration in water was recorded during the fifth sampling period (56^{th} day of culture) (Fig. 7.1a) whereas in soil it was during the third sampling period (28^{th} day of culture) (Fig. 7.2a). Similarly, a gradual increase was observed in nitrite-N, nitrate-N contents of water with the progression of experiment (Fig. 7.1b and c). The total heterotrophic bacteria (Fig. 7.1d) in water and soil (Fig. 7.2d) were considerably higher towards the end of the experiment. The increase of soil nitrite-N and nitrate-N were shown strong direct correlation

with the dosage levels of fertilization applied indifferent treatments (Fig. 7.2b and c).

Periphyton and plankton biomass

Periphyton biomass recorded from various treatments are shown in Table 7.3. The results show that there exist significant difference ($P<0.05$) in the periphyton production among various treatment, showing the least values in treatment 1 B and 1 K while it was highest in ($P<0.05$) in the treatment 2 B with the production high quantity and quality algal periphyton (Table 7.3). The percentage of protein concentration was maximum in the order of treatments 2.5 B (37.22%) and 2 B (36.17%), 2.5 K (32.06%) and 2 K (31.17%), however, there was no significant variations ($P>0.05$) among these treatments. The pattern of periphyton development (dry matter and pheophytin-a) during the sampling periods showed variation with the level of fertilization application (Fig. 7.3a and c). Altogether 72 genera of periphyton were identified from various treatments and the numerical strength of various genera of periphyton encountered in different substrates based treatments are shown in Table 7.5.

In respect of periphyton dry matter, pheophytin–a, ash content, protein and nitrogen (%) and acid free dry matter, significant difference ($P<0.05$) was observed, commensurate with different levels of fertilization (Table 7.3). The difference in respect development of

periphyton dry matter, no significantly difference (P>0.05) was noticed between treatments 2.5 B (1.80 mg cm^{-2}) and 2.5 K (1.76 mg cm^{-2}). However, these treatments showed higher mean dry matter which can be attributed to the higher levels of fertilization application in these treatments. While comparing the periphyton production in the treatments subjected to the same level of application of fertilizers, bamboo (2 B) showed significantly higher (P<0.05) periphyton production, in contrast to other treatments. Chlorophyll-a content was significantly lower in 1 K, on the other hand, it was very high in 2.5 B and 2.5 K. Furthermore, the treatment 2 B and 2 K were not found significantly different (P>0.05) from 2.5 K (Table 7.2). Chlorophyll-a concentrations at different fertilization levels and different sampling periods are shown in Fig. 7.3b. In the case of pheophytin-a, the highest mean value was recorded in treatment 2 B (1.51 µg cm^{-2}) while it was lowest in 1 K (1.39 µg cm^{-2}). Lowest mean value of ash content was recorded in 2.5 K while the highest value was in 2 B. In ash free dry matter the highest mean value was observed in 2.5 B in contrast to the lowest mean value in 2.5 K (Table 7.3).

The genera wise abundance of plankton in control, two types of substrata and varied levels of fertilization are presented in Table 7.4. Although planktons were more abundant at higher levels of fertilization, the number of plankton recorded from various treatments

did not show any significant difference (P>0.05). 69 genera of phytoplankton belonging to Chlorophyceae (16 genera), Cyanophyceae (6 genera), Cryptophyceae (2 genera), Crysophyceae (10 genera), Euglenophyceae (5 genera), Pyrrhophyceae (14 genera), Rhodophyceae (11 genera) and in zooplankton, Crustacea (5 genera), Rotifer (7 genera) were identified, among them Pyrrhophyceae appeared as the most dominant group in the all treatments.

4. Discussion

Water and sediment quality parameters

Improving aquaculture sustainability and its accessibility by introducing artificial substrates in aquaculture systems is useful in increasing the surface area for attachment of natural food organisms. The results of the present study showed that the type of substrate used had no significant effect (P>0.05) on temperature, dissolved oxygen and salinity. The variations in water quality make severe impacts on shrimp health and survival (Boyd, 1990). For the best survival and growth of shrimp, the dissolved oxygen concentration above 4.0 mg l^{-1} is essential (Boyd and Fast, 1992; Hall and Van Hamm, 1998). In the present study, dissolved oxygen ranged from 6.69 – 6.81 mg l^{-1} in various treatments. Water pH showed significant difference among treatments and was in the range of 8.36 – 8.52. It has been reported that shrimps become stressed outside their optimal

pH range of 7.0 – 9.0 (Boyd and Fast, 1992; Binch et al., 1997 and Phillips, 1998). Both substrate wise and fertilization levels wise, secchi disk reading showed significant difference. Garg and Bhatnagar (1996) observed that ammonia-N, nitrite-N and secchi disk reading showed increase commensurate with increasing application of fertilizer dose and the results of the present study fully concur with this. Similarly, the inorganic concentration of water and soil also showed an increasing trend with different levels of application of fertilizers. In the culture of the experiment, maximum water TAN concentration was observed in treatment 2.5 K with value of 6.41 $\mu g\ l^{-1}$. Weitzel et al. (1979), Stevenson and Stoermer (1982) and Morin and Cattaneo (1992) have reported that TAN levels in water were in the range of 24.63 – 68.98 $\mu g\ l^{-1}$ during periphyton based culture. The inorganic nitrogen concentration in sediment showed very high values in treatments 2.5 K and 2.5 B when compared to other treatments. It may be inferred that the high level of fertilization application in 2.5 K and 2.5 B might have amplified pond productivity which in turn caused the accumulation of inorganic nitrogen in the pond bottom as opined by Bormann et al. (1968), Vitsousek et al. (1979), Schimel and Firestone (1989) and Dail et al. (2001). Sediment nitrite-N and nitrate-N level also showed significant increase in treatments having high level of fertilization application (2.5 B and 2.5 K). It would thus appear that the high rate of fertilization application might have promoted the

growth of organic and inorganic nutrient load in the culture system as reported by Poernomo and Singh (1982), Apud et al. (1989) and Boyd (1989). Interestingly, the soil pH values in treatment 2.5 B and 2.5 K were significantly higher when compared to other treatments. According to Dent (1986), Boyd and Teichert-Coddington (1994) and Munsiri et al. (1995) the accumulation of management inputs such as fertilizers and organic matter are responsible for the high soil pH in the aquaculture systems. Soil pH is an important variable and an ideal value in the range 6.5 – 8.5 is normally considered as acceptable (Boyd, 1995). High soil pH deteriorates the water quality and affects adversely the survival and growth of the cultured species (Banerjea, 1967; Boyd, 1974).

It is very interesting to observe that there exist an inverse relationship between of total heterotrophic bacteria population and biological oxygen demand concentration in the culture system during sampling periods. Moriarty (1997), Remesh et al. (1999) and Umesh et al. (1999) reported that low rate of oxygen in ponds is attributed to the consumption by the heterotrophic bacterial population. Bell and Ahlgren (1987) also strongly agrees with oxygen consumption of bacteria. The results obtained from the substrate based culture system in the present study revealed that there is a direct correlation between the level of fertilization application and population of heterotrophic bacteria in the substrate aquaculture ponds.

Middelburg and Nieuwenhuize (2000a), Benner (2002) and Bronk (2002) found that the presence of microbial community may uptake the different nitrogenous substrates produced in ponds. The uptake of nitrogen by the heterotrophic bacteria is mostly focused on dissolved inorganic nitrogen (DIN) form, especially ammonium-N (NH_4^+) and nitrate-N (NO_3^-) which are the important nitrogen sources (Antia et al., 1991; Middelburg and Nieuwenhuize, 2000b; Bronk, 2002; Zehr and Ward, 2002; Berman and Bronk, 2003). The significant increase in heterotrophic bacterial population in water and soil resulted in the utilization of inorganic nitrogen concentration which might have helped in to the plummeting of inorganic nitrogen level in treatments where high rate of fertilization were applied.

The results of the present study revealed that there was significant enhancement in the concentration of Chlorophyll-a and organic carbon in treatments commensurate with increased dosage of application of fertilization (Table 7.2). Dewan et al. (1991) and Ahmed (1993) reported that the chlorophyll-a concentration showed an increase with high dosage of fertilization application. According to Doering and Ovitatt (1986), Doering et al. (1986, 1987) the organic carbon level showed an increase with the pond production which is further dependent on the level of application of fertilizer. While comparing the water and soil quality parameters and level of application fertilizers in various treatments in the present study, it

can be concluded that the level of administration of fertilizer in 2 B treatment was ideal in providing the optimal soil and water quality parameters when compared to other treatments, followed by 2 K.

Periphyton biomass

Primary and secondary productivity were subjected to very series studies in extensive aquaculture ponds, particularly in tropical countries. The periphyton concentrations were measured by nitrogen (%), dry matter and pheophytin-a concentrations. In the present study, the periphyton biomass was found in Bamboo substrate (2.5 B) followed by 2 B. Konan-Brou and Guiral (1994) reported that the periphton biomass production was very in Bamboo substrate among various substrate studied. The results of present study are corroborated with Konan-Brou and Guiral (1994). According to Keshavanath et al. (2001a) maximum periphytic biomass level coincides with the high level of fertilization and among various substrates studied, bamboo was recommended as best substrate for the substrate based aquaculture. In the present study also higher dry matter was observed in the treatment 2.5 B which was characterized by high periphyton production, however there was no significant variation could be observed in treatments 2.5 K and 2 B. It would thus appear that the fertilization level can be optimized at 2 B level. Hem and Avit (1994) and Keshavanath et al. (2001a) were of the view

that among the available artificial substrate useful for aquaculture, bamboo is far superior for the production of periphyton and increasing fish productivity. The variation in the values in respect of dry matter, pheophytin-a, ash content and ash free dry matter in different treatments indicate that level of fertilization has an important bearing on the above parameters. Huchette et al. (2000) reported that the ash content of periphyton show variation among different levels of application of fertilization. Based on the results of the present study, Treatment 2B is very ideal for the high production and ecological sustainability in aquaculture farms when compared to other treatment studied and therefore can be recommended. According to Makarevich et al. (1993) and Huchette et al. (2000), the temporal increase in periphyton nitrogen (%), dry matter, ash content, pheophytin-a and ash free dry matter can be attributed to the non grazing effect by organisms. In the present study, no culture animals were maintained in the treatments and the results are comparable to non grazing situation. In the present study 72 genera of periphyton planktons were identified from each treatment. Azim et al. (2002) identified 60 periphyton genera from bamboo based fresh water aquaculture ponds and while comparing the present results with that of Azim et al (2002), generic strength of plankton in the present study is very high. Conversely, Huchette et al. (2000) identified only 32 species of diatom as periphyton along with other

micro and microorganisms from both animal and plant kingdoms on artificial substrates in Tilapia cages. The quantity of periphyton varied substantially with substrate type, fertilization level, environment conditions, grazing pressure and taxonomic composition (Paine and Vadas, 1969; Heaper, 1988; Makarevich et al., 1993; Napolitano et al., 1996; Ledger and Hildrew, 1998; Huchettu et al., 2000; Keshavanath et al., 2001a). Shrimp were absent in this experiment, and there was chance of very minor grazing by zooplankton, mollusks and other invertebrates (especially chironomid larvae) possibly in a lower extent as suggested by Huchette et al. (2000). In the present trial macrobenthic organisms especially chironomid larvae were observed moving around the surface of the bamboo and kanchi during the periphyton sampling.

Plankton biomass

The treatments with bamboo as the substrate showed higher plankton production when compared to other treatments and the plankton production varied with fertilization levels. In the present study, maximum plankton production was observed in 2 B treatment which was significantly higher. Reynolds (1984), Dempster et al. (1993), Delince (1992) established very strong correlation between plankton production chlorophyll-a. The results of chlorophyll-a (Table 7.2) showed that nutrient concentration (fertilization level) had a

strong correlation with phytoplankton productivity (Boyd, 1990). In the present study, 69 planktons were identified from each treatment. This is very higher when compared to Azim et al. (2002) who reported 43 plankton genera from the fresh water aquaculture pond provided with bamboo as substrate. The phytoplankton production showed significant increase due rich nutrient concentration due to the high level of fertilization application .In fish ponds, the nitrate-N accumulates in the systems (Heinsbroek and Kamstra, 1990; Kamstra et al., 1996), however, in the present study, the nitrate-N content showed no significant difference in treatments during the experimental periods. In the present study there was a steady increase in the phytoplankton production during the period of experiment and this is indicative of the healthiness of the system as opined by Mollah and Haque (1978) Dewan et al. (1991) and Wahab et al. (1999b).

From the practical point of view, the addition of substrates to aquaculture system increases the primary and secondary production due to the additive effect of periphyton and phytoplankton based components of production. Shrimps feed planktonic algae for there growth (Johnston et al., 1999; 2000a; 2002). Shrimps generally require food sources such as benthic algae, algal detritus or plant fodder, that can harvested more efficiently (Dempster et al., 1993; Yakupitiyage, 1993).

Due to the addition of substrates to water column the algae growing on substrates, associated bacterial and zooplankton biomass are exploited directly by shrimps (Reynolds, 1984; Prejs, 1984; Horn, 1989; Dempster et al., 1995; Huchette et al., 2000). This results in higher shrimp yield. No negative effect on water and soil quality was observed due to the incorporation of artificial substrate. In the present study, the maximum periphyton ash content was observed in treatment 2 B. The periphyton based pond production depends on the nutritional quality of periphyton, grazing efficiency of shrimp, availability of other food source in pond and environmental conditions.

In Conclusion, for the better periphyton production, bamboo is recommended as a substrate to fix at water column. The fertilizer dose with (Treatment 2 B and 2 K) cattle dung - 3000 kg ha^{-1}, urea - 150 kg ha^{-1}, and super phosphate - 100 kg ha^{-1} respectively is recommended for optimal periphyton growth. Among the periphyton substrates, both bamboo and kanchi will not make any adverse effect on water and sediment quality parameters of the grow outs. These substrates are easily available and are having the advantage of using repeatedly for a considerable span. These substrates based periphyton production can improve the sustainability and profitability of shrimp farming in the country.

Table 7.1

Daily water quality parameters of periphyton production with fertilisation level

Variable	Treatments (mean ± SD)						
	1 C	**1 B**	**1 K**	**2 B**	**2 K**	**2.5 B**	**2.5 K**
Temperature (°C)	30.17 ± 1.82^{a}	30.28 ± 1.78^{a}	30.44 ± 1.65^{a}	30.78 ± 1.44^{a}	30.67 ± 1.61^{a}	30.83 ± 1.50^{a}	30.78 ± 1.59^{a}
Water P^{H}	8.43 ± 0.26^{aa}	8.47 ± 0.25^{a}	8.44 ± 0.22^{aa}	8.38 ± 0.24^{aa}	8.52 ± 0.23^{a}	8.36 ± 0.19^{ca}	8.43 ± 0.23^{aa}
DO (mg l^{-1})	6.77 ± 0.24^{a}	6.69 ± 0.23^{a}	6.76 ± 0.21^{a}	6.78 ± 0.27^{a}	6.80 ± 0.30^{a}	6.81± 0.23^{a}	6.69 ± 0.24^{a}
Salinity (ppt)	20.89 ± 0.76^{a}	20.94 ± 0.73^{a}	21.00 ± 0.69^{a}	20.72 ± 0.83^{a}	21.00 ± 0.77^{a}	20.89 ± 0.76^{a}	20.78 ± 0.94^{a}
Secchi disk reading (cm)	57.83 ± 5.83^{cd}	58.00 ± 5.47^{cd}	56.67 ± 4.89^{c}	58.67 ± 5.56^{bd}	57.72 ± 4.55^{bc}	62.39 ± 5.25^{a}	61.17 ± 5.47^{a}

Results from ANOVA Two-factor without replication

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

Table 7.2
Effect of fertilisation level and periphyton development in water and sediment quality

	Treatments (mean ± SD)						
	1 C	**1 B**	**1 K**	**2 B**	**2 K**	**2.5 B**	**2.5 K**
Water quality variable							
BOD ($mg\ l^{-1}$)	3.01 ± 0.80^{o}	3.26 ± 0.67^{a}	3.38 ± 0.75^{a}	3.38 ± 0.73^{a}	3.25 ± 0.75^{a}	3.35 ± 0.69^{a}	3.25 ± 0.77^{a}
Alkalinity ($mg\ CaC0_3\ l^{-1}$)	43.44 ± 4.38^{c}	50.39 ± 4.46^{b}	52.89 ± 3.61^{a}	49.83 ± 2.60^{b}	50.78 ± 3.39^{b}	52.67 ± 3.79^{a}	53.50 ± 3.31^{a}
TAN ($ug\ l^{-1}$)	4.87 ± 1.22^{ef}	4.81 ± 0.77^{f}	4.95 ± 0.85^{e}	5.27 ± 0.98^{d}	5.61 ± 1.09^{c}	6.13 ± 1.23^{b}	6.41 ± 1.27^{a}
Nitrite-N ($ug\ l^{-1}$)	0.20 ± 0.04^{d}	0.20 ± 0.02^{d}	0.25 ± 0.04^{c}	0.26 ± 0.04^{c}	0.30 ± 0.08^{b}	0.33 ± 0.09^{a}	0.34 ± 0.08^{a}
Nitrate-N ($ug\ l^{-1}$)	0.63 ± 0.10^{e}	0.65 ± 0.08^{a}	0.65 ± 0.08^{a}	0.68 ± 0.07^{c}	0.72 ± 0.09^{o}	0.75 ± 0.09^{a}	0.76 ± 0.10^{a}
THB ($10^5 cfu\ ml^{-1}$)	71.11 ± 21.75^{d}	98.11 ± 35.13^{c}	99.22 ± 38.22^{bc}	112.22 ± 46.84^{ab}	107.00 ± 44.86^{ac}	119.11 ± 48.52^{a}	115.33 ± 49.23^{ab}
Chlorophyll-a ($ug\ cm^{-2}$)	39.61 ± 7.94^{d}	44.94 ± 9.59^{c}	43.89 ± 10.33^{c}	50.50 ± 10.92^{b}	48.67 ± 10.32^{b}	55.94 ± 11.97^{a}	52.33 ± 10.01^{ab}
Sediment quality variable							
Soil pH	8.34 ± 0.07^{b}	8.34 ± 0.08^{b}	8.32 ± 0.08^{b}	8.29 ± 0.08^{b}	8.33 ± 0.10^{b}	8.37 ± 0.08^{a}	8.42 ± 0.12^{a}
TAN ($ug\ l^{-1}$)	14.36 ± 2.35^{d}	15.19 ± 2.81^{d}	15.63 ± 3.50^{d}	16.11 ± 4.35^{d}	18.88 ± 5.45^{c}	28.89 ± 9.97^{b}	30.51 ± 10.47^{a}
Nitrite-N ($ug\ l^{-1}$)	0.09 ± 0.04^{c}	0.08 ± 0.04^{c}	0.08 ± 0.03^{c}	0.08 ± 0.03^{c}	0.08 ± 0.03^{c}	0.12 ± 0.06^{b}	0.14 ± 0.07^{a}
Nitrate-N ($ug\ l^{-1}$)	0.30 ± 0.10^{d}	0.30 ± 0.09^{d}	0.32 ± 0.09^{cd}	0.35 ± 0.10^{bc}	0.36 ± 0.10^{b}	0.41 ± 0.12^{a}	0.43 ± 0.13^{a}
Organic carbon ($ug\ l^{-1}$)	11.07 ± 0.92^{a}	15.81 ± 4.42^{c}	16.34 ± 4.54^{c}	16.06 ± 4.50^{ca}	16.81 ± 4.34^{oc}	17.79 ± 4.81^{ao}	18.03 ± 5.00^{a}
THB ($10'cfu\ ml^{-1}$)	89.33 ± 25.97^{a}	105.11 ± 45.48^{c}	114.89 ± 42.99^{oc}	128.89 ± 43.31^{ao}	138.56 ± 51.20^{a}	147.89 ± 48.26^{a}	144.22 ± 54.19^{aoc}

Results from ANOVA Two-factor without replication

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

Table 7.3

Mean values of periphyton biomass and proximate composition parameters scraped from substrates

Variable	Treatments (mean ± SD)					
	1 B	**1 K**	**2 B**	**2 K**	**2.5 B**	**2.5 K**
Dry matter (mg cm^{-2})	1.61 ± 0.35^{c}	1.37 ± 0.24^{d}	1.76 ± 0.35^{a}	1.66 ± 0.34^{b}	1.80 ± 0.34^{a}	1.76 ± 0.34^{a}
Pheophytin-a (ug cm^{-2})	1.46 ± 0.13^{a}	1.39 ± 0.09^{d}	1.51 ± 0.12^{a}	1.47 ± 0.11^{b}	1.47 ± 0.12^{c}	1.44 ± 0.11^{d}
Ash content (%)	42.33 ± 7.03^{ab}	40.28 ± 7.28^{bc}	43.11 ± 8.59^{ab}	40.33 ± 7.70^{bc}	42.28 ± 6.94^{ab}	38.28 ± 7.14^{c}
Ash free dry matter (mg cm^{-2})	2.36 ± 0.14^{abc}	2.33 ± 0.13^{abc}	2.45 ± 0.16^{a}	2.37 ± 0.12^{abc}	2.50 ± 0.15^{a}	2.31 ± 0.52^{c}
Protein (%)	30.11 ± 4.27^{b}	27.94 ± 3.92^{d}	36.17 ± 6.26^{a}	31.17 ± 6.14^{c}	37.22 ± 7.26^{a}	32.06 ± 7.12^{c}
Nitrogen (%)	4.67 ± 0.66^{d}	4.33 ± 0.61^{e}	5.61 ± 0.97^{b}	4.83 ± 0.95^{cd}	5.77 ± 1.13^{a}	4.97 ± 1.10^{c}

Results from ANOVA Two-factor without replication

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

	Group / Genus	1 C	1 B	1 K	3 B	3 K	3 A B	3 A K
	PHYTOPLANKTON							
1	**Chlorophyceae**	Avg	Avg	Avg	Avg	Avg	Avg	Avg
	Actinastrum	322.67± 60.30^{d}	377.33 ± 37.85^{b}	313.00 ± 61.25^{c}	575.33 ± 44.56^{a}	493.67 ± 63.51^{ab}	578.33 ± 43.65^{a}	577.00 ± 17.32^{a}
	Ankistrodesmus	353.67± 100.17^{ab}	484.00 ± 60.65^{a}	236.33 ± 121.06^{b}	567.00 ± 30.00^{a}	342.33 ± 105.70^{ab}	566.67 ± 23.59^{a}	447.33 ± 91.09^{ab}
	Botryococcus	287.00 ± 52.92^{a}	488.67 ± 64.53^{a}	407.67 ± 46.26^{a}	372.33 ± 281.95^{a}	473.33 ± 54.64^{a}	465.00 ± 121.11^{a}	550.33 ± 16.26^{a}
	Chlorella	4926.00 ± 579.53^{b}	7924.67 ± 720.25^{ab}	507.67 ± 43.84^{c}	10285.00 ± 1912.69^{a}	588.00 ± 53.02^{c}	10543.00 ± 2125.81^{a}	708.00 ± 48.12^{c}
	Closterium	68.33 ± 16.44^{b}	144.67 ± 24.03^{ab}	132.33 ± 43.59^{ab}	192.67 ± 24.17^{a}	179.33 ± 62.77^{a}	221.67 ± 24.58^{a}	204.67 ± 45.57^{a}
	Cosmarium	177.67 ± 57.73^{a}	369.33 ± 12.58^{bc}	323.00 ± 73.63^{c}	492.33 ± 35^{ab}	403.33 ± 42.74^{abc}	527.33 ± 41.50^{a}	530.00 ± 64.09^{a}
	Crucigenia	78.33 ± 8.33^{d}	154.00 ± 10.82^{cd}	146.67 ± 27.97^{cd}	206.67 ± 33.23^{bc}	286.00 ± 70.66^{b}	229.33 ± 24.85^{bc}	407.67 ± 46.26^{a}
	Gonatozygon	389.33 ± 58.62^{c}	714.67 ± 122.41^{ab}	393.67 ± 158.22^{bc}	821.67 ± 59.21^{a}	477.33 ± 141.60^{bc}	882.00 ± 50.24^{a}	597.33 ± 158.05^{abc}
	Oocystis	244.00 ± 110.08^{d}	485.00 ± 33.06^{bc}	412.00 ± 52.72^{c}	572.67 ± 31.56^{ab}	567.33 ± 60.58^{abc}	612.67 ± 29.01^{ab}	649.33 ± 33.26^{a}
	Pediastrum	181.00 ± 46.36^{a}	399.67 ± 49.36^{a}	300.67 ± 57.45^{a}	519.33 ± 49.52^{a}	1651.00 ± 2223.95^{a}	566.00 ± 50.47^{a}	2236.00 ± 2977.82^{a}
	Selenastrum	75.33 ± 16.29^{c}	203.67 ± 44.84^{bc}	203.67 ± 50.33^{bc}	289.00 ± 68.99^{ab}	320.67 ± 71.77^{ab}	313.00 ± 66.46^{ab}	460.00 ± 105.06^{a}
	Spirogyra	152.00 ± 20.78^{c}	146.67 ± 23.18^{c}	213.33 ± 75.63^{bc}	227.67 ± 27.23^{bc}	360.67 ± 100.65^{ab}	271.67 ± 26.56^{ab}	464.33 ± 100.20^{a}
	Synedra	72.33 ± 22.81^{c}	117.00 ± 27.07^{c}	250.67 ± 106.64^{bc}	204.00 ± 39.96^{bc}	362.33 ± 114.99^{ab}	255.33 ± 33.84^{bc}	500.67 ± 114.89^{a}
	Treubaria	411.00 ± 57.42^{c}	535.33 ± 104.99^{bc}	322.67 ± 65.85^{c}	754.00 ± 100.00^{ab}	443.00 ± 80.30^{c}	852.00 ± 120.88^{a}	554.33 ± 90.67^{bc}
	Tetraedron	367.33 ± 53.48^{b}	560.00 ± 130.64^{ab}	381.67 ± 34.43^{b}	668.67 ± 167.53^{ab}	470.00 ± 51.74^{b}	790.67 ± 164.63^{a}	634.00 ± 65.09^{ab}
	Zygnema	412.67 ± 191.85^{ca}	678.33 ± 91.31^{abc}	246.67 ± 120.50^{d}	891.67 ± 63.22^{ab}	377.00 ± 145.26^{cd}	1056.00 ± 154.15^{a}	543.67 ± 166.23^{bcd}
	Total	**8518.67 ± 1453.10^{ab}**	**13783.00 ± 1557.57^{ab}**	**4791.67 ± 1139.37^{b}**	**17640.00 ± 2969.35^{ab}**	**7795.33 ± 3443.87^{ab}**	**18730.67 ± 3101.32^{a}**	**10064.67 ± 4139.98^{ab}**
2	**Cyanophyceae**							
	Aphanocapsa	431.00 ± 54.84^{b}	522.33 ± 57.81^{ab}	389.00 ± 114.35^{b}	646.00 ± 104.93^{ab}	490.00 ± 107.64^{ab}	738.67 ± 96.62^{a}	699.33 ± 115.48^{a}
	Chroococcus	606.00 ± 72.55^{b}	794.67 ± 68.71^{ab}	521.67 ± 408.19^{b}	927.33 ± 63.51^{ab}	566.00 ± 61.83^{b}	1227.33 ± 308.45^{a}	730.67 ± 112.61^{ab}
	Gomphosphaeria	666.33 ± 83.81^{b}	830.67 ± 57.74^{ab}	674.67 ± 88.15^{b}	985.33 ± 234.57^{ab}	674.67 ± 88.15^{b}	1265.67 ± 358.89^{a}	810.00 ± 97.89^{ab}
	Merismopedia	328.33 ± 53.35^{a}	322.00 ± 55.43^{a}	468.33 ± 189.44^{a}	526.33 ± 89.94^{a}	473.00 ± 146.77^{a}	640.33 ± 89.63^{a}	662.67 ± 165.70^{a}
	Microcystis	10426.00 ± 865.96^{c}	14012.67 ± 1513.84^{abc}	11426.00 ± 1799.23^{bc}	16314.67 ± 2059.28^{ab}	13981.00 ± 2105.82^{abc}	18836.00 ± 2160.83^{a}	16884.00 ± 1566.94^{a}
	Oscillatoria	403.33 ± 70.12^{c}	471.33 ± 91.78^{bc}	524.33 ± 141.67^{bc}	616.33 ± 120.97^{abc}	617.00 ± 43.59^{abc}	742.00 ± 109.78^{ab}	861.33 ± 104.03^{a}
	Total	**12861.00 ± 1200.62^{a}**	**16953.67 ± 1845.31^{ab}**	**14004.00 ± 2741.02^{a}**	**20016.00 ± 2673.21^{ab}**	**16801.67 ± 2553.80^{ab}**	**23450.00 ± 3124.20^{a}**	**20648.00 ± 2162.65^{ab}**
3	**Cryptophyceae**							
	Chroomonas	659.67 ± 93.03^{a}	723.00 ± 42.93^{a}	731.67 ± 192.66^{a}	699.67 ± 139.97^{a}	909.67 ± 302.10^{a}	758.67 ± 147.30^{a}	1029.67 ± 311.76^{a}
	Cryptomonas	344.00 ± 84.26^{c}	503.67 ± 73.82^{bc}	414.33 ± 50.62^{c}	768.67 ± 74.11^{a}	499.67 ± 64.66^{bc}	854.00 ± 85.44^{a}	696.33 ± 58.00^{ab}
	Total	**1003.67 ± 177.28^{a}**	**1226.67 ± 116.75^{abc}**	**1146.00 ± 243.28^{ab}**	**1468.33 ± 214.08^{abc}**	**1409.33 ± 366.76^{abc}**	**1612.67 ± 232.74^{ab}**	**1726.00 ± 369.76^{a}**
4	**Crysophyceae**							
	Botrydiopsis	286.33 ± 60.37^{c}	411.67 ± 50.65^{bc}	320.00 ± 73.82^{c}	582.33 ± 55.30^{ab}	452.67 ± 92.34^{abc}	644.00 ± 38.20^{a}	564.67 ± 107.52^{ab}
	Bumilleriopsis	516.00 ± 55.43^{c}	588.33 ± 77.24^{bc}	593.67 ± 80.83^{bc}	786.00 ± 51.16^{ab}	723.67 ± 80.88^{abc}	849.67 ± 56.09^{a}	837.33 ± 141.89^{a}
	Chlorothecium	507.67 ± 72.95^{b}	584.67 ± 90.75^{ab}	556.67 ± 72.98^{b}	789.00 ± 58.90^{ab}	680.00 ± 166.71^{ab}	863.67 ± 88.64^{a}	751.67 ± 117.00^{ab}
	Dinobryon	349.33 ± 249.57^{a}	684.33 ± 60.93^{a}	405.67 ± 223..01^{a}	792.00 ± 58.03^{a}	506.67 ± 113.93^{a}	840.67 ± 81.94^{a}	463.00 ± 354.63^{a}
	Epipyxis	180.67 ± 61.34^{b}	223.67 ± 59.53^{b}	410.67 ± 217.81^{ab}	363.67 ± 94.04^{ab}	486.33 ± 138.35^{ab}	434.00 ± 90.54^{ab}	601.67 ± 150.54^{a}
	Heterococcus	274.33 ± 43.84^{b}	195.33 ± 83.58^{b}	350.67 ± 130.27^{ab}	368.00 ± 110.73^{ab}	453.67 ± 85.05^{ab}	441.67 ± 104.64^{ab}	614.00 ± 135.19^{a}
	Leuvenia	227.00 ± 61.73^{b}	188.00 ± 43.31^{b}	293.67 ± 113.27^{ab}	397.33 ± 52.54^{ab}	409.00 ± 156.12^{ab}	459.67 ± 64.61^{ab}	543.6 ± 166.23^{a}
	Perone	204.67 ± 73.32^{a}	145.33 ± 7.64^{a}	299.33 ± 159.33^{a}	378.00 ± 81.00^{a}	316.33 ± 240.59^{a}	427.67 ± 102.51^{a}	426.00 ± 159.14^{a}

