C/N-controlled periphyton-based freshwater prawn farming system: a sustainable approach to increase pond productivity

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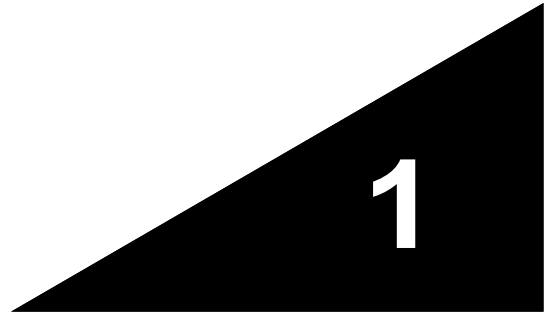on Monday 30 January 2012
at 4 p.m. in the Aula.

Dedicated to Jakia Sultana
(My partner of long journey)

Abstract

Three technologies showed to improve productivity and sustainability of pond production: (1) C/N ratio control, (2) providing substrates for periphyton development, and (3) fish driven re-suspension. The novelty of this PhD research is to combine these technologies, with the goal to raise pond productivity above levels obtained with each one of these technologies separately, and to increase the nutrient use efficiency in ponds above levels presently achieved, further enhancing sustainability. This combined technology is further referred to as **C/N controlled periphyton (C/N-CP)** technology. A series of experiments (Chapter 2-6) were conducted to develop such technology. The first step (Chapter 2) evaluated if increasing C/N ratio (from 10 to 20) in combination with providing vertical substrates for periphyton development in freshwater prawn monoculture ponds can enhance overall pond productivity. The results were encouraging due to the 75% increase of production; in addition it seemed that natural foods were underutilized by freshwater prawn. Therefore, the next step (Chapter 3) was further analysis of the above mentioned experiment investigating how C/N ratio control and addition of substrates influenced the natural food communities in freshwater monoculture ponds. This study suggested further investigation on the possibility of increasing stocking density of freshwater prawn and inclusion of tilapia due to its both sediment re-suspension and periphyton grazing activity. Therefore, in the third step (Chapter 4) increasing stocking densities of prawn (from 2 to 3 m^{-2}) and addition of different levels of tilapia (0, 0.5 and 1 individual m^{-2}) were tested. This study concludes that both stocking densities (2 and 3 juveniles m^{-2}) of prawn with the addition of 0.5 tilapia m^{-2} resulted in higher fish production, good environmental condition and economic return. In the fourth step (Chapter 5), the effects of addition of periphyton substrates and tilapia driven bioturbation were tested in C/N controlled (C:N=20) system. This study showed that addition of tilapia (0.5 individual m^{-2}) and periphyton substrates in C/N controlled ponds benefited freshwater prawn production and recommended that economic sustainability could still be further enhanced by identifying cheaper on-farm carbohydrate sources. Therefore, in the last step (Chapter 6) maize flour (*Zea mays*) is considered as a cheaper on-farm carbohydrate source and compared with tapioca starch. In addition, in this study considering the importance of rohu (*Labeo rohita*) as an indispensable species in south Asian aquaculture, both tilapia and rohu are considered to determine the suitability of either species in C/N-CP ponds. In added finfish (0.5 individual m^{-2}), 100% tilapia were found to be beneficial in C/N-controlled (C:N=20:1) prawn farming system compared to 50% tilapia+50% rohu or 100% rohu. In conclusion, a significant improvement of system environment, productivity and economic benefits was observed due to synergism among C:N ratio control, addition of periphyton substrates and tilapia driven bioturbation. Therefore, C/N-CP technology is a promising technology, improving the sustainability and productivity of present prawn farming by simple and affordable means.

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General Introduction

Globally, production from capture fisheries has leveled off and most of the main fishing areas are fully or over-exploited. Capture fisheries will, therefore, not be able to meet the growing global demand for aquatic food. About 30% of the global capture fisheries production is not used as human food, but to produce fishmeal and fish oil used in animal feeds. Concurrently, the demand for aquatic food grows due to population growth coupled with an increase in the per capita fish consumption. The present world population is 6.6 billion people (FAO, 2008) and expected to grow to 9 billion by 2050 (UN, 2000). Given the projected population growth over the next two decades, it is estimated that at least an additional 40 million tonnes of aquatic food will be required by 2030 to maintain the current per capita consumption (FAO, 2002). Therefore, due to the stagnating capture fisheries production, aquaculture is expected to play a major role in filling up the growing gap between global fish demand and supply. Today, aquaculture already accounts for 46.7 percent of the world's food fish supply (FAO, 2010). World aquaculture has grown tremendously during the last fifty years from a production of less than a million tonnes in the early 1950s to 52.5 million tonnes (excluding aquatic plants) in 2008, with a value of US$ 98.4 billion (FAO, 2010). Therefore, aquaculture has the potential to make a significant contribution to this increasing demand for aquatic food in most regions of the world; in order to achieve this; however, the sector (and aqua-farmers) faces significant challenges.

Potentials and role of aquaculture in Bangladesh

Being a country of rivers and floodplains, fish plays a very important role in the daily life of many people in Bangladesh. The Bengali expression "Mache Bhate Bengali", or "Fish and Rice make a Bengali," illustrates this importance. Historically people depended mainly on natural waters for supplies of fish; but as a result of declining catches of wild fish due to an increased fishing pressure by the growing population as well as environmental degradation, people began to culture fish in enclosed waters. At present, aquaculture has been expanding both vertically and horizontally as pond fish culture and crustacean (shrimp and freshwater prawn) farming offer tremendous potential. A broader selection of species is now cultured including high value crustacean species such as *Penaeus monodon* and *Macrobrachium rosenbergii*. In Bangladesh, during the last ten years the annual growth rate in total production was

around 5% whereas, the average annual growth in aquatic production through aquaculture was close to 10% (Figure 1).

Bangladesh is blessed with vast inland water bodies and has emerged as one of the leading nations in freshwater aquaculture production during recent years. In 2008, it was the sixth largest aquaculture producing country in the world, supplying 8.8% of global aquaculture production, excluding China (FAO, 2010). The country is situated on the deltaic plains with a large proportion of its area comprising the floodplain of three converging rivers, the Ganges, the Brahmaputra-Jamuna and the Meghna (GBM river system). Inland water resources comprise 305,025 ha of ponds and ditches, 5,488 ha of oxbow lakes, 217,877 ha of shrimp farms, 853,863 ha of rivers and estuaries, 114,161 ha of beels (shallow natural depression), 68,800 ha of man-made reservoirs (Kaptai lake) and 2,832,792 ha of floodplains (DOF, 2009). The total fish production in 2007-08 was 2.56 million metric tones of which 39.23% came from aquaculture; pond aquaculture contributed more than 90% to the total aquaculture production (DOF, 2009).
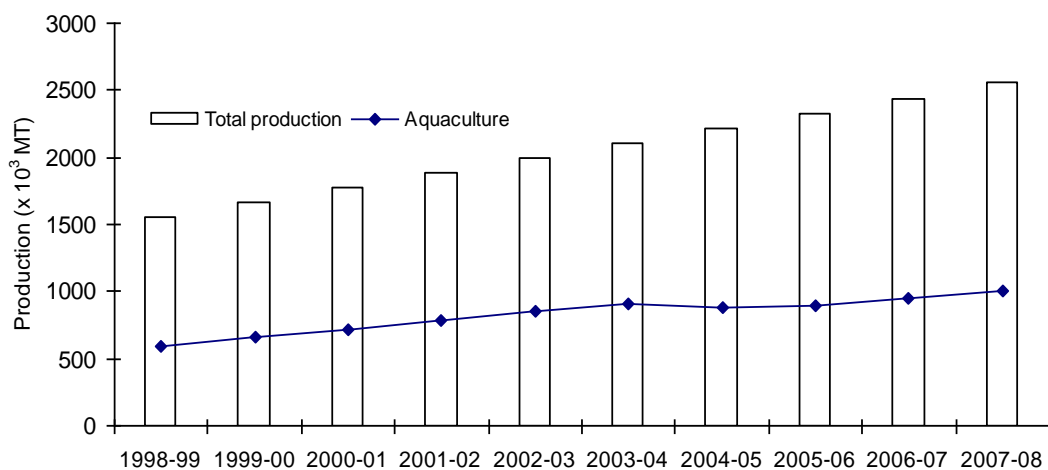
Figure 1. Trends of total production and aquaculture production over the last ten years in Bangladesh (Source: graph is prepared by using the data from DOF, 2009).

In Bangladesh, fisheries and aquaculture play a major role in nutrition, employment and foreign exchange earnings. About 12 million people are associated with the fisheries sector, of which 1.4 million people rely exclusively on fisheries related activities (DOF, 2005). Fish and fisheries products are contributing about 4.04% of annual export earning (DOF, 2009). Fish provides 58% of the animal protein intake in

Bangladesh and about 3.74% of national GDP, or 20.87% of the agriculture GDP (DOF, 2009).

In Bangladesh, aquaculture production systems are mainly extensive and extended extensive, with some semi-intensive and a few intensive systems. The average annual production is still very low compared to many fish producing countries. This is mainly because of the operation of intensive aquaculture which demands high investment and technical expertise are not affordable by resource-poor farmers. There is considerable potential for improvement of culture systems to intensify productivity. Therefore, novel, simple and affordable technologies are needed to improve livelihoods, including nutrition, food security and income in the aquaculture sector.

Status of freshwater prawn farming in Bangladesh

Freshwater prawn (*Macrobrachium rosenbergii*) is indigenous to South and South-East Asia, together with northern Australia and the western Pacific islands (New, 1988). It is an important aquaculture industry in many Asian countries, which together contributes over 98% of the global freshwater prawn production. The global farmed production of freshwater prawn (*Macrobrachium rosenbergii*) in 2007 was over 221,000 tones (FAO, 2009). Considering the giant freshwater prawn (*M. rosenbergii*) alone, the major producers in 2007 were China (56.3%), Thailand (12.3%), India (12.3%), Bangladesh (9.4%) and Taiwan (4.5%). The farmed production of *M. rosenbergii* increased 2.7 times globally and 4.0 times in Bangladesh during the last decade (Figure 2).

In Bangladesh, freshwater prawn farming is currently one of the most important sectors of the national economy and during the last two decades, its development has attracted considerable attention because of its export potential. This species is now considering as an emerging crustacean aquaculture species, receiving considerable attention in Bangladesh recently, and fetching attractive prices in both domestic and international markets. The contribution of freshwater prawn to Bangladesh shrimp[1] production increased from 10.6% in 1998 to 25.7% in 2008 (Figure 3). The shrimp sector in Bangladesh generated US $418 million in 2007, representing 3.4% of the total export value, with freshwater prawn contributing 20-25% (DOF, 2009). In Bangladesh, freshwater prawn farming areas increased from just 2200 ha in 1991 to

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[1] Bangladesh shrimp production includes both freshwater prawns and marine or brackish water shrimps.