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5 **Euglenophyceae**							
Colacium	310.67 ± 51.19^{c}	406.00 ± 58.95^{bc}	330.67 ± 63.80^{c}	541.33 ± 53.95^{ab}	416.67 ± 46.07^{bc}	588.33 ± 48.01^{a}	530.33 ± 63.51^{bc}
Euglena	342.00 ± 15.13^{c}	371.00 ± 48.57^{c}	371.00 ± 50.86^{c}	509.00 ± 36.59^{b}	511.00 ± 43.27^{b}	567.33 ± 42.52^{ab}	650.67 ± 71.77^{a}
Gymnodinium	286.00 ± 32.91^{a}	327.33 ± 52.25^{a}	287.00 ± 70.93^{a}	1857.33 ± 2372.49^{a}	472.00 ± 58.62^{a}	2239.33 ± 2596.42^{a}	746.00 ± 93.66^{a}
Phacus	469.00 ± 50.41^{b}	605.33 ± 70.44^{ab}	512.67 ± 131.90^{b}	765.67 ± 154.73^{ab}	611.33 ± 148.84^{ab}	861.67 ± 105.10^{a}	748.33 ± 100.38^{ab}
Trachalomonas	417.33 ± 56.13^{c}	444.33 ± 123.65^{bc}	567.67 ± 130.08^{abc}	550.67 ± 85.68^{abc}	676.33 ± 67.26^{ab}	691.33 ± 67.68^{ab}	789.00 ± 64.95^{a}
Total	**1825.00 ± 205.77^{a}**	**2154.00 ± 353.87^{a}**	**2069.00 ± 447.57^{a}**	**4224.00 ± 2703.44^{a}**	**2687.33 ± 364.06^{a}**	**4948.00 ± 2859.74^{a}**	**3464.33 ± 394.27^{a}**
6 **Pyrrhophyceae**							
Amphidinium	498.67 ± 78.26^{c}	612.00 ± 86.02^{bc}	745.00 ± 106.78^{abc}	830.67 ± 159.48^{ab}	857.33 ± 105.04^{ab}	887.33 ± 129.47^{ab}	931.6 ± 58.62^{a}
Ceratium	243.00 ± 13.08^{c}	278.33 ± 70.68^{bc}	329.67 ± 65.53^{bc}	463.33 ± 100.20^{ab}	404.00 ± 40.73^{abc}	543.67 ± 107.58^{a}	577.33 ± 9.07^{a}
Cystodinium	413.67 ± 51.32^{c}	489.67 ± 117.87^{bc}	322.67 ± 59.47^{c}	702.67 ± 144.14^{ab}	417.67 ± 46.06^{c}	785.67 ± 165.51^{a}	567.00 ± 0.00^{abc}
Glenodinium	368.33 ± 7679^{bc}	382.00 ± 34.07^{abc}	255.67 ± 99.01^{c}	553.00 ± 114.84^{ab}	347.00 ± 91.65^{bc}	658.33 ± 96.34^{a}	503.00 ± 163.22^{abc}
Gloeodinium	280.67 ± 64.29^{d}	381.00 ± 107.37^{bcd}	326.33 ± 60.12^{cd}	641.00 ± 124.72^{ab}	438.00 ± 63.41^{abcd}	706.67 ± 136.62^{a}	571.67 ± 117.07^{abc}
Gonyaulax	322.00 ± 154.51^{c}	573.00 ± 70.66^{bc}	796.00 ± 94.46^{ab}	717.00 ± 130.77^{ab}	896.67 ± 121.82^{ab}	804.33 ± 145.40^{ab}	1028.67 ± 198.80^{a}
Gymnodinium	275.67 ± 67.86^{c}	269.33 ± 76.01^{c}	357.00 ± 99.24^{bc}	396.00 ± 53.73^{abc}	463.67 ± 100.17^{abc}	540.67 ± 63.72^{ab}	608.67 ± 72.17^{a}
Gyrodinium	205.33 ± 80.98^{b}	187.33 ± 63.26^{b}	311.33 ± 171.04^{ab}	310.67 ± 110.73^{ab}	404.00 ± 156.39^{ab}	434.33 ± 114.90^{ab}	558.67 ± 129.70^{a}
Hemidinium	182.33 ± 72.86^{b}	318.33 ± 61.78^{ab}	347.00 ± 84.45^{ab}	450.33 ± 80.21^{ab}	495.67 ± 119.93^{ab}	559.33 ± 123.57^{a}	626.00 ± 204.97^{a}
Hypnodinium	211.00 ± 71.19^{d}	327.67 ± 52.54^{cd}	300.67 ± 57.45^{cd}	494.33 ± 63.80^{ab}	385.67 ± 32.33^{bc}	591.67 ± 40.99^{a}	492.00 ± 65.00^{ab}
Massartia	323.67 ± 58.59^{c}	484.00 ± 73.75^{bc}	494.33 ± 62.94^{abc}	662.67 ± 183.29^{ab}	564.67 ± 62.88^{abc}	787.33 ± 155.60^{a}	692.00 ± 58.51^{ab}
Oodinium	343.67 ± 85.05^{d}	491.33 ± 48.42^{bcd}	325.00 ± 52.85^{d}	657.00 ± 60.83^{ab}	417.00 ± 46.16^{cd}	752.00 ± 47.82^{a}	530.33 ± 67.86^{bc}
Peridinium	327.00 ± 60.83^{b}	343.67 ± 89.99^{b}	259.67 ± 93.03^{b}	492.00 ± 65.00^{ab}	353.33 ± 110.14^{b}	617.67 ± 69.29^{a}	475.33 ± 110.02^{ab}
Urococcus	224.33 ± 60.74^{c}	323.67 ± 49.33^{bc}	282.00 ± 77.90^{bc}	463.67 ± 100.17^{ab}	322.67 ± 74.20^{bc}	554.67 ± 91.13^{a}	426.00 ± 53.70^{abc}
Total	**4219.33 ± 996.33^{c}**	**5461.33 ± 1001.75^{c}**	**5452.33 ± 1184.27^{c}**	**7834.33 ± 1491.90^{c}**	**6767.33 ± 1170.91^{c}**	**9223.67 ± 1487.94^{a}**	**8588.33 ± 1308.72^{ab}**
7 **Rhodophyceae**							
Asterocystis	197.33 ± 51.68^{a}	223.33 ± 59.16^{a}	450.00 ± 193.94^{a}	360.33 ± 95.04^{a}	361.00 ± 318.89^{a}	468.00 ± 99.00^{a}	434.33 ± 328.04^{a}
Audouinella	210.00 ± 39.15^{e}	306.00 ± 44.19^{de}	370.67 ± 48.60^{cd}	407.33 ± 34.12^{bcd}	449.33 ± 23.71^{bc}	518.00 ± 59.23^{ab}	571.67 ± 8.08^{a}
Bangia	207.67 ± 69.30^{c}	287.67 ± 61.89^{bc}	350.00 ± 81.73^{bc}	394.67 ± 55.90^{abc}	453.67 ± 85.52^{ab}	476.67 ± 53.53^{ab}	570.33 ± 120.03^{a}
Batrachospermum	41.00 ± 20.66^{d}	43.33 ± 15.53^{d}	96.33 ± 29.50^{cd}	134.00 ± 26.29^{bc}	149.00 ± 15.72^{abc}	200.33 ± 35.12^{ab}	219.00 ± 44.44^{a}
Bostrychia	177.67 ± 61.89^{d}	357.00 ± 9.54^{c}	474.33 ± 81.12^{bc}	483.33 ± 35.57^{bc}	590.33 ± 40.41^{ab}	590.67 ± 40.99^{ab}	695.00 ± 57.03^{a}
Compsopogon	134.00 ± 30.27^{e}	257.00 ± 122.87^{de}	572.00 ± 98.85^{abc}	361.33 ± 95.57^{cde}	691.67 ± 109.70^{ab}	454.33 ± 102.38^{bcd}	833.00 ± 142.66^{a}
Lemanea	212.00 ± 43.03^{c}	333.33 ± 60.35^{bc}	210.33 ± 72.34^{c}	496.67 ± 72.98^{ab}	322.67 ± 74.20^{bc}	629.33 ± 65.16^{a}	453.00 ± 102.90^{ab}
Porphyridium	188.00 ± 59.51^{a}	416.00 ± 16.09^{a}	252.67 ± 98.62^{a}	436.00 ± 223.86^{a}	364.00 ± 95.54^{a}	517.33 ± 209.00^{a}	461.67 ± 100.43^{a}
Sirodotia	257.67 ± 93.18^{c}	412.00 ± 78.82^{bc}	329.33 ± 62.66^{bc}	540.33 ± 90.74^{ab}	417.00 ± 46.16^{bc}	676.67 ± 128.71^{a}	518.00 ± 59.63^{ab}
Thorea	224.67 ± 41.20^{c}	410.00 ± 142.31^{abc}	230.33 ± 64.38^{c}	515.00 ± 148.96^{ab}	315.67 ± 70.72^{bc}	631.00 ± 108.25^{a}	407.33 ± 34.96^{abc}
Tuomeya	156.33 ± 69.26^{a}	174.33 ± 57.19^{a}	125.67 ± 71.81^{a}	189.33 ± 134.52^{a}	180.00 ± 89.07^{a}	297.67 ± 203.08^{a}	217.33 ± 77.53^{a}
Total	**2006.33 ± 579.12^{c}**	**3220.00 ± 667.96^{c}**	**3461.67 ± 903.55^{c}**	**4318.33 ± 1013.55^{c}**	**4294.33 ± 969.65^{c}**	**5460.00 ± 1104.46^{c}**	**5380.67 ± 1075.73^{c}**
ZOOPLANKTON							
1 **Crustacea**							
Cyclops	841.67 ± 164.85^{a}	1541.67 ± 184.87^{a}	785.33 ± 59.47^{a}	7152.33 ± 9271.09^{a}	877.33 ± 76.17^{a}	13994.33 ± 10608.03^{a}	1039.00 ± 190.89^{a}
Daphnia	71.00 ± 23.30^{c}	131.67 ± 17.90^{c}	319.67 ± 58.02^{b}	299.33 ± 56.00^{b}	426.67 ± 51.68^{ab}	398.33 ± 51.68^{ab}	529.67 ± 64.66^{a}
Diaphanosoma	95.67 ± 52.44^{c}	140.00 ± 15.72^{c}	227.67 ± 56.15^{bc}	316.67 ± 69.40^{ab}	316.00 ± 45.53^{ab}	437.00 ± 33.78^{a}	414.33 ± 45.35^{a}

	Diaptomus	51.00 ± 11.79^{c}	83.33 ± 28.10^{bc}	137.67 ± 49.12^{bc}	128.67 ± 16.65^{bc}	193.00 ± 70.76^{ab}	195.33 ± 34.67^{ab}	288.33 ± 65.62^{a}
	Nauplius	922.67 ± 48.34^{bc}	1531.33 ± 329.07^{ab}	430.67 ± 222.15^{c}	1898.67 ± 482.33^{a}	525.33 ± 202.12^{c}	2182.33 ± 552.36^{a}	614.00 ± 212.05^{bc}
	Total	**1982.00 ± 300.72^{a}**	**3428.00 ± 575.65^{ab}**	**1901.00 ± 444.90^{a}**	**9795.67 ± 9895.48^{ab}**	**2338.33 ± 446.26^{a}**	**17207.33 ± 11280.52^{a}**	**2885.33 ± 578.57^{ab}**
2	**Rotifera**							
	Asplanchna	326.00 ± 60.11^{cd}	327.00 ± 60.83^{cd}	186.67 ± 41.30^{d}	526.00 ± 31.76^{ab}	276.33 ± 73.38^{cd}	634.33 ± 65.58^{a}	395.67 ± 52.27^{bc}
	Brachionus	477.00 ± 62.45^{a}	524.00 ± 74.48^{a}	485.67 ± 314.87^{a}	725.33 ± 145.45^{a}	586.33 ± 328.49^{a}	841.67 ± 134.89^{a}	732.00 ± 454.82^{a}
	Filinia	217.33 ± 43.02^{c}	285.33 ± 60.87^{bc}	194.33 ± 54.50^{c}	409.33 ± 55.19^{ab}	275.00 ± 68.43^{bc}	530.67 ± 62.93^{a}	398.00 ± 51.97^{ab}
	Keratella	212.67 ± 77.44^{a}	300.00 ± 155.21^{a}	311.33 ± 102.05^{a}	411.33 ± 165.21^{a}	425.00 ± 120.68^{a}	540.33 ± 161.66^{a}	517.33 ± 155.60^{a}
	Lecane	99.00 ± 75.48^{c}	239.67 ± 91.84^{bc}	322.00 ± 75.36^{abc}	374.33 ± 120.01^{ab}	416.67 ± 45.72^{ab}	501.00 ± 116.05^{a}	509.67 ± 47.65^{a}
	Polyarthra	97.33 ± 27.68^{b}	142.67 ± 48.21^{b}	323.67 ± 112.12^{ab}	290.33 ± 68.07^{ab}	427.00 ± 121.66^{a}	392.33 ± 55.52^{a}	512.33 ± 102.91^{a}
	Trichocerca	97.00 ± 24.64^{a}	206.67 ± 58.96^{ca}	254.33 ± 114.02^{bca}	417.33 ± 55.77^{abc}	360.00 ± 111.97^{abc}	570.33 ± 115.52^{a}	476.33 ± 113.42^{ab}
	Total	**1526.33 ± 370.81^{a}**	**2025.33 ± 550.40^{ca}**	**2078.00 ± 814.22^{ca}**	**3154.00 ± 641.46^{a}**	**2766.33 ± 870.33^{ac}**	**4010.67 ± 712.15^{a}**	**3541.33 ± 978.64^{ab}**
	G. Total	**36920.33 ± 6066.39^{a}**	**51814.00 ± 7312.33^{ca}**	**38687.67 ± 9070.45^{a}**	**73697.33 ± 22364.98^{ab}**	**49767.67 ± 11402.15^{ca}**	**90548.00 ± 24764.43^{b}**	**62236.33 ± 12534.39^{ac}**

Results from Tukey One-way ANOVA

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

Abundance of periphyton (cells or colonies / l) during the culture period of experiment. Numbers are means of three plots per treatment ± SD.

	Group / Genus	1 B	1 K	2 B	2 K	2.5 B	2.5 K
	PHYTOPLANKTON						
1	**Chlorophyceae**	Avg	Avg	Avg	Avg	Avg	Avg
	Actinastrum	1024.33 ± 203.16^{ab}	702.00 ± 149.97^{b}	1260.00 ± 95.06^{a}	968.33 ± 105.01^{ab}	1316.00 ± 56.43^{a}	1059.33 ± 169.46^{ab}
	Ankistrodesmus	1587.67 ± 290.34^{a}	1236.67 ± 392.53^{a}	2028.33 ± 385.22^{a}	2335.00 ± 407.91^{a}	2511.67 ± 1030.74^{a}	2166.67 ± 698.00^{a}
	Chlorella	13064.00 ± 3064.87^{ab}	4444 ± 5996.38^{b}	19139.67 ± 3021.53^{a}	20978.33 ± 4421.81^{a}	19176.33 ± 3854.00^{a}	20118.33 ± 5635.08^{a}
	Closterium	1558.00 ± 636.38^{a}	5339.67 ± 6333.33^{a}	1843.67 ± 624.21^{a}	2916.00 ± 673.37^{a}	2027.00 ± 733.69^{a}	1896.67 ± 325.05^{a}
	Cosmarium	445.00 ± 111.95^{b}	400.00 ± 127.42^{b}	657.67 ± 96.44^{ab}	901.00 ± 57.17^{a}	769.67 ± 76.16^{ab}	912.00 ± 252.06^{a}
	Crucigenia	184.33 ± 73.89^{b}	168.67 ± 71.28^{b}	300.33 ± 57.74^{ab}	514.33 ± 135.43^{a}	331.00 ± 103.79^{ab}	229.33 ± 170.05^{ab}
	Gonatozygon	1243.00 ± 256.84^{a}	1046.67 ± 166.90^{a}	1640.67 ± 252.71^{a}	1930.00 ± 456.63^{a}	1776.00 ± 268.10^{a}	1724.00 ± 571.21^{a}
	Oocystis	900.33 ± 231.80^{a}	800.33 ± 208.41^{a}	1200.00 ± 369.18^{a}	1399.67 ± 449.34^{a}	1366.67 ± 398.50^{a}	1271.67 ± 363.97^{a}
	Pediastrum	658.67 ± 83.58^{b}	582 ± 62.22^{b}	918.00 ± 210.82^{ab}	1657.00 ± 673.89^{a}	1082.67 ± 207.21^{ab}	1119.33 ± 147.71^{ab}
	Synedra	478.00 ± 145.12^{b}	389.67 ± 189.02^{b}	693.33 ± 175.82^{ab}	931.67 ± 58.62^{a}	762.33 ± 199.24^{ab}	783.00 ± 167.31^{ab}
	Treubaria	433.00 ± 85.63^{bc}	374.67 ± 108.61^{c}	645.67 ± 114.25^{bc}	951.33 ± 81.74^{a}	804.67 ± 140.80^{a}	711.33 ± 163.40^{ab}
	Tetraedron	1354.33 ± 105.03^{a}	1173.33 ± 128.07^{a}	1733.33 ± 119.97^{a}	2036.00 ± 73.02^{a}	2101.00 ± 230.36^{a}	5628.33 ± 5832.61^{a}
	Zygnema	1152.00 ± 190.20^{a}	1015.67 ± 160.62^{a}	1390.67 ± 235.39^{a}	1596.67 ± 323.79^{a}	1686.67 ± 259.10^{a}	1833.67 ± 855.38^{a}
	Total	**24082.67 ± 5478.80^{ab}**	**17673.33 ± 14094.77^{a}**	**33451.33 ± 5758.34^{ab}**	**39115.33 ± 7917.72^{a}**	**35711.67 ± 7558.12^{ab}**	**39453.67 ± 15351.29^{a}**
2	**Cyanophyceae**						
	Aphanocapsa	1257.00 ± 95.39^{a}	993.00 ± 29.46^{a}	1613.67 ± 40.41^{a}	1500.67 ± 321.25^{a}	1592.33 ± 319.69^{a}	1487.00 ± 836.84^{a}
	Chroococcus	924.33 ± 197.79^{a}	846.00 ± 174.73^{a}	1157.00 ± 200.75^{a}	1060.67 ± 196.40^{a}	1599.00 ± 325.27^{a}	856.33 ± 556.33^{a}
	Gloetrichia	1160.67 ± 199.85^{a}	1052.33 ± 190.71^{a}	1277 ± 176.10^{a}	1068.00 ± 200.50^{a}	1371.33 ± 104.57^{a}	1172.67 ± 64.38^{a}
	Gomphosphaeria	1117.00 ± 246.27^{a}	976.00 ± 156.69^{a}	13133 ± 10881.84^{a}	11288.33 ± 9279.55^{a}	14341.00 ± 11498.32^{a}	12419.33 ± 10002.11^{a}
	Lyngbya	12534.67 ± 2276.31^{a}	7824.33 ± 6118.63^{a}	16836.00 ± 3110.90^{a}	15336.00 ± 3532.36^{a}	17990.67 ± 3831.29^{a}	15324.00 ± 13339.53^{a}
	Microcystis	18945.33 ± 1416.62^{a}	15231.67 ± 3162.86^{a}	22246.67 ± 3105.94^{a}	22316.00 ± 1478.82^{a}	24785.33 ± 1904.14^{a}	19075.33 ± 9226.44^{a}
	Oscillatoria	921.33 ± 227.96^{ab}	684 ± 260.22^{b}	1342.67 ± 361.02^{ab}	1119.67 ± 347.86^{ab}	1733.67 ± 565.99^{a}	1720.33 ± 412.34^{ab}
	Rivularia	2099.67 ± 762.46^{a}	2007.33 ± 743.33^{a}	4147.33 ± 1661.03^{a}	3192.00 ± 1377.76^{a}	5769.67 ± 1982.95^{a}	3940.67 ± 2130.44^{a}
	Total	**38960.00 ± 5422.76^{ab}**	**29614.67 ± 10836.63^{a}**	**61753.33 ± 19537.99^{ab}**	**56881.33 ± 16734.51^{ab}**	**69183.00 ± 20532.22^{a}**	**55995.67 ± 36568.40^{ab}**
3	**Cryptophyceae**						
	Chroomonas	881.33 ± 135.07^{a}	806.67 ± 141.95^{a}	1297.33 ± 363.65^{a}	995.67 ± 255.21^{a}	1460.33 ± 326.52^{a}	1355.33 ± 213.74^{a}
	Cryptomonas	757.33 ± 62.07^{bc}	681.33 ± 87.20^{c}	1024.33 ± 125.26^{abc}	931.00 ± 118.65^{bc}	1326.00 ± 224.69^{bc}	1082.00 ± 104.89^{ab}
	Total	**1638.67 ± 197.14^{bc}**	**1488.00 ± 229.15^{c}**	**2321.67 ± 488.91^{ab}**	**1926.67 ± 373.87^{bc}**	**2786.33 ± 551.21^{a}**	**2437.33 ± 318.62^{ab}**
4	**Crysophyceae**						
	Botrydiopsis	1023.00 ± 202.19^{b}	926.33 ± 205.10^{b}	1327.33 ± 153.11^{ab}	1133.67 ± 155.05^{ab}	1500.67 ± 115.76^{a}	1363.67 ± 106.04^{ab}
	Bumilleriopsis	4689.00 ± 6632.84^{a}	899 ± 237.72^{a}	5974.33 ± 8416.86^{a}	4084.67 ± 5333.45^{a}	5163.33 ± 6253.05^{a}	4338.67 ± 4684.91^{a}
	Chlorothecium	754.00 ± 107.09^{a}	675.67 ± 95.11^{a}	992.33 ± 152.59^{a}	784.00 ± 165.98^{a}	1108.67 ± 218.82^{a}	1083.67 ± 427.01^{a}
	Dinobryon	728.33 ± 103.47^{a}	630.67 ± 91.28^{a}	947.67 ± 254.18^{a}	822.67 ± 168.17^{a}	1188.67 ± 205.46^{a}	1065.67 ± 407.14^{a}

	Heterococcus	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Lauvenia	571.33 ± 104.15^{a}	477.67 ± 115.80^{a}	822.33 ± 168.05^{a}	696.00 ± 201.67^{a}	948.67 ± 185.48^{a}	692.67 ± 127.59^{a}
	Perone	271.67 ± 73.19^{b}	234.67 ± 57.74^{b}	622.67 ± 216.55^{ab}	424.67 ± 170.87^{ab}	785.67 ± 166.61^{a}	1271.00 ± 572.07^{a}
	Uroglenopsis	974.33 ± 124.16^{a}	877.33 ± 120.83^{a}	1258.00 ± 100.50^{a}	999.00 ± 129.85^{a}	1500.33 ± 115.47^{a}	1136.33 ± 723.52^{a}
	Vaucheria	686.00 ± 157.04^{a}	609 ± 131.14^{a}	956.33 ± 373.17^{a}	692.00 ± 302.01^{a}	1132.67 ± 379.65^{a}	
	Total	**10311.67 ± 7703.43^{a}**	**5785.33 ± 1227.63^{a}**	**14050.33 ± 10021.94^{a}**	**10644.33 ± 6827.88^{a}**	**14772.00 ± 7990.66^{a}**	**13228.00 ± 7509.61^{a}**
5	**Euglenophyceae**						
	Colacium	836.00 ± 140.87^{b}	726.33 ± 146.47^{b}	1188.00 ± 204.27^{ab}	1025.00 ± 202.26^{ab}	1500.00 ± 237.69^{a}	1371.33 ± 183.05^{a}
	Euglena	450.33 ± 106.93^{a}	436.67 ± 168.84^{a}	711.67 ± 131.93^{a}	699.33 ± 89.49^{a}	818.33 ± 169.34^{a}	616.67 ± 335.33^{a}
	Phacus	1430.33 ± 173.10^{ab}	1186.67 ± 71.14^{b}	1813.67 ± 156.95^{a}	1598.67 ± 324.86^{ab}	1855.67 ± 284.59^{a}	1303.67 ± 106.04^{ab}
	Trachalomonas	7568.33 ± 2099.57^{a}	6960.00 ± 1809.65^{a}	10557.33 ± 1558.64^{a}	5793.00 ± 4042.06^{a}	11616.67 ± 3647.82^{a}	9593.67 ± 2637.93^{a}
	Total	**10285.00 ± 2520.47^{a}**	**9309.67 ± 2196.11^{a}**	**14270.67 ± 2051.80^{a}**	**9116.00 ± 4658.67^{a}**	**15790.67 ± 4339.44^{a}**	**12885.33 ± 3262.35^{a}**
6	**Pyrrhophyceae**						
	Amphidinium	1096.33 ± 177.20^{a}	1020.33 ± 147.67^{a}	1210.00 ± 46.77^{a}	1046.00 ± 83.45^{a}	1363.67 ± 202.01^{a}	1182.67 ± 304.79^{a}
	Ceratium	448.67 ± 76.84^{a}	375 ± 142.17^{a}	695.00 ± 57.03^{a}	480.67 ± 51.19^{a}	729.33 ± 179.16^{a}	564.00 ± 298.51^{a}
	Cystodinium	1066.00 ± 262.87^{a}	921.33 ± 210.74^{a}	1235.67 ± 237.18^{a}	992.00 ± 203.61^{a}	1556.67 ± 317.63^{a}	1461.00 ± 564.16^{a}
	Glenodinium	825.33 ± 135.49^{a}	703.00 ± 143.67^{a}	1055.67 ± 166.71^{a}	895.00 ± 188.61^{a}	1299.33 ± 307.14^{a}	1184.67 ± 542.31^{a}
	Gloeodinium	973.33 ± 79.69^{b}	859.33 ± 106.52^{b}	1166.33 ± 200.00^{ab}	920.33 ± 196.51^{b}	1670.33 ± 91.69^{a}	1659.67 ± 518.56^{a}
	Gonyaulax	865.00 ± 116.89^{a}	685.67 ± 156.87^{a}	1079.00 ± 97.00^{a}	862.67 ± 106.57^{a}	1468.67 ± 178.70^{a}	1549.67 ± 886.63^{a}
	Gyrodinium	728.33 ± 151.28^{a}	612 ± 148.36^{a}	859.67 ± 106.57^{a}	711.33 ± 127.03^{a}	1166.33 ± 202.00^{a}	1123.67 ± 476.91^{a}
	Hemidinium	860.00 ± 105.53^{a}	720.67 ± 55.16^{a}	1056.00 ± 156.76^{a}	881.00 ± 143.42^{a}	1356.00 ± 289.94^{a}	1219.00 ± 644.63^{a}
	Hypnodinium	573.67 ± 120.14^{a}	527 ± 201.78^{a}	686.33 ± 156.67^{a}	571.67 ± 140.36^{a}	877.33 ± 251.90^{a}	1096.33 ± 407.61^{a}
	Massartia	729.67 ± 150.98^{a}	577.33 ± 56.22^{a}	896.33 ± 58.62^{a}	685.33 ± 149.75^{a}	1064.00 ± 266.85^{a}	1125.00 ± 649.12^{a}
	Oodinium	822.67 ± 168.84^{a}	825.00 ± 61.83^{a}	1067.00 ± 264.58^{a}	850.67 ± 283.83^{a}	1096.00 ± 421.79^{a}	1170.67 ± 616.55^{a}
	Peridinium	653.67 ± 192.98^{a}	596.67 ± 164.41^{a}	889.33 ± 291.57^{a}	764.67 ± 275.83^{a}	1323.00 ± 411.79^{a}	1299.67 ± 736.14^{a}
	Urococcus	728.33 ± 150.15^{a}	664.33 ± 110.42^{a}	896.33 ± 56.00^{a}	727.33 ± 145.11^{a}	1200.00 ± 257.09^{a}	1227.00 ± 394.82^{a}
	Total	**10371.00 ± 1888.88^{a}**	**9087.67 ± 1705.81^{a}**	**12792.67 ± 1895.44^{a}**	**10388.67 ± 2095.26^{a}**	**16170.67 ± 3377.68^{a}**	**15863.00 ± 7040.76^{a}**
7	**Rhodophyceae**						
	Asterocystis	600.67 ± 126.06^{a}	508.00 ± 122.56^{a}	821.67 ± 167.56^{a}	673.00 ± 177.91^{a}	1008.67 ± 243.92^{a}	1051.33 ± 646.81^{a}
	Audouinella	726.33 ± 255.63^{a}	695.67 ± 219.51^{a}	1087.67 ± 303.40^{a}	902.00 ± 305.40^{a}	1189.33 ± 329.91^{a}	1208.33 ± 561.48^{a}
	Bangia	729.33 ± 151.51^{a}	647.00 ± 162.73^{a}	896.33 ± 117.20^{a}	726.00 ± 144.36^{a}	1020.33 ± 192.97^{a}	1043.33 ± 505.92^{a}
	Batrachospermum	201.33 ± 129.33^{a}	166.00 ± 115.17^{a}	486.00 ± 256.92^{a}	335.33 ± 208.33^{a}	651.33 ± 137.23^{a}	292.33 ± 245.08^{a}
	Bostrychia	552.33 ± 80.60^{a}	458.00 ± 94.40^{a}	795.67 ± 205.26^{a}	653.67 ± 173.78^{a}	942.00 ± 380.22^{a}	780.00 ± 374.34^{a}
	Compsopogon	464.33 ± 99.20^{a}	392.33 ± 94.33^{a}	650.00 ± 197.82^{a}	510.67 ± 159.63^{a}	933.67 ± 404.15^{a}	724.00 ± 450.12^{a}
	Lemanea	457.33 ± 89.50^{a}	411.33 ± 92.06^{a}	655.67 ± 189.69^{a}	458.00 ± 165.63^{a}	957.00 ± 373.23^{a}	892.00 ± 295.65^{a}
	Porphyridium	627.00 ± 206.64^{a}	541.33 ± 187.29^{a}	912.33 ± 253.48^{a}	771.33 ± 210.42^{a}	937.33 ± 386.40^{a}	879.67 ± 229.82^{a}
	Sirodotia	560.33 ± 92.92^{a}	457.33 ± 105.40^{a}	837.33 ± 141.89^{a}	687.67 ± 157.25^{a}	1022.67 ± 418.10^{a}	923.33 ± 320.15^{a}
	Thorea	483.00 ± 78.31^{a}	444.67 ± 56.58^{a}	678.33 ± 247.40^{a}	596.67 ± 145.14^{a}	934.33 ± 464.01^{a}	668.33 ± 654.68^{a}

	Tuomeya	193.33 ± 70.29^{bc}	148 ± 48.57^{c}	465.67 ± 100.03^{ab}	382.33 ± 123.13^{bc}	761.67 ± 158.41^{a}	718.00 ± 147.16^{a}
	Total	**5595.33 ± 1379.98^{cd}**	**4869.67 ± 1298.58^{d}**	**8286.67 ± 2180.66^{abc}**	**6696.67 ± 1970.97^{bcd}**	**10358.33 ± 3488.53^{a}**	**9180.67 ± 4431.21^{ab}**
	ZOOPLANKTON						
1	**Crustacea**						
	Diaphanosoma	493.67 ± 140.12^{a}	466 ± 101.50^{a}	513.33 ± 374.07^{a}	387.67 ± 232.17^{a}	611.00 ± 426.47^{a}	487.00 ± 373.30^{a}
	Diaptomus	147.67 ± 10.02^{ab}	124.00 ± 13.53^{b}	300.00 ± 55.43^{ab}	190.33 ± 59.97^{ab}	379.00 ± 127.59^{a}	308.67 ± 150.07^{ab}
	Monostyla	5655.33 ± 7881.60^{a}	4526.33 ± 6204.23^{a}	7597.33 ± 9889.56^{a}	6526.33 ± 7844.16^{a}	4706.67 ± 4416.16^{a}	4500.67 ± 4791.60^{a}
	Nauplius	109.00 ± 47.32^{a}	91.33 ± 46.88^{a}	297.33 ± 244.83^{a}	175.33 ± 174.09^{a}	406.67 ± 267.91^{a}	348.00 ± 190.55^{a}
	Total	**6405.67 ± 8079.05^{a}**	**5207.67 ± 6366.14^{a}**	**8708.00 ± 10563.89^{a}**	**7279.67 ± 8310.39^{a}**	**6103.33 ± 5238.13^{a}**	**5644.33 ± 5505.52^{a}**
2	**Rotifera**						
	Asplanchna	240.33 ± 106.93^{ab}	200.00 ± 77.97^{b}	509.00 ± 135.54^{ab}	413.67 ± 160.86^{ab}	651.00 ± 111.53^{a}	480.33 ± 260.66^{ab}
	Brachionus	472.33 ± 96.55^{a}	364.33 ± 85.80^{a}	600.67 ± 118.74^{a}	442.00 ± 180.55^{a}	637.33 ± 198.31^{a}	637.00 ± 108.50^{a}
	Filinia	51.00 ± 30.81^{a}	23.33 ± 15.95^{a}	99.67 ± 59.94^{a}	89.00 ± 67.55^{a}	168.00 ± 95.50^{a}	148.33 ± 127.64^{a}
	Keratella	240.00 ± 158.65^{a}	159.67 ± 69.51^{a}	440.33 ± 219.39^{a}	327.33 ± 196.57^{a}	548.67 ± 266.99^{a}	400.33 ± 152.75^{a}
	Lecane	187.33 ± 52.73^{a}	163.00 ± 91.00^{a}	423.00 ± 139.07^{a}	376.00 ± 219.01^{a}	591.67 ± 250.01^{a}	450.67 ± 232.92^{a}
	Polyarthra	186.67 ± 72.53^{b}	131.67 ± 16.62^{b}	427.67 ± 69.87^{ab}	353.33 ± 79.39^{ab}	708.33 ± 228.51^{a}	687.00 ± 206.57^{a}
	Trichocerca	264.67 ± 75.08^{ab}	180 ± 61.88^{b}	524.00 ± 74.48^{ab}	540.33 ± 161.66^{ab}	655.33 ± 198.86^{a}	514.67 ± 256.54^{ab}
	Total	**1642.33 ± 593.27^{c}**	**1222 ± 418.73^{c}**	**3024.33 ± 817.03^{a}**	**2541.67 ± 1065.58^{a}**	**3960.33 ± 1349.71^{a}**	**3318.33 ± 1345.59^{ab}**
	G. Total	**109292.33 ± 33263.79^{a}**	**84258.00 ± 38373.55^{a}**	**158659.00 ± 53316.01^{a}**	**144590.33 ± 49954.83^{a}**	**174836.33 ± 54425.69^{a}**	**158006.33 ± 81333.34^{a}**

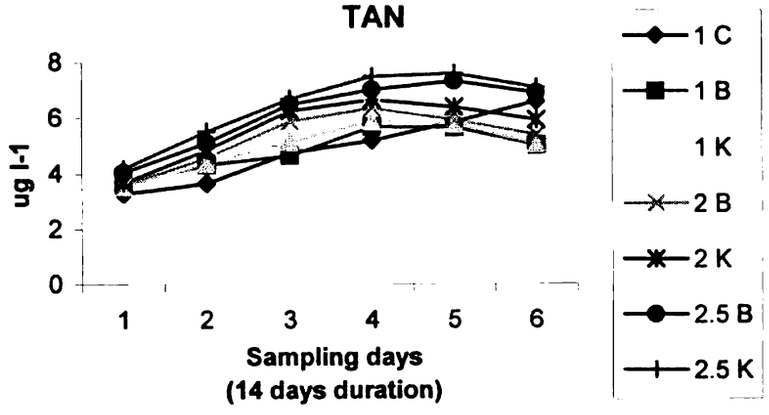
Results from Tukey One-way ANOVA

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

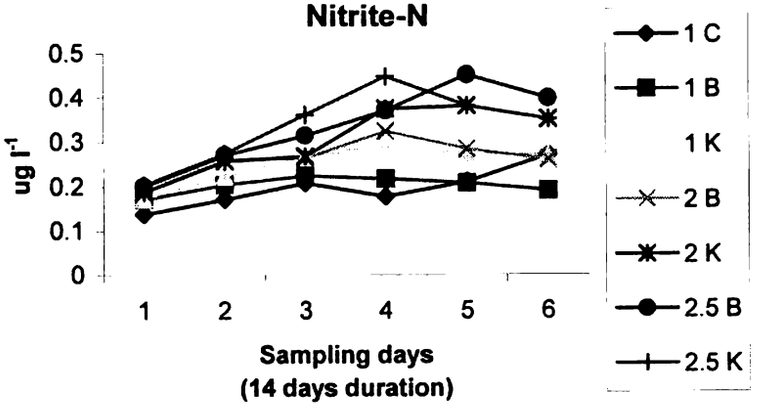
Fig. 7.1

The effect of fertilization and periphyton development on the water quality parameters

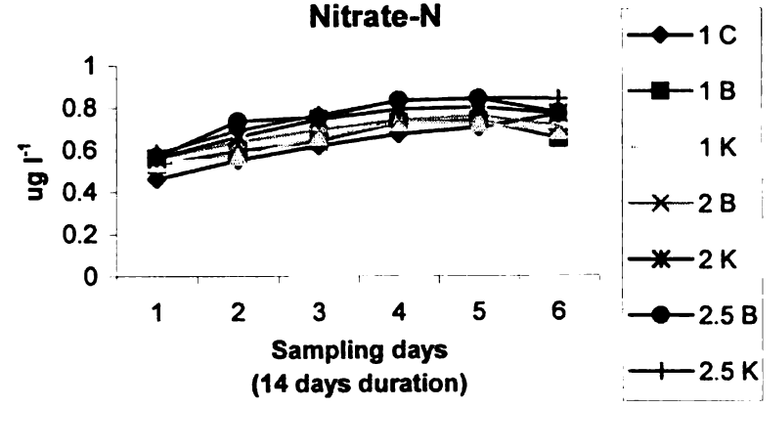
(a)



(b)



(c)



(d)

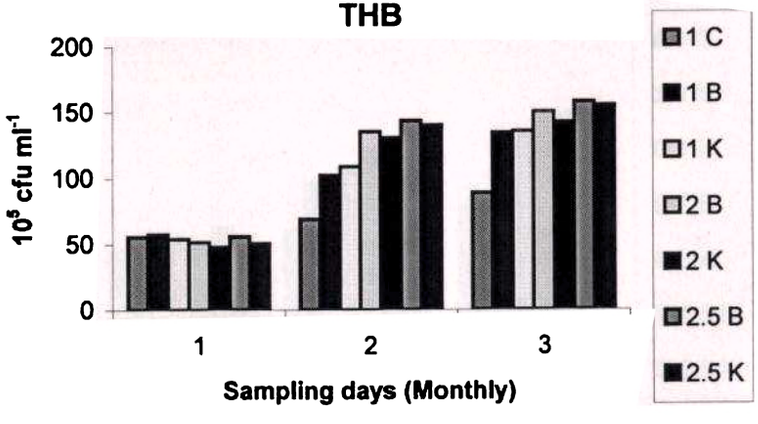
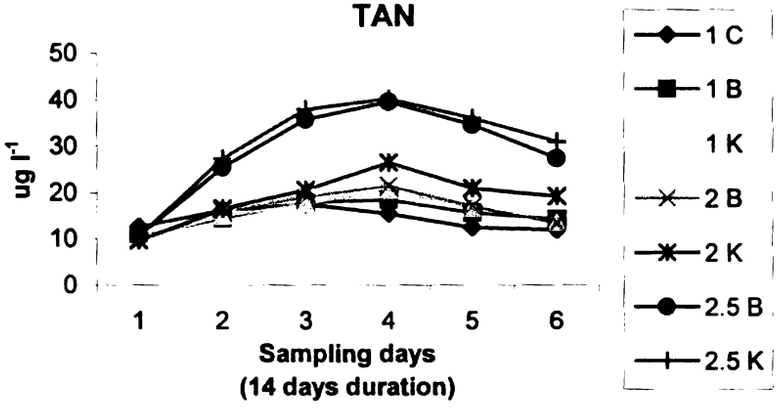


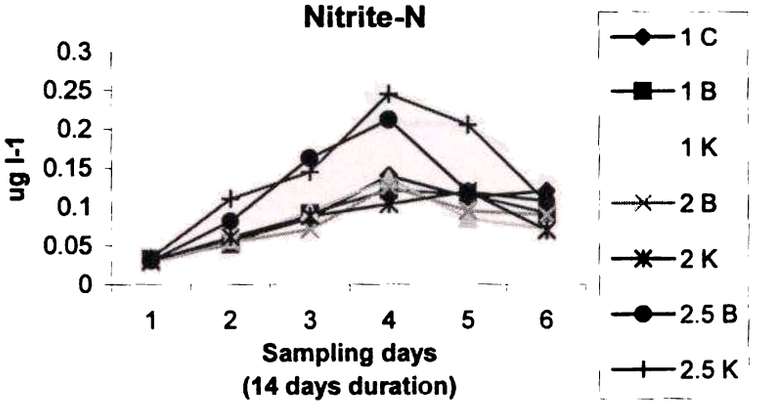
Fig. 7.2

The effect of fertilization and periphyton development on the sediment qauality parameters

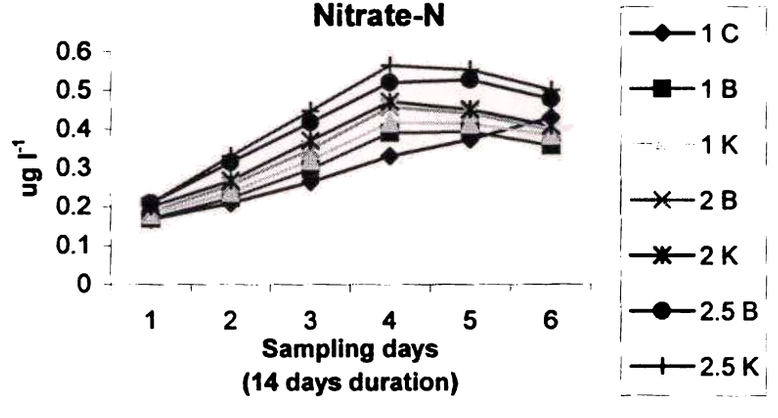
(a)



(b)



(c)



(d)

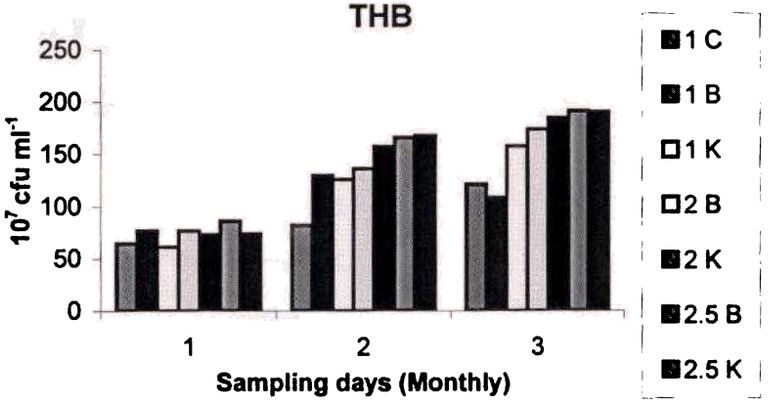
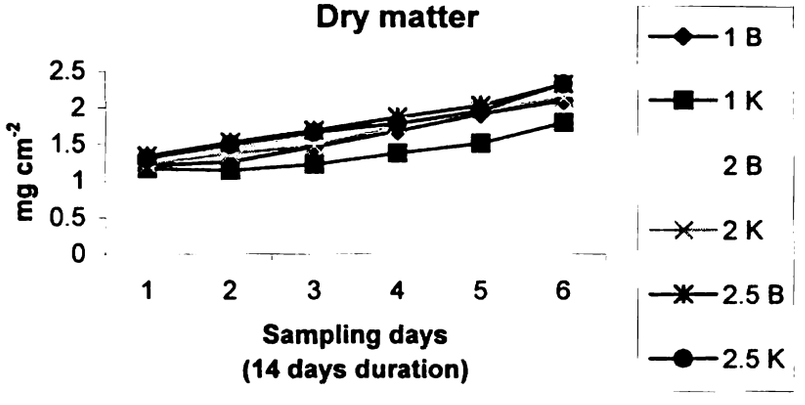


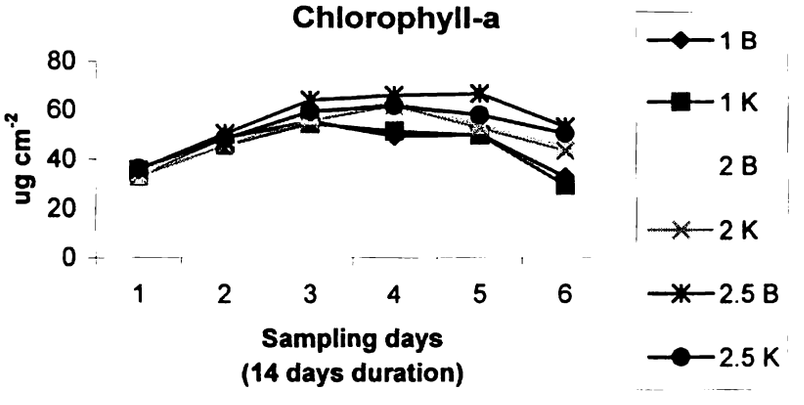
Fig. 7.3

Periphyton and phytoplankton biomass under different fertilization levels

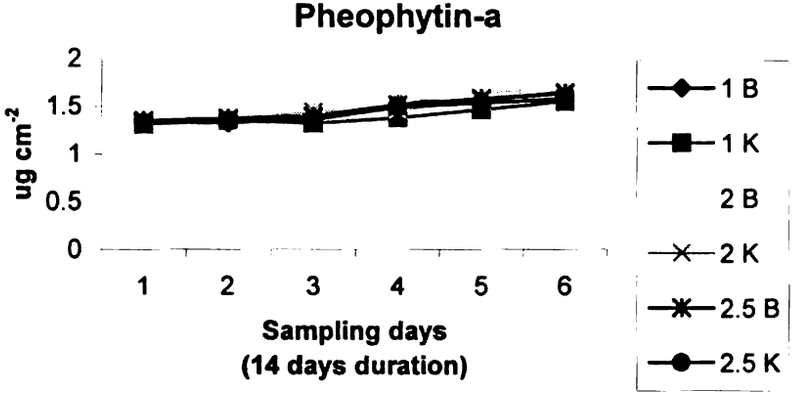
(a)



(b)



(c)



Chapter – 8

Effects of periphyton and addition of carbohydrate in the production and sustainability of *Penaeus monodon* (Fabricius)

1. Introduction

Penaeus monodon is the most widely farmed shrimp species in Southeast Asia under different farming practices. In recent years shrimp aquaculture production in Asia is suffering from a number of problems. One of the major factors confronting sustainable shrimp aquaculture is the rampant outbreak of diseases due to the accumulation of inorganic nitrogen in the pond bottom and the consequent environmental degradation and obsolete grow-out management practices (Primavera, 1998). The low survival and poor shrimp productivity are the consequent effects (de Graaf and Xuan, 1998; Johnston et al., 1999, 2000a). Ammonia-N is a highly toxic compound because it can easily cross most of the biological membranes and thus causing pH alterations which may reduce survival rates and impair various physiological mechanisms (Schmidt-Nielson, 1983; Campbell, 1991). The brackishwater shrimp aquaculture has been widely accepted an avocation for improving the financial benefits and poverty alleviation of local communities (Johnston et al., 2000b). The development of low cost technologies and their farm level adoption would help in improving the sustainability of aquaculture production and also mitigating risk associated with farming (Samocha et al., 1993; Peterson and Griffith, 1999). The costs of formulated feed coupled with labour required for feeding are major components for the increasing cost of shrimp

production (Lawrence and Lee, 1997). Optimizing the feeding strategy is a prime consideration in intensive shrimp pond management which involves knowledge on nutritional requirements, feed formulation and feed management. It is well known that the nutrient content of the feed will influence growth, survival and the amount of metabolic and excreted waste products entering the system (Smith et al., 2002). A major concern is the discharge of nutrients from shrimp farms into coastal waters, with the potential to contribute to the recurring algal blooms, oxygen depletion of bottom waters and reduced biodiversity. Most of the nutrients discharged from intensive shrimp farms originate from the formulated feed (Funge-Smith and Briggs, 1998). Therefore, efforts to improve feeding strategies must focus on both optimizing production and minimizing waste.

Periphyton based aquaculture is recognized as one of the essential means of increasing shrimp production and therefore artificial substrates have been added to the culture system for facilitating the periphyton production. The term 'periphyton' is applied to the complex of sessile biota attached to submerged substrata such as stones and sticks, and includes algae and invertebrates but also the associated detritus and microorganisms (Welcomme, 1972; Hem and Avit, 1994). The added surface area provided by the substrates enhance the colonization of epiphytic biota, which in turn provides a natural food supplement for the shrimp (Moss and Moss, 2004;

Burford et al., 2004a). Shrimps generally require food sources such as benthic animals, benthic algae, algal detritus or plant fodder, which can be harvested more efficiently (Dempster et al., 1993; Yakupitiyage, 1993). According to Taghon (1982), benthic invertebrates were able to ingest microscopic glass beads when they were coated with proteins. Information on shrimp diet of *Penaeus* species are enormous (Tiews et al., 1976; Marte, 1980, 1982; Smith et al., 1992) indicating that shrimps feed mostly on small planktons and zoobenthos. *Penaeus monodon also* have the ability to select these foods (Apud et al., 1981; El Hag, 1984).

Shrimp farming has undergone rapid development from traditional, extensive rearing systems to a large scale industrial and commercial production, and therefore considerable research efforts were made to develop artificial cheap protein rich diets. However, the knowledge on the contribution of such diets to shrimp growth in ponds is very scarce. To improve the sustainability of shrimp production, addition of organic carbon rich substrates (glucose, cassava and cellulose) to control the carbon / nitrogen ratio was attempted (Avnimelech, 1999). This technology was applied for the reduction of dissolved inorganic nitrogen in extensive and intensive systems. The application of organic carbon sources for adjustments in the carbon / nitrogen ratio in the feed was well practiced in aerated and circulated shrimp and fish aquaculture ponds (Avnimelech et al.,

1989; Avnimelech, 1999; Browdy et al., 2001; Hari et al., 2004, 2006). On the otherhand, Pardue (1973), Hem and Avit (1994), Wahab et al. (1999a, b), Azim et al. (2002), Keshavanath and Wahab (2001) and Keshavanath et al. (2001a, b) reported that artificial substrate based culture will substantially increase the production of pond harvestable biomass where compared to grow-outs without substrates.

Against this backdrop, a further step ahead is attempted in the development of a technology that combines the advantages of substrate based system (Bamboo and Kanchi) and carbohydrate source addition in the farming of shrimp under extensive system of culture.

The objectives of the present study are as follows:

1. To evaluate the periphyton and phytoplankton quality and quantity in with and with out substrates as well as addition of carbohydrate source.
2. To monitor the water and soil quality parameters in various treatments with and with out substrate as well as carbohydrate addition.
3. To compare the shrimp production and yield in different treatments as mentioned above.
4. To explore the possibility of minimizing the protein content of the supplementary feed of shrimp both in favor of periphyton and carbohydrate in the grow-outs.

2. Materials methods

Experimental design

The experiments were carried out in outdoor concrete tanks having an effective bottom area of $6m^2$. The experimental tanks were provided with a uniform sediment layer (7 cm thick) collected from the pokkali shrimp farm. Lime was added initially at 3 kg $tank^{-1}$. Bamboo and kanchi were used as substrate (*Bambusa sp.*) with and without carbohydrate source while addition of carbohydrate and without substrate have used as control. The treatments were maintained triplicate using complete randomized design. To stimulate the phytoplankton bloom the tanks were fertilized with the dose of cattle dung, urea and super phosphate @ 3000, 150 and 100 kg ha^{-1} respectively (based on the results of chapter 7), the treatment with addition of carbohydrate here in called control as treatment CH, addition of carbohydrate with bamboo substrate treatment called as B + CH, addition of carbohydrate with kanchi substrate treatment called as K + CH, without addition of carbohydrate with bamboo substrate treatment called as B and without addition of carbohydrate source with kanchi treatment called as K. The experimental tanks were planted with bamboo poles (mean length - 2.0 m the effective water area in 1.5 m and mean diameter – 5.5 cm) vertically in to the pond bottom, at a density of 9 poles m^{-2} while kanchi was planted horizontally at the pond @ 34 kanchi poles m^{-2} (mean length – 2.0 m

and mean diameter – 1.5 cm). The tanks were filled with 22 ppt saline water. The water level in the culture tank was maintained at 100 cm during the culture period. Twenty days old *Penaeus monodon* post larvae (0.015 ± 0.01 g) purchased from a commercial hatchery were stocked in the culture tank at a density of 7 post larvae m^{-2}. Shrimps were daily fed with 25% dietary protein feed @ 15% of their initial biomass and adjusted gradually to 6% towards the end of the culture. The tapioca powder used as the carbohydrate source was purchased from local market. The pelleted 25% protein feed was distributed evenly over the tank surface twice daily at 08.00 and 18.00 hours. The required quantity of tapioca flour was mixed with tank water in a beaker and applied to the water column uniformly followed by the feeding in the morning.

The required quantity of tapioca flour on a daily basis was estimated following Avnimelech (1999), Hari et al. (2004, 2006). In the substrate installed tanks periphyton dry matter and % of nitrogen production were measured for calculating the amount of carbohydrate. The quantities of tapioca powder added to in various treatments during the period of experiment are shown in Table 8.1.

Harvesting

Shrimps were harvested on 80th day of culture by total draining of water. The individual length and weight were measured using a dial

reading caliper from the tip of the rostrum to the tip of telson. Specific growth rate, net shrimp yield, and individual shrimp yield were recorded during the sampling days.

Water and sediment quality parameters

Water quality parameters such as temperature (mercury thermometer), salinity (hand refractometer), secchi 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 (2 cm diameter). Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. The water samples were filtered through GF/C Whatman glass fiber filter 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 (5 day 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). Total Kjeldahl nitrogen in the periphyton was estimated (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 unit (cfu).

Determination of periphyton biomass

From each tank three poles were selected randomly and sample of periphyton from 2 x 2 cm^2 area was taken from different depths per poles. The samples were collected on biweekly basis between 09.00 and 10.00 hours. The areas were carefully scraped with the scalpel blade to remove the periphyton. After the sampling the poles were kept back in the original place. The materials collected were pre weighed and dried at 105^{0}C until constant weight and kept in a dessiccator. The samples were then transferred to a muffle furnace and ashed at 450^{0}C for 6 hour and weighed. The dry matter, acid free dry matter and ash content were determined by weight difference. APHA (1995) standard methods were used for the determination.

The periphyton–a concentrations were determined following standard method APHA (1995). After removal, the material was immediately transferred to a tube containing 10 ml 90% acetone, sealed and transferred to the refrigerator for storing overnight. Following the samples collected in the morning were homogenized for

30 seconds with a tissue grinder and centrifuged for 10 min at 3000 rpm. The supernatant was transferred to the cuvettes and then acidified by the addition of three drops of 0.1 *N* HCl and absorption measured for the periphyton–a.

Species composition of periphyton and plankton

The periphyton samples were taken from randomly selected poles 2 x 2 cm^2 samples each of different depth per pole and pooled together. The samples were collected on biweekly basis after the substrate installation. Pooled samples were preserved in 5% buffered formalin and after the vigorous shaking, a 1 ml of sub sample was transferred to a Sedgewick-Rafter cell (S-R cell), the number of colonies was counted on 10 randomly selected field of chamber under a binocular microscope (Azim et al., 2001). The periphyton sample densities were calculated by the formula.

$$N = (P \times C \times 100)/S$$

Where *N* = number of periphyton cells per cm^2 surface area; *P* = number of periphytic units counted in ten fields; *C* = volume of final concentrate of the sample (ml); *S* = area of scraped surface cm^2.

For the taxonomic identification of plankton, the samples were collected by passing 5 l of water taken from the four locations of each tank and filtered through a 45 µ mesh size plankton net. The concentrated sample was then transferred to a 100 ml measuring

cylinder and made up to 100 ml with distilled water. Then the samples were preserved with 5% formalin solution. The 1 ml of plankton sub samples were estimated using Sedgewick-Rafter cell (S-R cell). The plankton densities were calculated by the formula.

$$N = (P \times C \times 100)/L$$

Where N = the number of plankton cells per liter of original water; P = the number of plankton counted in the ten fields; C = the volume of the final concentration of the sample (ml); L = the volume (liters) of the tank water sample.

Taxa were identified to genus level using keys of Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976), Bellinger (1992) and Sreekumar (1996).

Statistical analysis

Statistical analysis of daily and biweekly water, sediment quality parameters and periphyton biomass (dry matter, chlorophyll-a, pheophytin-a, ash content and ash free dry matter) were done by ANOVA: Two-Factor without Replication were performed using Microsoft Excel 2000. The periphyton and plankton taxonomic data were analyzed by SPSS 11.5 Tukey-HSD test One-way ANOVA. The shrimp yield, individual shrimp weight, SGR and the survival rate were statistically performed by SPSS 11.5 Tukey-HSD test One-way

ANOVA. Significant treatment effect was separated by calculating the least significant difference at 5% level.

3. Results

Water quality parameters

In the periphyton based culture of shrimp, temperature, water pH, dissolved oxygen (DO), and salinity did not showed significant difference ($P>0.05$) between treatments while secchi disk reading and soil pH were shown significant difference ($P<0.05$) among the treatments. The highest secchi disk reading was observed in the treatment CH (55.27 ± 1.88 cm) while it was lowest in K (52.59 ± 1.45 cm). In the case of soil pH, it was highest in treatment K (8.52 ± 0.11) whereas it was lowest in CH (8.44 ± 0.10). Mean value with standard deviation of these parameters are shown in Table 8.2 & 8.3.

The mean and standard deviation of various water quality parameters studied such as biological oxygen demand (BOD), total ammonia nitrogen (TAN), nitrite-N, nitrate-N, total heterotrophic bacteria (THB), chlorophyll-a and alkalinity are presented in Table 8.3. The alkalinity did not show significant difference ($P>0.05$) among the treatments. Significantly highest BOD values were recorded in the treatment K (3.21 ± 0.12 mg l^{-1}) while it was lowest in B + CH treatment (2.97 ± 0.29 mg l^{-1}). The pattern of variation of BOD values in various treatments during the culture period is shown in Fig. 8.1a.

In respect of water TAN (4.67 ± 0.99 ug l^{-1}) and nitrite-N (0.36 ± 0.06 ug l^{-1}) significantly higher values were recorded in treatment K while it was lowest in treatment CH (TAN 4.04 ± 0.84 ug l^{-1} and nitrite-N 0.28 ± 0.05 ug l^{-1}) (Table 8.3). Conversely higher value of nitrate-N was observed in treatment B (0.57 ± 0.08 ug l^{-1}) while it was least in treatment CH (0.49 ± 0.03 ug l^{-1}) (Table 8.3). The pattern of variation of water TAN, nitrite-N and nitrate-N concentrations during the culture period are shown in Fig. 8.1b, c and d. With regard to total heterotrophic bacterial count and chlorophyll-a, significantly higher values were observed in the treatment B + CH (141.63 ± 44.17 x 10^5 cfu ml^{-1} and 51.14 ± 8.05 ug cm^{-2}) while it was lowest in B (120.85 ± 46.45 x 10^5 cfu ml^{-1}) and K (45.28 ± 7.34 ug cm^{-2}) (Table 8.3). The total heterotrophic bacterial population count in water in various treatments during the culture period is depicted in Fig. 8.1e.

While examining the temporal variation of biological oxygen demand, it can be seen that there was a reduction of this parameter towards the end, from 3.36 – 2.49 mg l^{-1}. Commensurate with the decrease of BOD, the quantity of unicellular organism (THB) showed an increase trend during the experimental period, which registered an increase from 60.06 to 186.10 x 10^5 cfu ml^{-1}. The inorganic nitrogen concentration (TAN, nitrite-N and nitrate-N) showed it peak level at the sampling period three and four (Fig.8.1) and thenceforth, a reduction was noted towards the end of culture.

Sediment quality parameters

The variation of sediment Total ammonia nitrogen (TAN), nitrite-N, nitrate-N, organic carbon and total heterotrophic bacterial production are shown in Table 8.3. The nitrite-N concentration (0.09 - 0.20 ug l^{-1}) did not show significant variation ($P>0.05$) between treatments. B and K treatments showed significantly higher ($P<0.05$) TAN (17.46 ± 4.70 ug l^{-1} in treatment K), nitrate-N (0.18 ± 0.06 ug l^{-1} in treatment K) and organic carbon (14.05 ± 2.71 ug l^{-1} in treatment K) concentrations during the culture (Table 8.3). Significantly higher soil heterotrophic bacterial population was seen in treatment B + CH (187.65 x 10^7 cfu ml^{-1}) while it was lowest in treatment K (137.08 x 10^7 cfu ml^{-1}). The pattern of variation in respect of TAN, nitrite-N, nitrate-N, THB and organic carbon concentrations during the culture period are depicted in Fig. 8.2a, b, c, d and e.

During the culture period, the inorganic nitrogen level of soil TAN showed its peak in the fourth sampling period (23.57 ug l^{-1}) while the highest nitrate-N was observed in the fourth sampling day (on 42 th day) (0.23 ug l^{-1}) (Fig. 8.2). The nitrite-N, organic carbon and heterotrophic bacterial population level showed a gradual increase with the progression of culture and the values recorded from various treatments were not uniform (Fig. 8.2).

Taxonomic composition of periphyton

The qualitative and quantitative variation of periphyton in the bamboo and kanchi treatments with addition of carbohydrate source and without addition of carbohydrate source are presented in Table 8.4. Treatment bamboo with addition of carbohydrate showed higher periphyton production (45560.74 cells or colonies l^{-1}) during the culture which was significantly different from other treatments ($P<0.05$) while the lowest periphyton production was observed in the treatment kanchi (16245.25 cells or colonies l^{-1}). The periphyton community comprised of members of 7 groups of phytoplankton and 2 groups of zooplankton. 72 genera of plankton were identified of which 61 genera were represented by phytoplankton whereas the representation zooplankton was very low with 11 genera. Details showing the genera of various phyto and zooplankton identified under various groups, numerical strength of plankton in various treatments during the culture period with mean and standard deviation are shown in Table 8.4. The group wise details of phytoplankton belonging to periphyton are Chlorophyceae (13 genus), Cyanophyceae (8 genus), Cryptophyceae (2 genus), Crysophyceae (10 genus), Euglenophyceae (4 genus), Pyrrhophyceae (13 genus), and Rhodophyceae (11 genus). Zooplankton cells genera were divided into Crustacea (4 genus) and Rotifera (7 genus). Chlorophyceae and

Pyrrhophyceae were the most predominant groups of phytoplankton encountered under periphyton.

Taxonomic composition of phytoplankton

The abundance of plankton by genera and group wise (in cells or colonies l^{-1}) in various treatments during the culture is presented in Table 8.5. 7 groups of phytoplankton and 2 groups of zooplankton were identified from each treatment. Highest plankton production was observed in treatment B + CH addition (20491.68 cells or colonies l^{-1}) while it was lowest in treatment K (4893.69 cells or colonies l^{-1}). Statistically significant difference ($P<0.05$) was observed in plankton production among the treatments. 76 genera of plankton communities were identified of which 64 genera belonged to phytoplankton cells and 12 genera belonged to zooplankton cells (Table 8.5). Details of phytoplankton genera identified under various groups are as follows: Chlorophyceae - 16 genus, Cyanophyceae - 6 genus, Cryptophyceae - 2 genus, Crysophyceae - 10 genus, Euglenophyceae - 5 genus, Pyrrhophyceae -14 genus, and Rhodophyceae - 11 genera. Zooplankton cells under Crustacea were represented by 5 genus while 7 genera were identified under Rotifera. Chlorophyceae appeared as the most dominant group in the present study followed by Pyrrhophyceae, Rhodophyceae and Crysophyceae.

Periphyton biomass

Periphyton biomass in each treatment was estimated and the results are presented in Table 8.6. Periphyton dry matter, chlorophyll-a, pheophytin-a, nitrogen (%) and ash content showed significant difference ($P<0.05$) between treatments. Highest value of periphyton biomass dry matter was observed in the treatment B + CH (1.44 ± 0.05 mg cm^{-2}) and the lowest was in treatment K (1.30 ± 0.06 mg cm^{-2}). Ash free dry matter did not show any significant difference ($P>0.05$) between the treatment, but the highest values were recorded in the treatment B + CH followed by K + CH. Pheophytin-a concentrations showed an increase over time (Fig. 8.3) and highest level was observed in treatment B + CH. The temporal variation of Periphyton biomass in various treatments are presented in Fig. 8.3.

Proximate composition such as protein and nitrogen of periphyton were analyzed and the results are gives in Table 8.6. Protein content ranged from 30.89% in Kanchi to 38.61% in B + CH. Ash content was highest in B + CH with 32.17% whereas in other treatments it was in the range 29.22 - 31.06% . Nitrogen content was lowest in treatment K with 4.79 while it was highest in treatment B + CH with 5.99%.

Harvested shrimp

The individual shrimp weight, net yield, SGR and survival rate in various treatments are shown in Table 8.7. One-way ANOVA results showed that the individual shrimp weight was significantly different ($P<0.05$) among treatments. The highest individual shrimp weight was recorded in treatment B + CH (27.48 g) while it was lowest in treatment CH (24.43 g). The highest SGR was recorded in treatment B + CH (7.51) whereas it was lowest (7.40) in treatment CH. Net shrimp yield did not show significant difference ($P>0.05$) between treatments, the maximum yield was observed in treatment B + CH (161.83 g m^{-2}) followed by treatment K + CH (150.50 g m^{-2}) while it was lowest in treatment CH (130.31 g m^{-2}). Interestingly, there was no significant difference ($P>0.05$) was observed in survival rate among various treatments. The highest survival rate was recorded in B + CH treatment (84.13%) while it was lowest in treatment CH (76.19%).

4. Discussion

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., 2004b) 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. In the present study, tapioca flour was used as carbohydrate source for the direct application to the culture system. The biodegradable substrates like sugarcane bagasse, paddy strew, dried water hyacinth (*Eichornia crassipes*), kanchi, PVC pipes and bamboo poles were used in the culture system as artificial substrates for the periphyton production. Among them, highest periphyton growth was concentrated on bamboo poles (Ramesh et al., 1999; Umesh et al., 1999; Azim, 2001; Keshavanath et al., 2001a; Azim et al., 2002; Joice et al., 2002; Mridula et al., 2003). In the present study, B + CH, K + CH addition and B, K were the four treatments used for the study.

With regards to water quality parameters of various treatments, no significant difference was observed in the case of dissolved oxygen (DO) concentration in any of the treatments and this may be attributed to the addition of aeration to the culture system. On the contrary, the biological oxygen demand (BOD) showed variations among the treatments and this would manifest the differential rates of consumption pattern of oxygen by the microorganisms in different treatments. Several studies proved the reduction of BOD level in substrate installed tanks compared to non substrates tank (Ramesh

et al., 1999; Umesh et al., 1999; Keshavanath et al., 2001a, b, 2002, 2004; Dharmaraj et al., 2002; Joice et al., 2002; Mridula et al., 2003). In the present study also the BOD values were comparatively low in the substrate added treatments, a finding which fully concurs with the above. However, the BOD values were extremely low in the treatments which were provided with both artificial substrata and addition of carbohydrate. Interestingly, in respect of total heterotrophic bacterial population, a glaring difference was registered between substrate installed and non substrate added treatments, showing higher values in substrate addition and it was highest in treatment having both the addition of substratum and carbohydrate. According to Kalpan et al. (1987), Bender and Phillips (2004) and Garcia-Meza et al. (2005) periphyton mat consist of a solid matrix embedded with bacteria, algae, protozoa, fungi, zooplankton and small invertebrates. A significant reduction in total ammonia nitrogen (TAN) in both the water and soil recorded in the treatments of CH, B + CH and K + CH was observed and it concurs with the findings of Avnimelech and Mokady (1988), Avnimelech et al. (1994, 1989), Avnimelech (1999) and Hari et al. (2004, 2006). Cotner et al. (2000) are of the view that the addition of low concentration of carbohydrate was useful in reducing TAN concentrations and results increasing of THB in both the water and soil column.

Results of the ANOVA show that there exist significant variations in the nitrite-N nitrate-N and chlorophyll-a concentrations among the treatments. In ponds and lakes, phytoplankton productivity is positively correlated with nutrient concentrations (Boyd, 1990) and in periphyton based ponds, this relationship is interfered with by competition and interactions between periphyton and phytoplankton. Higher chlorophyll-a content in substrates treatment indicates the phytoplankton production, which is an indication of the positive effect on plankton nutritional quality (Azim et al., 2002). In the present study, organic carbon concentration is high in without carbohydrate addition substrate treatment when compared to carbohydrate added treatments. In shrimp farms, organic carbon is produced from the accumulation of both organic matter from the unconsumed food metabolites and remains of dead organisms at the pond bottom (Grave-Lizarraga, 1995). Moriarty (1997) reported that most of the bacteria in a pond are saprophytic heterotrops, which are using organic matter for growth and energy by decomposing detritus.

The periphyton biomass production was measured as dry matter, chlorophyll-a, pheophytin-a, ash content and ash free dry matter. The results in respect of these parameters showed that substrates with addition of carbohydrate produced higher periphyton organisms with highest production B + CH treatment (Bamboo +

Carbohydrate addition). Keshavanath et al. (2001a) and Azim et al. (2001) reported that bamboo produced higher periphyton biomass when compared to other artificial substrates. According to Azim et al. (2001) microbial organisms and meio-macrofauna grown in the periphyton may also be utilized by cultured organism and therefore, the need for artificial feed can be substantially reduced by providing the substrates in aquaculture ponds. In the present study, 72 genera of periphyton planktons were identified from the treatments. The quantity of periphyton varied substantially with substrate type, fertilization level, environment conditions and taxonomic composition (Paine and Vadas, 1969; Heaper, 1988; Makarevich et al, 1993; Napolitano et al, 1996; Ledger and Hildrew, 1998; Huchettu et al, 2000; Keshavanath et al, 2001a). B + CH treatment showed higher quantity of periphyton which was significantly different, from the other treatments which was followed by K + CH. According to Heaper et al. (1989) the basic changes of plankton food production may due to the variation of nutrients and environmental changes of culture system. Dharmaraj et al. (2002) also reported that plankton production was higher in substrate plus feed treatment than in substrate alone treatment, attributing the difference to the manuring effect of unconsumed feed and the faecal matter. Dewan et al. (1991) and Ahmed (1993) established the relationship between phytoplankton production of chlorophyll-a with fertilization effect.

The individual shrimp weight and net shrimp yield was higher in treatment B + CH followed by K + CH though it was not significantly different. Azim et al. (2002) reported that bamboo poles substrate based periphyton growth will help to increase individual weight at harvest as well as net yield of cultured organism. The highest growth was probably related to the highest abundance of periphyton phytoplankton and zooplankton and the ability of organism to utilize (Martinez-Cordova et al., 1998). The higher growth rate and shrimp yield can also be attributed to the low inorganic levels (Wahab et al., 2003) and increased heterotrophic production in the carbohydrate added ponds (Avnimelech, 1999; Burford et al., 2003; Burford et al., 2004b). Furthermore, lower TAN levels in water and sediment also positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). Among the treatments, there was no significant difference in the survival rate of shrimp though higher survival rate was observed in treatment B + CH followed by K + CH. This can be attributed to the improved water quality parameters primarily associated with the consistency of culture pond (Miller, 1976; Parker and Davis, 1981; Ayinla et al., 1994; Johnston et al., 2000b). The substrate with addition of carbohydrate performs better sustainable culture practice when compared with other traditional practices.

In conclusion, the aim of aquaculture is to maximize production of cultured organism in a sustainable manner. The experiment carried out involves substrates – periphyton – shrimp – environment relationship with and without carbohydrate addition interference. Periphyton grown on bamboo and kanchi poles is an excellent natural food for shrimp species and supports to further enhance the growth and survival of farmed organism. Manipulation carbon / nitrogen ratio by the addition of carbohydrate to the extensive shrimp culture system facilitated increase of heterotrophic bacterial production which in turn utilize the inorganic nitrogen species in the culture system, thus making the shrimp farming more ecologically sustainable by reducing inorganic nitrogen production and economically viable by minimizing the requirement of artificial feed. The demand for high quality dietary protein content in shrimp feed can also be substantially reduced in favor of carbohydrate addition to the water column, thus making the farming more ecologically friendly. This is a new novel approach to shrimp culture, the technology is appropriate under all circumstances, from the nursery to the grow-out systems, from the commercial level to resource poor and marginal shrimp farmers of the country.

Table 8.1
The quantity of carbohydrate added in each treatment during the culture period

Days of culture	Treatments	Quantity of carbohydrate added (g)
1 day - 15 days	CH	0.75
	B + CH	1.23
	K + CH	1.17
	B	1.15
	K	1.12
16 days - 30 days	CH	2.50
	B + CH	3.01
	K + CH	2.94
	B	2.95
	K	2.90
30 days - 45 days	CH	5.00
	B + CH	5.58
	K + CH	5.53
	B	5.51
	K	5.43
45 days - 60 days	CH	7.00
	B + CH	7.60
	K + CH	7.55
	B	7.54
	K	7.50
60 days - 75 days	CH	8.50
	B + CH	9.18
	K + CH	9.10
	B	9.13
	K	9.04
75 days - 100 days	CH	11.00
	B + CH	11.75
	K + CH	11.72
	B	11.69
	K	11.65

Table 8.2
Daily water quality parameters of carbohydrate addition and periphyton stocked with *Penaeus monodon*

Variable	Treatments (mean ± SD)				
	CH	**B + CH**	**K + CH**	**B**	**K**
Temperature (^{o}C)	30.33 ± 1.14^{a}	30.29 ± 1.20^{a}	30.45 ± 1.32^{a}	30.36 ± 1.24^{a}	30.37 ± 1.44^{a}
Water P^{H}	8.28 ± 0.12^{a}	8.26 ± 0.12^{a}	8.23 ± 0.13^{a}	8.25 ± 0.14^{a}	8.27 ± 0.12^{a}
DO (mg l^{-1})	6.58 ± 0.21^{a}	6.51 ± 0.25^{a}	6.52 ± 0.29^{a}	6.47 ± 0.24^{a}	6.52 ± 0.24^{a}
Salinity (ppt)	20.83 ± 0.99^{a}	20.78 ± 1.11^{a}	20.83 ± 1.10^{a}	20.89 ± 1.02^{a}	21.00 ± 1.03^{a}
Secchi disk reading (cm)	55.27 ± 1.88^{a}	54.71 ± 1.95^{a}	54.30 ± 1.96^{a}	53.11 ± 1.48^{b}	52.59 ± 1.45^{b}

Results from ANOVA Two-factor without replication

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

Table 8.3

Effect of carbohydrate addition and periphyton on the water and sediment quality

	Treatments (Mean ± SD)				
	CH	**B + CH**	**K + CH**	**B**	**K**
Water quality variable					
BOD (mg l^{-1})	3.11 ± 0.18^{c}	2.97 ± 0.29^{d}	3.13 ± 0.15^{b}	3.18 ± 0.11^{ab}	3.21 ± 0.12^{a}
Alkalinity (mg $CaC0_3$ l^{-1})	43.79 ± 3.49^{a}	44.49 ± 5.23^{a}	42.97 ± 6.04^{a}	42.31 ± 4.69^{a}	42.22 ± 7.85^{a}
TAN (ug l^{-1})	4.04 ± 0.84^{c}	4.13 ± 0.89^{c}	4.25 ± 1.02^{b}	4.64 ± 0.98^{a}	4.67 ± 0.99^{a}
Nitrite-N (ug l^{-1})	0.28 ± 0.05^{c}	0.30 ± 0.04^{b}	0.30 ± 0.04^{b}	0.35 ± 0.06^{a}	0.36 ± 0.06^{a}
Nitrate-N (ug l^{-1})	0.49 ± 0.03^{c}	0.52 ± 0.06^{b}	0.53 ± 0.07^{b}	0.57 ± 0.08^{a}	0.54 ± 0.09^{b}
THB (10^5cfu ml^{-1})	116.06 ± 38.86^{c}	141.63 ± 44.17^{a}	130.51 ± 44.15^{b}	120.85 ± 46.45^{c}	122.92 ± 50.48^{bc}
Chlorophyll -a (ug cm^{-2})	48.55 ± 7.96^{b}	51.14 ± 8.05^{a}	50.30 ± 7.74^{a}	46.62 ± 7.21^{c}	45.28 ± 7.34^{c}
Sediment quality variable					
Soil pH	8.44 ± 0.10^{b}	8.46 ± 0.09^{b}	8.44 ± 0.11^{b}	8.49 ± 0.11^{a}	8.52 ± 0.11^{a}
TAN (ug l^{-1})	12.96 ± 2.99^{c}	13.11 ± 2.70^{c}	13.21 ± 2.66^{c}	15.13 ± 4.82^{b}	17.46 ± 4.70^{a}
Nitrite-N (ug l^{-1})	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.14 ± 0.21^{a}	0.20 ± 0.28^{a}	0.15 ± 0.21^{a}
Nitrate-N (ug l^{-1})	0.14 ± 0.03^{b}	0.14 ± 0.03^{b}	0.15 ± 0.05^{b}	0.17 ± 0.06^{a}	0.18 ± 0.06^{a}
Organic carbon (ug l^{-1})	13.41 ± 2.04^{bc}	13.09 ± 2.19^{c}	13.40 ± 2.15^{bc}	13.86 ± 2.52^{ab}	14.05 ± 2.71^{a}
THB (10^7cfu ml^{-1})	172.39 ± 69.42^{a}	187.65 ± 71.73^{a}	140.35 ± 47.91^{b}	153.07 ± 49.04^{b}	137.08 ± 45.52^{b}

Results from ANOVA Two-factor without replication

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

Table 5.4
Abundance of periphyton (cells or colonies l⁻¹) during the culture period of experiment.
Number are means of three ponds per CH with and without addition