50,000 ha today, expanding on average 10-20% per annum (Khondaker, 2007).This figure is expected to rise with the expansion of prawn cultivation into new areas.
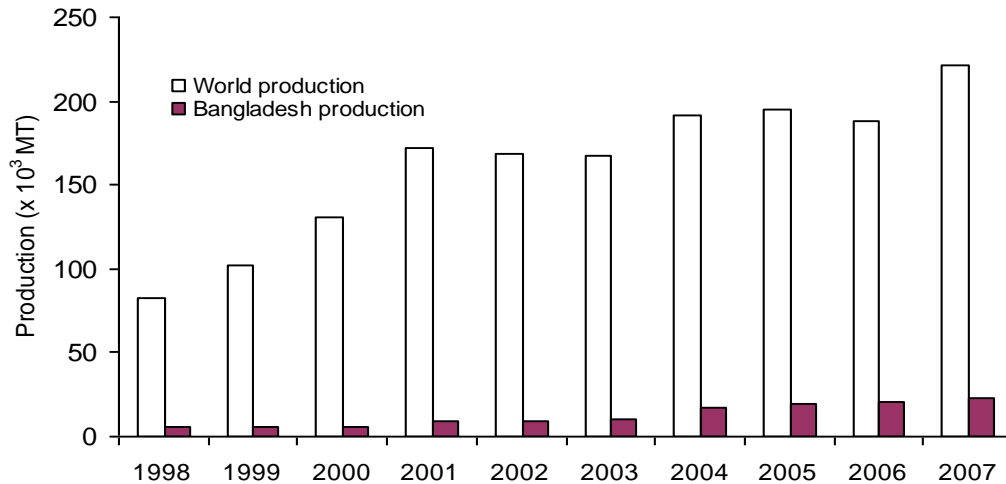
Figure 2. Production of farmed freshwater prawn (*Macrobrachium rosenbergii*) in the world and Bangladesh (Source: graph is prepared by using the data from FAO, 2009).
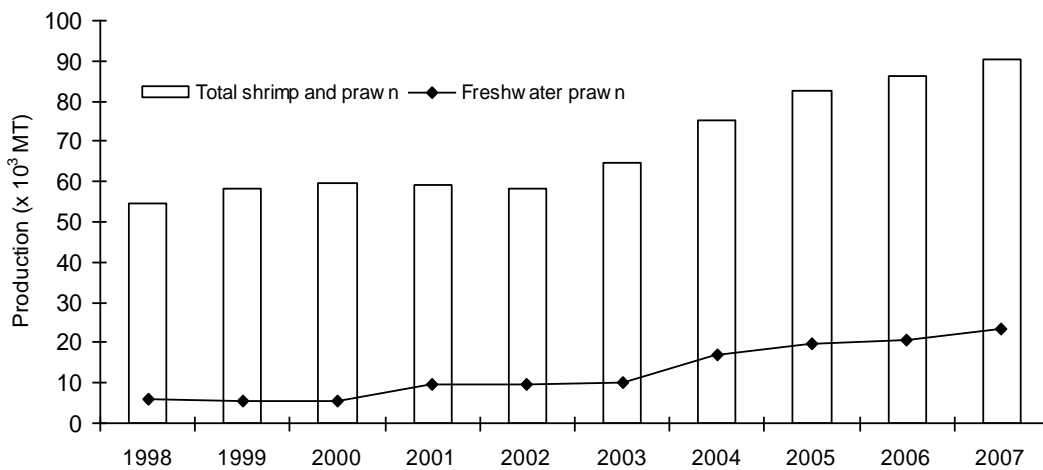
Figure 3. Contribution of freshwater prawn in total shrimp and prawn production over the last ten years in Bangladesh (Source: graph is prepared by using data from DOF, 2009; FAO, 2009)

There are two prawn farming systems in Bangladesh: pond and *gher*. In southwest Bangladesh, the cultivation of prawn in modified rice field is locally referred to as '*gher*' (Rutherford, 1994). Although prawn farming practice is still traditional and

extensive in nature, now many farmers are practicing improved methods where prawns are cultivated semi-intensively. Extensive production typically use slightly modified versions of traditional methods with low-density (10000-18000 postlarvae ha^{-1}) and relies mainly on natural productivity (e.g., phytoplankton, zooplankton and benthos) of the ponds and occasionally with supplementary diets consisting of a mixture of locally available feed ingredients, such as rice bran, wheat bran, oil cake and fish meal (Ahmed et al., 2008). Semi-intensive operations practice intermediate levels of stocking (18000-30000 post larvae ha^{-1}), applying manufactured pelleted feeds (Ahmed et al., 2008). Most of the farmers practice polyculture of freshwater prawn with various carp species having complementary feeding habits to make better use of the natural food available (Asaduzzaman et al., 2006a). Resource-poor farmers prefer semi-intensive polyculture because the capital needed to buy expensive artificial feeds is minimized, while the exploitation of natural foods in ponds is optimized. Nevertheless, there is a tendency by richer farmers to further increase production through the application of higher amounts of artificial feeds. However, on average yields from the extensive ponds in Bangladesh are in the range of 390 to 412 kg ha^{-1} year^{-1} and productivity is low compared with other countries (Table 1). Countries with a larger export market than Bangladesh use more intensive techniques and have significantly higher yields. So, Bangladesh urgently needs to increase freshwater prawn productivity to satisfy the future demand of aquatic products and to retain and expand the present export markets.

Table 1.

Comparison of prawn yields in Bangladesh and other producing countries (Source: modified from Ahmed et al., 2008).

Country	Prawn production (kg ha^{-1} year^{-1})	Reference
Bangladesh	390-412	Asaduzzaman et al. (2006a)
China	1,500	Weimin and Xianping (2002)
India	600-1,000	Raizada et al. (2005)
Taiwan	1,500	New (2005)
Thailand	2,338	Vicki (2007)
Vietnam	1,000-1,500	Ridmontri (2002)

Intensifying freshwater prawn production: needs and challenges

Until today, freshwater prawn production in Bangladesh increased primarily by expanding the culture area. This demand large additonal quantity of water and land area, both being scarce resources. Therefore, a more practical and sustainable way to raise prawn production is by increasing pond productivity per unit land area and water. Mostly, aquaculture intensification comes with higher stocking densities and greater use of water, feeds and fertilizers, leading to increased waste production (Beveridge et al., 1997). In addition, in many countries the increase in production, particularly in shrimp aquaculture, has recently seen the negative impacts of unsustainable production method with regard to environment and consumer safety. Therefore, raising pond productivity in an ecological, social and economic sustainable way is essential to feed future generations.

Higher yields can be obtained by applying more energy, capital and technology. Unfortunately, these resources require capital, which is out of reach to the majortity of the resource poor farmers in Bangladesh. Farmers need new pond production concepts, relying on locally available resources and requiring little investment, that are sustainable (see review of Azim and Little, 2006). To this end, several recent studies in many countries, demonstrated various low-cost technologies that can significantly raise pond productivity, but that were so far never tested in combination.

Major issues in optimizing productivity and sustainability in stagnant ponds

Stagnant ponds have mostly no inlet and drainage system. Such ponds provide the majority of crustacean and finfish production in Bangladesh. With no water exchange, the farmer relies on the intrinsic self-purification capacity of the pond. The major problem associated with aquaculture in stagnant ponds is rapid eutrophication, resulting from increasing concentrations of nutrients and organic matters during culture. In these stagnant ponds, formulated feeds are the principal nutrient input. To produce 1 kg live weight fish one needs 1-3 kg of dry weight feed (assuming a food conversion ratio about 1-3), depending on the culture species and the quality of the feed (Naylor et al., 2000). About 36% of the feed is not consumed and accumulates at the pond bottom in the form of organic waste (Brune et al., 2003). The microbial decomposition of organic matter in the system leads to an increased levels of TAN and nitrite, both harmful to fish even at low concentration (Meade, 1985; Jimenez-

Montealegre et al., 2002; Torres-Beristain et al., 2006). Bacteria present in water and sediment transform TAN into nitrite and nitrate by nitrification. However, in stagnant water ponds TAN tends to accumulate within the system due to insufficient nitrification activity (Grommen et al., 2002). Deteriorated water quality has resulted in disease outbreaks and heavy financial losses and in criticism from various environmental organizations as being environmentally irresponsible. In addition, organic residues create sites with a high biological oxygen demand in stagnant ponds. The oxygen supply to the pond bottom is limited, even in the periods of natural mixing and surface re-aeration due to strong winds. Low oxygen availability affects the benthic community's diversity and structure, and reduced sediments are avoided by shrimps and prawns (Gray et al., 2002; Buzzelli et al., 2002). Therefore, the production potential of aquaculture in stagnant ponds is limited and often associated with poor water quality, disease outbreak, high production cost and low economic benefit (Figure 4).
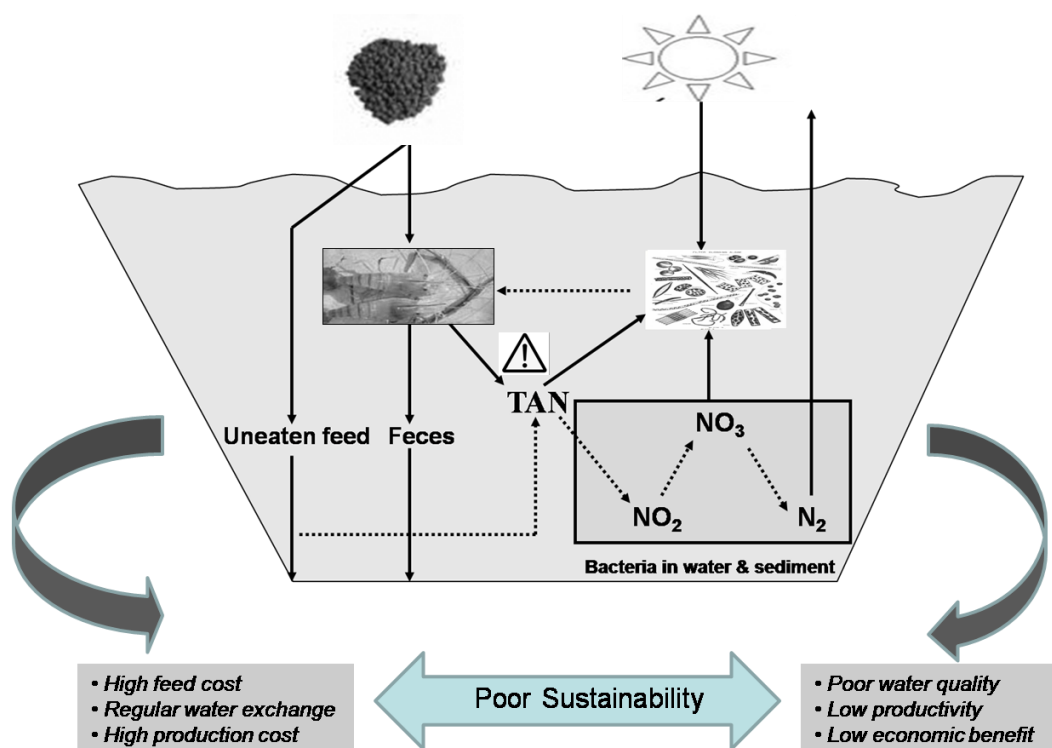

Figure 4. Sustainability issues of freshwater prawn farming in stagnant ponds

The dependency on the use of fishmeal and fish oil as prime feed ingredients in shrimp farming is also not sustainable (Naylor et al. 2000). Manufactured feeds for fish culture represent 50% or more in the production cost, primarily due to the cost of

the protein component (Bender et al., 2004). Only 20% to 30% of the feed is retained in fish biomass, the rest potentially polluting the culture environment (Briggs and Funge-Smith, 1994; Jackson et al., 2003; Thakur and Lin, 2003). Therefore, to make fish farming more sustainable in stagnant ponds, pond management should be geared towards improving nutrient retention.

Means for Intensifying productivity in stagnant ponds

Recently, several studies in many countries demonstrated various low-cost technologies that can significantly raise pond productivity. Among these low-cost technologies, C/N ratio control through carbohydrate addition (Avnimelech, 1999; Hari et al., 2004; Avnimelech, 2007), providing substrates for periphyton development (van Dam et al., 2002; Tidwell et al., 2000, 2002, 2005; Azim et al., 2003a, 2003b; Keshavanath et al., 2001; Milstein et al., 2009) and fish driven re-suspension (Riise and Roos, 1996; Jimenez-Montealegre et al., 2002; Ritvo et al., 2004; Milstein et al., 2002) seems to be promising options for resource poor farmers. A brief overview of these technologies and their role in productivity are discussed below.

(1) C/N ratio control

C/N ratio control through carbohydrate addition seems to be relatively cheap and simple way to intensify aquaculture. Microbial control of water quality and heterotrophic production of single cell protein (biofloc) by manipulating C:N ratio in both biofloc technology (BFT) ponds and extensive ponds are rapidly expanding (McIntosh, 2000; Hari et al., 2004; Hargreaves, 2006; Crab et al., 2007; Avnimelech, 2007). Fish and shrimp, in general, utilize just 20-25% of feed proteins. This implies that one has to supply with the feed, 4 times the amount of protein as harvested with the fish. The non-utilized protein is excreted as ammonium that often limits fish growth and even leads to mortality. This problem can be overcome in heterotorphic systems by the addition of carbonaceous substrates. At high carbon to nitrogen ratios (C:N) heterotrophic microorganisms would dominate over autotrophic microorganisms and would assimilate total ammonia nitrogen, nitrite and nitrate, to produce cellular proteins that can serve as supplemental feed source for the culture fish and shrimps (Avnimelech, 1999; Moss et al., 1999; Browdy et al., 2001; Burford

and Lorenzen, 2004). This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006). Again, the conversion of ammonium to microbial protein needs less dissolved oxygen compared to oxygen requirement for nitrification (Avnimelech, 2006; Ebeling et al., 2006) suggesting the preference of heterotrophic community rather than nitrifying bacteria in C/N controlled system. In a heterotrophic microbial based production system, bacterial flocs provide more stable water quality than does a phytoplankton-based production system (Boyd and Clay, 2002). C/N ratio control can increase nitrogen retention from the added feed by 7% (Schneider et al., 2005) to 13% (Hari et al., 2004). Therefore, nitrogen retention from the added feed can be increased approximately from 25% to the 32-38%. In summary, C/N ratio control benefits aquaculture by improving water quality through reducing toxic inorganic nitrogen content such as ammonia and nitrite, improving nutrient utilization efficiency, reducing nutrient discharge and finally improving overall sustainability of aquaculture.

(2) Providing vertical substrates for periphyton development

Another means to intensify production in aquaculture ponds is through stimulating periphyton development. Extensive work was conducted and published during the last decade on periphyton's role and ecology in aquaculture ponds (Azim et al., 2005a). Vertical surfaces (bamboo poles, plastic stripes etc.) placed in ponds are colonized with microbial communities, including bacteria, algae, protozoa and fungi embedded in an extra-cellular polysaccharide matrix. The assemblage of attached organisms on submerged surfaces, including associated non-attached fauna are referred to as periphyton (van Dam et al., 2002). This community is actively metabolizing organic residues and significantly enlarges the pond's food base. In fed ponds, roughly 3 times the amount of organic matter that was retained in fish production settles to the pond bottom, creating an anoxic zone characterized by inefficient recycling of organic wastes. An important benefit of periphyton communities is their ability to absorb dissolved and suspended matter, inclusive organic matter from the water column, reducing bottom accumulation while maximizing the percentage of organic matter remaining exposed to aerated conditions in the water column. Besides entrapping organic detritus, periphyton removes nutrients from the water column and helps to

control the dissolved oxygen concentration and the pH of the surrounding water (Azim et al., 2002; Dodds, 2003; Bender et al., 2004). Supplying substrates improves the nitrogen-related processes (nitrification), thus keeping ammonia level low (Langis et al., 1988). In a traditional fish pond, phytoplankton is the most important component for energy fixation and fuelling the food web. When substrates are installed in the pond, inorganic nutrients can also follow the extra periphyton loop (Azim, 2001). This adds a third natural food source existing of periphytic microorganisms that can be consumed by the fish and also dead periphyton contributes to the detrital mass in the ponds (van Dam and Verdegem, 2005). However, unlike dead phytoplankton, dead periphyton remains attached to substrates, providing a rich source of organic nutrients for heterotrophic microorganisms. Processing of this organic matter yields inorganic nutrients that can be utilized by living algae again (Wetzel, 1983). For freshwater finfish, the reported increase in production associated with substrates ranged from 30-115% in carp monoculture and 30-210% in carp polyculture, depending on amount and types of substrate used, cultured species, nature of ponds (on-station or on-farm), and other management aspects such as feeding and/or fertilization (see review of Azim and Little, 2006). It has been reported that both survival and growth of shrimps and freshwater prawn were significantly higher due to provision of substrates as compared to traditional production system without substrates (Cohen et al., 1983; Tidwell and Bratvold, 2005; Uddin et al., 2006). In summary, the benefits exerted from periphyton-based ponds are periphyton as additional natural food, substrates as shelter to minimize territorial effects and improved water quality through trapping suspended solids, organic matter breakdown and enhanced nitrification.

(3) Fish driven re-suspension

Still another means to raise aerobic microbial breakdown of organic matter in the ponds is through re-suspension. This can be done mechanically, but also very effectively through the action of fish, especially sediment browsing species like tilapia. Most of these fish species are specialized to feed on benthic organisms and in doing so affect water transparency, nutrient cycling, and phytoplankton, zooplankton, and benthic macroinvertebrate abundances (Northcote, 1988). Even a relatively short perturbation of bottom sediments can lead to significant changes of organic matter transformations and may even oxidize pond bottoms. By digging and sieving of

sediments, benthivorous fishes increase oxygen availability in the sediment and cause re-suspension of bottom particles, which in turn has a large impact on the abiotic and biotic properties of the overlying water column (Phan-Van et al., 2008; Jiménez-Montealegre et al., 2002). In fed ponds, organic matter in the form of uneaten feed, feces, dead plankton settles to pond bottom, creating an anoxic zone where nutrients remain trapped (Avnimelech and Zohar, 1986). By fish driven re-suspension, the bottom nutrients are exposed to aerobic conditions in the water column and better mineralized, stimulating the natural food web (Jiménez-Montealegre et al., 2002). Rivto et al. (2004) demonstrated that fish driven re-suspension leads to an appreciable mixing and oxidation of sediments. The digging and sieving of sediments by benthivorous fish also increased diffusion rates across the sediment-water interface (Hohener and Gachter, 1994), which in turn increases nutrient availability in the overlying water. Stocking bottom browsing species in polyculture ponds is a traditional world-wide applied methodology to enhance pond productivity. In most cases fish driven re-suspension significantly improved production. In polyculture ponds, total production increased almost twice in the presence of 0.5 benthivorous fish (common carp) m^{-2} (Rahman, 2006). In summary, fish driven re-suspension leads to better nutrients retention in combination with increased production, thereby improve farm productivity and sustainability.

C/N-controlled periphyton-based system

The proposed C/N-controlled periphyton-based system (C/N-CP) combines and upgrades the previously described three approaches. The first is microbial control of water quality and recycling of protein through the adjustment of C/N ratio in the pond. The second is based upon the application of vertical substrates and development of periphyton, improving water quality and providing shelter and additional food for the cultured species and thereby improving productivity. The third one is fish driven re-suspension, improving nutrients retention and farm productivity. Although the effects of C/N control, substrate addition, and fish driven re-suspension on pond ecology and production are well documented, their combined effects on productivity have never been investigated in stagnant ponds. Previous studies showed that each of these techniques enhanced production in stagnant ponds, and further enhanced production might be obtained through synergism between the various techniques.

The above technology requires installation of hard substrates and application of cheap carbohydrates, resources which can be produced within the farmers' traditional agricultural systems. The combination of fish drive re-suspension with vertical substrates in C/N ratio control ponds may be even more efficient, due to the possibility that the re-suspended organic particles will be trapped by the periphyton communities. With this technology, the utilization of the aquatic food web is optimized by encouraging bacteria and epiphytic production, hence recycling nutrients and enlarging the microbial based food web. The proposed C/N-CP system carries a number of environmental advantages as well. The system is based upon the induction of an efficient food web that utilizes natural food sources and recycle waste components. In addition, less wastes accumulate in the pond. An important environmental advantage is the ability to recycle nitrogen and raise protein utilization.

Addition of tilapia and/or rohu in C/N-CP freshwater prawn farming system

In order to fulfill our research objective, we should have to choose an additional species which has re-suspension activity and can effectively graze on periphyton and plankton. Avnimelech et al. (1999) reported that tilapias effectively re-suspend sediment, and such activity is more pronounced in large fish. In addition of re-suspension activity, tilapia can effectively graze on the periphyton (Uddin, 2007; Azim et al., 2003a; Dempster et al., 1993; Milstein et al., 2009) and phytoplankton (Perschbacher and Lorio, 1993). Again, Uddin (2007) showed that in mixed culture the feeding niches of tilapia and prawn only partially overlap, and recommended this duo-culture as an alternative to polyculture of Chinese and Indian carps. Moreover, it is found in almost all the countries of the world, and farmers prefer tilapia as culture species due to its adaptation to a wide range of environments, good taste, fast growth, easy reproduction and versatile feeding behavior.

Of all species stocked in polyculture, fish farmers in south Asia like to stock a native major carp, commonly known as rohu, because it fetches the highest market price and has the highest consumer preference (Dey et al., 2005). This species is a column feeder mainly living on plankton (Jhingran and Pullin, 1985) and periphyton (Azim et al., 2003c) but sediment re-suspension with rohu has not been reported as for tilapia (Costa-Pierce and Pullin, 1989; Riise and Roos, 1996; Avnimelech et al., 1999; Jimenez-Montealegre et al., 2002). Therefore, in this study rohu is used to determine

the suitability of either species with freshwater prawn in C/N-controlled periphyton-based system.

Objectives, hypothesis and outline of the thesis

The present research aims to develop a sustainable methodology for stagnant ponds without a massive investment common to many intensive system. The overall objective is to combine heterotrophic pond management, periphyton technology and fish driven re-suspension into a low cost technology, further referred to as **C/N-controlled periphyton-based (C/N-CP) technology**, applicable by small scale farmers. To reach this goal special attention was given to 1) enhancing heterotrophic bacteria activity, improving feed utilization efficiencies and raising crop yields; 2) optimizing periphyton development and quality through C:N ratio control; 3) minimizing the development of anoxic bottom conditions through proper pond preparation and fish bioturbation. The development of such a methodology is of high priority to satisfy future demands for aquatic products, while providing the opportunity to resource poor farmers to participate and benefit significantly from the growth of aquaculture production. The present research explores the hypothesis that combination of C/N ratio control, providing substrates for periphyton development and fish driven re-suspension, will leads to a substantial increase of average farm productivity and sustainability in stagnant ponds.