	Group / Genus	B + CH	K + CH	B	K
	PHYTOPLANKTON				
1	**Chlorophyceae**	**Avg**	**Avg**	**Avg**	**Avg**
	Actinastrum	359.80 ± 27.66^{a}	277.08 ± 30.05^{b}	137.53 ± 14.91^{c}	129.01 ± 9.73^{c}
	Ankistrodesmus	582.52 ± 110.63^{a}	668.14 ± 116.72^{a}	331.64 ± 57.94^{b}	207.68 ± 39.44^{b}
	Chlorella	5496.75 ± 867.76^{a}	6002.73 ± 1265.25^{a}	2979.52 ± 628.02^{b}	1959.73 ± 309.38^{b}
	Closterium	529.48 ± 179.27^{ab}	834.38 ± 192.68^{a}	414.15 ± 95.64^{b}	188.77 ± 63.91^{b}
	Cosmarium	188.88 ± 27.70^{b}	257.81 ± 16.36^{a}	127.97 ± 8.12^{c}	67.34 ± 9.87^{a}
	Crucigenia	86.25 ± 16.58^{ab}	147.17 ± 38.75^{a}	73.05 ± 19.23^{b}	30.75 ± 5.91^{b}
	Gonatozygon	471.19 ± 72.57^{ab}	552.25 ± 130.66^{a}	274.11 ± 64.85^{bc}	167.99 ± 25.87^{c}
	Oocystis	344.63 ± 106.03^{ab}	400.50 ± 128.57^{a}	198.79 ± 63.82^{ab}	122.87 ± 37.80^{b}
	Pediastrum	263.64 ± 60.54^{ab}	474.13 ± 192.83^{a}	235.34 ± 95.71^{ab}	93.99 ± 21.59^{b}
	Synedra	196.45 ± 54.42^{ab}	266.59 ± 16.77^{a}	132.32 ± 8.33^{bc}	70.99 ± 18.00^{c}
	Treubaria	185.43 ± 32.81^{b}	272.21 ± 23.39^{a}	135.12 ± 11.61^{b}	66.11 ± 11.70^{c}
	Tetraedron	497.80 ± 34.45^{b}	582.58 ± 20.89^{a}	289.17 ± 10.37^{c}	177.48 ± 12.28^{a}
	Zygnema	399.39 ± 67.60^{a}	456.87 ± 92.65^{a}	226.77 ± 45.99^{b}	142.39 ± 24.10^{b}
	Total	**9602.19 ± 1658.03^{a}**	**11192.44 ± 2265.57^{a}**	**5555.49 ± 1124.54^{b}**	**3425.11 ± 589.60^{b}**
2	**Cyanophyceae**				
	Aphanocapsa	463.43 ± 11.61^{a}	429.40 ± 91.92^{a}	213.14 ± 45.63^{b}	165.22 ± 4.14^{b}
	Chroococcus	332.28 ± 57.65^{a}	303.50 ± 56.20^{a}	150.64 ± 27.89^{b}	118.47 ± 20.55^{b}
	Gloetrichia	366.74 ± 50.58^{a}	305.60 ± 57.37^{a}	151.69 ± 28.48^{b}	130.75 ± 18.03^{b}

	Gomphosphaeria	3771.68 ± 3125.17[a]	3230.04 ± 2685.25[a]	1603.26 ± 1317.30[a]	1404.70 ± 1164.39[a]
	Lyngbya	4835.15 ± 893.42[a]	4388.24 ± 1010.75[a]	2178.15 ± 501.70[b]	1723.85 ± 318.53[b]
	Microcystis	6389.05 ± 892.00[a]	6385.49 ± 423.15[a]	3169.50 ± 210.04[b]	2277.86 ± 318.02[b]
	Oscillatoria	385.60 ± 103.68[a]	320.38 ± 99.54[ab]	159.02 ± 49.41[b]	137.48 ± 36.97[b]
	Rivularia	1191.08 ± 477.03[a]	913.36 ± 394.23[a]	453.35 ± 195.68[a]	424.65 ± 170.07[a]
	Total	**17735.02 ± 5611.14[a]**	**16276.00 ± 4788.40[a]**	**8078.76 ± 2376.77[b]**	**6322.98 ± 2000.51[b]**
3	**Cryptophyceae**				
	Chroomonas	372.58 ± 104.44[a]	284.90 ± 73.03[ab]	141.41 ± 36.25[b]	132.84 ± 37.23[b]
	Cryptomonas	294.18 ± 35.97[a]	266.40 ± 33.95[a]	132.23 ± 16.85[b]	104.88 ± 12.83[b]
	Total	**666.76 ± 140.41[a]**	**551.30 ± 106.98[a]**	**273.64 ± 53.10[b]**	**237.72 ± 50.06[b]**
4	**Crysophyceae**				
	Botrydiopsis	381.20 ± 43.97[a]	324.39 ± 44.37[a]	161.01 ± 22.02[b]	135.91 ± 15.68[b]
	Bumilleriopsis	1715.78 ± 2417.25[a]	1168.78 ± 1526.11[a]	580.14 ± 757.50[a]	611.72 ± 861.81[a]
	Chlorothecium	284.99 ± 43.82[a]	224.33 ± 47.49[a]	111.35 ± 23.57[b]	101.61 ± 15.62[b]
	Dinobryon	272.16 ± 73.00[a]	235.40 ± 48.12[ab]	116.84 ± 23.88[bc]	97.03 ± 26.03[c]
	Epipyxis	186.20 ± 20.36[a]	130.58 ± 24.07[b]	64.81 ± 11.95[c]	66.38 ± 7.26[c]
	Heterococcus	143.88 ± 33.33[ab]	157.76 ± 33.40[a]	78.30 ± 16.58[bc]	51.30 ± 11.88[c]
	Leuvenia	236.17 ± 48.26[a]	199.15 ± 57.70[ab]	98.85 ± 28.64[bc]	84.20 ± 17.21[c]
	Perone	178.82 ± 62.19[a]	121.51 ± 48.89[ab]	60.31 ± 24.27[b]	63.76 ± 22.17[b]
	Uroglenopsis	361.29 ± 28.86[a]	285.85 ± 37.16[b]	141.89 ± 18.44[c]	128.81 ± 10.29[c]
	Vaucheria	274.65 ± 107.17[a]	198.01 ± 86.42[a]	98.28 ± 42.89[a]	97.92 ± 38.21[a]
	Total	**4035.13 ± 2878.21[a]**	**3045.76 ± 1953.73[a]**	**1511.80 ± 969.75[a]**	**1438.63 ± 1026.16[a]**
5	**Euglenophyceae**				
	Colacium	341.18 ± 58.67[a]	293.29 ± 57.87[a]	145.58 ± 28.73[b]	121.64 ± 20.92[b]
	Euglena	204.38 ± 37.89[a]	200.11 ± 25.61[a]	99.33 ± 12.71[b]	72.87 ± 13.51[b]
	Phacus	520.87 ± 45.07[a]	457.44 ± 92.96[a]	227.07 ± 46.14[b]	185.70 ± 16.07[b]

	Trachelomonas	[illegible]	[illegible]	[illegible]	[illegible]
	Total	**4098.41 ± 589.26^{a}**	**2608.45 ± 1333.03^{ab}**	**1294.73 ± 661.66^{b}**	**1461.19 ± 210.69^{b}**
6	**Pyrrhophyceae**				
	Amphidinium	347.50 ± 13.43^{a}	299.30 ± 23.88^{b}	148.56 ± 11.85^{c}	123.89 ± 7.79^{c}
	Ceratium	199.60 ± 16.38^{a}	137.54 ± 14.65^{b}	68.27 ± 7.27^{c}	71.16 ± 5.84^{c}
	Cystodinium	354.87 ± 68.12^{a}	283.85 ± 58.26^{a}	140.89 ± 28.92^{b}	126.52 ± 24.29^{b}
	Glenodinium	303.18 ± 47.88^{a}	256.09 ± 53.97^{a}	127.12 ± 26.79^{b}	108.09 ± 17.07^{b}
	Gloeodinium	334.96 ± 57.44^{a}	263.34 ± 56.23^{a}	130.71 ± 27.91^{b}	119.42 ± 20.48^{b}
	Gonyaulax	309.88 ± 27.86^{a}	246.84 ± 30.49^{b}	122.52 ± 15.14^{c}	110.48 ± 9.93^{c}
	Gyrodinium	246.89 ± 30.60^{a}	203.54 ± 36.35^{a}	101.03 ± 18.04^{b}	88.02 ± 10.91^{b}
	Hemidinium	303.27 ± 45.02^{a}	252.09 ± 41.04^{a}	125.13 ± 20.37^{b}	108.12 ± 16.05^{b}
	Hypnodinium	197.11 ± 44.59^{a}	163.58 ± 40.16^{ab}	81.19 ± 19.93^{bc}	70.27 ± 16.04^{c}
	Massartia	257.42 ± 16.84^{a}	196.10 ± 42.85^{a}	97.34 ± 21.27^{b}	91.78 ± 6.00^{b}
	Oodinium	306.43 ± 75.98^{a}	243.41 ± 81.21^{ab}	120.82 ± 40.31^{b}	109.25 ± 27.09^{b}
	Peridinium	255.41 ± 83.74^{a}	218.80 ± 78.92^{ab}	108.60 ± 39.18^{ab}	91.06 ± 29.85^{b}
	Urococcus	257.42 ± 16.08^{a}	208.12 ± 41.52^{a}	103.30 ± 20.16^{b}	91.78 ± 5.73^{b}
	Total	**3673.94 ± 544.35^{a}**	**2972.61 ± 599.54^{b}**	**1475.49 ± 297.59^{c}**	**1309.85 ± 194.08^{c}**
7	**Rhodophyceae**				
	Asterocystis	235.98 ± 48.12^{a}	192.57 ± 50.91^{ab}	95.59 ± 25.27^{bc}	84.13 ± 17.16^{c}
	Audouinella	312.37 ± 87.13^{a}	258.10 ± 87.39^{ab}	128.11 ± 43.37^{b}	111.37 ± 31.07^{b}
	Bangia	257.42 ± 33.66^{a}	207.74 ± 41.31^{a}	103.11 ± 20.50^{b}	91.78 ± 12.00^{b}
	Batrachospermum	139.57 ± 73.79^{a}	95.95 ± 59.61^{a}	47.63 ± 29.59^{a}	49.76 ± 26.31a
	Bostrychia	228.51 ± 58.95^{a}	187.04 ± 49.73^{ab}	92.84 ± 24.68^{b}	81.47 ± 21.02^{b}
	Compsopogon	186.67 ± 56.81^{a}	146.12 ± 45.68^{ab}	72.53 ± 22.67^{b}	66.55 ± 20.26^{b}
	Lemanea	188.30 ± 54.48^{a}	131.05 ± 47.39^{ab}	65.05 ± 23.52^{b}	67.13 ± 19.42^{b}
	Porphyridium	262.01 ± 72.80^{a}	220.71 ± 60.21^{ab}	109.55 ± 29.89^{b}	93.41 ± 25.95^{b}

	Sirodotia	240.47 ± 40.75^{a}	196.77 ± 44.99^{a}	97.67 ± 22.23^{b}	85.74 ± 14.53^{b}
	Thorea	194.81 ± 71.05^{a}	170.73 ± 41.53^{ab}	84.74 ± 20.61^{b}	69.46 ± 25.33^{b}
	Tuomeya	133.74 ± 28.73^{a}	109.40 ± 35.23^{ab}	54.30 ± 17.49^{b}	47.68 ± 10.24^{b}
	Total	**2379.86 ± 626.27^{a}**	**1916.18 ± 563.97^{b}**	**951.12 ± 279.93^{c}**	**848.48 ± 223.28^{c}**
	ZOOPLANKTON				
1	**Crustacea**				
	Diaphanosoma	147.42 ± 107.43^{a}	110.93 ± 66.43^{a}	55.06 ± 32.97^{a}	52.56 ± 38.30^{a}
	Diaptomus	86.16 ± 15.92^{a}	54.46 ± 17.16^{ab}	27.03 ± 8.52^{b}	30.72 ± 5.68^{b}
	Monostyla	2181.89 ± 2840.19^{a}	1867.44 ± 2244.52^{a}	926.92 ± 1114.09^{a}	777.90 ± 1012.60^{a}
	Nauplius	85.39 ± 70.31^{a}	50.17 ± 49.81^{a}	24.90 ± 24.73^{a}	30.44 ± 25.07^{a}
	Total	**2500.86 ± 3033.86^{a}**	**2083.00 ± 2377.93^{a}**	**1033.92 ± 1180.31^{a}**	**891.62 ± 1081.65^{a}**
2	**Rotifera**				
	Asplanchna	146.18 ± 38.93^{a}	118.37 ± 46.03^{ab}	58.75 ± 22.85^{b}	52.12 ± 13.88^{b}
	Brachionus	172.51 ± 34.10^{a}	126.47 ± 51.66^{ab}	62.78 ± 25.64^{b}	61.50 ± 12.16^{b}
	Filinia	28.62 ± 17.21^{a}	25.47 ± 19.33^{a}	12.64 ± 9.59^{a}	10.20 ± 6.14^{a}
	Keratella	126.46 ± 63.01^{a}	93.66 ± 56.25^{a}	46.49 ± 27.92^{a}	45.09 ± 22.46^{a}
	Lecane	121.48 ± 39.94^{a}	107.59 ± 62.67^{a}	53.40 ± 31.11^{a}	43.31 ± 14.24^{a}
	Polyarthra	122.82 ± 20.07^{a}	101.10 ± 22.72^{a}	50.18 ± 11.28^{b}	43.79 ± 7.15^{b}
	Trichocerca	150.49 ± 21.39^{ab}	154.61 ± 46.26^{a}	76.74 ± 22.96^{bc}	53.65 ± 7.63^{c}
	Total	**868.56 ± 234.65^{a}**	**727.27 ± 304.90^{a}**	**360.99 ± 151.34^{b}**	**309.66 ± 83.66^{b}**
	G. Total	**45560.74 ± 15316.18^{a}**	**41373.01 ± 14294.05^{b}**	**20535.92 ± 7095.00^{c}**	**16245.25 ± 5459.08^{d}**

Results from Tukey One-way ANOVA

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

Table 8.5
Abundance of plankton (cells or colonies l^{-1}) during the culture period of experiment.
Number are means of three ponds

	Group / Genus	CH	B + CH	K + CH	B	K
	PHYTOPLANKTON					
1	**Chlorophyceae**	**Avg**	**Avg**	**Avg**	**Avg**	**Avg**
	Actinastrum	103.00 ± 18.85^{ab}	161.41 ± 19.69^{a}	124.34 ± 28.58^{ab}	90.29 ± 27.74^{bc}	41.94 ± 9.64^{c}
	Ankistrodesmus	117.89 ± 33.39^{ab}	155.36 ± 29.05^{a}	92.55 ± 28.58^{abc}	78.86 ± 20.12^{bc}	31.22 ± 9.64^{c}
	Botryococcus	95.67 ± 17.64^{a}	96.21 ± 71.97^{a}	127.97 ± 14.77^{a}	44.36 ± 33.18^{a}	41.01 ± 8.10^{a}
	Chlorella	1478.67 ± 245.99^{b}	2763.71 ± 912.38^{a}	144.65 ± 20.36^{c}	1274.34 ± 420.70^{bc}	48.79 ± 6.87^{c}
	Closterium	22.78 ± 5.48^{ab}	49.29 ± 16.27^{a}	48.48 ± 16.97^{a}	22.73 ± 7.50^{ab}	16.35 ± 5.72^{b}
	Cosmarium	59.22 ± 19.24^{bc}	148.74 ± 10.73^{a}	89.19 ± 30.05^{b}	68.58 ± 4.95^{bc}	26.65 ± 10.74^{c}
	Crucigenia	26.11 ± 2.78^{b}	57.59 ± 16.19^{ab}	74.81 ± 21.99^{a}	26.55 ± 7.47^{b}	25.24 ± 7.42^{b}
	Gonatozygon	129.78 ± 19.54^{b}	248.24 ± 17.89^{a}	129.05 ± 38.28^{b}	107.28 ± 12.11^{b}	43.53 ± 12.91^{c}
	Oocystis	81.33 ± 36.69^{b}	160.35 ± 21.02^{a}	150.04 ± 20.28^{a}	73.94 ± 9.69^{b}	46.22 ± 13.52^{b}
	Pediastrum	57.78 ± 16.09^{a}	156.90 ± 14.96^{a}	439.15 ± 607.62^{a}	72.35 ± 6.90^{a}	148.13 ± 204.96^{a}
	Selenastrum	21.56 ± 9.67^{b}	87.31 ± 20.84^{a}	79.43 ± 16.18^{a}	35.66 ± 14.27^{b}	26.79 ± 5.46^{a}
	Spirogyra	47.11 ± 13.09^{bc}	68.78 ± 8.23^{ab}	97.51 ± 27.21^{a}	26.75 ± 6.64^{c}	32.89 ± 9.18^{bc}
	Synedra	24.11 ± 7.60^{b}	61.63 ± 12.07^{a}	97.96 ± 31.09^{ab}	28.42 ± 5.57^{bc}	33.04 ± 10.49^{c}
	Treubaria	126.11 ± 23.17^{b}	227.79 ± 30.12^{a}	119.77 ± 21.71^{b}	105.04 ± 13.93^{b}	40.40 ± 7.32^{c}
	Tetraedron	122.44 ± 17.83^{b}	202.01 ± 50.61^{a}	127.07 ± 13.99^{ab}	86.13 ± 33.25^{bc}	42.86 ± 4.72^{c}
	Zygnema	101.89 ± 16.17^{b}	269.39 ± 19.10^{a}	101.93 ± 39.27^{b}	122.07 ± 11.46^{b}	34.38 ± 13.25^{c}
	Total	**2615.45 ± 503.21^{b}**	**4914.73 ± 893.68^{a}**	**2043.91 ± 609.07^{bc}**	**2263.35 ± 436.49^{b}**	**679.44 ± 214.50^{c}**
2	**Cyanophyceae**					
	Aphanocapsa	132.67 ± 17.03^{b}	202.00 ± 32.81^{a}	143.05 ± 30.70^{ab}	82.99 ± 17.92^{bc}	47.03 ± 10.09^{c}
	Chroococcus	202.00 ± 24.18^{b}	289.97 ± 19.86^{a}	166.61 ± 26.51^{bc}	117.86 ± 8.65^{c}	54.77 ± 8.71^{d}
	Gomphosphaeria	196.33 ± 54.67^{ab}	308.11 ± 73.35^{a}	207.09 ± 27.06^{ab}	130.67 ± 31.11^{bc}	68.08 ± 8.89^{c}
	Merismopedia	109.44 ± 17.78^{ab}	164.58 ± 28.12^{a}	145.19 ± 45.05^{a}	69.80 ± 11.93^{b}	47.73 ± 14.81^{b}

	Microcystis	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Oscillatoria	125.67 ± 17.50^{ao}	192.72 ± 37.83^{a}	170.71 ± 26.63^{a}	73.93 ± 26.79^{bc}	[illegible]
	Total	**4024.44 ± 756.84^{a}**	**5428.33 ± 1138.17^{a}**	**4983.82 ± 983.24^{a}**	**2286.61 ± 497.69^{b}**	**1638.38 ± 324.22^{b}**
3	**Cryptophyceae**					
	Chroomonas	199.78 ± 49.77^{ao}	213.70 ± 42.75^{ao}	276.49 ± 100.62^{a}	86.85 ± 22.54^{o}	81.91 ± 39.97^{o}
	Cryptomonas	109.33 ± 28.37^{oc}	219.18 ± 36.19^{a}	142.78 ± 24.17^{o}	87.43 ± 22.85^{oc}	46.53 ± 7.88^{c}
	Total	**309.11 ± 78.14^{c}**	**432.88 ± 78.94^{a}**	**142.78 ± 24.17^{c}**	**174.28 ± 45.39^{c}**	**128.45 ± 47.85^{c}**
4	**Crysophyceae**					
	Botrydiopsis	95.44 ± 20.12^{bc}	159.63 ± 15.16^{a}	113.52 ± 23.16^{ab}	50.32 ± 16.45^{c}	54.91 ± 11.20^{c}
	Bumilleriopsis	166.66 ± 27.24^{a}	204.60 ± 28.14^{a}	181.49 ± 20.28^{a}	76.37 ± 10.51^{b}	84.71 ± 9.52^{b}
	Chlorothecium	160.42 ± 16.85^{a}	216.28 ± 16.15^{a}	170.53 ± 41.81^{a}	72.44 ± 18.07^{b}	82.48 ± 20.22^{b}
	Dinobryon	106.86 ± 78.51^{ab}	203.84 ± 33.79^{a}	127.07 ± 28.57^{ab}	76.09 ± 12.61^{b}	61.46 ± 13.82^{b}
	Epipyxis	60.22 ± 20.45^{abc}	99.69 ± 25.78^{ab}	121.97 ± 34.70^{a}	37.21 ± 9.62^{c}	58.99 ± 16.78^{bc}
	Heterococcus	91.44 ± 14.61^{ab}	100.88 ± 30.35^{ab}	113.77 ± 21.33^{a}	37.66 ± 11.33^{c}	55.03 ± 10.32^{bc}
	Leuvenia	63.16 ± 19.50^{ab}	108.92 ± 14.40^{a}	102.57 ± 39.15^{a}	34.29 ± 14.48^{b}	49.61 ± 18.94^{ab}
	Perone	68.22 ± 24.44^{a}	103.62 ± 22.20^{a}	79.33 ± 60.34^{a}	38.68 ± 8.29^{a}	38.37 ± 29.18^{a}
	Uroglenopsis	62.49 ± 24.35^{ao}	105.96 ± 41.25^{ao}	116.50 ± 22.13^{a}	39.55 ± 15.40^{o}	56.35 ± 10.70^{ao}
	Vaucheria	71.88 ± 15.70^{aoc}	99.87 ± 26.32^{a}	87.62 ± 22.17^{ao}	37.28 ± 9.83^{c}	42.38 ± 10.72^{oc}
	Total	**946.80 ± 261.76^{c}**	**1403.28 ± 253.54^{a}**	**1214.38 ± 313.64^{b}**	**499.90 ± 126.60^{d}**	**584.30 ± 151.41^{d}**
5	**Euglenophyceae**					
	Colacium	91.42 ± 29.78^{bc}	170.84 ± 17.02^{a}	117.46 ± 12.99^{b}	63.18 ± 11.04^{cd}	44.36 ± 4.90^{d}
	Euglena	103.14 ± 15.78^{b}	146.61 ± 19.79^{a}	142.07 ± 11.85^{a}	56.83 ± 7.67^{c}	51.55 ± 7.80^{c}
	Gymnodinium	95.33 ± 10.97^{a}	586.14 ± 748.72^{a}	133.06 ± 16.52^{a}	192.13 ± 229.49^{a}	50.25 ± 6.24^{a}
	Phacus	150.17 ± 25.83^{aoc}	229.98 ± 42.63^{a}	169.99 ± 45.65^{ao}	89.14 ± 16.52^{oc}	64.20 ± 17.24^{c}
	Trachalomonas	133.24 ± 19.92^{o}	173.78 ± 27.04^{ao}	189.53 ± 20.00^{a}	67.36 ± 10.48^{c}	71.57 ± 7.55^{c}
	Total	**573.30 ± 102.28^{ab}**	**1307.35 ± 855.19^{a}**	**752.11 ± 107.01^{ab}**	**468.63 ± 275.20^{ab}**	**281.93 ± 43.73^{b}**
6	**Pyrrhophyceae**					
	Amphidinium	151.17 ± 39.27^{ab}	225.84 ± 43.36^{a}	231.86 ± 28.41^{a}	75.66 ± 14.53^{b}	87.25 ± 10.69^{b}
	Ceratium	81.00 ± 4.36^{bc}	125.97 ± 27.24^{a}	109.26 ± 11.02^{ab}	42.20 ± 9.13^{cd}	33.89 ± 10.89^{d}
	Cystodinium	137.89 ± 17.11^{ab}	191.04 ± 39.19^{a}	112.96 ± 12.46^{bc}	64.00 ± 13.13^{cd}	42.51 ± 4.69^{d}

	Glenodinium	122.78 ± 25.60^{bc}	150.35 ± 41.22^{ab}	183.85 ± 24.79^{a}	84.84 ± 15.32^{bc}	44.58 ± 6.45^{c}
	Gloeodinium	93.56 ± 21.43^{bc}	163.68 ± 45.74^{a}	118.46 ± 17.15^{ab}	56.19 ± 23.26^{c}	84.85 ± 11.80^{c}
	Gonyaulax	107.33 ± 51.50^{bc}	194.94 ± 35.55^{ab}	225.49 ± 31.35^{a}	32.08 ± 10.17^{b}	47.19 ± 10.19^{b}
	Gymnodinium	81.34 ± 35.70^{ab}	107.66 ± 14.61^{a}	125.40 ± 27.09^{a}	25.26 ± 13.80^{b}	41.12 ± 15.92^{ab}
	Gyrodinium	63.49 ± 24.41^{ab}	84.46 ± 30.10^{ab}	109.26 ± 42.29^{a}	41.02 ± 7.31^{c}	47.88 ± 14.78^{c}
	Hemidinium	60.78 ± 24.29^{bc}	122.44 ± 21.81^{ab}	127.25 ± 39.27^{a}	42.68 ± 8.58^{b}	32.13 ± 14.27^{b}
	Hypnodinium	67.10 ± 21.87^{ab}	127.39 ± 25.62^{a}	85.38 ± 37.91^{ab}	54.14 ± 23.61^{b}	54.88 ± 9.23^{b}
	Massartia	107.89 ± 19.53^{ab}	180.17 ± 49.83^{a}	145.84 ± 24.54^{a}	46.14 ± 21.95^{c}	42.44 ± 4.70^{c}
	Oodinium	114.56 ± 28.35^{b}	178.62 ± 16.54^{a}	112.78 ± 12.48^{b}	41.20 ± 10.43^{b}	35.96 ± 11.21^{b}
	Peridinium	109.00 ± 20.28^{a}	133.76 ± 17.67^{a}	95.56 ± 29.79^{a}	42.23 ± 9.12^{bc}	29.83 ± 6.80^{c}
	Urococcus	74.78 ± 20.25^{bc}	126.06 ± 27.23^{a}	79.27 ± 18.07^{ab}		
	Total	**1372.65 ± 353.93^{c}**	**2112.38 ± 425.72^{a}**	**1772.61 ± 356.61^{b}**	**664.04 ± 191.28^{d}**	**659.82 ± 140.94^{d}**
7	**Rhodophyceae**					
	Asterocystis	61.71 ± 21.68^{a}	98.80 ± 26.06^{a}	97.60 ± 86.21^{a}	41.03 ± 10.82^{a}	33.99 ± 30.02^{a}
	Audouinella	65.44 ± 20.49^{b}	111.69 ± 9.36^{a}	121.48 ± 6.41^{a}	40.75 ± 8.74^{b}	42.30 ± 2.23^{b}
	Bangia	67.26 ± 21.90^{bc}	108.22 ± 15.33^{ab}	122.65 ± 23.12^{a}	44.94 ± 6.37^{c}	42.71 ± 8.05^{c}
	Batrachospermum	13.67 ± 6.89^{ab}	36.74 ± 7.21^{a}	32.98 ± 14.82^{ab}	12.71 ± 6.70^{b}	11.48 ± 5.16^{b}
	Bostrychia	59.22 ± 20.63^{b}	128.35 ± 14.15^{a}	148.60 ± 26.28^{a}	41.23 ± 15.53^{b}	51.75 ± 9.15^{b}
	Compsopogon	44.67 ± 10.09^{bc}	99.08 ± 26.21^{b}	166.87 ± 32.83^{a}	41.15 ± 10.88^{c}	58.11 ± 11.43^{bc}
	Lemanea	70.67 ± 14.34^{b}	136.18 ± 20.01^{a}	79.24 ± 18.06^{b}	56.56 ± 8.31^{bc}	24.34 ± 11.08^{c}
	Porphyridium	62.67 ± 19.84^{a}	119.55 ± 61.38^{a}	98.41 ± 25.83^{a}	49.65 ± 25.49^{a}	34.27 ± 8.99^{a}
	Sirodotia	85.89 ± 31.06^{bc}	148.16 ± 24.88^{a}	112.74 ± 12.48^{ab}	61.53 ± 10.33^{bc}	39.26 ± 4.35^{c}
	Thorea	67.83 ± 19.26^{b}	137.16 ± 44.77^{a}	79.06 ± 18.29^{ab}	56.96 ± 18.59^{b}	27.53 ± 6.37^{b}
	Tuomeya	52.11 ± 23.09^{a}	42.00 ± 31.97^{a}	48.67 ± 24.08^{a}	17.44 ± 13.28^{a}	16.95 ± 8.39^{a}
	Total	**651.13 ± 209.27^{b}**	**1165.93 ± 281.32^{a}**	**1108.31 ± 288.42^{a}**	**463.96 ± 135.05^{c}**	**382.70 ± 105.22^{c}**
	ZOOPLANKTON					
1	**Crustacea**					
	Cyclops	270.61 ± 52.88^{a}	2113.31 ± 2703.46^{a}	271.00 ± 34.26^{a}	732.19 ± 961.78^{a}	100.84 ± 12.75^{a}
	Daphnia	23.67 ± 7.77^{c}	85.62 ± 24.50^{b}	129.10 ± 15.20^{a}	28.84 ± 10.48^{c}	44.13 ± 11.34^{c}
	Diaphanosoma	28.00 ± 16.95^{b}	97.21 ± 21.30^{a}	101.16 ± 14.58^{a}	32.20 ± 6.87^{b}	37.64 ± 5.42^{b}

	Diaptomus	17.00 ± 3.93^{b}	39.50 ± 5.11^{ab}	61.79 ± 22.65^{a}	11.44 ± 2.99^{b}	22.99 ± 8.43^{b}
	Nauplius	285.90 ± 33.76^{b}	582.87 ± 148.07^{a}	168.18 ± 64.71^{bc}	205.11 ± 52.11^{bc}	62.58 ± 24.08^{c}
	Total	**625.17 ± 115.29^{a}**	**2918.50 ± 2902.45^{a}**	**731.23 ± 151.39^{a}**	**1009.79 ± 1034.23^{a}**	**268.19 ± 62.02^{a}**
2	**Rotifera**					
	Asplanchna	110.11 ± 21.61^{ab}	136.54 ± 8.25^{a}	68.97 ± 26.03^{bc}	41.49 ± 7.86^{c}	26.05 ± 9.83^{c}
	Brachionus	156.71 ± 23.74^{ab}	188.28 ± 37.75^{a}	158.58 ± 88.84^{ab}	63.57 ± 12.75^{ab}	59.91 ± 33.56^{b}
	Filinia	72.44 ± 14.34^{ab}	95.86 ± 13.57^{a}	67.58 ± 26.59^{abc}	30.30 ± 7.35^{bc}	25.53 ± 10.05^{c}
	Keratella	70.89 ± 25.81^{ab}	106.77 ± 42.88^{ab}	114.94 ± 32.64^{a}	36.05 ± 14.48^{b}	39.54 ± 17.03^{ab}
	Lecane	33.00 ± 25.16^{b}	97.17 ± 31.15^{a}	112.69 ± 12.37^{a}	32.81 ± 10.52^{b}	42.57 ± 4.67^{b}
	Polyarthra	30.19 ± 12.91^{b}	75.36 ± 17.67^{ab}	115.48 ± 32.90^{a}	25.45 ± 5.97^{b}	40.12 ± 16.66^{b}
	Trichocerca	29.60 ± 12.36^{b}	108.33 ± 14.48^{a}	97.36 ± 30.28^{a}	36.58 ± 4.89^{b}	36.78 ± 11.44^{b}
	Total	**502.94 ± 135.94^{a}**	**808.31 ± 165.75^{a}**	**735.61 ± 249.66^{a}**	**266.25 ± 63.81^{c}**	**270.50 ± 103.24^{c}**
	G. Total	**11620.99 ± 2516.66^{a}**	**20491.68 ± 6994.76^{a}**	**13484.75 ± 3086.20^{a}**	**8096.81 ± 2805.73^{a}**	**4893.69 ± 1193.13^{a}**

Results from Tukey One-way ANOVA

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

Table 8.6

Mean values of periphyton biomass and proximate composition parameters of periphyton scraped from substrates

Variable	Treatments (mean ± SD)			
	B + CH	**K + CH**	**B**	**K**
Dry matter (mg cm^{-2})	1.44 ± 0.05^{a}	1.36 ± 0.08^{b}	1.42 ± 0.05^{a}	1.30 ± 0.06^{c}
Pheophytin-a (ug cm^{-2})	1.42 ± 0.02^{a}	1.39 ± 0.04^{b}	1.38 ± 0.03^{bc}	1.37 ± 0.04^{c}
Ash content (%)	32.17 ± 3.11^{a}	29.94 ± 3.15^{bc}	31.06 ± 3.42^{ab}	29.22 ± 3.23^{c}
Ash free dry matter (mg cm^{-2})	2.21 ± 0.05^{a}	2.21 ± 0.06^{a}	2.20 ± 0.05^{a}	2.20 ± 0.06^{a}
Protein (%)	38.61 ± 6.43^{a}	35.06 ± 6.78^{b}	34.61 ± 6.75^{b}	30.89 ± 6.43^{c}
Nitrogen (%)	5.99 ± 1.00^{a}	5.43 ± 1.05^{b}	5.37 ± 1.05^{b}	4.79 ± 1.00^{c}

Results from ANOVA Two-factor without replication

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

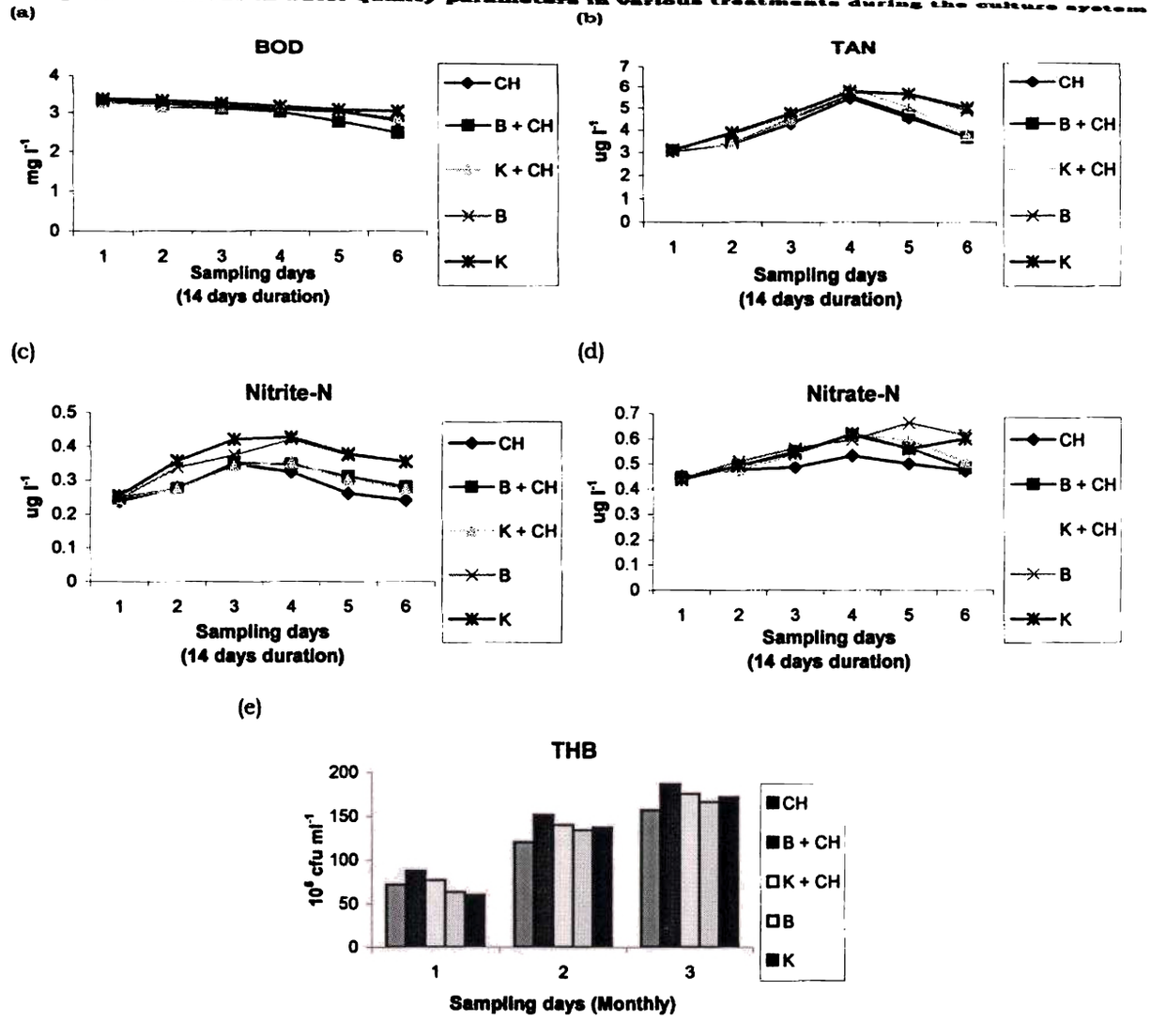
Table 8.7
Weight, shrimp yield, SGR, FCR and survival of *Penaeus monodon* cultured in various treatments