This PhD thesis starts with a general introduction (this Chapter) and concludes with a general discussion (Chapter 7). The research (Chapter 2-6) followed a step-wise approach. The first step (Chapter 2) evaluated if increasing C/N ratio (from 10 to 20) in combination with providing vertical substrates for periphyton development in freshwater prawn monoculture ponds can enhance overall pond productivity. The results were encouraging due to the 75% increase of production; in addition it seemed that natural foods were underutilized by freshwater prawn. Therefore, the next step (Chapter 3) was further analysis of the above mentioned experiment investigating how C/N ratio control and addition of substrates influenced the natural food communities in freshwater monoculture ponds. This study suggested further investigation on the possibility of increasing stocking density of freshwater prawn and inclusion of tilapia due to its both sediment re-suspension and periphyton grazing

activity. Therefore, in the third step (Chapter 4) increasing stocking densities of prawn (from 2 to 3 m^{-2}) and addition of different levels of tilapia (0, 0.5 and 1individual m^{-2}) were tested. This study concludes that both stocking densities (2 and 3 juveniles m^{-2}) of prawn with the addition of 0.5 tilapia m^{-2} resulted in higher fish production, good environmental condition and economic return. In the fourth step (Chapter 5), the effects of addition of periphyton substrates and tilapia driven bioturbation were tested in C/N controlled (C:N=20) system. This study showed that addition of tilapia (0.5 individual m^{-2}) and periphyton substrates in C/N controlled ponds benefited freshwater prawn production and recommended that economic sustainability could still be further enhanced by identifying cheaper on-farm carbohydrate sources. Therefore, in the last step (Chapter 6) maize flour (*Zea mays*) is considered as a cheaper on-farm carbohydrate source and compared with tapioca starch. In addition, in this study considering the importance of rohu as an indispensable species in south Asian aquaculture, both tilapia and rohu are considered to determine the suitability of either species in C/N-CP ponds. In the general discussion (Chapter 7), major conclusions of the previous chapters were integrated and interpreted, strengths and weaknesses of the followed approaches were outlined and suggestions for further studies were given.

2

C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds

Chapter 2

C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds

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Abstract

The present research investigated the effect of carbon/nitrogen ratio (C/N ratio) control in ponds with or without substrate addition for periphyton development on production of giant freshwater prawn. C/N ratios of 10, 15 and 20 were investigated in 40 m^2 ponds stocked with 2 prawn juveniles (5.023±0.02 g) m^{-2} with or without added substrates for periphyton development. The various treatment combinations of C/N ratio and periphyton substrate addition are abbreviated as 'CN10', 'CN15', 'CN20', 'CN10+P', 'CN15+P' and 'CN20+P', P representing periphyton substrate. A locally formulated and prepared feed containing 30% crude protein with C/N ratio 10 was applied. Tapioca starch was used as carbohydrate source for manipulating C/N ratio and applied to the water column separately from the feed. Increasing the C/N ratio from 10 to 20 reduced ($P<0.001$) the total ammonia-nitrogen (TAN), nitrite–nitrogen (NO_2–N) and nitrate–nitrogen (NO_3–N) in water column and total Kjeldahl nitrogen (TKN) in sediment. The addition of substrates only influenced the NO_2–N concentration in the water column ($P<0.001$). Increasing the C/N ratio raised the total heterotrophic bacterial (THB) population in the water column, sediment and periphyton ($P<0.001$). It also increased the dry matter (DM), ash free dry matter (AFDM), and chlorophyll *a* content of periphyton ($P<0.001$). The lowest specific growth rate (SGR), the highest food conversion ratio (FCR), and the lowest protein efficiency ratio (PER) were recorded in treatment CN10 ($P<0.05$). The addition of substrates did not influence size at harvest ($P>0.05$) but improved the survival from 62.8 to 72% ($P<0.001$). Increasing the C/N ratio from 10 to 20 increased the net yield by 40% and addition of substrate increased the net yield by 23%. The combination of C/N ratio control and substrate addition increased the net yield by 75% from 309 (CN10) to 540 (CN20+P) kg ha^{-1} (120 days)$^{-1}$. This 75% higher production concurred with (1) a lower inorganic nitrogen content in the water column, (2) a higher THB abundance supplying additional single cell protein to augment the prawn production, and (3) an improved periphyton productivity and quality.

1 Introduction

Freshwater prawn (M*acrobrachium rosenbergii*) is indigenous to South and South-East Asia, together with northern Australia and the western Pacific islands (New, 1988). It is an important aquaculture industry in many Asian countries, which together contributes over 98% of the global freshwater prawn production. In Bangladesh, freshwater prawn farming areas increased from just 2200 ha in 1991 to 35,000–40,000 ha today (DOF, 2006). There is a great potential for further development of freshwater prawn farming in ponds and extensive low lying agricultural lands throughout the country. On average, yields from extensive ponds are in the range of 300–600 kg ha^{-1} year^{-1} (Asaduzzaman et al., 2006). Raising of freshwater prawn production through expansion of pond area would demand large additional quantities of water and land area, both are very scarce resources. In consequences, the most practical way to raise freshwater prawn production is by increasing pond productivity per unit land area and water. The challenge is to do this sustainably. Aquaculture intensification, however, comes with higher stocking densities and greater use of water, feeds and fertilizers, leading to increased waste production (Beveridge et al., 1997). Operation of intensive aquaculture also demands high investment and technical expertise, which are not affordable by resource-poor farmers of Bangladesh. Efforts are needed to intensify aquaculture by using the resources derived from other agricultural systems and manipulating natural food thereby maximizing overall nutrient retention (Azim and Little, 2006).

To this end, the use of periphyton substrates and manipulation of C:N ratio in freshwater finfish and prawn production in extensive ponds have been found promising (see reviews of van Dam et al., 2002; Hargreaves, 2006; Azim and Little, 2006). These techniques require installation of hard substrates or application of cheap carbohydrates, resources which could potentially be produced within the farmers' traditional agricultural systems. It has been reported that both survival and growth of freshwater prawn were significantly higher due to provision of substrates as compared to traditional production system without substrates (Cohen et al., 1983; Tidwell and Bratvold, 2005; Uddin et al., 2006). The benefits exerted from periphyton-based ponds are periphyton as additional natural food, substrate as shelter to minimize territorial effects and improved water quality through trapping suspended solids, organic matter breakdown and enhanced nitrification. On the other hand, microbial

control of water quality and heterotrophic production of single cell protein (biofloc) by manipulating C:N ratio in both biofloc technology (BFT) ponds and extensive ponds are rapidly expanding especially in producing penaeid shrimp (McIntosh, 2000; Hari et al., 2004; Hargreaves, 2006; Crab et al., 2007; Avnimelech, 2007). Generally, C:N ratio manipulations work in BFT and in extensive ponds. In the latter, it is assumed that development of biofilm on the bottom takes on the role of bioflocs in BFT.

However, although the effects of substrate addition and C:N control on finfish and shellfish production are well documented, their combined effects on productivity have never been investigated in extensive ponds. The goal of the present research is to quantify the single and combined effects of C:N ratio manipulation and substrate addition on prawn production. Attention was also given to the effect of C:N ratio manipulation on (1) periphyton quantity and quality and (2) the heterotrophic bacterial activity in the water column, sediment and periphyton.

2 Materials and Methods

2.1 Experimental design

An on-station trial was conducted with a 3×2 factorial design with three levels of C:N ratio (10, 15 and 20) as first factor, and with and without substrates addition for periphyton development as second factor. The treatments without periphyton substrates are referred to as 'CN10', 'CN15' and 'CN20', while the treatments with periphyton substrates are referred to as 'CN10+P', 'CN15+P' and 'CN20+P'. Treatments were executed in triplicate and assigned randomly between ponds.

2.2 Experimental site and pond preparation

The experiment was carried out at the Fisheries Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh for a period of 120 days. A 81×8.9 m pond was drained completely and partitioned by galvanized iron sheets into 18 small ponds of 40 m^2 each. The ponds were rain-fed and fully exposed to prevailing sunlight. Before starting the experiment, ponds were manually cleaned of aquatic vegetation. All unwanted fishes were eradicated by rotenone application at the rate of 100 g pond^{-1}. Lime (CaCO$_3$) was applied to all

ponds at the rate of 250 kg ha^{-1} on Day 1. On Day 2, ponds were filled with water from the nearby deep tube-well. On Day 4, 15 bamboo *kanchi* (side shoots of bamboo) per m^2 water surface area, with a mean diameter of 2.8 cm were posted vertically into the bottom mud in substrate treatment ponds, excluding a 0.5 m wide perimeter. This resulted in an additional area of 40 m^2 for periphyton development equaling about 100% of the pond surface area. On the Day 5, all ponds were fertilized with semi decomposed cattle manure, urea and triple super phosphate (TSP) at the rates of 3000, 100 and 100 kg ha^{-1}, respectively. After fertilization, the ponds were left for 10 days to allow plankton development in the water column and periphyton growth on substrates, and subsequently stocked.

2.3 Prawn stocking and pond management

Juveniles of *M. rosenbergii* (5.023±0.02 g) purchased from a nearby commercial hatchery were stocked in the ponds at a density of 2 juveniles m^{-2}. A locally formulated and prepared pellet feed (2 mm) containing 30% protein with C/N ratio close to 10 was used. The proximate composition of the diet and tapioca starch is given in Table 1. The daily feeding rates were 5% body weight at the start of experiment, and declined gradually to 3% body weight at the end of the culture period with assuming 80% survival of total stock in each pond. Feed was distributed evenly over the pond's surface, twice daily at 07:00 and 18:00 h. Weights of 10% of total number of prawn were measured individually in every month to estimate the prawn biomass and adjust the feeding rate. The prawns were sampled using a cast net after removing some bamboo *kanchi*. After sampling, bamboo *kanchi* were put back to their original positions.

Table 1. Proximate composition of the prepared feed and tapioca starch.

Component	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	NFE[*]
Prepared feed	11.6	29.9	8.1	4.8	13.1	32.5
Tapioca starch	12.9	1.6	0.9	5.4	5.2	74.0

Locally purchased tapioca starch was used as carbohydrate source for manipulating the C/N ratio. In order to raise the C/N ratio to 15 and 20 in the respective ponds, additional 0.45 and 0.9 kg tapioca starch were applied for each kg of formulated feed, respectively. The pre-weighed tapioca starch was mixed in a beaker with pond water

and uniformly distributed over the ponds' surface directly after the feed application at 07:00 h.

2.4 Prawn harvesting and estimation of yield parameters

Prawns were harvested after draining the ponds. Individual length (wooden measuring board) and weight (Denver-xp-3000; precision=0.1 g) were recorded. Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and net yields 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

Net yield = total biomass at harvest − total biomass at stocking

2.5 Determination of water quality parameters

Water quality parameters, temperature (Celsius thermometer), dissolved oxygen (YSI digital DO meter, model 58), pH (CORNING 445 pH meter) and Secchi depth (Secchi disc) were monitored *in situ* at 06:00 and 18:00 h on weekly basis. Water samples were collected using a horizontal water sampler from three locations of each pond and pooled together. Total alkalinity (titrimetric method) and NO_2–N, NO_3–N, NH_3-N and PO_4–P concentrations (HACH kit model DR 2010) were measured on a fortnightly basis (APHA, 1992). Before nutrient analysis, water samples were filtered through microfibre glass filter paper (Whatman GF/C), using a vacuum pressure air pump. The filtered water was used for nutrient analysis. The filter paper was kept in a test tube containing 10 ml of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 h. Later, chlorophyll *a* was determined using a spectrophotometer (Milton Roy Spectronic, model 1001 plus) at 664- and 750-nm wave length, following Boyd (1979).

2.6 Determination of sediment quality parameters

Sediment samples were collected from three locations of each pond using PVC pipes (having 4 cm diameter and sampling depth 10 cm) were monitored on biweekly basis between 09:00 and 10:00 h. The samples were dried, ground and sieved with a 2mm sieve (Soil and Plant Analysis Council, 1999). Soil pH was determined by a direct reading digital pH meter (CORNING 445 pH meter) with soil water ratio 1:2.5

(McLean, 1982). Organic matter of sediment was determined by ignition method (Page et al., 1989). Total nitrogen of sediment was determined by the common Micro-Kjeldahl digestion method following Page et al., 1989. Total phosphorus of sediment samples were determined by acid digestion method (Jones and Case, 1990; Watson and Issac, 1990).

2.7 Determination of periphyton biomass

The periphyton biomass, in terms of dry mater (DM), ash free dry matter (AFDM) and pigment concentrate (chlorophyll *a*), growing on bamboo *kanchi* were determined monthly following standard methods (APHA, 1992), beginning from the 15th day of the substrate installation and continued at monthly intervals. From each pond, three poles were selected randomly and two 2×2 cm^2 samples of periphyton were taken at each of three depths (25, 50 and 75 cm below from the water surface) per pole. At the time of periphyton collection, care was taken not to remove any of the substrate itself. After sampling, the poles were replaced in their original positions, marked and excluded from subsequent samplings. One of the two samples was used to determine total DM and ash content. The materials from each pole were collected on pre-weighed and labeled pieces of aluminum foil, dried at 105 °C until constant weight (24 h in a Memmert stove, Model UM/BM 100–800), and kept in a desiccators until weighed (BDH 100A; precision 0.0001 g). Dry samples from depth and poles per pond were pooled, transferred to a muffle furnace and ashed at 450 °C for 6 h and weighed. The dry matter (DM) and ash free dry matter (AFDM) were determined by weight differences (APHA, 1992).

Another sample was used to determine chlorophyll *a* concentrations following standard methods (APHA, 1992). Collected materials were immediately transferred to labeled tubes containing 10 ml of 90% acetone, sealed and stored overnight in a refrigerator. The following morning, samples were homogenized for 30 s with a tissue grinder, refrigerated for 4 h, and then centrifuged for 10 min at 2000–3000 rpm. The supernatant was carefully transferred to 1 cm glass cuvette and absorption measured at 750 and 664 nm using a spectrophotometer (Milton Roy Spectronic, model 1001 plus). Chlorophyll *a* concentration was calculated using the equation given in APHA (1992).

2.8 Assessment of bacterial load in water, sediment and periphyton

Total bacterial load of pond water, sediment and periphyton were determined on monthly basis between 09:00 and 10:00 h. All samples were collected from 5 different locations, mixed homogenously and collected with sterile glass bottles for bringing to the Bacteriological Laboratory, Department of Microbiology and Hygiene, Faculty of Veterinary Science, BAU, Mymensingh, Bangladesh. One ml water sample was transferred with a sterile pipette to a test tube containing 9.0 ml of phosphate buffered saline (PBS) and the tube was shaken thoroughly whereas 5.0 g of sediment and periphyton samples were weighed and transferred to a sterile conical flask and made up to 50 ml with phosphate buffered saline (PBS) and the contents mixed thoroughly to prepare a stock solution. Serial dilution of up to 10^{-6} for water and 10^{-8} for sediment and periphyton were prepared with PBS. Volumes (0.1 ml) of each dilution were spread over the surface of duplicate plates of tryptone soya agar (TSA; Difco, Detroit, MI, USA) with incubation at 30 °C for 24–48 h. Plates with 30–300 colony forming units (CFU) were counted with a Leica Quebec Darkfield Colony Counter (Leica, Inc., Buffalo, NY, USA) and expressed as colony forming units.

2.9 Statistical analysis

Growth and yield parameters (prawn growth, yield, FCR, SGR, PER and survival) were analyzed by a two-way ANOVA with addition of substrate (P and noP) and C/N ratio (10, 15 and 20) as main factors. Sediment, water quality and THB counts data were compared by splitplot/repeated measures ANOVA with addition of substrate (P and noP) and C/N ratio (10, 15 and 20) as main factors and time as the sub-factor (Gomez and Gomez, 1984). The data were checked for normality, and transformed if necessary. Especially percentage and ratio data were arcsine transformed. All ANOVA were performed using SAS 6.21 program (SAS Institute, Cary, NC 27513, USA). If a main effect is significant, the ANOVA was followed by Tukey's test at *P*<0.05 level of significance.

Table 2. Effects of different C/N ratio and addition of periphyton substrates on different water quality parameters based on two-way ANOVA.

Variables	Means (Tukey test)					Significance (P value)		
	C/N ratio			Substrate				
	CN10	CN15	CN20	Yes	No	C/N	P	C/N×P
Temp. (°C) at 6 AM	27.32	27.28	27.28	27.32	27.27	NS	NS	NS
Temp. (°C) at 6 PM	30.7	30.63	30.59	30.6	30.68	NS	NS	NS
DO (mg L^{-1}) at 6 AM	4.64[c]	5.02[b]	5.31[a]	4.98	5.0	***	NS	NS
DO (mg L^{-1}) at 6 PM	6.11[c]	6.35[b]	6.65[a]	6.36	6.38	***	NS	NS
pH range at 6 AM	7.1-8.4	7.0-8.2	6.9-8.3	6.9-8.3	7.1-8.4	-	-	-
pH range at 6 PM	7.3-9.6	7.1-9.4	7.3-9.4	7.3-9.4	7.1-9.6	-	-	-
Transparency (cm)	34.15[a]	30.46[b]	27.58[b]	30.83	30.86	**	NS	NS
Total Alkalinity (mg L^{-1})	136.4	134.3	130.8	135.2	132.5	NS	NS	NS
Chlorophyll-a (µg L^{-1})	119.5[b]	159.1[ab]	205.2[a]	165.2	157.0	***	NS	NS
NO$_2$-N (mg L^{-1})	0.011[a]	0.008[b]	0.007[b]	0.007[b]	0.011[a]	**	***	NS
TAN (mg L^{-1})	0.238[a]	0.107[b]	0.078[b]	0.117	0.165	***	NS	NS
NO$_3$-N (mg L^{-1})	0.068[a]	0.042[b]	0.033[b]	0.047	0.048	***	NS	NS
PO$_4$-P (mg L^{-1})	0.67	0.67	0.77	0.75	0.65	NS	NS	NS

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 15=treatments with C/N ratio 15; 20=treatment with C/N ratio 20; Yes=treatments with the addition of periphyton substrates; No=treatments without periphyton substrates; P=Periphyton substrates; CN×P=Interaction of different C/N ratio and periphyton substrates. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *P<0.05; **P<0.01; ***P<0.001; NS, Not significant.

3 Results

3.1 Water quality parameters

Water quality parameters and outcomes of ANOVA are presented in Table 2. Temperature and pH of the water were similar among the treatments both in morning and evening. Increasing the C/N ratio from 10 to 20 increased the dissolved O_2 content of water from 4.6 to 5.3 mg l^{-1} in the morning and from 6.1 to 6.7mg l^{-1} in the evening. It also reduced the water transparency. The addition of substrates for periphyton development did not influence the dissolved O_2 content and transparency in the water column. C/N ratio control had no effect on total alkalinity and PO_4–P concentration of water column but it increased the chlorophyll *a* content. The ANOVA result showed that increasing C/N ratio reduced the nitrite–nitrogen, total ammonia-nitrogen and nitrate–nitrogen of pond water. On the other hand, the addition of periphyton substrates reduced the nitrite–nitrogen concentration of the water column with no effect on any other water quality parameters.

3.2 Sediment quality parameters

The sediment quality parameters are summarized in Table 3. The addition of carbohydrate for increasing C/N ratio increased the organic matter content in the sediment. Total nitrogen concentration in the sediment was also reduced by increasing C/N ratio. But C/N ratio control had no effect on pH and total phosphorus content of the sediment. The ANOVA result showed that the addition of periphyton substrates had only effect for reducing organic matter content of the sediment.

3.3 Effects on bacterial load of water, sediment and periphyton

The mean total heterotrophic bacterial load of water, sediment and periphyton was summarized in Table 4. The result of the ANOVA showed that the C/N ratio control influenced the THB count and promoted the growth of THB population in water column, sediment and periphyton whereas the addition of periphyton substrates had no effects on them. The THB count in the water column, sediment and periphyton increased during the culture period (Table 5).

Table 3. Effects of different C/N ratio and addition of periphyton substrates on different sediment quality parameters based on two-way ANOVA

Variables	Means (Tukey test)					Significance (*P* value)		
	C/N ratio			Substrate				
	CN10	CN15	CN20	Yes	No	C/N	P	C/N×P
pH range	6.7-8.0	6.5-8.0	6.2-8.1	6.2-8.1	6.5-8.0	-	-	-
Organic matter (%)	2.25[c]	2.55[b]	2.72[a]	2.44[b]	2.56[a]	***	*	NS
Total nitrogen (%)	0.173[a]	0.142[b]	0.120[c]	0.143	0.147	***	NS	NS
Total phosphorus (mg L^{-1})	17.33	19.75	19.91	19.89	18.1	NS	NS	NS

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 15=treatments with C/N ratio 15; 20=treatment with C/N ratio 20; Yes=treatments with the addition of periphyton substrates; No=treatments without periphyton substrates; P=Periphyton substrates; C/N×P=Interaction of different C/N ratio and periphyton substrates. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, Not significant

Table 4. Effects of different C/N ratio and addition of periphyton substrates on total heterotrophic bacterial (THB) load of water, sediment and periphyton based on two-way ANOVA

Variables	Means (Tukey test)					Significance (P value)		
	C/N ratio			Substrate				
	CN10	CN15	CN20	Yes	No	C/N	P	C/N×P
Water THB ($\times 10^5$ cfu ml^{-1})	3.41[c]	4.66[b]	5.80[a]	4.64	4.61	***	NS	NS
Sediment THB ($\times 10^7$ cfu g^{-1})	5.03[c]	5.90[b]	6.84[a]	5.90	5.94	***	NS	NS
Periphyton THB ($\times 10^7$ cfu g^{-1})	2.97[c]	3.46[b]	4.15[a]	-	-	***	-	NS

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 15=treatments with C/N ratio 15; 20=treatment with C/N ratio 20; Yes=treatments with the addition of periphyton substrates; No=treatments without periphyton substrates; P=Periphyton substrates; C/N×P=Interaction of different C/N ratio and periphyton substrates. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. $P<0.05$; **$P<0.01$; ***$P<0.001$; NS, Not significant.

Table 5. Total heterotrophic bacterial load of water, sediment and periphyton over sampling periods[Ψ].