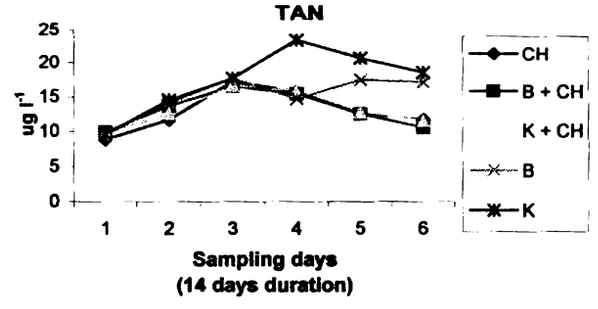
Variable	Treatments (Mean ± SD)				
	CH	**B + CH**	**K + CH**	**B**	**K**
Individual shrimp weight gain (g)	24.43 ± 0.02^{e}	27.48 ± 0.01^{a}	26.56 ± 0.06^{b}	25.76 ± 0.02^{c}	25.03 ± 0.04^{d}
Net shrimp yield (g m^{-2})	130.31 ± 16.36^{a}	161.83 ± 13.95^{a}	150.50 ± 9.12^{a}	143.09 ± 13.70^{a}	134.86 ± 14.73^{a}
Specific growth rate (SGR)	7.40 ± 0.00^{c}	7.51 ± 0.00^{a}	7.48 ± 0.00^{b}	7.45 ± 0.00^{c}	7.42 ± 0.00^{d}
Survival rate (%)	76.19 ± 9.52^{a}	84.13 ± 7.27^{a}	80.95 ± 4.76^{a}	76.37 ± 7.65^{a}	76.98 ± 8.36^{a}

Results from Tukey One-way ANOVA

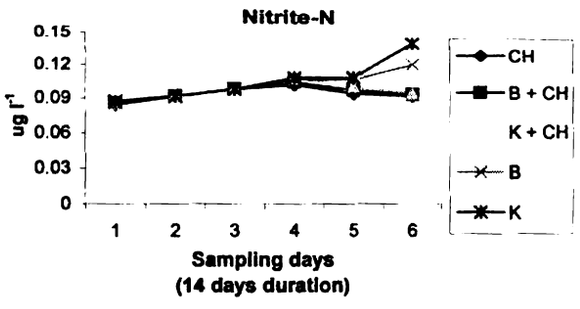
Treatments with mean values in same row with different superscripts differ significantly ($P<0.05$)

Fig. 5.1
Temporal variations in water quality parameters in various treatments during the culture system

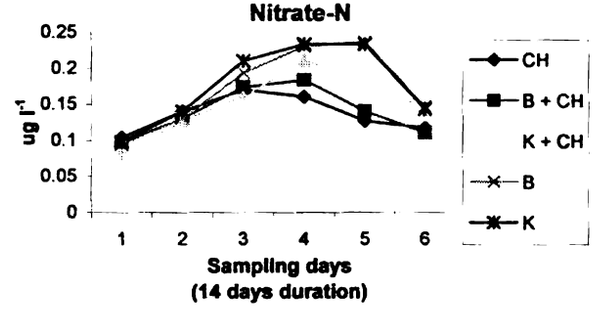



TAN
25
20
15
10
5
0
ug l-1
1
2
3
4
5
6
Sampling days
(14 days duration)
CH
B + CH
K + CH
B
K

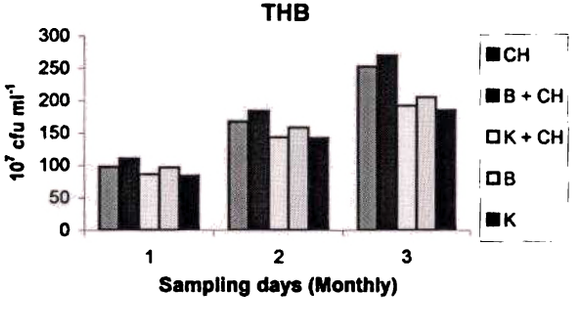
(c)


Nitrite-N
0.15
0.12
0.09
0.06
0.03
0
ug l-1
1
2
3
4
5
6
Sampling days
(14 days duration)
CH
B + CH
K + CH
B
K

(d)


Nitrate-N
0.25
0.2
0.15
0.1
0.05
0
ug l-1
1
2
3
4
5
6
Sampling days
(14 days duration)
CH
B + CH
K + CH
B
K

(e)


THB
300
250
200
150
100
50
0
10-7 cfu ml-1
1
2
3
Sampling days (Monthly)
CH
B + CH
K + CH
B
K

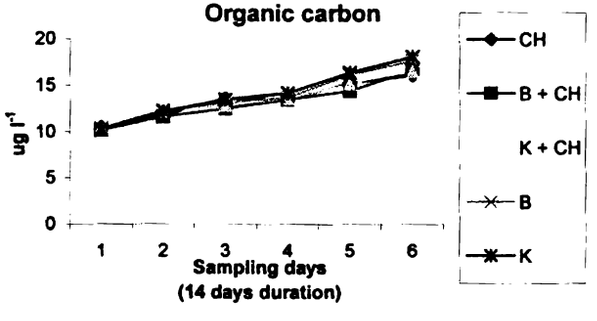
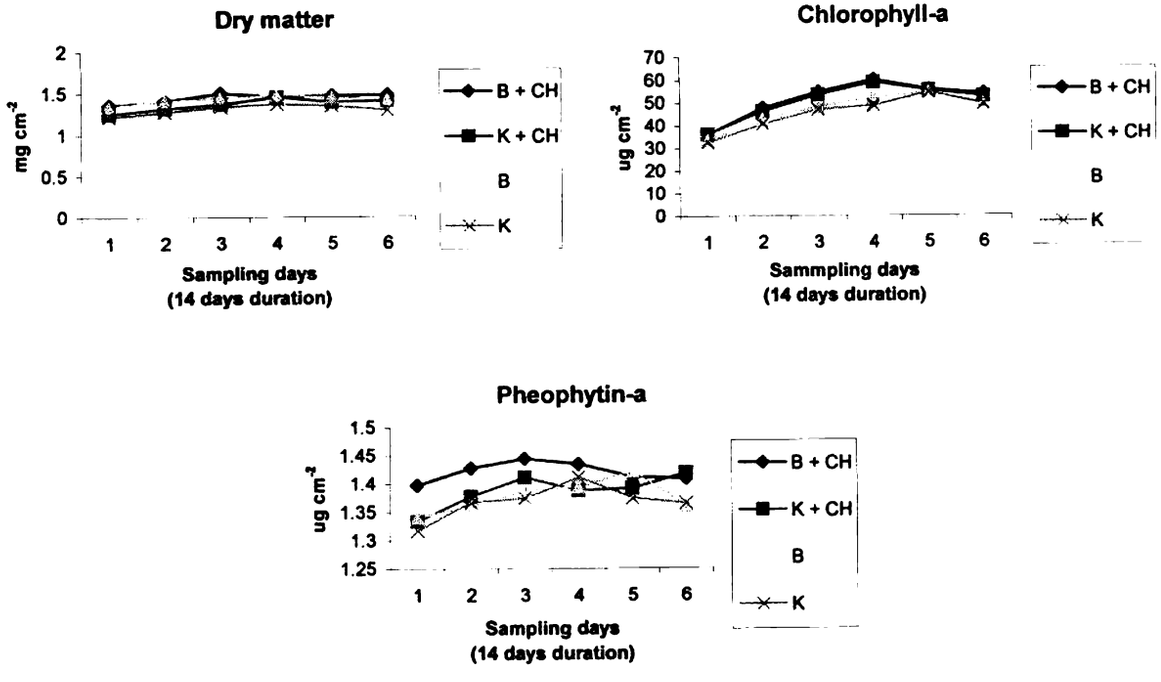

Organic carbon
20
15
10
5
0
ug l-1
1
2
3
4
5
6
Sampling days
(14 days duration)
CH
B + CH
K + CH
B
K

Fig. 8.3
Periphyton and phytoplankton biomass during sampling days



Chapter – 9

Summary

Aquaculture production systems used across the world differ widely depending on the species being cultured and on the geographical location and socio-economic context. Tiger shrimp, *Penaeus monodon* (Fabricius) is the most widely used candidate species for shrimp farming in South-East Asia. Worldwide aquaculture has been increasing rapidly in the last decade; approximately at an average rate of more than 10% per year (Muir, 1995; Tacon, 1997; Pedini and Shehadeh, 1997; World Bank, 1998; FAO, 2001). Asian countries contribute to 91% of the world shrimp production (FAO, 2002). Of the global shrimp production, 90% comes from extensive and modified extensive types of farming (FAO, 2001). A sound knowledge on the biology of this species, its rapid growth, bigger size and good demand in both domestic and export markets have made it the most dear species for culture in the brackishwater grow-out of India. One of the major problems encountered in shrimp aquaculture system is the accumulation of toxic inorganic species (NH_4^+ and NO_2^-) in the pond sediment. The prime source of ammonium to the farming system is typically from the protein rich feed. Only 20 - 40% of the nitrogen is incorporated to the shrimp tissue, while the remaining is lost to the pond and ultimately to the environment. As such no effective mechanism is available to remove the nitrogenous metabolites accumulation in the pond bottom. The high feed input and shrimp stocking density applied in the shrimp

aquaculture system result in the enrichment of the water with the ammonium and other inorganic nitrogenous species. The management of such system thus calls for developing methods to remove these compounds from the pond. One of the common solutions used to remove the excessive nitrogen is by resorting to frequent exchange of water. However, this approach is becoming highly regulated in order to maintain environmental sustainability by avoiding excessive release of nutrients into environment, the danger of introducing pathogens to the environment during water exchange, high operational cost incurred in pumping etc. Another approach would be encouraging adoption of a technology for enhancing nitrification process of the ammonium and nitrite to the relatively inert nitrate species. This is often done by employing bio-filters, however, the high cost and surface area involved in treating and digest a large mass of feed residue is one of the main problems associated with this technology. An additional strategy that is presently giving attention is the removal of ammonium from the water through its assimilation in to microbial protein by addition of carbonaceous material to the system (Avnimelech, 1999). The addition of properly adjusted carbohydrates could potentially eliminate the problem of inorganic nitrogen accumulation. An added advantage of this technology is the potential utilization of microbial protein as a source of protein for fish and shrimp. The technology developed based

on the introduction of artificial substrates in the culture system for increasing the production of natural food attached to surface areas (periphyton) was also found to be effective in the harvesting of nutrients. In the present study, a pioneer attempt was made to develop a technology for the reduction of inorganic nitrogen in water and sediment and feed protein wastage by the addition of carbohydrate, and also to reduce damage to the ecosystem and to convert nitrogen in to harvestable product by both the addition of carbohydrate and introduction of substrates based aquaculture. The developing eco-friendly, economically sustainable and scientific culture practice incorporating innovative management strategies will be useful in improving the marketable yield and income from the farming of the tiger prawn.

This study encompasses the results of seven experiments which are presented in 7 chapters. The topic is adequately introduced in the first chapter and an exhaustive review of the relevant literature on the subject is made in Chapter 1. The optimal dietary protein level was assessed for the addition of carbohydrate in *Penaeus monodon*, with a view to know whether the addition of carbohydrate will helps in increasing the shrimp production and reducing the feed cost. Two dietary protein levels 25% and 40% (P25 and P40) with or without carbohydrate source addition (P25 + CH and P 40 + CH) were compared at a stocking density @ 6 post larvae (PL 20) of *Penaeus*

monodon m^{-2} (0.357 ± 0.01 g). The quantity of carbohydrate was calculated following Avnimelech (1999); i.e. 390 g and 620 g tapioca flour kg^{-1} of the 25% and 40% diet fed, respectively. The addition of carbohydrate source was found useful in significantly ($P<0.05$) reducing the total ammonia nitrogen (TAN) in the water and sediment. Protein level in the diet had a significant effect ($P<0.05$) in the inorganic nitrogen concentration in the sediment. Treatment P40 showed higher ($P<0.05$) water TAN, nitrite-N and nitrate-N level, while it was lowest in the treatment P25 + CH. Total heterotrophic bacterial (THB) population both in the water column and sediment increased ($P<0.05$) during the period. Lower specific growth rate (SGR), and higher feed conversion ratio (FCR) were recorded in P25 treatment when compared to other treatments. Higher shrimp yield was recorded in P25 + CH when compared to P40. Survival of shrimp was not effected by the treatments. The results revealed that the demand for dietary protein level 40% can be lowered to 25% in favour of carbohydrate addition to the water column without compromising shrimp production. Furthermore, addition of carbohydrate was useful in reducing toxic inorganic nitrogen levels in the pond as well as effluents.

The effect of various mode of carbohydrate application and diets having various protein levels were evaluated in the production and sustainability of *Penaeus monodon*. Five treatments viz. carbohydrate

added to the diet (CHD), 25% protein diet (P25), 25% protein diet with carbohydrate source addition to the water column (P25 + CH), 40% protein diet (P40) and 40% protein diet with carbohydrate source addition to the water column (P40 + CH) were compared. The experiments were carried out in 6 m^2 concrete tanks, with a muddy bottom and stocking density of 7 post larvae (PL 20) of *Penaeus monodon* m^{-2} (0.015 ± 0.01 g). Tapioca flour was used as carbohydrate source and applied to the water column after the first feeding during the day. A 40% dietary protein feed was pulverized and repelletised after adding carbohydrate source at 620g kg^{-1} of feed. While the addition of carbohydrate was resulted in changing the carbon / nitrogen ratio of the feed at par with a 25% protein diet. Addition of carbohydrate to the water column significantly reduced ($P<0.05$) the concentration of total ammonia nitrogen (TAN), in treatment P25 + CH (5.91 µg l^{-1}) in contrast to the addition of carbohydrate to the diet (7.04 µg l^{-1}) while the treatment P25 registered high TAN concentration 9.79 µg l^{-1}. THB population showed significant increase ($P<0.05$) during the culture period. No significant variation ($P>0.05$) in chlorophyll-a was observed during the culture period. Higher specific growth rate (SGR) and lower feed conversion ratio (FCR) were recorded in P25 + CH treatment among other treatments ($P<0.05$). Higher shrimp yield was recorded in P25 + CH (148.4 g m^{-2}) than P25 (113.9 g m^{-2}) and CHD (124.9 g m^{-2})

($P<0.05$). Net protein value (%) and protein efficiency ratio (PER) were significantly higher ($P<0.05$) in P25 + CH treatment. Survival of the shrimps was not affected by treatments ($P>0.05$). The application of carbohydrate through the diet had no significant effect in reducing the toxic inorganic nitrogen production and in increasing the shrimp yield. Addition of carbohydrate to the water column will be helpful in increasing heterotrophic bacterial growth, which will supply bacterial protein to enhance the shrimp production, thus, a reduction of percentage of protein in diet from 40% to 25% is possible without compromising shrimp production. This technology is highly useful in reducing the feed cost. A reduction of toxic inorganic nitrogen levels in the pond as well as effluents is also useful in making the farming more ecologically sustainable and eco-friendly.

On-farm trails were carried out to prove the viability of carbohydrate addition in an extensive shrimp farming system of *Penaeus monodon* in improving sustainability, reducing feed cost and growth performance when fed with a low protein feed. 25% dietary protein with carbohydrate (P25 + CH) and 40% dietary protein (P40) were compared in earthen ponds each having 250 m^2 area. The stocking density was maintained @ 6 post larvae (PL 20) of *Penaeus monodon* m^{-2} (0.016 ± 0.01 g). Tapioca flour was used as carbohydrate source which was directly applied to the water column after the first feeding during the day. Total ammonia nitrogen (TAN) concentration

in water and sediment in treatment P25 + CH was significantly lower ($P<0.05$) when compared to P40. Highest THB value was observed in the treatment P25 + CH (57.04 x 10^5 cfu ml^{-1}) due to the addition of carbohydrate. Low protein diet with addition of carbohydrate to the water column showed a significant effect ($P<0.05$) in the shrimp yield in P25 + CH when compared to P40 and the values were 64.4 and 44.8 g m^{-2} respectively. Chlorophyll-a showed significant increase ($P<0.05$) during the culture period and the value ranged from 9.7 – 45.1 µg l^{-1}. The variables PER, FCE, ADG and NPV were higher in the treatment P25 + CH and it was significantly different ($P<0.05$) from P40. Survival of shrimp was not affected ($P>0.05$) by the treatment and it was 35.5% and 42.2% in P40 and P25 + CH respectively. Total variable cost was much higher in treatment P40 (IRS. 97, 420.0/-) when compared to P25 + CH (IRS. 73, 302.0/-). Cost of feed and carbohydrate source for P25 + CH was lower than P40 since the latter is costly due to high protein content. Total revenue from the harvested shrimp (ha^{-1}) was 54% higher in treatment P25 + CH than in P40 due to the combined effect of better yield and higher price of shrimp due to their precarious size. Net profit from the P25 + CH was IRS. 1,14,292.0/- which was significantly higher than P40 (IRS. 20,422.0/-). The benefit cost ratio was significantly ($P<0.05$) higher in treatment P25 + CH (1.4) than to P40 (0.2). By applying the technology, the farming can become more economically viable due to

the reduction of dietary protein level by the addition of carbohydrate to the water column. Farming on the basis of this technology is very useful in improving the ecological sustainability of extensive shrimp farming system. The technology would benefit the extensive farming practices by increasing production; reducing feed cost and production of low level of inorganic nitrogen in the pond and furthermore, low discharge of nutrients into the environment.

Stocking density and carbohydrate addition relationship in the yield and sustainability of *Penaeus monodon* (Fabricius) were studied. 25% dietary protein feed with and without carbohydrate addition were the treatments used in the study. Carbohydrate was applied to the water column followed by the first feeding during morning. The experiments were carried out in $6m^3$ concrete tanks stocked with 3, 7 and 12 post larvae m^{-2} of *Penaeus monodon* (PL.20) (0.015 ± 0.01 g). TAN concentrations of water and sediment showed a cumulative increase in treatment with out carbohydrate when compared with treatments 3 + CH, 7 + CH, 12 + CH. Low TAN concentration observed in carbohydrate added treatments are clear manifestation of its ability to reduce the concentration of inorganic nitrogen. The result shows that there exist a direct correlation between stocking density and level of toxic inorganic nitrogen irrespective of carbohydrate addition. A significant increase in the total heterotrophic bacterial (THB) count of both water and soil was observed due to the addition of carbohydrate.

In the present study, higher shrimp yield and FCE were recorded in the carbohydrate added treatments when compared to control. The survival rate of shrimp was similar between treatments. It may be concluded that, the manipulation of carbon / nitrogen ratio due to the carbohydrate addition is possible in varying stocking densities. Addition of cheap carbohydrate to the system is proved to be very useful in producing microbial protein, thus enabling more yields from shrimp culture system. 25% dietary protein with addition of carbohydrate is a better combination for the farming of shrimp. The level of inorganic nitrogen showed significant reduction both in water and sediment, thus making grow-out more ecologically sustainable. The results of the present study were found to be highly useful in improving the sustainability and profitability from the intensive and modified extensive shrimp farming systems.

The efficiency of various type of carbohydrate in the control of inorganic nitrogen and increasing production of *Penaeus monodon* (Fabricius) have been evaluated. The experiments were carried out in 6 m^2 tanks having muddy bottom. 25% dietary protein feeds with addition of each carbohydrate sources such as tapioca flour, wheat flour, rice flour, potato flour and yam flour as different carbohydrate source were used for comparison. Stocking density @ 7 post larvae (PL 20) of *Penaeus monodon* 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. Nitrate-N ranged from 2.97 – 3.22 $\mu g\ l^{-1}$ in the treatments studied, where as rice and wheat flour treatments showed significant difference ($P<0.05$) from potato flour. During the culture period, sediment TAN values were in the range 14.71 – 17.18 $\mu g\ l^{-1}$ whereas THB values ranged from 69.04 – 86.21 x 10^5cfu ml^{-1} and among the treatments rice and tapioca flour showed significant difference ($P<0.05$). No significant difference ($P>0.05$) was observed in individual shrimp weight, shrimp yield, SGR, and average daily weight between treatments. Survival of the shrimp was not affected by the treatments ($P>0.05$). The addition of carbohydrate was useful in reducing toxic concentration of nitrogenous species, whereby the environmental conditions can be made highly favorable for shrimp farming, thus showing a high survival rate, thus becoming shrimp farming more sustainable and economically viable.

The efficiency of artificial substrates in the periphyton production, evaluation of quantity and quality based on the nature of substrate and optimization of fertilization rates in the production of periphyton in the absence of shrimp grazing pressure were investigated. The experiment was carried out in $6m^2$ concrete tanks with muddy bottom having artificial substrate either with poles of

bamboo and kanchi. The effect of three dose of fertilizer application, the first fertilizer dose of cattle dung – 1500 kg ha^{-1}, urea – 100 kg ha^{-1}, and super phosphate – 50 kg ha^{-1} (treatment 1 C), first fertilizer dose with substrate bamboo (treatment 1 B), first fertilizer dose with substrate kanchi (treatment 1 K), second fertilization dose of cattle dung – 3000 kg ha^{-1}, urea – 150 kg ha^{-1}, and super phosphate – 100 kg ha^{-1} with substrate bamboo (treatment 2 B), second fertilization dose with substrate kanchi (treatment 2 K) and third fertilization dose of cattle dung – 4500 kg ha^{-1}, urea – 200 kg ha^{-1}, and super phosphate – 150 kgha^{-1} with substrate bamboo (treatment 2.5 B), third fertilization dose with substrate kanchi (treatment 2.5 K). Bamboo poles and kanchi poles at a density of 9 poles m^{-2} and 34 poles m^{-2} were studied. 2.5 B showed significantly higher ($P<0.05$) periphyton biomass in terms of dry matter, ash content and ash free dry matter when compared to 1 K and there is no significant difference with ($P>0.05$) 2 B. Depending on the fertilization level and substrate type, water and soil TAN, nitrite-N, organic carbon and water chlorophyll-a were showed significant variation ($P<0.05$) during the experimental period. Percentage of periphyton protein was found to be significantly higher in 2.5 B and 2 B treatment followed by 2.5 K. 72 genera of periphyton and 69 genera of phytoplankton were identified. In 2.5 B, fertilization dose was found very effective in producing higher phytoplankton and periphyton quantity, however

there was no significant difference (P>0.05) with 2 B treatment. 2 B treatment exhibit the rapid development of a relatively stable community showing slight difference with that of treatment 2 K. The fertilizer dose with (Treatment 2 B and 2 K) cattle dung – 3000 kg ha^{-1}, urea – 150 kg ha^{-1}, and super phosphate – 100 kg ha^{-1} respectively is recommended as optimal for periphyton growth, on the basis of high quantity of periphyton production and the superior quality of the periphyton. Bamboo is recommended as a better substrate for periphyton production. Periphyton substrates, bamboo and kanchi did not have any adverse effect on water and sediment quality parameters of the experimental tanks.

The combined effect of periphyton and addition of carbohydrate in the production and sustainability of *Penaeus monodon* (Fabricius) were studied. Tapioca flour was used as carbohydrate source and applied to the water column immediately after the first feeding during the day. Poles of either bamboo or kanchi at a density of 9 poles m^{-2} and 34 poles m^{-2} were used as artificial substrates. Bamboo or kanchi with 25% protein feed (B, K), addition of carbohydrate source (B + CH, K + CH) and without substrate with addition of carbohydrate was used as control (CH), were compared. Stocking density was maintained @ 7 post larvae (PL 20) of *Penaeus monodon* m^{-2} (0.015 ± 0.01 g). The application of fertilizer with cattle dung @ 3000 kg ha^{-1}, urea 150 kg ha^{-1} and super phosphate 100 kg ha^{-1} were used for

enhancing the productivity. The experiment was carried out in 6m^2 concrete tanks with sediment layer. Bamboo poles and kanchi poles at a density of 9 poles m^{-2} and 34 poles m^{-2} erected vertically and horizontally respectively for the periphyton production. While comparing those of carbohydrate added treatments with non carbohydrate added ones, TAN showed significant reduction while total heterotrophic bacterial production in water and soil showed a significant increase ($P<0.05$)in treatments with carbohydrate. B + CH treatment showed significantly higher ($P<0.05$) periphyton production followed by the treatment K + CH. Treatment B + CH showed higher periphyton production (45560.74 cells or colonies l^{-1}) with progression of culture which was significantly different ($P<0.05$) from other treatments, the lowest periphyton production was observed in treatment K (16245.25 cells or colonies l^{-1}). The individual shrimp weight, net shrimp yield, SGR and survival rate were higher in treatment B + CH followed by K + CH. Addition of carbohydrate together with bamboo as the artificial substrate were found to be effective in reducing the inorganic nitrogen concentration and enhancing the shrimp yield. Periphyton grown on bamboo and kanchi poles is an excellent natural food for shrimp species which might have helped in increasing both growth and survival of the cultured species. Based on the results of the present study, it can be concluded that the sustainability and yield of *Penaeus monodon* can be substantially

improved by planting bamboo poles as artificial substrates and addition of carbohydrate to optimizing the carbon / nitrogen ratio in the extensive culture.

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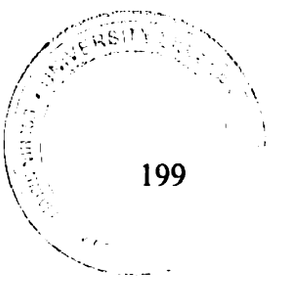

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Effects of carbohydrate addition on production in extensive shrimp culture systems

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Abstract

One indoor and one on-farm trial were conducted to evaluate the effect of control of carbon/nitrogen ratio (C/N ratio) by addition of carbohydrate to the water column in extensive types of shrimp culture systems. In the indoor experiment, 25% and 40% dietary protein ('P25' and 'P40') with or without carbohydrate source addition ('P25+CH' and 'P40+CH') were compared in fiber reinforced plastic tanks of 1200-l capacity stocked with 6 *Penaeus monodon* juveniles (0.357±0.01 g) m^{-2}. In the on-farm trial, 25% dietary protein with carbohydrate ('P25+CH') and 40% dietary protein ('P40') were compared in 250-m^2 earthen ponds stocked with 6 post-larvae of *P. monodon* m^{-2}. Tapioca flour was used as carbohydrate source and applied to the water column followed by the first feeding during the day in both experiments. The addition of carbohydrate significantly ($P<0.001$) reduced the total ammonia nitrogen (TAN) in the water and sediment in both experiments. It significantly ($P<0.05$) increased the total heterotrophic bacterial (THB) population both in water column and sediment. In the indoor experiments, lower specific growth rate (SGR) and higher feed conversion ratio (FCR) values were recorded in the 'P25' treatment compared to shrimps in other treatments ($P<0.05$). Higher shrimp yield was recorded in 'P25+CH' (64.43 g m^{-2}) when compared to 'P40' (44.79 g m^{-2}) ($P<0.001$) in the on-farm trial. The FCR value was lower ($P<0.05$) in the 'P25+CH' treatment than in the 'P40' treatment. The nitrogen retention (%) and protein efficiency ratio (PER) were higher ($P<0.001$) in the 'P25+CH' treatment when compared to other treatments in both experiments. Survival of the shrimps was not affected by treatment

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(P>0.05). In the on-farm trial the benefit cost ratio was higher in 'P25+CH' treatment than 'P40' (1.3 against 0.2) and the profit increased 400% in'P25+CH' treatment. A 35% reduction of feed cost and 54% increase in the revenue from shrimp was recorded in the 'P25+CH' treatment when compared to the 'P40'. Control of C/N ratio by the addition of a carbohydrate source to the pond water column benefited the extensive shrimp culture practices in three ways (1) increased heterotrophic bacterial growth supplying bacterial protein to augment the shrimp production, (2) reduced demand for supplemental feed protein and subsequent reduction in feed cost and (3) reduced toxic inorganic nitrogen levels in the pond as well as effluents.

Keywords: C/N ratio; Extensive shrimp culture; Carbohydrate; Heterotrophic bacteria; *Penaeus monodon*

1. Introduction

Shrimp farming in ponds is one of the major aquaculture activities in the tropical and subtropical countries contributing 26.1% of the global shrimp landing (Tacon, 2003). Extensive and modified extensive types of the culture, which are characterized by low to no inputs (feed, fertilizer, etc.) and low stocking densities contribute the bulk of the global shrimp production (90%) (FAO, 2001). However, the adverse environmental impacts resulting from the intensification of shrimp culture practices in many of the coastal regions in the tropics and subtropics (Naylor et al., 1998) have drawn attention to the development of sustainable shrimp faming techniques.

One of the potential management measures to improve production and nutrient retention in shrimp culture systems is the addition of organic carbon rich substrate (glucose, cassava, sorghum meal or cellulose) to control the carbon/nitrogen ratio (C/N ratio) (Avnimelech, 1999). The C/N ratio has been widely used as an index of the rate at which organic matter will decompose (Alexander, 1961). If the organic matter is low in nitrogen content (i.e. high C/N ratio), some of the nitrogen for the microbial growth must be obtained from the water column and will become immobilized as microbial protein (Boyd, 1996). This phenomenon was utilized in the reduction of dissolved inorganic nitrogen in intensive, well aerated and circulated aquaculture ponds by the application of organic carbon sources and adjustments in the C/N ratio in the feed (Avnimelech et al., 1989; Avnimelech, 1999; Browdy et al., 2001). Previous studies have shown that immobilization of inorganic nitrogen only occurs when the C/N ratio of the organic matter is higher than 10 (Lancelot and Billen, 1985). The manipulation in the C/N ratio may result in a shift from an autotrophic to a heterotrophic system (Avnimelech, 1999; Browdy et al., 2001). The heterotrophic bacterial population utilizes the inorganic N to synthesize bacterial protein and new cells (single cell protein) and it may be utilized as a food source by carp, tilapia (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993) or shrimp (Burford et al., 2004), thus lowering the demand for supplemental feed protein (Avnimelech, 1999).

Studies that investigated the effect of addition of organic carbon sources on the reduction of inorganic nitrogen in aquaculture ponds concentrated on intensive or super intensive systems with constant mixing and aeration (Avnimelech, 1999). An investigation

on the effect of addition of organic carbon to extensive stagnant shrimp ponds on water quality and production has not been done, because keeping suspended solids distributed in the water column is believed to contribute to the success of the technique. However, because the bulk of cultured shrimps are still grown in extensively managed stagnant water ponds, even a small effect of carbohydrate addition on production will have a major effect on global shrimp production.

The present study aimed to reduce the inorganic nitrogen accumulating in an extensive shrimp culture system by (1) increasing the C/N ratio of the feed through reducing its protein content and by (2) increasing the C/N ratio further through carbohydrate addition to the ponds. These manipulations should facilitate the development of heterotrophic bacteria and the related in situ protein synthesis, which in turn will contribute to the protein intake of the shrimps.

2. Materials and methods

One indoor and one on-farm trial were conducted. The indoor experiment had a 2×2 factorial design with two levels of dietary protein (25% and 40%) as first factor and with and without carbohydrate source addition to the water column as second factor. The treatments without carbohydrate addition are abbreviated as 'P25' and 'P40', while the treatments with carbohydrate source are abbreviated as 'P25+CH' and 'P40+CH'. Based on the results of the indoor experiment (similar performance of shrimp but a large difference in water quality parameters and nitrogen input), the 'P25+CH' and 'P40' treatments were selected for comparison in the on-farm trial.

2.1. Experimental setup

2.1.1. Indoor experiments

The experiment was carried out in 1200-l fiber reinforced plastic (FRP) tanks having an effective bottom area of 1.86 m^2. All the tanks were provided with a uniform sediment layer (4 cm thick) taken from an extensive shrimp culture pond. Culture tanks were filled with 26 ppt saline water from the Cochin estuary, Kerala State, India, which was pumped into a concrete tank and kept for 1 week. The water level was maintained at 50 cm without water exchange during the 60-day experiment. Twenty-day-old post-larvae of *Penaeus monodon* (PL 20) purchased from a commercial hatchery were nursed for 30 days in FRP tanks at a stocking density of 250 m^{-2}. The PLs were purchased from a reliable provider and visually checked for signs of diseases or parasites, which were not found. No tests for disease screening were performed. After 30 days, uniformly sized (0.357±0.01 g) juveniles were selected and initial length and batch weight were recorded before stocking at a density of 6 juveniles m^{-2} in the culture tanks. To stimulate phytoplankton bloom, culture tanks were fertilized with urea and super phosphate at the rate of 4 and 1 g m^{-2} $week^{-1}$ during the first 3 weeks of the experiment. Two shrimp diets were prepared to provide 25% and 40% crude protein with C/N ratios of 12.9 and 8.1, respectively. The crude lipid content of both the diets is 7.3%. Locally purchased tapioca flour (flour of dried roots of tapioca plant *Manihot esculenta*, which contain 74–76% nitrogen-free

extracts and <2% crude protein) was used as carbohydrate source. Twenty grams of carbohydrate was added per gram of N-NH_4^+ released. The amount of N-NH_4^+ released was estimated assuming that the added carbohydrate contains 50% carbon and that 50% of the dietary protein input was converted to ammonia. In consequence, 0.39 and 0.62 kg tapioca flour were applied for each kg of the 25% and 40% dietary protein feed administrated, respectively. The photoperiod was maintained at 12-h dark and 12-h light for the whole experimental period.

Triplicate tanks were maintained for each treatment and the tank allocation for each treatment was completely randomized. Shrimps were fed with experimental feed at 15% of initial weight and adjusted gradually to 6% at the end of the culture. The pelleted sinking feed was distributed evenly over the tank's surface, twice daily at 08:00 and 18:00 h. The pre-weighed tapioca flour was mixed with tank water in a beaker and applied to the water column uniformly followed by the first feeding during the day. Shrimps were harvested after draining the tanks: individual length, weight and survival were recorded. Specific growth rate (SGR), feed conversion rate (FCR), protein efficiency ratio (PER) and nitrogen retention (%) were calculated as follows:

SGR=[(ln final weight−ln initial weight)×100]/days of experiment
FCR=feed consumed (dry weight)/live weight gain (wet weight)
PER=live weight gain/protein consumed
Nitrogen retention (%)=(nitrogen in shrimp biomass×100)/nitrogen in feed given

2.1.2. On-farm trial

Eight 250-m^2 earthen ponds, part of a locally traditional shrimp–rice alternating farming system, were selected for the field experiment. The ponds were prepared following the usual pre-stocking procedures of an extensive shrimp farm, which includes draining up of the ponds after rice cultivation, cleaning the aquatic weeds and the remains of the rice crop, and strengthening of the dike and periphery. Lime was applied at 2000 kg ha^{-1}. Elimination of the wild fishes was performed by the application of tea seed cake at 60 kg ha^{-1}. Saline water used to fill the ponds was filtered through a 300-μm nylon screens to eliminate fish eggs and larvae. Water depth was maintained at 1 m throughout the experimental period. Initially, cow dung was applied at 1000 kg ha^{-1} $crop^{-1}$. However, urea and single super phosphate were added biweekly to water column at 80 and 20 kg ha^{-1}, respectively, during the first two months of culture to initiate an algal bloom in the ponds. Lime was added to the ponds at 5 kg $pond^{-1}$ on a biweekly basis to maintain the pH and alkalinity. PL-20 of *P. monodon* (0.016±0.001 mg) purchased from a commercial hatchery were stocked at 6 PL m^{-2}.

Four randomly allocated ponds were used for each treatment. Commercial 25% and 40% crude protein sinking pelleted shrimp feeds (Higashimaru Feeds India Limited) were used for the experiment. Shrimps were fed at 20% of their initial biomass and adjusted gradually to 4% at the end of the culture. Tapioca flour was pre-weighed at 0.39 kg tapioca flour for each kilogram of 25% dietary protein feed, and applied uniformly over the pond surface on a daily basis. Shrimps were harvested on 94th day of culture, total shrimp yield from each ponds were estimated, and subsamples were weighed and counted for calculating the average weight and

shrimp survival. Shrimp and feed samples were analyzed for moisture and nitrogen (AOAC, 1990).

2.2. Water and sediment quality parameters

Water quality parameters, temperature (mercury thermometer), dissolved oxygen (portable DO meter-Eutech instruments, Singapore for indoor experiment; Winkler method (APHA, 1995) for on-farm trial), salinity (hand refracto meter; Atago, Japan), pH (pH-Scan-Eutech instruments, Singapore) and transparency (Secchi disc) were measured in situ at 09:00 h on daily basis. Water samples were collected using a horizontal water sampler from three locations of each tank/pond and pooled together. Sediment samples were collected from three locations using PVC pipes (having 1.5 cm diameter for indoor experiment, 4 cm for on-farm trial). Both water and sediment samples were transported to the laboratory within 2 h after collection in iceboxes and analyzed. Sediment and water samples were collected on biweekly basis between 09:00 and 10:00 h. Composite water column samples were filtered through a GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrate-N (NO_3^--N) (cadmium reduction), nitrite-N (NO_2^--N), total ammonia nitrogen (TAN) (phenol hypochlorite method) and soluble reactive phosphate (SRP) (ascorbic acid method) (Grasshoff et al., 1983). Chlorophyll-*a* in non-filtered water column samples was estimated following standard method (APHA, 1995). The total nitrogen and phosphate in the water sample was estimated following persulphate digestion (Grasshoff et al., 1983). Biological oxygen demand (5-day BOD for indoor and 2-day BOD for on-farm trial) and chemical oxygen demand of water samples were estimated following APHA (1995). The organic carbon in the sediment was determined following EI Wakeel and Riley (1957). Exchangeable TAN, NO_2^--N, NO_3^--N in the sediment were measured (Mudroch et al., 1996). Total Kjeldahl nitrogen in the water (on-farm trial) (APHA, 1995) and sediment (Mudroch et al., 1996) was also estimated. Total heterotrophic bacteria (THB) count in the water and sediment was estimated following the standard procedures (APHA, 1995) and expressed as colony forming units (*cfu*). Records were maintained for the feed, carbohydrate source, lime, fertilizer input and water exchange. The feed and carbohydrate cost per hectare and revenue from the shrimps for each treatment were computed. The cost per kilogram of the carbohydrate source, 25% and 40% protein feed were Rs. 10, 26 and 46, respectively. The market prices for 40 and 50 counts (number of shrimps per kilogram) were Rs. 280 and 300 kg^{-1}.