Variables		Sampling periods					Significance [Φ]
		Period 1	Period 2	Period 3	Period 4	Period 5	P value
Water THB ($\times 10^5$ cfu ml^{-1})	Mean	2.93[e]	4.36[d]	4.92[c]	5.28[b]	6.64[a]	***
Sediment THB ($\times 10^7$ cfu g^{-1})	Mean	4.38[e]	5.38[d]	6.02[c]	6.62[b]	7.23[a]	***
Periphyton THB ($\times 10^7$ cfu g^{-1})	Mean	2.16[e]	3.20[d]	3.76[c]	4.13[b]	4.50[a]	***

Mean values in the same row with different superscript differ significantly ($P<0.05$).
[Ψ] One sampling period is 30 days.
[Φ] Results from split-plot two way ANOVA. *** $P<0.001$.

3.4 Periphyton biomass

Periphyton dry matter (DM), ash free dry matter (AFDM) and chlorophyll *a* concentration per unit substrate surface area are given in Table 6. The result of ANOVA showed that the C/N ratio control influenced all of these parameters. All of the parameters of periphyton biomass increased during the culture period (Figure 1). Mean values of all of these parameters were the highest in the CN20+P treatment, intermediate in CN15+P treatment and the lowest in CN10+P treatment (Figure 1).

Table 6. Means of periphyton biomass scraped from bamboo *kanchi* in different treatments

Variables		Treatments			Significance
		CN10+P	CN15+P	CN20+P	*P* value
DM (mg cm^{-2})	Mean±SE	2.92±0.06^b	3.42±0.17^a	3.63±0.19^a	**
AFDM (mg cm^{-2})	Mean±SE	1.88±0.08^b	2.30±0.15^a	2.49±0.16^a	**
Chlorophyll-*a* (µg cm^{-2})	Mean±SE	12.59±0.23^b	13.34±0.42^a	14.72±0.59^a	**

Values are the means of 5 sampling dates, three depths, three poles and three ponds (N=135). DM: Dry matter, AFDM: Ash free dry matter. Mean values in the same row with different superscript differ significantly ($P< 0.05$). ** $P< 0.01$.

3.5 Freshwater prawn growth and yield parameters

The yield parameters of freshwater prawn in different treatments are presented in Table 7. The ANOVA result showed that increasing C/N ratio increased the individual prawn weight at harvest but the addition of periphyton substrates had no effect on it. The SGR value was also increased with increasing C/N ratio. Both C/N ratio control and addition of periphyton substrates had effect on the protein efficiency ratio. The FCR was decreased by increasing of C/N ratio and the addition of periphyton substrates. The ANOVA result showed that C/N ratio had no effect on the survival of prawn but the addition of periphyton substrates increased the survival of prawn from 63 to 72%. Both the C/N ratio control and addition of periphyton substrates influenced the gross and net yield of prawn. The C/N ratio control (i.e. increasing C/N ratio from 10 to 20) increased net yield of prawn from 342 to 480 kg ha^{-1}120d^{-1} (40%) and addition of periphyton substrates increased net yield from 370 to 456 kg ha^{-1}120d^{-1} (23%). The interaction of C/N ratio control and addition of periphyton substrates was not significant for net yield of prawn. Therefore, the effect of C/N ratio control is additive to substrate addition for periphyton development, both increasing the net yield of prawn.

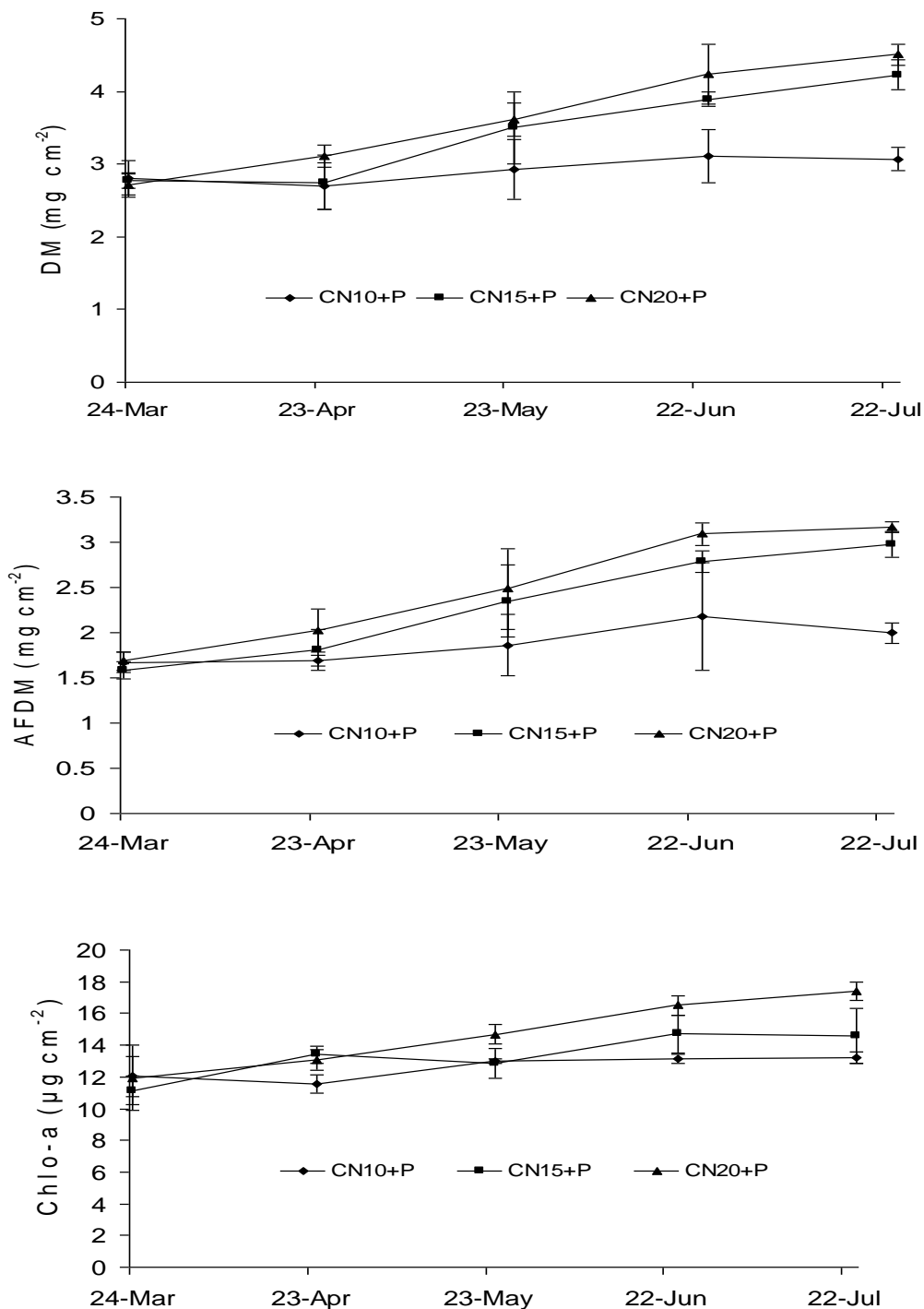**Figure 1.** Quantity of periphyton biomass per unit surface area during the experimental period. Values are means (±S.D.) of three replicates (each replicates contain three poles and three depth samples) per sampling date in each treatment. CN10+P=C/N ratio 10 + addition of periphyton substrates; CN15+P=C/N ratio 15 + addition of periphyton substrates; CN20+P=C/N ratio 20 + addition of periphyton.

Table 7. Effects of different C/N ratio and addition of periphyton substrates on growth and yield parameters of freshwater prawn based on two-way ANOVA.

Variables	Means (Tukey test)					Significance (P value)		
	C/N ratio			Substrate				
	CN10	CN15	CN20	Yes	No	C/N	P	C/N×P
Individual stocking weight (g)	5.1	5.2	5.1	5.2	5.1	NS	NS	NS
Individual harvesting weight (g)	33.4[c]	38.5[b]	42.0[a]	38.8	37.2	***	NS	NS
Individual weight gain (g)	28.3[c]	33.3[b]	36.9[a]	33.6	32.0	***	NS	NS
Specific growth rate (% bw d^{-1})	1.56[c]	1.67[b]	1.75[a]	1.67	1.65	***	NS	NS
Protein efficiency ratio	1.13[b]	1.33[a]	1.40[a]	1.39[a]	1.18[b]	***	***	NS
Food conversion ratio	2.97[a]	2.54[b]	2.40[b]	2.41[b]	2.85[a]	***	***	NS
Survival (%)	65.2	67.7	69.3	72.1[a]	62.8[b]	NS	***	NS
Gross yield (kg ha^{-1} 120 d^{-1})	445[c]	522[b]	583[a]	560[a]	473[b]	***	***	NS
Net yield (kg ha^{-1} 120 d^{-1})	342[c]	418[b]	480[a]	456[a]	370[b]	***	***	NS

C/N ratio=Carbon/Nitrogen ratio; 10=treatments with C/N ratio 10; 15=treatments with C/N ratio 15; 20=treatment with C/N ratio 20; Yes=treatments with the addition of periphyton substrates; No=treatments without periphyton substrates; P=Periphyton substrates; C/N×P=Interaction of different C/N ratio and periphyton substrates. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *P<0.05; ***P<0.001; NS, Not significant.

4. Discussion

In freshwater prawn culture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon, 1982; Diab and Shilo, 1988). Manipulation of C/N ratio by addition of carbohydrate significantly reduced inorganic N concentrations in the water column and total nitrogen in the sediment. The findings are in the agreement with Hari et al, (2004); Avnimelech and Mokady (1988); Avnimelech et al. (1989) and Avnimelech (1999) who reported that the addition of carbohydrate to the production systems will reduce the TAN concentration through immobilization by bacterial biomass. It is reported that fish in a pond assimilate only 15–30% of the nitrogen added in the feed (Acosta-Nassar et al., 1994; Gross et al., 2000; Davenport et al., 2003), the remainder being lost to the system as ammonia and organic N in feces and feed residue, which also undergoes decomposition and eventually produces ammonia. Therefore, higher dietary protein levels resulted in significantly higher TAN and NO_2–N concentrations in the water column. Li and Lovell (1992) reported that the ammonia concentration increased with increasing dietary protein concentration and protein feeding rate. In the present study, tapioca starch was used for increasing the C/N ratio of the feed resulting in a significant increase in the THB count, together with observed lower TAN concentrations in water. It 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). Thus, the reduction in nitrogenous compound (NO_3–N, NO_2–N and TAN) could be attributed to the addition of carbonaceous substrates that lead to an increased microbial biomass, which immobilized TAN for the synthesis of new bacterial cells (Hari et al., 2004) and uptake of the nitrogenous compounds by phytoplankton. In general, nitrogen is needed to produce the protein rich microbial cells. Inorganic nitrogen is immobilized into bacterial cells when metabolized organic substrates have a high C:N ratio.

The addition of substrates for periphyton development significantly reduced the NO_2–N and also lowering the TAN (0.165 mg l^{-1} without periphyton substrates and 0.117 mg l^{-1} with periphyton substrates). This is because in substrate-based ponds, nitrifying bacteria develop on the substrates which are located in the water column where more oxygen is available than at the water-sediment interface. Periphytic biofilm enhance

nitrification (Langis et al., 1988), keeping NO_2–N and TAN levels low. Therefore, accumulation of toxic inorganic nitrogen can be prevented by maintaining a high C/N ratio together with the addition of periphyton substrates and inducing uptake of ammonium by the microbial and periphyton algal community. The significantly higher bacterial load in the water column, sediment and periphyton in C/N20 ponds revealed that heterotrophic bacteria utilized the added carbon source resulting in higher productivity (Hari et al., 2004). This increased bacterial load led to higher decomposition rates releasing inorganic nutrients that in turn further stimulate bacterial development (Avnimelech et al., 1989). Under aerobic condition, microbial breakdown of organic matter leads to the production of new bacterial cells, amounting to the 40–60% of the metabolized organic matter (Avnimelech, 1999). Therefore, increased bacterial population function both as a bioreactor controlling water quality and as a protein food source for prawn.

The periphyton biomass in terms of DM and AFDM increased steadily during the culture period and the rate of increase was higher in higher C/N ratio treatments. This might be because of low grazing pressure on periphyton by the overall low biomass of prawns and an increased periphyton density in the C/N15 and C/N20 treatments. The reported stocking densities of freshwater prawn were as high as 120,000 ha^{-1} in substrate based systems (Tidwell and Bratvold, 2005) which was 6 times higher than the density maintain in the present study. The higher periphyton chlorophyll *a* in CN20+P treatment is mainly because of higher rate of nutrient cycling within the periphyton biomass itself (Wetzel, 1983). With the higher C:N ratio, the decomposition rate by bacteria in periphyton substrates in the well-oxygenated water column is increased, resulting in more nutrients which were subsequently reutilized by the bacteria and algae. Generally, bacteria compete with algae on available inorganic nutrients. But, periphyton is a complex mixture of autotrophic and heterotrophic organism and cannot simply be regarded as an attached equivalent of phytoplankton, although it certainly performs similar functions, such as oxygen production and the uptake of inorganic nutrients. There is an intense exchange of inorganic and organic solutes between autotrophic and heterotrophic components within the periphyton assemblage, and suspended solids can be trapped by the periphytic biofilm (Verdegem et al., 2005). Therefore, there is a tight coupling between autotrophs and heterotrophs in the periphyton mat. The periphytic algae

supply organic matter (trapped OM and dead periphyton) to the heterotrophs, the latter inorganic nutrients (after recycling) to the autotrophs. Again in ponds with substrates, organic matter and nutrients derived from feed and carbohydrates are partly trapped by periphyton (van Dam et al., 2002) and had a fertilization effect on autotrophic periphyton in higher C/N ratio treatments. Hence, a better growth and turn over of bacteria in the periphyton, also means more inorganic nutrients for the algae in higher C/N ratio treatments.

The highest net and gross yields of freshwater prawn were recorded in ponds maintained with higher C:N ratio and provided with periphyton substrates. The net yield of freshwater prawn increased by 40% due to increasing C/N ratio from 10 to 20. Addition of periphyton substrates further increased net yield by 23%. This increase in net yield was mainly due to the increased survival since periphyton substrates did not have an effect on individual weight at harvest. Addition of substrates might have minimized territoriality of freshwater prawn. It provides additional shelter and natural food in the form of periphyton colonized on bamboo *kanchi* substrates along with improvements of environmental conditions through a range of ecological and biological process (Tidwell et al., 2000; Tidwell et al., 2002; van Dam et al., 2002; Milstein et al., 2003). However, there was no interaction effect of C/N ratio control and addition of substrates on net yield indicating that the effect of C/N ratio control is additive to substrate addition. Concurrently, similar survival rates in C/N controlled treatments without periphyton substrates addition showed that water and sediment quality were favorable for the freshwater prawn culture (Hariati et al., 1996) and suggested that differences in production are related to food quality and food availability. The FCR was the lowest and PER and SGR was the highest in higher C/N ratio and periphyton substrates added treatments. Nevertheless, environmental parameters (increased abundance of plankton & periphyton biomass) indicate that the natural foods were underutilized by freshwater prawn in the present experiment. This suggests further investigation on the possibility of decreasing artificial feeding rate or increasing in stocking density of culture animals. Inclusion of a periphyton grazing fish species in this system could further increase the production and improve the system environment. Uddin et al. (2006) explored the potential of mixed culture of tilapia and freshwater prawn in periphyton-based system. Again, tilapia has bioturbation effect. So, it is being hoped that it will also improve nutrient cycling in extensive stagnant ponds of C/N-controlled periphyton-based system.

5. Conclusion

The new technology could be referred to as C/N-controlled periphyton-based (C/N-CP) system. This system of freshwater prawn farming reduced the potentially toxic TAN and NO_2–N concentrations in the water column. The increasing C/N ratio facilitated increased THB growth in water, sediment and periphyton. Such type of THB production is an important component of natural food in ponds stocked with freshwater prawn. The THB population converts inorganic nitrogen into protein rich microbial cell, thus lowering the inorganic nitrogen content in water and sediment. Concurrently, the quality and quantity of periphyton of the added substrates was increased with increasing C/N ratio. The above result of the present study could be useful in improving the sustainability of freshwater prawn framing. In summary, the C/N-CPP system of freshwater prawn farming system benefited the freshwater prawn farming by (1) reducing toxic inorganic nitrogen content of pond water, (2) increasing THB and algal abundance supplying additional single cell protein to augment the prawn production, and (3) improved periphyton productivity and quality leading to a substantial increase of average farm production of prawns.

3

Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture Ponds

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Chapter 3

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Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture Ponds

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Abstract

An on-station trial was conducted to investigate the effects of three C/N ratios (10/1, 15/1 and 20/1) along with substrate presence or absence on natural food communities in freshwater prawn culture ponds. The experiment was carried out in 40 m^2 ponds stocked with a stocking density of 2 prawn juveniles (5.023±0.02 g) m^{-2}. A locally formulated and prepared feed containing 30% crude protein with C/N ratio 10 was applied to all ponds. In order to raise the C/N ratio of the feed input to 15 and 20, tapioca starch was applied separately as a source of carbohydrate in addition to the artificial feed. Under substrate treatments, bamboo side shoots were posted vertically in pond bottoms resulting in 100% additional surface area as periphyton substrates. The treatments with different C/N ratios are referred to as 'CN10', 'CN15' and 'CN20'. Increasing the C/N ratio from 10 to 20 significantly increased the biovolume of phytoplankton, crustaceans and rotifers in the water column by 15%, 6% and 11%, respectively. The biovolume of periphytic plankton was 50% higher in treatment CN20 compared to treatment CN10. Increasing the C/N ratio from 10 to 20 raised the biovolume of total heterotrophic bacteria (THB) in the water column (70%), sediment (36%) and periphyton (40%). The chironomids biovolume was also significantly higher (28%) in treatment CN20 compared to treatment CN10. The addition of substrates decreased the biovolume of water column plankton by 14% but the combined biovolume (plankton + periphytic plankton) was almost double in substrate-added ponds. The biovolume of plankton, periphytic plankton and THB increased significantly with culture time duration whereas the biovolume of benthic macroinvertebrates decreased significantly with culture time indicating that freshwater prawn grazed on them. A significant interaction between C/N ratios and substrate presence or absence was only observed for plankton biovolume in the water column. This study demonstrated that plankton, periphyton and microbial biofloc communities were underutilized by the freshwater prawn in treatment CN20. This leaves room for increasing the stocking density of prawn and/or inclusion of periphyton grazing fish species to improve nutrient utilization efficiency and overall sustainability.

Keywords: C/N ratio, Substrates addition, Freshwater prawn, Natural food community, Plankton, Periphyton, Heterotrophic bacteria, Benthic macroinvertebrates

1 Introduction

The ecology of aquaculture ponds consists of a number of interrelated physical, chemical and biological processes. Among them, following three basic processes are important: production, consumption and decomposition. The primary productivity is based on the use of solar energy to convert carbon dioxide into plant biomass through photosynthesis. Phytoplankton, periphytic algae and submerged plants all contribute to this primary productivity on which the food web in ponds is partially based. In aquaculture ponds, the food web is enhanced by added organic matter in the form of manure and artificial feed. In the consumption process, both autochthonous and added organic matters are eaten directly or indirectly by aquatic animals and used as building blocks of biomass and a source of energy. The decomposition of *in situ* produced and added organic matter is mediated by mainly heterotrophic micro-organisms that break down and/or decompose organic matter producing detritus and inorganic nutrients. The released inorganic nutrients stimulate primary production, and broaden the base of autotrophic food webs.

Pond aquaculture of finfish and crustaceans contributes bulk (47.4% and 6.2%, respectively) of the world aquaculture production (FAO, 2006). The majority of ponds are operated extensively or semi-intensively, strongly depending on the natural food production in the pond, but driven by external nutrient inputs. Artificial diet in prawn/shrimp aquaculture accounts 50-70% of total operating cost, and therefore, optimizing the natural productivity would be the most efficient strategy to optimize the cost of production. Therefore, better integration between various resources available on the farm and optimization of natural productivity of food webs is essential to improve on-farm efficiency. During the last decades, several attempts (polyculture and /or pond fertilization) have been made to increase and utilize the pond communities, which serve as natural food items for cultured fish species in aquaculture ponds. To this end, developments such as (1) C/N ratio control (Avnimelech, 1999; Hari et al., 2004; Avnimelech, 2007; Asaduzzaman et al., 2008) and (2) providing substrates for periphyton development (van Dam et al., 2002; Tidwell et al., 2000, 2002; Azim et al., 2003a, 2003b; Keshavanath et al., 2001; Milstein et al., 2009) have been found promising to increase natural food communities in aquaculture ponds, the former mainly increasing heterotrophic bacteria, the later mainly increase autotrophic organisms.

The C:N ratio of most of the feeds used in semi-intensive aquaculture ponds is around 10:1, but bacteria require about 20 units of carbon per unit of nitrogen assimilated (Avnimelech, 1999). Therefore, with such a low C:N ratio in the feed, carbon is the limiting nutrient for heterotrophic bacteria populations in aquaculture ponds. So, the bacterial population will not expand beyond a certain point due to the limited availability of carbon. The C:N ratio in the pond can be increased by adding different locally available cheap carbon sources (for review see Hargreaves, 2006). If the C:N ratio is increased by adding a carbohydrate source such as tapioca starch in addition to the regular feed, the increased availability of carbon allows the heterotrophic bacterial population to grow to a dense mass. Therefore, 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 bacteria population utilizes the ammonium in addition to the organic nitrogenous wastes to synthesize new cells (single cell microbial protein) (Schneider et al., 2005), and it may be utilized as a natural food source by carps, tilapias (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993), shrimps (Burford et al., 2004) or freshwater prawn (Asaduzzaman et al., 2008).

The principle of periphyton-based aquaculture is to increase the natural food production by adding hard substrate materials into the water column. In a traditional fish pond, phytoplankton is the most important component for energy fixation and fuelling the food web. When substrates are installed in the pond, inorganic nutrients can also follow the extra 'periphyton loop' (Azim, 2001). This adds a third natural food source existing of periphytic microorganisms that can be consumed by the fish and also dead periphyton contributes to the detrital mass in the ponds (van Dam and Verdegem, 2005). However, unlike dead phytoplankton, dead periphyton remains attached to substrates, providing a rich source of organic nutrients for heterotrophic microorganisms. Processing of this organic matter yields inorganic nutrients that can be utilized by living algae again (Wetzel, 1983).