2.3. Statistical analysis

All non-repeatedly measured variables (shrimp growth, yield, FCR, SGR and PER, survival and nitrogen retention) were analyzed by a one-way ANOVA for the on-farm trial and by a two-way ANOVA for the indoor experiment. Survival of shrimp and nitrogen retention in shrimps was analyzed using arcsine-transformed data, however, non-transformed data are presented in the tables. Daily and biweekly sediment and water quality parameters including THB counts were compared by split-plot ANOVA with treatments as main factor and time as the subfactor (Gomez and Gomez, 1984). All the

ANOVA were performed using the SAS 6.21 program (SAS Institute, Cary, NC 27513, USA). If a main effect was significant, the ANOVA was followed by Tukey's test at $P<0.05$ level of significance.

3. Results

3.1. Indoor experiment

3.1.1. Water and sediment quality parameters

The mean temperature, pH, salinity, Secchi disc visibility and dissolved oxygen content of the treatments were in the range of 28.1–29.8 °C; 7.8–7.9, 26.3–26.7 ppt; 45.9–51.4 cm; 6.0–6.1 mg O_2 l^{-1}, respectively. There were no significant differences ($P>0.05$) in any of the above water quality parameters among the different treatment tanks. Dietary protein level and carbohydrate addition had no significant effect on the BOD_5, COD, total alkalinity and SRP. The values ranged between 3.5 and 4. 5 mg O_2 l^{-1}, 213.7 and 230.2 mg O_2 l^{-1}, 51.0–51.9 mg $CaCO_3$ l^{-1} and 4.2–6.3 μmol l^{-1}, respectively. The monthly observation on the chlorophyll-*a* concentration (24.6–28.4 μg l^{-1}) showed no significant variation between treatments.

The water and sediment quality parameters were summarized in Table 1. The addition of carbohydrate to the water column ($P<0.001$) reduced the TAN and nitrite-N levels in the experimental tanks. The total nitrogen concentration in the water was also reduced ($P<0.001$) by the addition of carbohydrate. The ANOVA results showed that the protein level in the diet was having a significant effect ($P<0.01$) on the water TAN, nitrite-N and total nitrogen concentrations. The treatment had no significant effect on the water nitrate-N concentrations; however, its levels in the tanks increased with time ($P<0.001$) (Table 2). TAN, nitrite-N and total nitrogen concentration in the water column of the different treatments varied with time ($P<0.001$) (Table 2).

The organic carbon content of the sediment increased ($P<0.001$) with time (Table 2); however, there was no treatment effect, both in terms of protein level and carbohydrate addition (Table 1). The addition of carbohydrate to the tanks facilitated significant reduction in TAN in the sediment (Table 1). Bulk density and TKN in the sediment were not affected by the application of carbohydrate (Table 1).

The THB ranged from 12.1 to 26.9×10^4 cfu ml^{-1} in the water column and from 24.8 to 62.1×10^6 cfu g^{-1} in the sediment. The results of the ANOVA showed that the addition of the carbohydrate source had a significant ($P<0.05$) effect on the THB count and it promoted the growth of THB population both in water column and sediment whereas the different protein levels had no effect on it. The THB count in the water increased ($P<0.05$) with culture period while the bacterial population in the sediment remained stable during the culture period (Table 2).

3.1.2. Yield parameters, feed conversion and nitrogen efficiencies

The SGR varied significantly ($P<0.01$) and higher values were recorded in treatments 'P40+CH', 'P40' and 'P25+CH' than in 'P25' (Table 3). The dietary protein level had an effect ($P<0.01$) on the SGR of shrimps. The results of the multi-

Table 1
Effect of carbohydrate addition and dietary protein levels on the water and sediment quality in indoor and on-farm trial

Variable	Treatments				S.E.M.[ψ]	Significance (P-value)[†]		
	P25	P25+CH	P40	P40+CH		P	CH	P×CH[‡]
Indoor experiment								
Water quality variable								
TAN (μg l^{-1})	4.7^{b}	3.1^{b}	9.0^{a}	3.7^{b}	0.516	**	**	**
Nitrite (μg l^{-1})	2.2^{b}	1.0^{c}	3.6^{a}	1.5^{bc}	0.171	**	***	NS
Nitrate (μg l^{-1})	5.0	2.0	7.5	3.6	1.112	NS	NS	NS
Total nitrogen (μg l^{-1})	168.9^{b}	102.6^{c}	341.1^{a}	199.6^{b}	8.88	**	***	**
THB ($\times 10^{4}$ *cfu* ml^{-1})	17.3^{ab}	23.9^{a}	12.1^{b}	26.9^{a}	16.34	NS	**	NS
Sediment quality variable								
TAN (μg g^{-1})	36.2^{a}	32.9^{ab}	36.2^{a}	31.1^{b}	0.812	NS	**	NS
TKN (μg g^{-1})	188.7^{ab}	177.0^{b}	218.2^{a}	202.0^{ab}	5.976	**	NS	NS
Organic carbon (μg g^{-1})	13.6	16.1	14.5	14.6	0.666	NS	NS	NS
Bulk density (g cm^{-3})	0.91	0.92	0.92	0.9	0.008	NS	NS	NS
THB ($\times 10^{6}$ *cfu* ml^{-1})	33.8^{ab}	41.3^{ab}	24.8^{b}	62.1^{a}	4.976	NS	**	NS
On-farm trial								
Water quality variable								
TAN (μg l^{-1})		1.5^{b}	3.5^{a}		0.889			**
Nitrite (μg l^{-1})		0.14	0.16		0.006			NS
Nitrate (μg l^{-1})		0.79	0.95		0.087			NS
TKN (μg l^{-1})		49.4^{b}	68.1^{a}		5.368			*
SRP (μg l^{-1})		0.6	0.6		0.066			NS
Chlorophyll-*a* (μg l^{-1})		18.9	18		3.463			*
THB ($\times 10^{5}$ *cfu* ml^{-1})		57.1^{a}	40.6^{b}		1.270			**
Sediment quality variable								
TAN (μg g^{-1})		29.7^{a}	25.8^{b}		1.014			*
Nitrite (μg g^{-1})		0.08	0.08		0.006			NS
Nitrate (μg g^{-1})		0.08^{b}	0.14^{a}		0.028			NS
TKN (μg g^{-1})		333.1	337.8		23.020			NS
Organic carbon (μg g^{-1})		20.4	19.6		0.657			NS
Bulk density (g cm^{-3})		0.73	0.76		0.009			NS
THB ($\times 10^{7}$ *cfu* ml^{-1})		53.9^{a}	41.5^{b}		1.366			**

Mean values in same row with different superscript differ significantly ($P<0.05$).

[ψ] SEM: Standard Error Mean value.

[†] Results from split-plot two way ANOVA; P=Protein level; CH=Carbohydrate addition; P×CH=Protein level×Carbohydrate addition interaction.

[‡] 'P×CH' notation for indoor experiment is the interaction of protein level and carbohydrate addition; for on-farm trial it represent the treatment effect of P25+CH vs. P40.

comparison (Tukey's test) showed that FCR of shrimps in 'P40+CH', 'P40' and 'P25+CH' (2.4–3.0) were significantly lower than the 'P25' treatment (6.4). The corrected FCR values were calculated by considering the quantity of carbohydrate source added to the tanks as feed. On comparing the corrected FCR values the 'P40' treatment showed the lowest FCR (3.0), while the difference between 'P25+CH' (4.1) and 'P40+CH' (3.9) was not significant. The nitrogen retention (%) and PER was

Table 2
Water and sediment quality in indoor and on-farm trial over sampling periods†

Variable	Sampling period								S.E.M.Ψ	Significance‡
	Initial	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7		*P*-value
Indoor experiment										
Water quality variable										
TAN (μg l^{-1})	0.1^{c}	0.2^{c}	7.1^{b}	13.5^{a}	4.6^{a}		–		0.67	***
Nitrite (μg l^{-1})	0.7^{c}	1.1^{c}	2.5^{b}	4.9^{a}	1.0^{c}	–	–	–	0.35	***
Nitrate (μg l^{-1})	0.6^{b}	0.2^{b}	1.3^{b}	8.1^{a}	12.4^{a}		–		1.22	***
Total nitrogen (μg l^{-1})	58.1^{c}	86.8^{c}	205.1^{b}	348.9^{b}	316.1^{a}		–		12.39	***
THB ($\times 10^{4}$ *cfu* ml^{-1})	–	–	14.5^{b}	–	25.6^{a}	–	–	–	23.33	***
Sediment quality variable										
TAN (μg g^{-1})	11.8^{a}	22.9^{d}	40.4^{c}	45.2^{b}	50.3^{a}	–	–	–	1.18	***
TKN (μg g^{-1})	118.7^{d}	171.4^{c}	212.6^{b}	222.2^{ab}	257.4^{a}	–		–	9.88	***
Organic carbon (μg g^{-1})	11.0^{c}	13.6^{bc}	15.97^{ab}	16.46^{ab}	16.6^{a}				0.72	***
Bulk density (g cm^{-3})	0.96	0.89	0.9	0.9	0.9	–	–	–	0.01	**
THB ($\times 10^{6}$ *cfu* ml^{-1})			37.7		43.2				5.54	NS
On-farm trial										
Water quality variable										
TAN (μg l^{-1})	3.2^{ab}	4.6^{a}	2.2^{b}	2.0^{b}	1.6^{b}	1.4^{b}	2.6^{ab}	2.3^{b}	0.51	***
Nitrite (μg l^{-1})	0.06^{c}	0.11^{b}	0.12^{b}	0.12^{b}	0.25^{a}	0.16^{b}	0.22^{a}	0.14^{b}	0.01	***
Nitrate (μg l^{-1})	1.3^{a}	1.1^{ab}	0.6^{bc}	1.6^{a}	1.3^{a}	0.1^{c}	0.6^{bc}	0.4^{bc}	0.16	***
TKN (μg l^{-1})	8.5^{d}	41.5^{cd}	50.7^{bc}	59.1^{abc}	61.2^{abc}	68.2^{abc}	85.8^{ab}	95.0^{a}	8.44	***
SRP (μg l^{-1})	1.49^{a}	1.58^{a}	0.57^{bc}	0.79^{b}	0.04^{d}	0.03^{d}	0.21^{cd}	0.20^{cd}	0.11	***
Chlorophyll-*a* (μg l^{-1})	9.8^{b}	5.5^{b}	7.0^{b}	10.6^{b}	20.0^{ab}	22.5^{ab}	27.5^{ab}	45.2^{a}	6.89	**
THB ($\times 10^{5}$ *cfu* ml^{-1})	28.0^{d}	29.6^{d}	35.5^{d}	39.6^{cd}	52.9^{bc}	61.9^{ab}	69.8^{a}	73.3^{a}	3.44	***
Sediment quality variable										
TAN (μg g^{-1})	17.1^{c}	32.5^{b}	31.2^{b}	20.2^{c}	17.9^{c}	23.3^{c}	36.4^{ab}	43.2^{a}	1.59	***
Nitrite (μg g^{-1})	0.03^{c}	0.05^{bc}	0.11^{ab}	0.05^{bc}	0.09^{abc}	0.07^{abc}	0.14^{a}	0.1^{abc}	0.02	***
Nitrate (μg g^{-1})	0.14^{a}	0.08^{a}	0.09^{a}	0.07^{a}	0.17^{a}	0.04^{a}	0.11^{a}	0.16^{a}	0.04	NS
TKN (μg g^{-1})	121.7^{d}	291.1^{c}	324.6^{bc}	347.0^{abc}	355.7^{abc}	391.8^{ab}	410.3^{ab}	441.4^{a}	21.66	***
Organic carbon (μg g^{-1})	10.8^{d}	18.9^{bc}	19.5^{bc}	19.7^{bc}	19.5^{bc}	22.3^{abc}	24.3^{ab}	25.1^{a}	1.13	***
Bulk density (g cm^{-3})	0.94^{a}	0.67^{b}	0.77^{b}	0.76^{b}	0.71^{b}	0.66^{b}	0.72^{b}	0.72^{b}	0.03	***
THB ($\times 10^{7}$ *cfu* ml^{-1})	17.0^{b}	46.3^{a}	51.5^{a}	51.4^{a}	51.0^{a}	58.0^{a}	53.4^{a}	53.0^{a}	3.17	***

Mean values in same row with different superscript differ significantly ($P<0.05$).

† One sampling period is 14 days.

Ψ SEM: Standard Error Mean value.

‡ Results from split-plot two way ANOVA.

higher ($P<0.001$) in 'P25+CH' treatment when compared to other treatments. Survival of the shrimps did not vary ($P>0.05$) among the different treatments (80–88.9%).

3.2. On-farm trial

3.2.1. Water and sediment quality parameters

Water temperature (32.7–32.7 °C), pH (7.7–7.7), salinity (13.0–13.2 ppt), Secchi disc transparency (59.5–60.9 cm) and dissolved oxygen (5.8–8.9 mg O_2 l^{-1}) did not vary ($P>0.05$) between the treatments but did vary over time ($P<0.01$). Treatment had no significant effect ($P>0.05$) on BOD_2 and COD of the water and the values ranged from 1.6 to 2.0 mg O_2 l^{-1} and from 384.5 to 386.0 mg O_2 l^{-1}, respectively. The total alkalinity was low and varied with culture period; however, it did not show difference among the treatments and mean values for '25%+CH' and '40%' were 8.8 and 8.6 mg $CaCO_3$ l^{-1}, respectively.

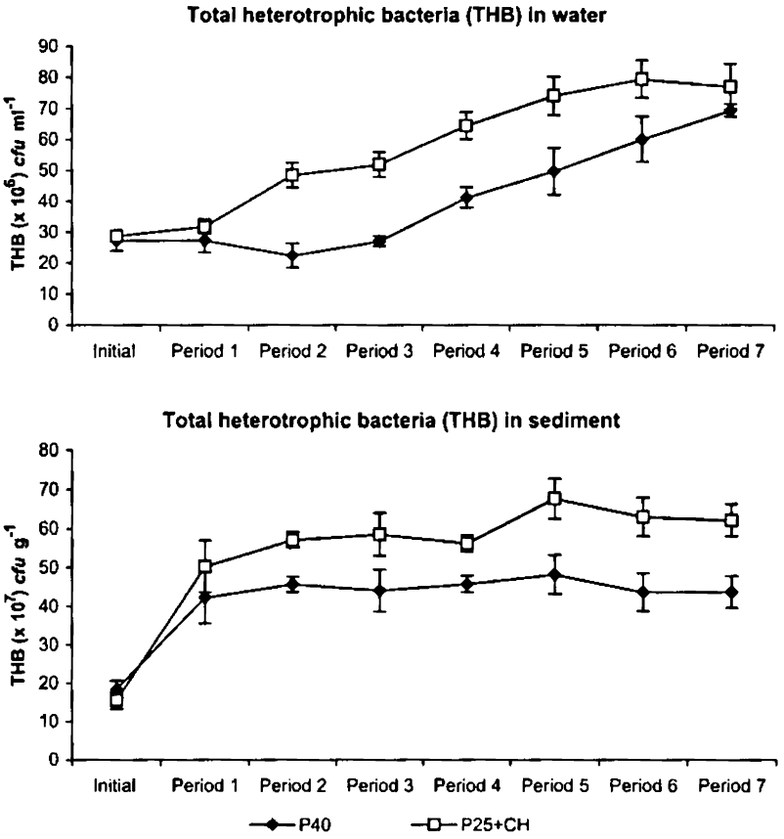

Fig. 1. Total heterotrophic bacteria (mean±S.E.) in the pond water and sediment of the on-farm trial. Number of replicates (n)=4 and each period represents biweekly sampling.

TAN concentrations varied significantly ($P<0.01$) among the treatments and lower level was recorded in 'P25+CH' than 'P40'. Nitrite-N and nitrate-N together with TAN showed significant variation with respect to time. TKN concentration was significantly lower in 'P25+CH' treatment when compared to 'P40' and the TKN values showed an increasing trend over time. The addition of carbohydrate to the water column had a profound effect on the THB population of pond water and the results of the split-plot ANOVA showed a significant variation ($P<0.01$) among the treatments and an increase with time (Fig. 1 and Table 2).

The addition of carbohydrate significantly ($P<0.05$) reduced the TAN concentration in the pond sediment from 29.7 ('P40') to 25.8 μg g^{-1} ('P25+CH'). TAN in the sediment showed variation with respect to time, resulting in a net accumulation (Table 2). Nitrite-N, TKN, organic carbon and bulk density of the sediment were not affected ($P>0.05$) by the

Table 3
Effect of carbohydrate addition and protein levels on weight, shrimp yield, SGR, FCR, nitrogen retention (%), PER and survival (%) of *P. monodon* in indoor and on-farm trial

	Treatments (mean±S.D.)				S.E.M.[ψ]	Significance (P-value)[†]		
	P25	P25+CH	P40	P40+CH		P	CH	P×CH[‡]
Indoor experiment								
Individual shrimp weight gain (g)	1.4 ± 0.3^{b}	2.4 ± 0.1^{a}	2.5 ± 0.3^{a}	3.0 ± 0.5^{a}	0.13	**	**	NS
Net shrimp yield (g m^{-2})	6.4 ± 0.5^{c}	13.5 ± 0.8^{b}	13.7 ± 0.7^{b}	17.0 ± 1.9^{a}	0.46	***	***	*
Specific growth rate	2.6 ± 0.3^{b}	3.4 ± 0.1^{a}	3.4 ± 0.2^{a}	3.8 ± 0.2^{a}	0.09	**	**	NS
Feed conversion ratio	6.4 ± 0.5^{a}	3.0 ± 0.2^{b}	3.0 ± 0.2^{b}	2.4 ± 0.3^{b}	0.13	***	***	**
Corrected feed conversion ratio	6.4 ± 0.5^{a}	4.1 ± 0.2^{b}	3.0 ± 0.2^{c}	3.9 ± 0.5^{bc}	0.02	***	*	**
Nitrogen retention in shrimp (%)	16.3 ± 0.5^{c}	28.9 ± 2.1^{a}	17.1 ± 1.3^{c}	22.4 ± 2.0^{b}	0.44	*	***	**
Protein efficiency ratio	0.6 ± 0.05^{c}	1.3 ± 0.1^{a}	0.9 ± 0.1^{c}	1.1 ± 0.1^{b}	0.03	NS	***	**
Survival rate (%)	80.6±17.3	88.9±4.8	88.9±12.7	88.9±9.6	5.77	NS	NS	NS
On-farm trial								
Individual shrimp weight gain (g)		25.7 ± 1.7^{a}	21.1 ± 0.8^{b}		0.67			**
Net shrimp yield (g m^{-2})		64.4 ± 12.2^{a}	44.8 ± 7.8^{b}		5.12			*
Total biomass harvested (g m^{-2})		83.5 ± 14.4^{a}	61.9 ± 7.9^{b}		5.82			*
Specific growth rate of shrimps		7.9 ± 0.1^{a}	7.7 ± 0.1^{b}		0.03			**
Feed conversion ratio		1.6 ± 0.3^{b}	2.2 ± 0.3^{a}		0.15			*
Corrected feed conversion ratio		2.3±0.4	2.2±0.3		0.18			NS
Nitrogen retention (%) in shrimps		45.3 ± 8.1^{a}	19.8 ± 3.2^{b}		1.90			**
Protein efficiency ratio		2.5 ± 0.4^{a}	1.2 ± 0.2^{b}		0.16			**
Survival rate (%)		42.2±10.5	35.5±7.0		2.62			NS

Mean values in same row with different superscript differ significantly ($P<0.05$).

[ψ] SEM: Standard Error Mean value.

[†] Results from split-plot two way ANOVA; P=Protein level; CH=Carbohydrate addition; P×CH=Protein level×Carbohydrate addition interaction.

[‡] 'P×CH' notation for indoor experiment is the interaction of protein level and carbohydrate addition; for on-farm trial it represent the treatment effect of P25+CH vs. P40.

treatment. Mean THB count of the sediment varied ($P<0.01$) from 41.5×10^7 ('40%') to 53.9×10^7 cfu g^{-1} ('P25+CH') revealing the positive effect of carbohydrate addition on the bacterial population of the pond sediment. The number of bacteria showed a significant increase from the initial sampling date to the second and then stabilized for the entire culture period (Fig. 1, Table 2).

3.2.2. Shrimp yield, FCR, nitrogen retention, PER and survival (%)

Individual shrimp weight at harvest was higher ($P<0.01$) in treatment 'P25+CH' than 'P40' (Table 3). The addition of carbohydrate to the water column even with low protein diet had a significant effect ($P<0.05$) on the shrimp production and the shrimp yield was 64.4 and 44.8 g m^{-2} in 'P25+CH' and 'P40', respectively. Some wild fishes were also

Table 4
Cost (in Rs.[a]) and economic analysis of on-farm experiment (per hectare)

Particulars	Quantity	Rate (Rs.[a])	Treatments		Significance (P-value)
			P25+CH	P40	
Variable cost					
Pond preparation	20 man days	150.0	3000.0	3000.0	
Eradication	60 kg tea seed cake	30.0	1800.0	1800.0	
Labour charge for eradication	4 man days	150.0	600.0	600.0	
Lime	3200 kg	1.2	3840.0	3840.0	
Cowdung	1000 kg	0.5	500.0	500.0	
Urea	320 kg	1.5	480.0	480.0	
Super phosphate	80 kg	3.0	240.0	240.0	
Seed	60,000 nos.	0.3	15,000.0	15,000.0	
Shrimp feed (25% crude protein)	1320 kg	26.0	34,320.0	0.0	
Shrimp feed (40% crude protein)	1320 kg	46.0	0.0	60,720.0	
Tapioca flour	130 kg	10.0	5200.0	0.0	
Salary of farm assistant	3 months	2500.0	7500.0	7500.0	
Power cost	200 units	1.5	300.0	300.0	
Harvest cost (Rs. kg^{-1})		5.0	3222.0	2240.0	
Fuel cost	44 l	27.3	1200.0	1200.0	
Total variable costs (Rs.)			77,202.0	97,420.0	
Fixed costs					
Interest			4500.0	4500.0	
Depreciation			1500.0	1500.0	
Total fixed costs			6000.0	6000.0	
Production					
Total shrimp yield (kg ha^{-1})			644.3[a]	447.9[b]	**
Price of shrimp (Rs. kg^{-1})			300.0	280.0	
Economic analysis					
Total production costs			83,202.0	103,420.0	
Gross return (Rs.)			193,275.0[a]	125,406.0[b]	*
Net profit (Rs.)			110,073.0[a]	21,986.0[b]	**
Benefit/cost ratio			1.3[a]	0.2[b]	**

Mean values in same row with different superscript differ significantly ($p<0.05$).
[a] US$ 1=44.65 Indian Rupees (Rs.).

harvested from the ponds and their quantity is included in the 'total biomass harvested' in Table 3. A higher ($P<0.01$) specific growth rate was recorded in treatment 'P25+CH' than in 'P40'. The 45.3% nitrogen retention in treatment 'P25+CH' was higher than the 19.8% nitrogen retention in treatment 'P40' ($P<0.01$). The shrimp survival was not affected ($P>0.05$) by the treatments and it ranged between 35.5% and 42.2%. The FCR was lower ($P<0.05$) in treatment 'P25+CH' than 'P40', while the corrected FCR values did not differ between treatments. The protein efficiency ratio was higher ($P<0.01$) in treatment 'P25+CH' than in 'P40'.

The extrapolated cost of feed and carbohydrate source (ha^{-1}) for 'P25+CH' was lower than 'P40' due to the higher cost of high protein shrimp feed (Rs. 46 against Rs. 26 kg^{-1}). The harvested shrimps from P25+CH and P40 came under 40 and 50 counts, respectively. The total revenue from the harvested shrimp (ha^{-1}) was 54% higher in treatment 'P25+CH' than in 'P40' due to the combined effect of better yield and high price of shrimps according to their marketable size (Table 4). A 35% reduction of feed cost was recorded in the 'P25+CH' treatment when compared to 'P40'. The net profit from the 'P25+CH' treatment was 400% higher than for 'P40'. The benefit cost ratio was significantly higher in treatment 'P25+CH' than in 'P40' (1.3 against 0.2) (Table 4).

4. Discussion

In the present study, the addition of carbohydrate to the water column increased the C/N ratio and resulted in a significant increase in the total heterotrophic bacterial count. Similar results were found by Burford et al. (2003). A significant reduction in the TAN in both sediment and water column was recorded in treatments with carbohydrate addition and concurs with findings of Avnimelech and Mokady (1988), Avnimelech et al. (1989, 1994) and Avnimelech (1999). However, the 0.15–13.5 μg l^{-1} TAN levels in the water column in the present study were low compared to 500–3000 μg l^{-1} TAN levels reported in other studies (Hopkins et al., 1993). In spite of these low concentrations, the addition of carbohydrate further reduced the TAN concentrations, and resulted in a higher THB in the water column and sediment. These finding concur with Cotner et al. (2000) who showed that glucose addition to water samples with a TAN concentration of 7.4–17.1 μg l^{-1} collected from Florida Bay enhanced microbial growth.

Shrimp rely on natural foods even in fed ponds. Studies using stable isotope have shown that the natural biota can contribute to shrimps nutrition in less intensive systems (Parker and Anderson, 1989; Cam et al., 1991; Burford, 2000). Focken et al. (1998) found 71% natural food in stomachs of *P. monodon* in semi-intensively managed fed pond. Four to ten percent of ^{15}N-enriched natural biota were retained by shrimp within 48 h (Burford, 2000). In the present study, the contribution of the phytoplankton to the natural food did not vary significantly among the treatments as indicated by the chlorophyll-*a* content.

The utilization of microbial protein depends on the ability of the target animal to harvest the bacteria and its ability to digest and utilize the microbial protein (Avnimelech 1999). The higher yield in the carbohydrate added treatments of the present study showed that *P. monodon* can well utilize the additional protein derived

from the increased bacterial biomass as a result of carbohydrate addition. Burford et al. (2004) suggested that 'flocculated particles' rich in bacteria and phytoplankton could contribute substantially to the nutrition of the *Litopenaeus vannamei* in intensive shrimp ponds.

The survival rates of shrimps were similar among the treatments in both experiments showing that carbohydrate addition to the ponds did not have a significant effect. This may be due to the fact that the water quality parameters recorded for both the experiments fell in the favorable limits of *P. monodon* (Chen et al., 1990; Hariati et al., 1996). However, the shrimp survival was 56% lower in on-farm trial when compared to the indoor experiments. This can be attributed to the presence of crabs and predatory fishes in the experimental ponds. The initial dose of tea seed cake applied proved to be inefficient to eliminate all the wild fishes. The occurrence of crabs and wild fishes are one of the constrains in the extensive shrimp ponds in Kerala Sate, India, affecting the shrimp survival and yield (Kurian, 1982). Another reason for the lower survival recorded in the on farm trial when compared to the indoor culture might be the size of the shrimps at the time of stocking. *P. monodon* PL 20 were stocked directly in the on-farm trial against 30-day-old nursery reared juveniles in the indoor trial.

The net shrimp yield from the indoor tanks was significantly higher in carbohydrate added treatments. The on-farm results were even more encouraging and showed that 25% protein diet together with carbohydrate addition to the water column resulted in a 43% higher shrimp yield, 116% higher protein efficiency ratio and a 128% higher nitrogen retention. No differences were observed between the corrected FCR values. Similarly, better feed and protein conversion coefficients were recorded in tilapia, fed with low protein and carbohydrate source in intensive ponds (Avnimelech, 1999).

The results of the indoor experiment showed that dietary protein level had a significant effect on the concentration of toxic inorganic nitrogen species. Li and Lovell (1992) reported that ammonia was increased in response to dietary protein concentration and total protein fed. In both the experiments, the toxic inorganic nitrogen species such as NH_4^+ and NO_2^--N (indoor experiment) in water column were significantly reduced by the addition of carbohydrate. The higher growth rate and shrimp yield can be attributed to the low inorganic nitrogen levels (Wahab et al., 2003) and increased heterotrophic production in the carbohydrate added ponds (Avnimelech, 1999; Burford et al., 2004; Burford et al., 2003). Furthermore, lower TAN in the sediment positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). Protein conversion efficiency of the shrimps in carbohydrate added ponds were higher; revealing that the input feed protein along with the microbial protein were effectively converted by shrimps into biomass. The feed conversion ratio recorded in the indoor experiment are relatively higher than on-farm trial, which may be due to the controlled laboratory condition and the values are comparable with those of Shariff et al. (2001). The corrected FCR values in the on-farm trial showed that the addition of carbohydrate along with low protein diet did not significantly add much quantity of feed material to the pond. The nitrogen retention in the shrimps in the carbohydrate added treatment was 28.9% in the indoor tanks and 45.3% in the on-farm ponds, which revealed that input nitrogen was very effectively utilized by the shrimps and converted into harvestable products in the carbohydrate treatments. These values are higher than that of Thakur and

Lin (2003) who recorded 22.8–30.7% of nitrogen retention in *P. monodon* reared in closed intensive culture systems.

The higher bacterial population and reduced levels of inorganic nitrogen in the carbohydrate added treatments revealed that the tapioca flour is a good source of organic carbon as it was well utilized by the heterotrophic bacterial population. In previous studies, several other carbohydrate sources like glucose and cassava meal cellulose powder (Avnimelech and Mokady, 1988; Avnimelech et al., 1989; Avnimelech et al., 1994; Avnimelech, 1999), molasses (Burford et al., 2004) were used in fish and shrimp ponds to reduce the TAN. In the present study, a more practical approach was made by selecting the tapioca flour as carbohydrate source, due to its low cost (Rs. 10 kg^{-1}), easy availability, low protein content and wide acceptance by the farmers as one of the potential feed ingredients (Hari, 2000).

The realistic economic analysis performed in the on-farm trial showed that the added carbohydrate with low protein feed substantially reduced the feed cost when compared to high protein feed. Furthermore, the combined effect of high shrimp yield and higher market prize for the shrimps increased the revenue by 54% and the net profit by 400% when compared to the high protein feed. Thus, this technology benefited the extensive shrimp culture practices in three ways: (1) increased production, (2) reduced feed cost and (3) reduced inorganic nitrogen in the pond.

In conclusion, manipulation C/N ratio by the addition of carbohydrate to the extensive shrimp culture system performed equally well as observed in intensive ponds with high levels of aeration and mixing. The demand for dietary protein level can be significantly reduced in favor of carbohydrate addition to the water column without compromising shrimp production, making farming more economically viable. The added carbohydrate facilitated increased heterotrophic bacterial growth while augmenting shrimp production. A direct link between the addition of carbohydrate and improved shrimp production was found. Concurrently, the levels of inorganic nitrogen species in the water column were lower due to uptake by heterotrophic bacteria, making farming also more sustainable.

The result of the present study could be useful in improving the sustainability of shrimp farming in extensive and modified extensive shrimp culture systems. There exists scope for further improvement of this management measure by optimizing the quantity of carbohydrate addition at various intensities of culture, and also comparing the potential of other carbohydrate sources. Radio or stable isotope studies are also needed to trace the organic carbon and inorganic nitrogen utilization by the pond microbial flora and also to quantify the contribution of microbial communities to total pond production.

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The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems

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Abstract

Water quality and shrimp production were monitored in extensively managed ponds which were fed a 25% (P25) or 40% (P40) dietary protein, each diet complemented with or without carbohydrate (CH) addition. The experiment was carried out in 6-m^3 concrete tanks, with a mud bottom and stocked with 7 post larvae (PL 20) of *Penaeus monodon* per m^2. Tapioca flour was used as carbohydrate source. CH addition reduced total ammonia nitrogen (TAN) and nitrite-nitrogen (NO_2^--N) in the water column and TAN in the sediment ($P<0.001$). CH addition also increased the total heterotrophic bacterial (THB) count in water column and sediment ($P<0.05$). Lower specific growth rate (SGR) and higher feed conversion ratio (FCR) were recorded in P25, compared to all other treatments ($P<0.05$). The 160 g m^{-2} shrimp yield in treatment P25+CH was similar to the 157 g m^{-2} yield in treatment P40, which was much higher than the 114 g m^{-2} yield in treatment P25 ($P<0.001$). CH addition to treatment P40, did not result in a higher yield ($P>0.05$). The protein efficiency ratio (PER) was higher ($P<0.001$) in treatment P25+CH compared to other treatments. Survival of the shrimps was not affected by treatment ($P>0.05$). A system nitrogen budget revealed that 16% to 21% of the total nitrogen input was retained in the shrimp, 0.22% to 0.49% in the water, 67% to 71% in the sediment, and 2.1% to 2.7% was lost through water exchange. The quantity of nitrogen not retained in shrimp biomass to produce 1 kg of shrimp ranged between 109.2 and 164.0 g N. The total water based N-loss (final pond water N+exchange N-loss) from an extensive type of shrimp culture system was within the range of 2.7% to 3.2% of the total input nitrogen. The percentage non-retained nitrogen was reduced by CH addition ($P<0.01$). In summary, CH addition to the water column under extensive shrimp culture conditions (1) increased the nitrogen retention in harvested shrimp biomass (2) reduced the demand for feed protein (3) reduced the concentration of

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TAN and NO_2^-–N in the pond, and (4) reduced nitrogen discharge making extensive shrimp farming more ecologically sustainable and economically viable.

Keywords: Carbohydrate addition; Extensive shrimp culture; Nitrogen budget; Heterotrophic bacteria; Water quality; Sustainability; *Penaeus monodon*

1. Introduction

Shrimp farming is the major aquaculture industry in Asian countries contributing 91% of the world shrimp production (FAO, 2001). There is, however, also a growing concern about the ecological sustainability of shrimp farming. Waste generated during culture, mainly feces and unconsumed feed, settle on the bottom. Mineralization of accumulated organic matter under anaerobic conditions leads to the formation of toxic metabolites like NH_4^+ and NO_2^-, spoiling the living environment of the shrimps (Fast and Boyd, 1992; Hopkins et al., 1994; Avnimelech and Ritvo, 2003). In addition, with the expansion of the shrimp industry, the discharge of nutrient rich effluents from culture ponds to the coastal waters is also becoming a major environmental concern (Folke and Kautsky, 1992; Naylor et al., 1998; Shang et al., 1998).

Of the nitrogen input to shrimp ponds, 20% to 30% is retained in shrimp biomass, the rest potentially polluting the culture environment (Briggs and Funge-Smith, 1994; Jackson et al., 2003; Thakur and Lin, 2003). Therefore, to make shrimp farming more sustainable, pond management should be geared towards improving nutrient retention. As a result fewer nutrients will be discharged or lost, improved water quality and the living environment of the shrimps will become healthier.

Dissolved inorganic nitrogen can be immobilized by bacteria in intensively, well aerated and continuously mixed fish or shrimp ponds by the addition of an organic carbon source (Avnimelech et al., 1989; Avnimelech, 1999). The resulting bacterial production (single cell protein) may be utilized as a food source by carp or tilapia (Schroeder, 1987; Beveridge et al., 1989; Rahmatullah and Beveridge, 1993) and shrimp (Burford et al., 2004; Hari et al., 2004), thus lowering the demand for protein in the supplemental feed (Avnimelech, 1999). Studies on the effect of carbohydrate (CH) addition concentrated on intensive or super intensive systems with constant mixing and aeration of pond water (Avnimelech, 1999). However, recent research showed that carbohydrate addition in extensive shrimp ponds improved the nitrogen retention efficiency and had a positive effect on production (Hari et al., 2004). Further improving the technique of carbohydrate addition in extensive production systems can have a major impact on the sustainability of shrimp farming, especially when considering that the vast majority of shrimp production in Asia still is harvested from extensively managed ponds.

Manipulations of shrimp culture systems to improve water quality and to enhance production require a thorough understanding of the physical, chemical and biological processes taking place (Boyd, 1986). Still little is known about these processes in extensive shrimp ponds. Therefore, the present study investigated the effect of CH addition and dietary protein level in extensively managed *P. monodon* ponds on (i) water quality, (ii) the partitioning of N-inputs in the pond system and (iii) shrimp production. A system N-budget was constructed to visualize the compartmentalization of N-inputs in the culture system.

2. Materials and method

One outdoor experiment with a 2-way factorial design with dietary protein level (25% and 40%) as first factor and CH addition (with or without) as second factor was conducted at the School of Industrial Fisheries, Cochin University of Science and Technology (CUSAT) in India. A 25% and 40% protein diet were used, further abbreviated as P25 and P40. The treatments that received carbohydrate addition are further referred to as P25+CH and P40+CH.

2.1. Experimental setup

The experiment was carried out in 7.2-m^3 concrete tanks having an effective bottom area of 6 m^2 (3 × 2 m). All the tanks were provided with a uniform 7-cm thick sediment layer taken from an extensive shrimp culture pond. Lime was added initially at 3 kg $tank^{-1}$. Culture tanks were filled with 22 ppt saline water from the Cochin estuary, Kerala State, India. A large closed concrete storage tank was filled to be able to replace water losses from the culture tanks. The water level in the culture tanks was maintained at 1 m height during the whole culture period to provide a water volume of 6 m^3. Overflow pipes facilitated the runoff of rainwater keeping mixing with tank water minimal. In addition, it was assumed that the influence of rain on the results is similar for all treatments. To stimulate phytoplankton development, culture tanks were fertilized with urea and super phosphate (Fertilizers and Chemicals Travancore Limited, Udyogamandal, India, Pin 683 501) at the rate of 4 and 1 g m^{-2} $week^{-1}$ during the first 6 weeks of culture. Cow dung was also added to the tanks at 5, 2, 3 and 2 kg dry matter $tank^{-1}$ at the onset of the 2nd, 3rd, 5th and 7th week of culture, respectively. Twenty-day-old post larvae (PL 20) of *Penaeus monodon* (0.016 ± 0.001 mg) purchased from a commercial hatchery were stocked in the tanks at a density of 7 PL m^{-2}. Sinking pelleted shrimp feeds containing 25% and 40% crude protein (Higashimaru Feeds India Limited, Amalgam House, Plot No.9, Bristow Road, Cochin, India, Pin 682 003) were used during the experiment. Tapioca flour (flour of dried roots of tapioca plant *Manihot esculenta*; supplied by Ramakrishna Stores, Market Road, Cochin, India, Pin 682 035) was used as carbohydrate source.

The quantity of carbohydrate (CH) added was calculated following Eq. (1) (Avnimelech, 1999), and assuming that the added carbohydrate contains minimum 50% carbon, the CH addition needed (ΔCH) to reduce total ammonia nitrogen concentration by 1 g N m^{-3} is 20 g m^{-3}.

$$\Delta CH = \Delta N/0.05. \quad (1)$$

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 50% of the feed nitrogen (Avnimelech, 1999):

$$\Delta N = \text{Quantity of feed} \times \%N \text{ in feed} \times \%N \text{ excretion}. \quad (2)$$

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

$$\Delta CH = \text{Quantity of feed} \times \%N \text{ in feed} \times \%N \text{ excretion}/0.05. \quad (3)$$

In consequence of the Eq. (3), 0.39 kg tapioca flour was administrated per kg of the 25% protein diet and 0.62 kg tapioca flour per kg of the 40% protein diet fed. The proximate composition of the experimental feeds and the tapioca flour are given in Table 1.

Treatments were executed in triplicate and were assigned randomly to 12 tanks. Check trays were used to collect shrimps from each tank and wet weight was recorded on biweekly basis. Feeding rate was calculated from the average weight of shrimp for each period. Shrimp's daily feeding rates were 15% body weight at the start of the experiment, and declined gradually to 3% at the end of the culture period. Feed was distributed evenly over the tank's surface, twice daily at 08.00 and 18.00 hours. The preweighed tapioca flour was mixed in a beaker with tank water and uniformly distributed over the tank's

Table 1
Proximate composition (%) of the experimental feeds and carbohydrate source (tapioca flour)

	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)†	Moisture (%)
Tapioca flour	1.8 ± 0.3	0.6 ± 0.1	2.5 ± 0.2	4.6 ± 0.3	76.6 ± 0.9	13.9 ± 0.6
P25	25.3 ± 0.4	4.4 ± 0.2	10.9 ± 0.6	4.7 ± 0.2	43.4 ± 0.3	11.4 ± 0.8
P40	40.1 ± 0.6	6.6 ± 0.2	8.9 ± 0.3	3.8 ± 0.4	30.2 ± 1.8	10.3 ± 0.7

P25: 25% dietary protein.
P40: 40% dietary protein.
† Nitrogen free extract.

surface directly after the feed application at 8.00 a.m. Shrimps were harvested after draining the tanks 122 days after stocking. Individual length, weight and survival were recorded. Triplicate samples of each batch of fertilizers, shrimp feeds and shrimp carcass at final harvest were analyzed for moisture and nitrogen content (AOAC, 1990).

2.2. Water quality and sediment parameters

Temperature (mercury thermometer), dissolved oxygen (portable DO meter-Eutech instruments, Singapore); salinity (hand refracto-meter; Atago, Japan), pH (pH-Scan-Eutech instruments, Singapore) and transparency (Secchi disc) were measured daily in situ at 09.00 a.m. Sediment and water samples were collected on biweekly basis between 09.00 and 10.00 hours. Water samples were collected using a horizontal water sampler from three locations of each tank and pooled together before analysis. Sediment samples were collected from six locations in each tank using 2-cm diameter PVC pipes. Composite water column samples were filtered through a GF/C Whatman glass fiber filter and the filtrate was analyzed for nitrite–N (NO_3^-–N) (cadmium reduction), nitrite–N (NO_2^-–N) and total ammonia nitrogen (TAN) (phenol hypochlorite method), soluble reactive phosphate (SRP) (ascorbic acid method) (Grasshoff et al., 1983). Chlorophyll-*a* in non-filtered water column samples was analyzed following standard methods (APHA, 1995). The total nitrogen in the water sample was estimated following persulphate digestion (Grasshoff et al., 1983). The organic carbon in the sediment was determined following El Wakeel and Riley (1957). Exchangeable TAN, NO_2^-–N, NO_3^-–N and total Kjeldahl nitrogen in the sediment were measured according to Mudroch and Azcue (1996). Total heterotrophic bacteria (THB) count in the water and sediment was estimated following the standard procedures (APHA, 1995) and expressed as colony forming units (*cfu*). Records were maintained of the feed, carbohydrate and fertilizer inputs and of the volume of water exchanged.

2.3. Nitrogen budget

Nitrogen inputs were measured in the initial water, sediment, the water replaced during exchange, organic and inorganic fertilizers, feed and the PLs stocked. The nitrogen from tapioca flour was included in the budget as feed based nitrogen. Nitrogen outputs were measured in the harvested shrimp, drained water during exchange, and water and sediment at the day of harvest. Nutrient inputs and outputs through water exchange were calculated by multiplying the nutrient concentration with the water volume. While calculating the input nitrogen the rainwater nitrogen was not included because the contribution of rainwater to the total N-budget was negligible small. Sediment samples were collected on the day of stocking and harvesting by taking randomly six cores from each tank and mixing them homogenously into a composite sample before analysis. The amounts of the different N-species in the sediment were estimated by multiplying nitrogen concentration by sediment mass. The total sediment mass was calculated based on mean bulk density. The amount of nitrogen that accumulated in the sediment during the culture cycles was measured as the difference between the nitrogen present at stock-

Table 2
Water quality parameters in the outdoor tanks stocked with *Penaeus monodon*

	Treatments (Mean ± SD)			
	P25	P25+CH	P40	P40+CH
Temperature (°C)	29.6 ± 1.9	29.9 ± 1.5	29.3 ± 1.6	29.6 ± 1.4
pH	8.1 ± 0.4	8.1 ± 0.4	8.1 ± 0.4	8.2 ± 0.5
Surface dissolved oxygen (mg l^{-1})	5.6 ± 2.5	6.4 ± 3.2	6.3 ± 2.7	6.8 ± 3.3
Bottom dissolved oxygen (mg l^{-1})	4.3 ± 1.9	4.5 ± 2.0	4.5 ± 1.9	4.9 ± 2.0
Salinity (ppt)	21.6 ± 2.6	22.2 ± 2.8	21.5 ± 2.0	21.8 ± 2.2
Secchi disc reading (cm)	62.4 ± 23.4	63.9 ± 23.6	58.4 ± 21.9	61.6 ± 22.1
Alkalinity (mg $CaCO_3$ l^{-1})	79.4 ± 34.9	84.6 ± 33.1	68.2 ± 28.1	86.7 ± 31.1

P25: 25% dietary protein; P25+CH: 25%dietary protein+carbohydrate addition.
P40: 40% dietary protein; P40+CH: 40%dietary protein+carbohydrate addition.

ing and harvest. (Briggs and Funge-Smith, 1994; Teichet-Coddington et al., 2000; Thakur and Lin, 2003).

2.4. Statistical analysis

All variables measured at harvest (shrimp growth, yield, FCR, SGR and PER, survival) and various components in the nitrogen budgets were analyzed by two-way ANOVA to determine the effect of dietary protein level, CH addition and their interaction. Survival of shrimp was analyzed using arcsine-transformed data. However, non-transformed data are presented in the tables. Daily and biweekly sediment and water quality parameters including THB counts were compared by split-plot ANOVA with treatments as main factor and time as the sub-factor (Gomez and Gomes, 1984). Subsequently, a 2-way ANOVA was carried out to determine the effect of dietary protein level, CH addition and their interaction term. All the ANOVA were performed using the SAS 6.21 program (SAS Institute, Cary, NC 27513, USA). If a main effect was significant (alpha=0.05), the ANOVA was followed by Tukey's test at $P<0.05$ level of significance.

3. Results

3.1. Water and sediment characteristics

3.1.1. Water column

The water quality parameters temperature, pH, surface and bottom dissolved oxygen, salinity, Secchi disc visibility and alkalinity were not affected by dietary protein level and CH addition ($P>0.05$; Table 2).

The addition of CH reduced water column TAN to 5.9 μg l^{-1} (Table 3). The interaction between protein level and CH addition did not affect any water or sediment variables. The results of the two-way ANOVA showed that besides carbohydrate addition, the dietary protein level significantly influenced the water TAN concentration (Table 3). The mean TAN concentration over the days of sampling peaked at 31st day (Fig. 1a). The addition of CH reduced ($P<0.001$) NO_2^-–N concentration. The highest NO_2^-–N concentrations also depended on protein level ($P<0.001$). The mean NO_2^-–N concentration showed two definite peaks on 46th and 76th day of rearing (Fig. 1b). The fluctuation in NO_2^-–N concentration in time was considerably ($P<0.001$), but all treatments peaked at the same days of sampling.

Table 3
Effect of carbohydrate addition and dietary protein levels on the water and sediment quality in outdoor tanks stocked with *Penaeus monodon*

Variable	Treatments				Significance[†]		
	P25	P25+CH	P40	P40+CH	P	CH	P×CH
Water quality variable							
TAN (μg l^{-1})	9.8 ± 6.9^{b}	5.9 ± 5.0^{c}	14.7 ± 15.8^{a}	8.5 ± 10.2^{b}	***	***	NS
Nitrite–N (NO_2^-–N) (μg l^{-1})	2.02 ± 3.0^{b}	0.8 ± 1.1^{c}	3.4 ± 5.0^{a}	1.9 ± 2.9^{b}	***	***	NS
SRP (μg l^{-1})	7.8 ± 8.5^{b}	$9.0 \pm 5.8^{a,b}$	$12.4 \pm 8.7^{a,b}$	14.4 ± 9.4^{a}	*	NS	NS
THB ($\times 10^4$ cfu ml^{-1})	20.8 ± 6.9^{b}	37.1 ± 14.3^{a}	$29.7 \pm 9.6^{a,b}$	34.3 ± 12.2^{a}	NS	**	NS
Sediment quality variable							
TAN (μg g^{-1})	20.2 ± 8.2^{b}	17.8 ± 6.7^{b}	25.9 ± 10.0^{a}	21.5 ± 8.9^{b}	**	*	NS
TKN (μg g^{-1})	224.6 ± 84.8^{b}	$228.4 \pm 80.2^{a,b}$	254.0 ± 105.2^{a}	249.9 ± 97.6^{a}	**	NS	NS
THB ($\times 10^6$ cfu ml^{-1})	45.2 ± 29.0^{b}	74.6 ± 50.7^{a}	41.5 ± 9.6^{b}	72.5 ± 12.2^{a}	NS	**	NS

Mean values in same row with a different superscript differ significantly ($p<0.05$).

[†] Results from split-plot two way ANOVA; P=Protein level; CH=Carbohydrate addition; P×CH=Protein level×carbohydrate addition interaction.

NS: not significant ($P>0.05$).

P25: 25% dietary protein; P25+CH: 25% dietary protein + carbohydrate addition.

P40: 40% dietary protein; P40+CH: 40%dietary protein + carbohydrate addition.

TAN: Total ammonia nitrogen; TKN: Total Kjeldahl nitrogen; SRP: Soluble reactive phosphate; THB: Total heterotrophic bacteria.

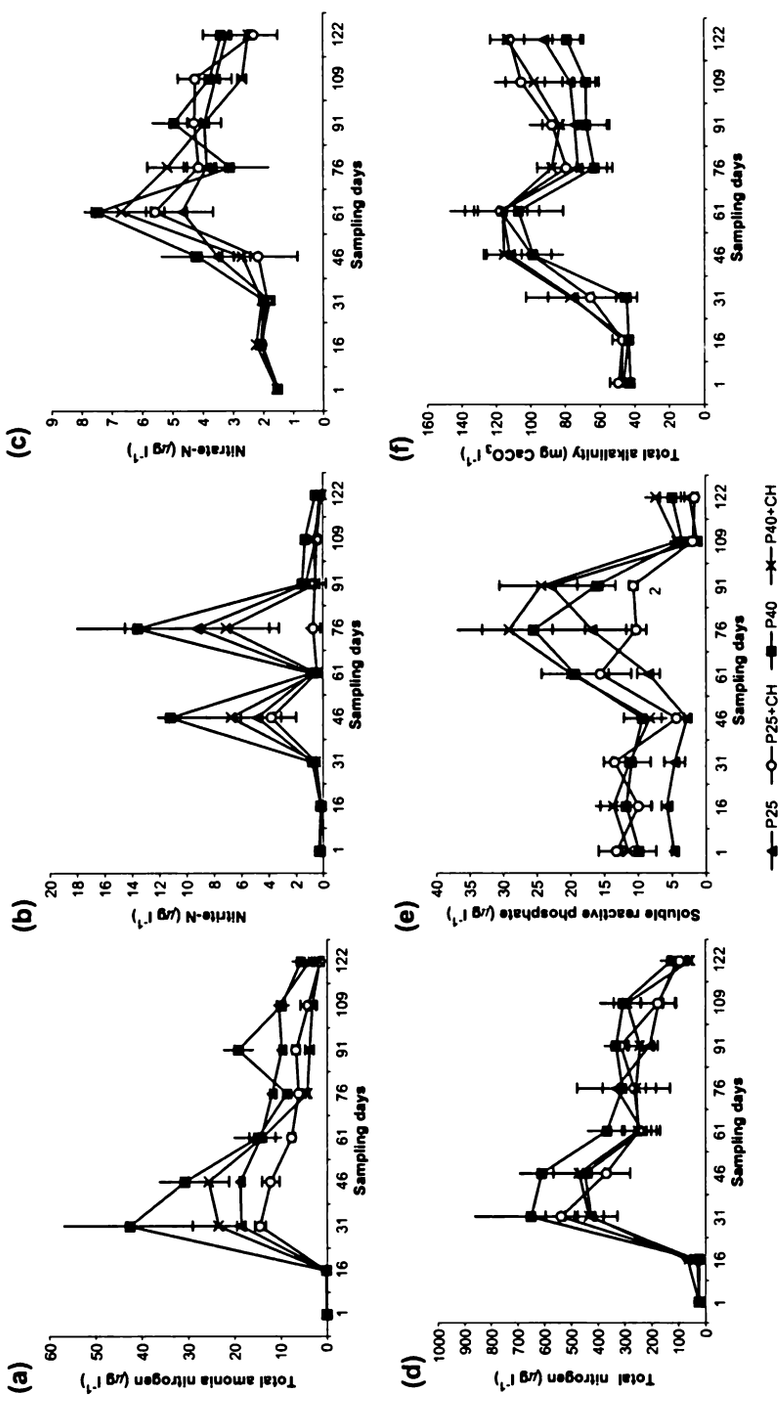

Fig. 1. The effect of carbohydrate addition and dietary protein levels on the water quality parameters in outdoor tanks stocked with *Penaeus monodon* P25: 25% dietary protein; P25+CH: 25% dietary protein+carbohydrate addition; P40: 40% dietary protein; P40+CH: 40% dietary protein+carbohydrate addition.

Dietary protein level and CH addition had no effect on the NO_3^-–N concentration in the water column. The mean NO_3^-–N concentration of the different treatments fluctuated between 3.1 and 3.6 μg l^{-1} (Fig. 1c). The total Kjeldahl nitrogen concentration was not influenced by CH addition or dietary protein level (Fig. 1d). The total Kjeldahl nitrogen showed a gradual decrease towards the end of culture after a sharp increase on 31st day of the experiment ($P<0.001$).

The dietary protein level significantly ($P<0.05$) influenced the SRP concentration. The variation in SRP concentration in time was highly significant ($P<0.001$) and highest values were recorded on 76th day of rearing (Fig. 1e). Neither the addition of carbohydrate or variation in protein level had affected the total alkalinity of the rearing water ($P>0.05$). A sharp increase in the total alkalinity was recorded in all treatment after 16th day of culture, except in treatment P40, where it increased only after 31st day. A decrease in the total alkalinity was recorded on 76th day in all treatments and thereafter a gradual increase was recorded till the end of culture (Fig. 1f). Slightly lower mean total alkalinity was recorded in P40 when compared to other treatments, however this difference was not statistically significant ($P>0.05$) (Table 2; Fig. 1f). Neither addition of carbohydrate or protein level had any effect on the chlorophyll-*a* concentration ($P>0.05$). Moreover, the observations on monthly chlorophyll-*a* concentration during the rearing period did not show any variation ($P>0.05$) (Fig. 2a). CH addition increased the THB count ($P<0.001$), while the dietary protein level did not have any effect ($P>0.05$) (Table 3). The THB count in the water column increased in time ($P<0.001$) (Fig. 2b).

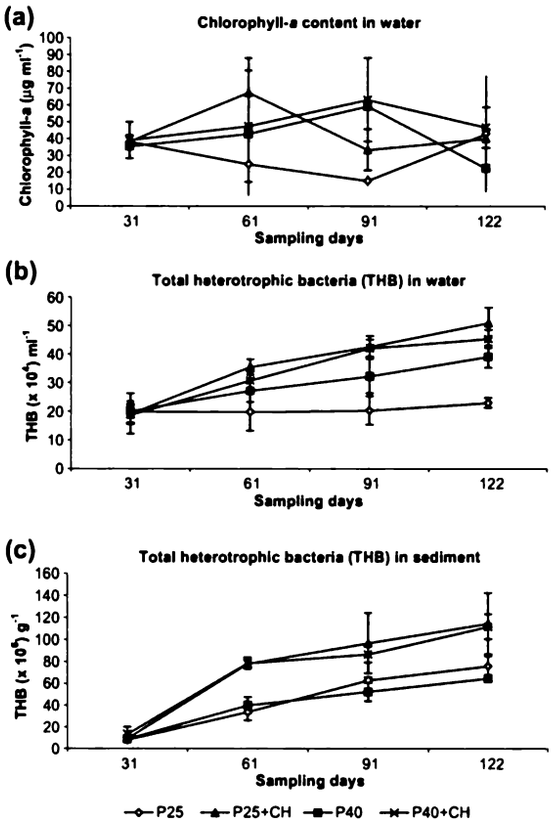

Fig. 2. Effect of carbohydrate addition and protein levels on the chlorophyll-*a* content in water and total heterotrophic bacteria (THB) (Mean ± S.E.) of the outdoor tanks stocked with *Penaeus monodon* P25: 25% dietary protein; P25+CH: 25% dietary protein+carbohydrate addition; P40: 40% dietary protein; P40+CH: 40% dietary protein+carbohydrate addition.

3.1.2. Sediment

The sediment TAN levels in the experimental tanks were affected by CH addition ($P<0.05$) and dietary protein level ($P<0.01$) (Table 3, Fig. 3a). CH addition to the 40% protein diet reduced the sediment TAN concentration ($P<0.05$) (Table 3). However, a similar effect of CH addition was not found with the 25% protein diet ($P>0.05$), even though the mean TAN concentration in the sediment in treatment P25+CH was slightly lower than the mean TAN concentration in P25 (17.8 vs. 20.2 μg g^{-1}) (Table 3). TAN gradually accumulated in the sediment during the culture period ($P<0.001$) (Fig. 3a). The NO_2^-–N and NO_3^-–N levels in the sediment were low when compared to TAN concentration and were not affected by the dietary protein level and CH addition ($P>0.05$). However, NO_2^-–N and NO_3^-–N levels in the sediment increased in time ($P<0.001$) (Fig. 3b and c).

The total Kjeldahl nitrogen in the sediment was influenced by dietary protein level ($P<0.01$) (Table 3). The sediment TKN gradually accumulated in time ($P<0.001$) (Fig. 3d). The organic carbon content in the sediment more than doubled between stocking and harvesting from 7.5 to 16.5 μg g^{-1} ($P<0.001$) (Fig. 3e). Neither addition of carbohydrate or protein level

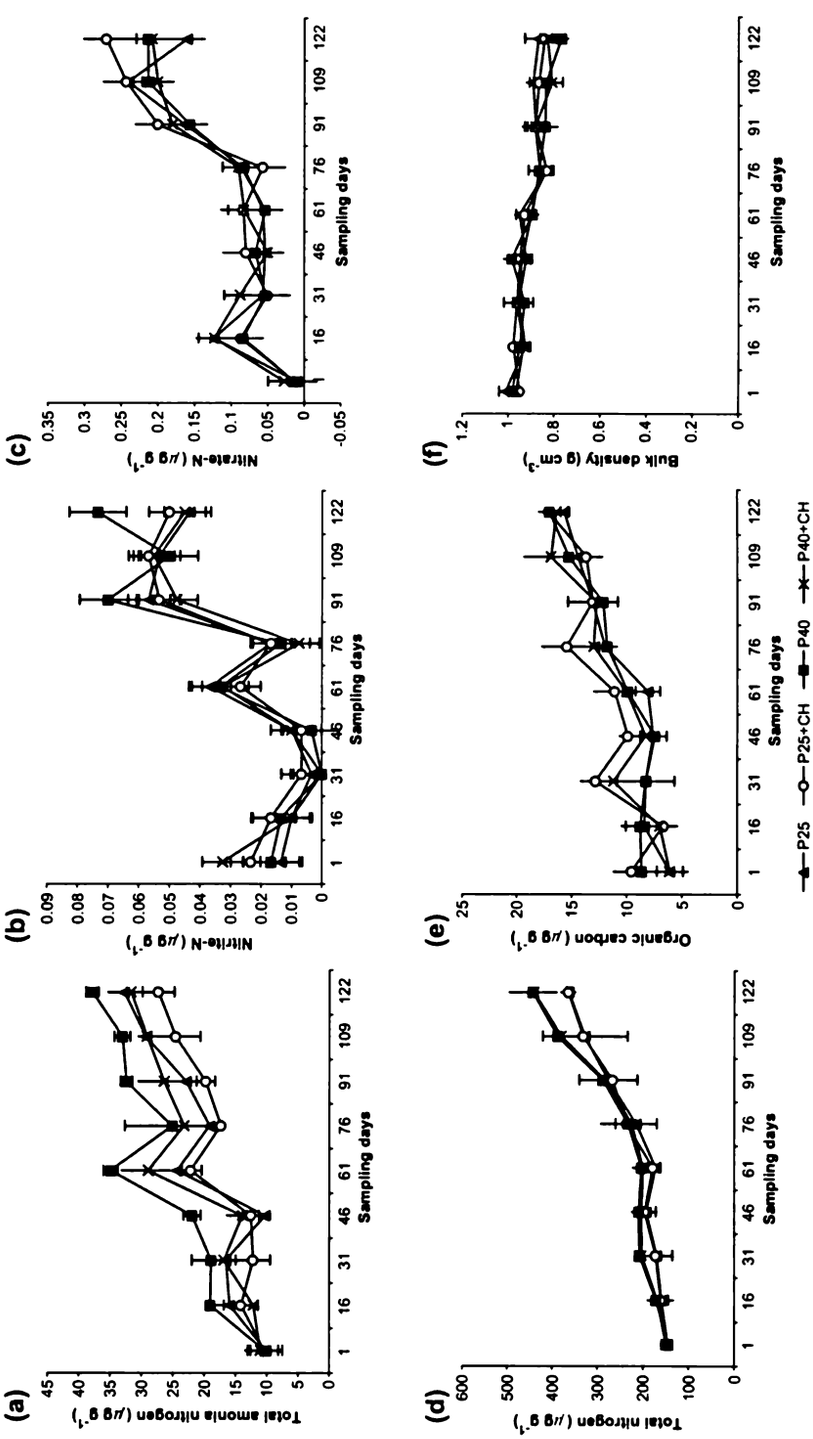

Fig. 3. The effect of carbohydrate addition and dietary protein levels on the sediment quality parameters in outdoor tanks stocked with *Penaeus monodon* P25: 25% dietary protein; P25+CH: 25% dietary protein+carbohydrate addition; P40: 40% dietary protein; P40+CH: 40% dietary protein+carbohydrate addition.

Table 4
Results of two-way ANOVA on the effect of carbohydrate addition and dietary protein levels on weight gain, net yield, SGR, FCR, PER and survival (%) of *Penaeus monodon* reared in outdoor tanks

	Significance		
	P	CH	P×CH
Specific growth rate (SGR)	*	***	NS
Individual shrimp weight gain (g)	NS	***	NS
Net shrimp yield (g m^{-2})	*	*	*
Feed conversion ratio (FCR)	**	**	*
Corrected feed conversion ratio	NS	**	**
Protein efficiency ratio (PER)	**	**	*
Survival rate (%)	NS	NS	NS

PER and survival (%) of *Penaeus monodon* reared in outdoor tanks P=Protein level; CH=Carbohydrate addition; P×CH=Protein level × carbohydrate addition interaction. NS: Not significant ($P>0.05$).

had any effect on the organic carbon content in the sediment ($P>0.05$). The mean bulk density of the sediment by treatment was 0.9 g cm^{-3} for all treatments ($P>0.05$), while it reduced in time ($P<0.001$) (Fig. 3f). Higher sediment THB counts were recorded in CH added treatments (72.5 and 74.6×10^6 cfu g^{-1}) compared to the non-CH-added treatments (41.5 to 45.2×10^6 cfu g^{-1}) ($P<0.001$) (Table 3) and the mean THB count increased in time ($P<0.001$) in all treatments (Fig. 2c).

3.2. Shrimp growth, yield, FCR, PER and survival (%)

SGR was more influenced by CH addition ($P<0.001$) than with dietary protein level ($P<0.05$), while the interaction was not significant (Table 4). SGR and individual shrimp weight gain were higher ($P<0.05$) in CH added treatments than P40 (Fig. 4a and b). CH addition, dietary protein level, and their interaction term all had a significant effect on net shrimp yield, FCR, corrected FCR and PER (Table 4). Higher net shrimp yield ($P<0.001$) was recorded in treatment P25+CH (160.0 g m^{-2}) than in treatment P25 (113.9 g m^{-2}), and this higher yield was comparable to the yield in treatments P40 (157.0 g m^{-2}) and P40+CH (161.1 g m^{-2}) (Fig. 4c). The mean FCR of treatment P25+CH (1.1) was lower ($P<0.05$) than the mean FCR of treatment P25 (1.5) and was the same in treatments P40 (1.1) and P40+CH (1.1) (Fig. 4d). Corrected FCR values were calculated by adding the weight of CH addition to the weight of pellets fed. The corrected FCR was similar for treatments P25, P25+CH and P40+CH, which were higher than for treatment P40 ($P<0.05$) (Fig. 4e). The PER was 3.6 in treatment P25+CH, which was higher than for other treatments where the PER varied between 2.2 and 2.6 ($P<0.001$) (Fig. 4f). Shrimp survival ranged between 77% and 83% and was similar between treatments ($P>0.05$).

3.3. Nitrogen budget

The nitrogen contribution from the tapioca flour was included in the nitrogen budget as feed nitrogen input. The feed nitrogen input accounted for about 30% and 41% of total N-input in the 25% and 40% dietary protein treatments, respectively (Table 5). The contribution to the total N-input of the initial water ranged between 0.38 and 0.41 g N $tank^{-1}$ (0.24% to 0.31%), while the contribution of sediment ranged between 42 and 44 g N $tank^{-1}$ (27.5% to 33.2%). The PL's contributed an insignificant amount to the N-input budget (0.01%). Among the input nitrogen sources, only the feed originated nitrogen showed significant variation ($P<0.001$).

On average, 16.1% to 21.1% of the N-input was retained as harvested shrimp (Table 5). To produce 1 kg shrimp, 109.2 g of the total N-input was not harvested in treatment P25+CH. This was 164.0 g N in treatment P25, 132.1 g N in treatment P40+CH and 137.5 g N in treatment P40. The major share of the N-inputs, 67.3% to 71.3%, remained in the sediment, and therefore, was not completely lost. Real losses, which are the sum of water-N, N-exchange loss and unaccounted-N fluctuated between 9.0% of total N-input in treatment P25+CH and 15.6% in treatment P40. CH addition and dietary protein level did not influence ($P<0.05$) nitrogen accumulation in the sediment, water column and the exchange loss. However, CH addition ($P<0.001$) and dietary protein level ($P<0.01$) had an effect on nitrogen retention in shrimp biomass. Nitrogen retention was 21.1% in treatment P25+CH and 16.1% in P25. The retention in treatment P25 was similar to the 16.7% retention in P40, but worse than the 18.5% retention in treatment P40+CH. In the nitrogen budget, some of the nitrogen went unaccounted. The unaccounted-N (6.2% to

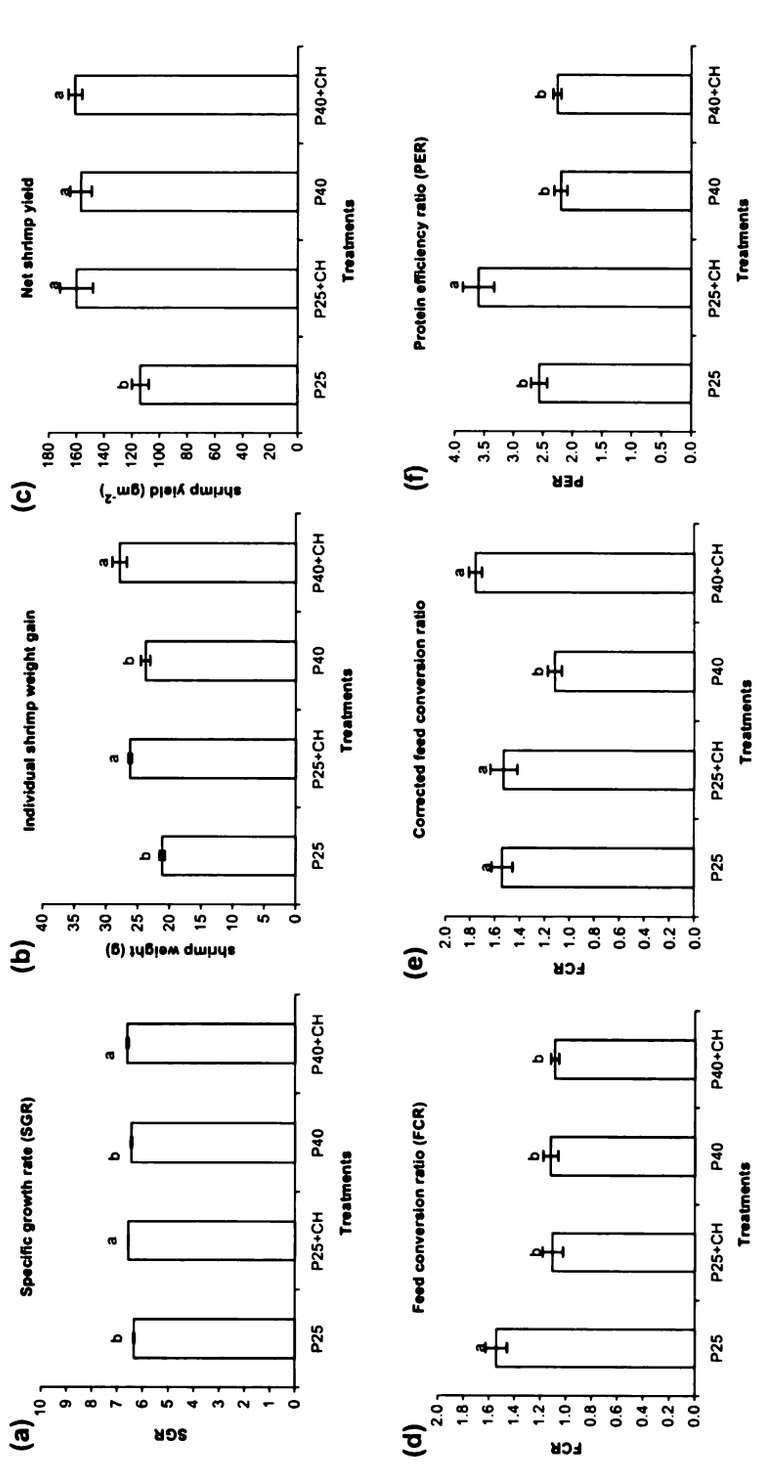

Fig. 4. Effect of carbohydrate addition and protein levels on weight, net shrimp yield, SGR, FCR and PER of *Penacus monodon* reared in outdoor tanks Mean values were presented with SE as error bars; means with different script differ significantly ($P<0.05$) P25: 25% dietary protein; P25 + CH: 25% dietary protein + carbohydrate addition; P40: 40% dietary protein; P40 + CH: 40% dietary protein + carbohydrate addition.

Table 5
Nitrogen budget for different treatments during a 122 day growth experiment on *Penaeus monodon* stocked in outdoor tanks

Treatments	Nitrogen inputs (g N tank^{-1})						Nitrogen outputs (g N tank^{-1})					
	Water	Sediment	Fertilizers	Feed	Shrimp	Total input	Water	Sediment	Exchange loss	Shrimp	Total output	Unaccounted
P25	0.41	44.0	48.5	39.5	0.02	132.4	0.63	94.4	3.0	21.3	119.3	13.2
(%)†	0.31	33.2	36.7	29.8	0.01	100.0	0.48	71.3	2.3	16.1	90.1	9.9
P25+CH	0.39	42.8	48.5	39.9	0.02	131.7	0.58	91.8	3.0	27.7	123.1	8.6
(%)†	0.30	32.5	36.9	30.3	0.01	100.0	0.44	69.9	2.3	21.1	93.8	6.2
P40	0.40	42.5	48.5	63.1	0.02	154.6	0.76	104.6	4.2	25.9	135.4	19.2
(%)†	0.26	27.5	31.4	40.8	0.01	100.0	0.49	67.7	2.7	16.7	87.6	12.4
P40+CH	0.38	43.4	48.5	63.9	0.02	156.2	0.34	104.7	3.2	28.7	137.0	19.2
(%)†	0.24	27.8	31.1	40.9	0.01	100.0	0.22	67.3	2.1	18.5	88.1	11.9
Significance (P value)‡												
P	NS	NS	–	***	–	***	NS	NS	NS	**	**	NS
CH	NS	NS	–	–	–	NS	NS	NS	NS	***	NS	NS
P×CH	NS	NS	–	*	–	NS	NS	NS	NS	NS	NS	NS

† Percentage of total input nitrogen.
‡ Results from two-way ANOVA.
P=Protein level; CH=Carbohydrate addition; P×CH=Protein level×carbohydrate addition interaction.
P25: 25% dietary protein; P25+CH: 25%dietary protein+carbohydrate addition.
P40: 40% dietary protein; P40+CH: 40%dietary protein+carbohydrate addition.

12.4%) was not influenced by CH addition or dietary protein level ($P>0.05$) (Table 5).

4. Discussion

4.1. Reduction of toxic inorganic compounds

The shrimp growth recorded in the present study was not limited by any of the water quality parameters as they fell in the favorable limits for *P. monodon* production (Chen et al., 1990; Hariati et al., 1996). TAN concentrations were low (5.9–14.7 μg l^{-1}) in the present study, compared to earlier reports by Chen and Tu (1991) (6.5 mg l^{-1}) and Thakur and Lin (2003) (198–519.1 μg l^{-1}) for *P. monodon* rearing systems. TAN levels peaked on 31st day of culture followed by an increase in NO_2^-–N nitrite concentration on 46th day, showing that it took 5 weeks to establish the nitrification process. This duration was slightly shorter than the 8 weeks needed to establish the nitrification process in *P. monodon* concrete culture tanks by Thakur and Lin (2003).

In this study, higher dietary protein levels resulted in significantly higher TAN and NO_2^-–N concentrations in the water column, and higher TAN concentrations in the sediment. Li and Lovell (1992) reported that the ammonia concentration increased with increasing dietary protein concentration and protein feeding rate. By adding CH to the culture tanks, TAN and NO_2^-–N concentrations in the water column were significantly reduced. This agrees with Avnimelech and Mokady (1988), Avnimelech et al. (1989, 1994) and Avnimelech (1999) who reported that the addition of carbohydrate to intensively well-mixed production systems will reduce the TAN concentration through immobilization by bacterial biomass. In the present study, extensively managed tanks were used, which means a minimum water exchange and no mixing of the water column. Under such extensive conditions, CH addition to the water column also resulted in a significant increase in the THB count, together with observed lower TAN concentrations in water and sediment. CH addition also caused a significant reduction in NO_2^-–N concentration in the water column, which can be attributed to low availability of TAN as substrate for nitrification and hence the production of NO_2^-–N (Avnimelech, 1999; Hari et al., 2004). The higher nitrite and nitrate values recorded in the P40 treatment also revealed the possibility of more nitrification. The insignificant decrease in the alkalinity in P40 tanks might be due to the increased nitrification;

where the H^+ ions liberated during nitrification negatively affected the alkalinity in order to buffer the change in pH (Boyd, 1990; Hargreaves, 1998). The nitrification ultimately leads to the formation of NO_3^-–N, so one would expect also to find differences in nitrate concentration as a result of CH addition. However, the nitrate level was not influenced by CH addition, and the underlying mechanism needs further study.

In shrimp culture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon, 1982; Diab and Shilo, 1988). In the present study no differences were observed in chlorophyll-*a* concentration between treatments. In addition, the chlorophyll-*a* concentration remained stable over time. Thus the reduction in TAN and NO_2^-–N levels observed in CH added treatments could only be attributed to the increased THB population, which immobilized TAN for the synthesis of new bacterial cells (Hari et al., 2004).

SRP concentrations recorded in the water column varied with dietary protein level. Higher phosphorous concentrations in the high protein diets could be the reason. Unfortunately, the experimental diets were not analyzed for their phosphate content. This topic requires further research as phosphorous accumulation in minimum water exchange systems, threatens long-term sustainability (Funge-Smith and Briggs, 1998).

4.2. Increased shrimp yields due to CH addition

The comparable net shrimp yield and FCR in treatments P25+CH and P40 shows the possibility of reducing the dietary protein level in favor of addition of CH to the water column without any significant reduction in shrimp production (Hari et al., 2004). This was further supported by the interaction between the protein level and addition of CH to the pond in most of the production parameters. The use of low protein feed led to a significant reduction in the feed based inorganic nitrogen accumulation in the pond (Li and Lovell, 1992). Furthermore the addition of CH enhances the THB in the pond, which in turn result further reduction in inorganic N. Thus the low toxic inorganic N levels in the pond (Wahab et al., 2003) and utilization of microbial cells as feed act as favorable factors for the augmented shrimp production in P25+CH treatment (Avnimelech, 1999; Burford et al., 2003, 2004). Furthermore, lower TAN in the sediment positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). Similar survival rates in all treatments showed that water and sediment quality were favorable for *P. monodon* cultivation (Hariati et al., 1996), and suggest that differences in production are related to food quality and food availability.

Allan et al. (1995) recorded faster growth of prawns in well-prepared ponds with an abundant meiofanua. In the present study, a similar phytoplankton biomass was present in all treatments as indicated by chlorophyll-*a* concentrations, but THB counts, both in the water column and the sediment, were higher in CH added ponds. Utilization of microbial protein depends upon the ability of the target animal to harvest the bacteria and to digest and utilize the microbial protein (Avnimelech, 1999; Burford et al., 2003, 2004; Hari et al., 2004). The higher shrimp yield in the CH added treatments of the present study showed that *P. monodon* can well utilize the additional bacterial protein as a result of CH addition. Studies using stable isotope have shown that the natural biota contribute to shrimps nutrition in less intensive systems (Parker and Anderson, 1989; Cam et al., 1991; Burford, 2000).

4.3. Increased nitrogen retention

The PER in the treatment P25+CH was 3.6 g wet body weight per gram dietary protein, compared to 2.2–2.6 g g^{-1} found in the other treatments. Compared to treatment P40, addition of CH had no effect on the PER, indicating that shrimps are limited in the amount of protein they can deposit in body tissue. Even though more single cell protein was present in treatment P40+CH than in P40, this did not result in additional shrimp production. A reduced demand in dietary protein (25%) was resulted by the addition of the CH in the extensive type of the shrimp culture systems irrespective of the higher protein requirements of *P. monodon* (35–50%) assessed in earlier studies (Table 6). However, O'Keefe (1998) suggested a reduced dietary protein level (25–30%) for *P. monodon* in extensive type of shrimp culture systems against 30–40% and 40–50% in semi-intensive and intensive type of culture, respectively. The lipid com-

Table 6
Nutritional requirements of *Penaeus monodon*

Requirement of dietary component	Percentage of diet	Reference
Dietary protein	45–50%	Lee (1971)
	40–44%	Alava and Lim (1983)
	40–50%	Bautista (1986)
	40–44%	Shiau et al. (1991)
	35–45%	Bages and Solane (1981)
	36–40%	Shiau and Chou (1991)
	40–50%	Chen (1993a)
Dietary lipid	6%	Wu (1986)
	4–11%	Sheen et al. (1994a)
HUFA	0.5–1%	Chen and Tsai (1986)
Linolenic acid and DHA	1.44%	Merican and Shim (1997)
Cholesterol	0.50%	Chen (1993b)
	0.2–0.8%	Sheen et al. (1994b)
Dietary carbohydrate	20%	Alava and Pascual (1987)
	20–30%	Shiau and Peng (1992)

ponent in both the experimental diets (P25 and P40) fell with in the range (4–11%) suggested for *P. monodon* by Sheen et al. (1994a). The carbohydrate content (estimated in the form of nitrogen free extracts) in P40 agrees with the recommended level (Table 6) for *P. monodon* (Shiau and Peng, 1992) while it was comparatively higher in P25 diet (43%).

Depending on treatment, 16% to 21% of the total amount of nitrogen available in the system was retained in shrimp biomass. These values concur with 14% retention in semi intensive *P. vannamei* ponds (Teichet-Coddington et al., 2000), 18% retention in semi-intensive Thai shrimps ponds (Briggs and Funge-Smith, 1994) and 21–22% retention in intensive *P. monodon* shrimps ponds (Jackson et al., 2003). However, in closed intensive *P. monodon* rearing systems, a 23% to 31% N recovery was recorded (Thakur and Lin, 2003). In these closed intensive systems, the feed contributed 76% to 92% of the total N-input, compared to 30% and 41% feed contribution to the total N-input in the 25% and 40% protein diet treatments, respectively, in the present study.

4.4. Reduction of N-waste per kilogram shrimp production

The 6.2% to 12.4% of unaccounted nitrogen in the present N-budget concurred with other extensive shrimp culture systems. Martin et al. (1998) for ponds stocked at 4 shrimps m^{-2} and Briggs and Funge-Smith (1994) for semi-intensive ponds, both reported 10% unaccounted. However, in intensive systems, losses as high as 36% have been reported in concrete bottomed tanks (Thakur and Lin, 2003) or 66% in shrimp farms at Gulf of California (Paez-Osuna et al., 1999). Denitrification and ammonia volatilization are difficult to measure in open tanks or ponds. Therefore, in most studies, denitrification and ammonia volatilization are assumed to be part of the unexplained section of the N-budget, which is the difference between the measured nitrogen inputs and outputs (Martin et al., 1998; Jackson et al., 2003; Thakur and Lin, 2003). The combination of these findings suggests that more denitrification takes place in intensive than in extensive production systems. This subject needs more research before definite conclusions can be reached.

The amount of nitrogen accumulated in the sediment and lost through water exchange was not significantly affected by CH addition. Sixty seven to 71% of the total N-input accumulated in the sediment which was much more than the 18% reported by Briggs and Funge-Smith (1994), the 38% reported by Martin et al. (1998) and the 14% reported by Jackson et al. (2003). Thakur and Lin (2003) reported an accumulation of 40.9% to 52.8% of the N-input in the sediment and emphasized the importance of the sediment bottom in minimizing water born nitrogen loss.

High nitrogen retention in shrimp and sediment concurred with a very low 2.1–2.7% N-loss through water exchange. The principal reason is the low water exchange applied in this study. Only 150% of the total rearing water volume was exchanged during the entire culture period, which is much lower than normal water exchange in extensive type of rearing systems with daily 5% to 25% water exchange. Jackson et al. (2003) and Teichet-Coddington et al. (2000) recorded 57% to 80% nutrient loss through water exchange. Briggs and Funge-Smith (1994) emphasized that in culture systems with low water exchange, water born loss of nutrients is less important than N-accumulation in the sediment. This is also true for nearly closed systems, where apparently more nutrients are also lost in intensive systems than in extensive ones. The total water based N-loss in this study (final pond water

N+exchange N-loss) was with 2.7% to 3.2% still considerably smaller than the 14% to 18% water based N-loss reported by Thakur and Lin (2003) in the intensive closed suspension ponds. In consequence, extensive shrimp farming with low water exchange pollutes less surrounding surface waters than all other shrimp farming systems.

5. Conclusion

Carbohydrate addition to extensive shrimp culture ponds reduced the levels of potentially toxic TAN and NO_2^-–N in the water column and TAN in the sediment. The protein level in the diet can be reduced from 40% to 25%, without compromising shrimp production, if CH is added to the water column to enhance heterotrophic bacterial protein production. The addition of carbohydrate affected the nitrogen partitioning in the culture system and more input nitrogen was retained in harvested shrimp. The nitrogen budget constructed in the present study revealed that 16% to 21% of the total nitrogen input was retained in the shrimp, 0.22% to 0.49% in the water, 67% to 71% in the sediment, and 2.1% to 2.7% was lost through water exchange. The quantity of nitrogen not retained in shrimp biomass to produce 1 kg of shrimp ranged between 109.2 and 164.0 g N. The percentage non-retained nitrogen was reduced by CH addition ($P<0.01$). In summary, CH addition in combination with reduction of the dietary protein level improved the sustainability of shrimp farming in extensive shrimp culture systems through (1) increased nitrogen retention in harvested shrimp biomass (2) reduced demand for feed protein (3) reduced concentrations of potentially toxic TAN and NO_2^-–N in the system, and (4) reduced water based nitrogen discharge to the environment.

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dded Carbohyd d Production Extensive hrimp Culture

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Small dams were built in water channels to make the 250-m² test ponds.

Summary:
Although already proven in intensive aerated shrimp culture systems, tank and pond trials in India confirmed that the addition of carbohydrates to extensive shrimp ponds benefited production by increasing yield and reducing inorganic nitrogen in the water. The carbohydrates raised the levels of protein-rich bacteria in ponds to allow the use of cheaper, lower-protein feeds.

Adding a carbon source to intense shrimp production systems can ntrol total ammonia nitrogen (TAN) els and improve feed conversion. dies have shown that immobilizan of inorganic nitrogen only ocrs when the carbon:nitrogen (C:N) io of the organic matter is higher an 10.

Immobilization of TAN results in production of bacterial cells rich single-cell protein that can be utied as food by carp, tilapia, and rimp. As a result, the feed-conversn ratio of the supplemental feed plied to ponds decreases, making possible to use lower-cost, lowerotein feeds.

Although commonly applied in tensive aquaculture, the technique C:N ratio control is not used in exsively managed ponds, even though ese systems support much of the rmed shrimp production worldide. The authors recently evaluated the effects on water quality and roduction of adding organic carbon extensive stagnant shrimp ponds in India?).

Continuous mixing to keep dislved and particulate matter dislved in the water column is a key ctor in the success of C:N ratio maipulation in intensive systems. The challenge, therefore, was to determine if the added carbohydrates would positively impact water quality before precipitating to the bottom.

Dual Trials

The goals of the study were to evaluate the combined effects of reducing the protein content in supplemental feeds and carbohydrate addition in extensively managed ponds containing black tiger shrimp, *Penaeus monodon*. To reduce costs, an indoor trial in small replicate tanks was conducted to develop the technique before applying it to farmers' ponds.

In both experiments, 20 g of tapioca flour was added for each g of N-NH4+ (spell out) released. The amount of N-NH4+ released was estimated assuming that the added carbohydrate contained 50% carbon, and 50% of the dietary protein input was converted to ammonia.

In consequence, 0.39 kg of tapioca flour was added for each kg of the 25%-protein feed used in the trials. For one kg of the 40%-protein diet used, 0.62 kg tapioca flour was added. In the indoor experiment, the photoperiod was maintained at 12 hours dark and 12 hours light.

Table 1. Effects of carbohydrate addition and protein levels on *P. monodon* performance in indoor and farm experiments.

	Treatment Means			
	P25	P25+CH	P40	P40+CH
Indoor Trial				
Specific growth rate (% bw/day)	2.6^{c}	3.4^{b}	3.4^{b}	3.8^{a}
Feed-conversion ratio	6.4^{a}	3.0^{b}	3.0^{b}	2.4^{b}
Nitrogen retention (%)	16.3^{c}	28.9^{a}	17.1^{c}	22.4^{b}
Protein efficiency ratio	0.6^{c}	1.3^{a}	0.9^{c}	1.1^{b}
Survival rate (%)	81	89	89	89
Farm Trial				
Specific growth rate (% bw/day)		7.9^{a}	7.7^{b}	
Feed-conversion ratio		1.6^{b}	2.2^{a}	
Nitrogen retention (%)		45.3^{a}	19.8^{b}	
Protein efficiency ratio		2.5^{a}	1.2^{b}	
Survival rate (%)		42	36	

Mean values in same row with different superscripts differ significantly ($P < 0.05$).

oor Experiment

he indoor experiment was car- out in triplicate 1,200-l tanks for of four diet treatments. Twen- ve and 40% crude protein diets e applied, with or without carbo- rate addition, resulting in treat- ts referred to as P25, P40, P25+CH, P40+CH. Shrimp were fed initial- t 15% body weight, which was dually reduced to 6% toward the of the culture. The sinking feed ets were distributed evenly over tank surfaces twice daily.

All tanks were filled with 26-ppt ne water and provided a uniform n sediment layer taken from an ensive shrimp culture pond. The er level in the tanks was main- ed at 50 cm. Uniformly sized 0.36-g imp juveniles were stocked at a sity of 6 animals/m^2. The tanks e fertilized with urea and super sphate at rates of 4 and 1 g/m^2/ k, respectively, during the first ee weeks of the experiment.

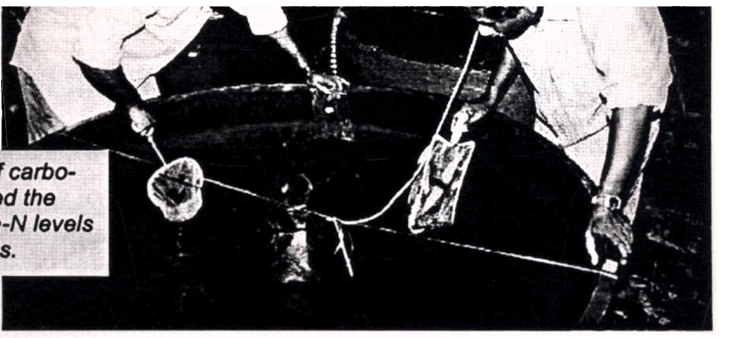
door Results

The addition of carbohydrate re- ed the TAN and nitrite-N levels he experimental tanks. The high- tein diet resulted in higher levels TAN, nitrite-N, and total nitrogen ncentrations. There was no effect the organic carbon content of the iment, but the addition of carbo- rate caused a significant reduc- n of sediment TAN. The total het- trophic bacteria count in the water umn and sediment were higher in atments with added carbohydrate.

Table 1 shows the effects of carbo- rate addition and protein levels various yield parameters. The spe- ic growth rate and feed conversion io were similar in the P40 and +CH treatments. The protein effi- ncy ratio was highest in treatment P25+CH. The survival was 80-88% and did not vary between treatments.

Table 2. Economic analysis of farm experiment (U.S. $/ha – rounded).

	Treatment P25+CH	P40
Variable costs	$1,729	$2,182
Fixed costs	$134	$134
Production costs	$1,863	$2,316
Shrimp yield (kg)	644.3	447.9
Shrimp price	$7	$6
Gross return	$4,329	$2,809
Net profit	$2,465	$492
Benefit:cost ratio	1.3	0.2



The addition of carbohydrate reduced the TAN and nitrite-N levels in the test tanks.

Pond Experiment

In the farm trial, eight 250-m^2 earthen ponds were stocked at 6 PL_{20}/m^2. Treatments P40 and P25+CH were applied to four replicate ponds each. Initially, lime was applied at 2,000 kg/ha and cow dung at 1,000 kg/ha. Urea and single super phosphate were added biweekly to the water column at 80 and 20 kg/ha, respectively, during the first two months of culture to initiate algal blooms in the ponds. Lime was added to the ponds at 5 kg/ pond biweekly.

Pond Results

TAN concentrations in the water column and sediment were lower in treatment P25+CH than P40. The addition of carbohydrate had a profound effect on the heterotrophic bacteria count (Figure 1). Shrimp yield and individual shrimp weight at harvest were higher in treatment P25+CH than P40. Growth rates, feed conversion, and protein efficiency were also better for P25+CH. Survival was 36-42 % and not different between treatments (Table 1).

Cost Computations

The combined cost of feed and carbohydrate for treatment P25+CH was lower than for P40 due to the higher cost of high-protein shrimp feed – Rs. 46 (U.S. $1.03) against Rs. 26 ($.58)/ kg. The harvested shrimp from P25+CH came under count 40 compared to count 50 for treatment P40.

The revenue/ha from the harvested shrimp was 54% higher in P25+CH than P40 due to the combined effect of better yield and higher prices for bigger shrimp (Table 2). A 35% reduction in feed cost was recorded in the P25+CH treatment when compared to treatment P40. The benefit:cost ratio was significantly higher in treatment P25+CH than P40.

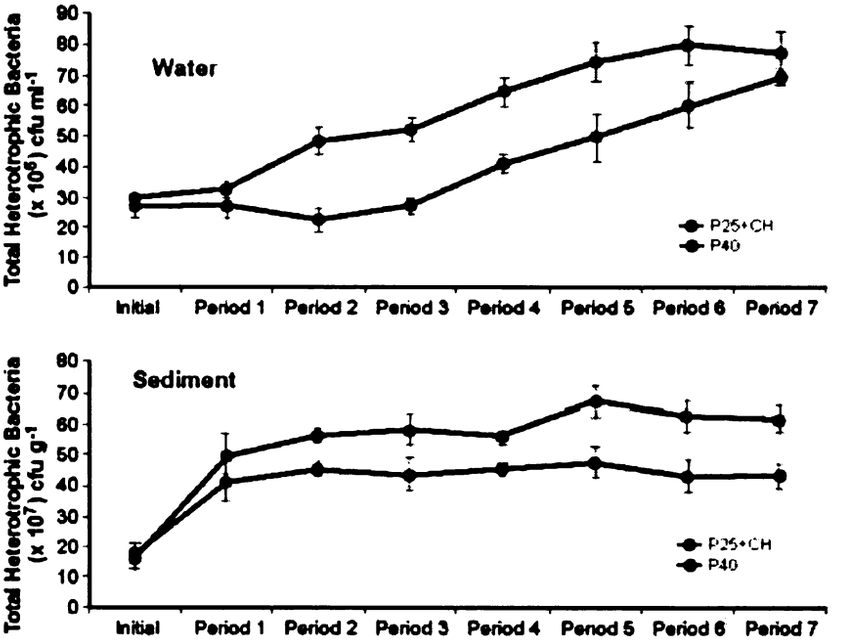


Figure 1. *Total heterotrophic bacteria count in pond water and sediment of the farm trial. Number of replicates = 4. Each period represents biweekly sampling.*

Improved ecological sustainability and shrimp yield in extensive shrimp culture system by the addition of carbohydrate

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Abstract

Experiments were conducted in the indoor and outdoor tanks in order to evaluate the effect of direct addition of carbohydrate source to the water column in the water quality and soil nutrients and carbon /nitrogen ratio in replicating extensive types of shrimp culture system. In the indoor experiments, 25 and 40% dietary protein ('P25' and 'P40') with or without carbohydrate source addition ('P25+CH' and 'P40+CH') were compared by stocking @ 6 post larvae of *Penaeus monodon* (0.357±0.01 g) per m^2. In the out door experiments, 25% and 40% dietary protein ('P25' and 'P40') with or without carbohydrate addition (25+CH and 40+CH) were compared in $6m^3$ concrete tanks having an effective bottom area of $6m^2$ stocked @ 7 post larvae of *Penaeus monodon* (PL 20) per m^2. Tapioca flour was used as carbohydrate source. Followed by the first feeding during the day, tapioca powder was applied directly to the water column in both experiments. The addition of carbohydrate was found significantly ($P<0.001$) reducing the toxic total ammonia nitrogen (TAN) and nitrate nitrogen in the water and sediment in both the experiments. Besides a significant ($P<0.05$) increase in the total heterotrophic bacterial (THB) population in water column and sediment were also observed. In the indoor experiments, lower SGR and higher FCR values were recorded in the 'P25' treatment compared to shrimp in other treatments ($P<0.05$). In the outdoor experiments,

significantly higher SGR ($\underline{P}$<0.05) and lower FCR values were recorded in treatments other than 'P25'. Higher shrimp yield ($\underline{P}$<0.001) was recorded in 'P25+CH' (160.0 g m^{-2}) when compared to 'P25' (113.9 g m^{-2}) however; it was not different from 'P40' (157.0 g m^{-2}) and 'P40+CH' (161.1 g m^{-2}). The nitrogen retention (%) and protein efficiency ratio were significantly higher ($\underline{P}$<0.001) in 'P25+CH' treatment when compared to other treatments. Survival of the shrimps was not affected ($\underline{P}$ >0.05) in both the treatments. The major sink of nitrogen was the sediment (67.3-71.3 %) and nitrogen loss to environment via water exchange was comparatively low (2.3 to 2.7 %). The study revealed that addition of carbohydrate to the pond could enhance the recovery of nitrogen in the form of harvestable products while accumulation in the sediment and losses through the water was not affected. The results show that the control of carbon /nitrogen ratio by the addition of carbohydrate sources to the pond water column benefited the extensive shrimp culture practices possible in four ways viz. 1) Increased nitrogen retention as harvested shrimp biomass 2) reduced demand in feed protein 3) reduction of toxic inorganic nitrogen species in the pond 4) reduction of nitrogen waste generated per unit shrimp production thus making the farming more ecologically sustainable and economically viable.

Key wards: C/N ratio; extensive shrimp culture; carbohydrate; Penaeus monodon; sustainability.

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SAQ.B.12

Effect of carbohydrate addition in the culture of *Penaeus monodon* (Fabricius) having varying stocking density

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Abstract

C / N ratio experiments were conducted to evaluate the effect of carbohydrate addition in different stocking density shrimp culture system. Tapioca flour was used as carbohydrate source. 40 % dietary protein feed with or without carbohydrate source addition, carbohydrate source was applied to the water column followed by the first feeding during morning. The experiments were carryout in $6m^3$ concrete tank having an effective bottom area of $6m^2$ stocked with 3 post larvae m^{-2}, 7 post larvae m^{-2} and 12 post larvaem^{-2} of *Penaeus monodon* (PL.20) (0.015 ± 0.01 gm^{-2}). The carbohydrate added tank shows significant reduction of total ammoniacal nitrogen (TAN) in the water ($P<0.05$) and sediment ($P<0.05$). In the carbohydrate without addition tanks shows significant increase of TAN in water and sediment ($P<0.05$). Total heterotrophic bacterial (THB) population increase significantly ($P<0.05$) both in the water and sediment. Compared to treatment there is no significant increase in THB carbohydrate without addition tanks. Significant increase in shrimp yield was recorded ($P<0.05$) in carbohydrate added tanks compared to other treatments. According to the stocking density the survival of the shrimp affected by each carbohydrate with and without addition ($P<0.05$). The study revels that the addition of carbohydrate to the pond could enhance nitrogen in the form of harvestable products and reduced toxic inorganic nitrogen level in the pond by making the farming more economically viable and ecologically sustainable.

Key words: C /N ratio, *Penaeus monodon*, Sustainability, stocking density, carbohydrate, aquaculture.

Utilization of different carbohydrate sources for the control of C /N ratio and the production of *Penaeus monodon* (Fabricius)

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Abstract

Addition of different low protein cheap carbohydrate source to water column for evaluating the effect of C /N ratio was studied. 25% dietary protein feed with or without different carbohydrate source addition were compared in $6m^2$ concrete tank stocked with 7 post larvae/m^2 of *Penaeus monodon* (PL.20) (0.015 ± 0.01 g/m^2). Potato flour, yam flour, rice flour, wheat flour and tapioca flour were used as carbohydrate sources. 25% dietary protein feed was applied first followed by the application of above carbohydrate sources to the water column directly. The addition of different carbohydrate sources was effective in reducing ($P<0.05$) the total ammoniacal nitrogen (TAN) in water and sediment, however there was no significant difference in any of the treatments. Increase in the total heterotrophic bacterial population (THB) ($P<0.05$) recorded in various carbohydrate sources applied treatments was also significant, however the difference could be observed among the treatments during the culture period there is significant reduction ($P<0.05$) in BOD commensurate with the increase noted in THB in all treatment. There is no significant difference ($P>0.05$) between net shrimp yield, individual shrimp weight, FCR, SGR, and ADG among various treatments. Survival of the shrimp was also uniform in all treatment ($P>0.05$). The study revealed that the addition of above different carbohydrate source to the pond was equally effective in reducing the toxic inorganic nitrogen, and increasing shrimp growth rate and survival, there by improving the sustainability and production of shrimp farm. It can be concluded that Potato flour, yam flour, rice flour, wheat flour and tapioca flour are equally effective in the control of Carbon /Nitrogen ratio of the shrimp farm.

Key words: *Penaeus monodon*, sustainability, aquaculture, carbohydrate source, C/ N ratio.

S10-03

EFFECT OF CARBON / NITROGEN RATIO IN IMPROVING PRODUCTION, SUSTAINABILITY AND REDUCING FEED COST IN SHRIMP FARMING SYSTEM

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The effect of optimisation of carbon / nitrogen was evaluated in improving the yield and sustainability of *Penaeus monodon* (Fabricius) under extensive system. 25% dietary protein with carbohydrate (P25 + CH) and 40% dietary protein (P40) were compared in earthen ponds with 250 m^2 area. The stocking density was marked @ 6 post larvae (PL 20) of *Penaeus monodon* m^{-2} (0.016 ± 0.01 g). Tapioca flour was used as carbohydrate source and applied to the water column immediate after the first feeding during the day. The addition of carbohydrate significantly reduced ($P<0.05$) the water and soil total ammonia nitrogen (TAN) in the P25 + CH treatment. The total heterotrophic bacterial population in the treatment (P25 + CH) in water and sediment showed significant increase ($P<0.05$) during both the culture period and treatment wise. The results of the chlorophyll-a shows significant increase ($P<0.05$) during the culture period, the value ranged for 9.7 – 45.1 μg l^{-1}. The organic carbon concentration also showed significant increase ($P<0.05$) during the culture period (10.7 – 25.0 μg l^{-1}). Higher shrimp yield was recorded in treatment P25 + CH (64.43 g m^{-2}) when compared to treatment P40 (44.79 g m^{-2}) ($P<0.05$). The FCR value was lower ($P<0.05$) in the P25 + CH treatment than to P40 treatment. Survival of the shrimps was not affected by treatment ($P>0.05$). The benefit cost ratio was higher in P25 + CH treatment than P40 (1.4 against 0.2) and the profit increased 400% in P25 + CH treatment. A 35% reduction of feed coast and 54% increase in the revenue from the shrimp was recorded in the P25 + CH treatment when compared to the P40 treatment. The addition of carbohydrate to the water column approach seems to be: (1) Practical and inexpensive means of reducing the accumulation of inorganic nitrogen in the pond. (2) Nitrogen control is induced by feeding bacteria with carbohydrates and through the subsequent uptake of nitrogen from the water, by the synthesis of microbial proteins. (3) Reduce the dietary protein level by the addition of carbohydrate in the commercial shrimp culture system. (4) Reduce the feed cost and higher revenue from the harvested shrimp. (5) Increase the profitability of shrimp farming operation by the addition of carbohydrate. (6) Improve the sustainability of extensive shrimp culture system.

Keywords: C / N ratio, *Penaeus monodon*, sustainability, carbohydrate, aquaculture

S10-09

EFFECT OF PERIPHYTON AND CONTROL OF CARBON / NITROGEN RATIO IN THE PRODUCTION AND SUSTAINABILITY OF *PENAEUS MONODON* (FABRICIUS) UNDER EXTENSIVE SYSTEM

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The yield and sustainability of *Penaeus monodon* was evaluated in a periphyton supplemented grow-out system with and without addition of carbohydrate sources. Tapioca flour was used as the carbohydrate source in a feed diverse extensive farming system. Artificial substrates of the grow-out were prepared either by bamboo or kanchi in separate treatments. The treatment so compared were bamboo or kanchi with 25% protein feed (B, K) without carbohydrate addition, with carbohydrate addition (B + CH, K + CH), without substrate with addition of carbohydrate as control (C). Stocking density @ 7 PL m^{-2} (0.015 ± 0.01 g) maintained in all treatments. Cattle dung 3000 kg ha^{-1}, urea 150 kg ha^{-1} and super phosphate 100 kg ha^{-1} were used as fertilizers. Bamboo poles were used @ 9 poles m^{-2} either vertically while kanchi pole was placed @ 34 poles m^{-2} horizontally for facilitating periphton production. The carbohydrate added treatments were compared to other treatment without carbohydrate addition. TAN showed significant reduction and total heterotrophic bacterial production in water and soil showed significant increase ($P<0.05$) in carbohydrate added treatments. B + CH treatment showed significantly higher ($P<0.05$) periphyton production followed by the treatment K + CH. Treatment B + CH showed higher periphyton production (45560.74 cells or colonies l^{-1}) during the culture which was significantly different ($P<0.05$) from other treatments, the lowest periphyton production was observed in treatment K (16245.26 cells or colonies l^{-1}). The individual shrimp weight, net shrimp yield, SGR and survival rate were higher in treatment B + CH followed by K + CH. Addition of carbohydrate together with bamboo as the artificial substrate were found to be effective in reducing the inorganic nitrogen concentration and enhances the shrimp yield. It is concluded that the sustainability and yield of *Penaeus monodon* can be substantially improved by planting bamboo poles as artificial substrates and addition of carbohydrate to optimizing the carbon / nitrogen ratio in the extensive culture.

Keywords: Periphyton, C/N ratio, sustainability, aquaculture, *Penaeus monodon*.

THE POTENTIAL OF PERIPHYTON SUBSTRATE BASED FARMING OF *Penaeus Monodon* (FABRICIUS) WITH THE CONTROL OF CARBON / NITROGEN RATIO

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Production of tiger prawn, *Penaeus monodon* (Fabricius), in a periphyton based carbohydrate added and without addition were compared to evaluate the effect of control of carbon / nitrogen ratio in the culture. Tapioca flour was used as carbohydrate source followed by the feeding. Poles of either bamboo or kanchi were used as artificial substrates. Bamboo or kanchi with 25% protein feed (B, K) without carbohydrate source, with carbohydrate source addition (B + CH, K + CH) and without substrate with addition of carbohydrate used as control (C) were compared. Stocking density was @ 7 post larvae (PL 20) of *Penaeus monodon* m^{-2} (0.015 ± 0.01 g). Fertilizer used was cattle dung 3000 kg ha^{-1}, urea 150 kg ha^{-1} and super phosphate 100 kg ha^{-1}. The experiment was carried out in $6m^2$ concrete tanks with sediment layer. Bamboo poles and kanchi poles at a density of 9 poles m^{-2} and 34 poles m^{-2} were used vertically and horizontally for the periphyton production. The carbohydrate added treatments showed significant ($P<0.05$) reduction in the inorganic nitrogen production when compared to other treatment carbohydrate without addition. Total heterotrophic bacterial production in water and soil showed significant increase ($P<0.05$) in carbohydrate added treatments. B + CH treatment showed significantly higher ($P<0.05$) periphyton production followed by the treatment K + CH. Treatment B + CH showed higher periphyton production (45560.74 cells or colonies l^{-1}) during the culture which is significantly different ($P<0.05$) from other treatments, the lowest periphyton production was observed in treatment K (16245.26 cells or colonies l^{-1}).The individual shrimp weight, net shrimp yield, SGR and survival rate are higher in treatment B + CH followed by K + CH (Table 1). Addition of carbohydrate with artificial substrate bamboo reduces the inorganic nitrogen concentration and enhances the shrimp production followed by kanchi and it is an appropriate combination for a sustainable periphyton based culture.

ble 1

ect of with and without periphyton and with CH and without CH in shrimp culture on weight, shrimp yield d survival of *Penaeus monodon*

	Treatments (Mean ± SD)				
	C	B+CH	K+CH	B	K
liuidual shrimp weight gain (g)	24.43 ± 0.02^e	27.48 ± 0.01^a	26.56 ± 0.06^b	25.76 ± 0.02^c	25.03 ± 0.04^d
t shrimp yield (g/m^2)	130.31 ± 16.36^a	161.83 ± 13.95^a	150.50 ± 9.12^a	143.09 ± 13.70^a	134.86 ± 14.73^a
ecific growth rate (SGR)	7.4 ± 0.00^e	7.51 ± 0.00^a	7.48 ± 0.00^b	7.45 ± 0.00^c	7.42 ± 0.00^d
rvival rate (%)	76.19 ± 9.52^a	84.13 ± 7.27^a	80.95 ± 4.76^a	76.37 ± 7.65^a	76.98 ± 8.36^a

sults from Tukey One-way ANOVA

:atments with mean values in same row with different superscripts differ significantly ($P<0.05$)

EFFECT OF VARIOUS CARBOHYDRATE ADDITION IN THE EXTENSIVE CULTURE OF *Penaeus monodon* IN THE CANTROL OF C / N RATIO

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The experiment was carried out in 6 m^2 concrete tanks, with a mud bottom and stocked with 7 post larvae (PL 20) of *Penaeus monodon* m^{-2} (0.015 ± 0.01 g). 25% dietary protein feed with addition of each carbohydrate source, tapioca flour (T), wheat flour (W), rice flour (R), potato flour (P) and yam flour (Y) were mixed with pond water and apply to the water column followed by the first feeding during morning. The results reveled that the additions of various type of carbohydrate was useful in reducing the total ammonia nitrogen (TAN) and nitrate-N production in the water and sediment through no significant variations (P>0.05) were observed among treatments. Addition of various carbohydrate increased the total heterotrophic bacteria (THB) population in the water and soil. Higher specific growth rate (SGR) and feed conversion ratio (FCR) were recorded in various treatment. The survival of the shrimp was not affected by the treatments (P>0.05) (Table 1). The results of the addition of carbohydrate to the water column proved that the cheap carbon source used has the ability to control inorganic nitrogen production and it benefited to the extensive culture practice. The carbon metabolism and nitrogen immobilizing bacterial processes by microorganism use carbohydrate as a food to produce proteins and new cells it helps to (1) increase shrimp yield in culture (2) reduced the feed cost by low demand for supplemental feed protein (3) reduce the inorganic nitrogen level by increasing the survival rate.

Table 1
Effect of carbohydrate addition on weight, shrimp yield, SGR, FCR, and survival of *Penaeus monodon* in outdoor trial

	Treatments (Mean ± SD)				
	P	Y	R	W	T
Individual shrimp weight gain (g)	25.9 ± 0.3[a]	25.8 ± 0.5[a]	25.8 ± 0.4[a]	26 ± 0.4[a]	25.7 ± 0.3[a]
Net shrimp yield (g/m^2)	147 ± 7.9[a]	143.7 ± 11.5[a]	142.1 ± 6.1[a]	145.7 ± 13.1[a]	141.4 ± 7.8[a]
Specific growth rate (SGR)	6.76 ± 0.01[a]	6.75 ± 0.02[a]	6.75 ± 0.02[a]	6.76 ± 0.02[a]	6.75 ± 0.01[a]
Feed convertion ratio (FCR)	1.19 ± 0.06[a]	1.22 ± 0.10[a]	1.23 ± 0.05[a]	1.2 ± 0.11[a]	1.24 ± 0.07[a]
Protein efficiency ratio (PER)	3.3 ± 0.18[a]	3.23 ± 0.26[a]	3.2 ± 0.14[a]	3.28 ± 0.30[a]	3.18 ± 0.18[a]
Feed convertion effect (%)	84.33 ± 4.51[a]	82.4 ± 6.60[a]	81.49 ± 3.51[a]	83.58 ± 7.50[a]	81.09 ± 4.48[a]
Average daily weight gain (ADG)	0.216 ± 0.002[a]	0.215 ± 0.003[a]	0.215 ± 0.003[a]	0.216 ± 0.003[a]	0.214 ± 0.002[a]
Survival rate (%)	80.95 ± 4.8[a]	79.37 ± 5.0[a]	78.57 ± 2.4[a]	80.16 ± 6.0[a]	78.57 ± 4.1[a]

Results from Tukey One-way ANOVA
Treatments with mean values in same row with different superscripts differ significantly (P<0.05)