Recently, we investigated the combined effects of C/N ratio control and periphyton substrates (referred to as C/N-CP technology) on freshwater prawn production in extensive ponds (Asaduzzaman et al., 2008). Although the effects of C/N ratio control and substrate addition on the finfish and shellfish production are well documented,

their combined effects on natural communities, part of which serve as natural diet for aquacultured species, have never been investigated. This paper is further analysis of the above mentioned experiment investigating how C/N ratio control and presence and absence of added substrates influence the natural food communities in aquaculture ponds.

2 Materials and Methods

2.1 Experimental design

The experiment had a 3×2 factorial design with three levels of C:N ratio (10, 15 and 20) and two levels of substrate (with and without substrates). Treatments with different C/N ratio are referred to as 'CN10', 'CN15' and 'CN20'. Treatments were executed in triplicate and assigned randomly between ponds.

2.2 Experimental site and pond preparation

The experiment was carried out at the Fisheries Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh for a period of 120 days. A 81×8.9 m earthen pond was drained completely and partitioned by galvanized iron sheets into 18 small ponds of 40 m^2 each with an average water depth of 1 m. The ponds were rain-fed and fully exposed to prevailing sunlight and used before for research. Before starting the experiment, ponds were manually cleaned of aquatic vegetation. All unwanted fishes were eradicated by rotenone application at the rate of 100 g $pond^{-1}$. Lime ($CaCO_3$) was applied to all ponds at the rate of 250 kg ha^{-1} (Day 1). On Day 2, ponds were filled with water from the nearby deep tube-well. On Day 4, 15 bamboo *kanchi* (side shoots of bamboo) per m^2 water surface area, with a mean diameter of 2.8 cm were posted vertically into the bottom mud in substrate treatment ponds, excluding a 0.5 m wide perimeter. This resulted in an additional area of 40 m^2 for periphyton development equaling about 100% of the pond surface area. On Day 5, all ponds were fertilized with semi decomposed cattle manure (3000 kg ha^{-1}), urea (100 kg ha^{-1}) and triple super phosphate (100 kg ha^{-1}). After fertilization, the ponds were left for 10 days to allow plankton development in the water column and periphyton growth on substrates, and were subsequently stocked.

2.3 Prawn stocking and pond management

Juveniles of *M. rosenbergii* (5.023±0.02 g) purchased from a nearby commercial hatchery were stocked in the ponds at a density of 2 juveniles m^{-2}. A locally formulated and prepared pellet feed (2 mm) containing 30% protein with C/N ratio close to 10 was applied. The daily feeding rate was 5% body weight at the start of experiment, and declined gradually to 3% body weight at the end of the culture period with assuming 80% survival of total stock in each pond. Feed was distributed evenly over the ponds' surface, twice daily at 07:00 and 18.00 h. Weights of 10% of total number of prawn were measured individually in every month to adjust the feeding rate. The tapioca starch was used as carbohydrate source for manipulating the C/N ratio. In order to raise the C/N ratio from 10 (as control) to 15 and 20, 0.45 and 0.9 kg tapioca starch were applied for each kg of formulated feed in the CN15 and CN20 treatment ponds, respectively. The pre-weighed tapioca starch was mixed in a beaker with pond water and uniformly distributed over the ponds' surface directly after the feed application at 07:00 h.

2.4 Assessment of the plankton in water column

Plankton samples were collected monthly by pooling 10 liter of water from five different locations in each pond and passing them through a 45 μm mesh plankton net. The concentrated samples were preserved in small plastic bottles with 5% buffered formalin. Qualitative and quantitative estimations of plankton were done using a Sedgewick-Rafter (S-R) cell containing 1000 1-mm^3 cells. A 1 ml sample was put in the S-R cell and was left 15 min undisturbed to allow plankton to settle. The plankton in 10 randomly selected cells were indentified up to genus level and counted under a binocular microscope (Swift, M-4000). Planktons were identified using keys by Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976), and Bellinger (1992). Plankton abundance was calculated using the following formula:

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

where *N* is the number of plankton cells or units per liter of original water; *P*, the number of plankton counted in 10 fields; *C*, the volume of final concentrate of the sample (ml); *L*, the volume (l) of the pond water sample.

2.5 Assessment of periphytic plankton

From each pond, three bamboo *kanchi* were selected randomly and 2×2 cm² samples of periphyton were taken at each of three depths (25, 50 and 75 cm below from the water surface) per pole on a monthly basis starting after 7 days of substrate installation. Periphytic plankton samples from different depths and different bamboo *kanchi* were pooled and preserved in a labeled plastic vial containing 5% buffered formalin. After vigorous shaking, a 1 ml sub-sample was transferred in a S-R cell and the periphytic plankton number was estimated in 10 randomly selected cells under a binocular microscope (Swift, M-4000). Taxa were identified to genus level using the similar keys as plankton. Periphytic plankton density was calculated using the following formula:

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

where N is the number of periphytic plankton cells or units per cm² surface area; P, the number of periphytic plankton units counted in 10 fields; C, the volume of final concentrate of the sample (ml); S, the area of scraped surface (cm²).

2.6 Assessment of bacterial load in water, sediment and periphyton

Total bacterial load of pond water, sediment and periphyton were determined on monthly basis between 09:00 and 10:00 h. All samples were collected from 5 different locations, mixed homogenously and collected with sterile glass bottles for bringing to the Bacteriological Laboratory, Department of Microbiology and Hygiene, Faculty of Veterinary Science, BAU, Mymensingh, Bangladesh. One ml water sample was transferred with a sterile pipette to a test tube containing 9.0 ml of phosphate buffered saline (PBS) and the tube was shaken thoroughly whereas 5.0 g of each sediment and periphyton samples were weighed and transferred to a sterile conical flask and made up to 50 ml with phosphate buffered saline (PBS) and the contents mixed thoroughly to prepare a stock solution. Serial dilution of up to 10^{-6} for water and 10^{-8} for sediment and periphyton were prepared with PBS. Volumes (0.1 ml) of each dilution were spread over the surface of duplicate plates of tryptone soya agar (TSA; Difco, Detroit, MI, USA) with incubation at 30 °C for 24–48 h. Plates with 30–300 colony forming units (CFU) were counted with a Leica Quebec Darkfield Colony Counter (Leica, Inc., Buffalo, NY, USA) and expressed as CFU.

2.7 Assessment of benthic macroinvertebrates

The benthic macroinvertebrates samples were collected monthly with an Ekman grab (area: 225 cm^2). In each pond, bottom mud samples were collected from 3 different locations, which were then combined into a composite sample. Benthic macroinvertebrates were collected after filtering sediments through a 250 μm mesh sieve and preserved in a plastic vial containing 10% buffered formalin. Identification keys used for benthic macroinvertebrates were Brinkhurst (1971), and Pinder and Reiss (1983). Benthic macroinvertebrates density was calculated using the formula,

$$N = Y \times 10000/3A$$

with N = the number of benthic organisms (number m^{-2}); Y = total number of benthic organisms counted in 3 samples; A = area of Ekman dredge (cm^2).

2.8 Data calculation and analysis

The biovolumes of plankton, periphytic plankton and benthic macroinvertebrates were calculated according to Rahman et al. (2006). The biovolumes of heterotrophic bacteria were calculated using the value of Nakano and Kawabata (2000). The biovolumes of plankton, periphytic plankton, THB and benthic macroinvertebrates were analyzed by repeated measures ANOVA with addition of substrate and C/N ratio as main factors and time as the sub-factor (Gomez and Gomez, 1984). The data were checked for normality, and percentage and ratio data were arcsine transformed. All ANOVA were performed using 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 Effects on plankton biovolume

The plankton communities in pond water consisted of four groups of phytoplankton and two groups of zooplankton in all treatments. Forty four genera of phytoplankton belonging to Bacillariophyceae (13 genera), Chlorophyceae (21 genera), Cyanophyceae (7 genera) and Euglenophyceae (3 genera) were found (Table 1). Seventeen genera of zooplankton, including nine genera of Crustacea and eight genera of Rotifera were also identified.

Table 1.

List of plankton and periphyton genera recorded from the experimental ponds.

Group	Genus	Plankton	Periphytic plankton
Bacillariophyceae	*Actinella*	√	×
	Asterionella	√	×
	Coscinodiscus	√	√
	Cyclotella	√	√√
	Diatoma	√	√√
	Fragillaria	√√	√√
	Melosira	√√	√
	Navicula	√√	√√
	Nitzschia	√√	√
	Rhizosolenia	√	×
	Surirella	√	√
	Synedra	√√	√√
	Tabellaria	√√	√√
Chlorophyceae	*Actinastrum*	√	√
	Ankistrodesmus	√	√
	Botryococcus	√	√
	Chaetophora	√	√
	Chlorella	√√	√√
	Closterium	√	√
	Coelastrum	√√	√
	Draparnaldia	√	√
	Gonatozygon	√	√
	Microspora	×	√√
	Oedogonium	√	√
	Oocystis	√	√√
	Palmella	√√	√√
	Pediastrum	√√	√√
	Scenedesmus	√√	√√
	Sphaerocystis	√√	√√
	Spirogyra	√	×
	Stigeoclonium	√√	√
	Tetraedron	√	√
	Ulothrix	√√	√√
	Volvox	√	√
	Zygnema	√	√
Cyanophyceae	*Anabaena*	√√	√√
	Anacystis	√	√
	Aphanizomenon	√	√√
	Aphanocapsa	√	√√
	Gomphosphaeria	√√	√√
	Microcystis	√√	√√
	Oscillatiria	√	√
Euglenophyceae	*Euglena*	√√	√√
	Phacus	√√	√√
	Trachelomonas	√	×
Rotifera	*Asplanchna*	√√	√√
	Brachionus	√√	√√
	Filinia	√	√√
	Keratella	√	×
	Lecane	√	√
	Trichocerca	√√	√
	Polyarthra	√	×
	Notholca	√	×
Crustaceans	*Ceriodaphnia*	√	×
	Cyclops	√√	×
	Daphnia	√	×
	Diaphanosoma	√√	×
	Diaptomus	√	×
	Lepotodora	√	×
	Moina	√	×
	Nauplius larvae	√√	√√
	Sida	√	×

"√" indicates presence; "√√" indicates dominating genera "×" indicates absence

In all treatments the same genera of plankton were found. Among phytoplankton *Synedra*, *Tabellaria*, *Fragillaria*, *Melosira*, *Navicula*, and *Nitzschia* (Bacillariophyceae), *Chlorella*, *Coelastrum*, *Palmella*, *Pediastrum*, *Sphaerocystis*, *Stigeoclonium*, *Ulothrix* and *Scenedesmus* (Chlorophyceae), *Microcystis*, *Anabaena* and *Gomphosphaeria* (Cyanophyceae), *Euglena* and *Phacus* (Euglenophyceae), and among zooplankton *Cyclops*, *Diaphanosoma* and crustacean nauplii, and *Brachionus*, *Asplanchna* and *Trichocerca* (Rotifera) were the dominating genera.

The results of the ANOVA on the biovolume of major groups of plankton are shown in Table 2. C/N ratio control influenced the biovolume of all the major groups of plankton (except Chlorophyceae and Cyanophyceae). The mean total biomass of Bacillariophyceae, Euglenophyceae and total phytoplankton were higher in treatment CN20 than in treatment CN10. In the case of Crustacea, Rotifera, total zooplankton and total plankton, the mean total biomass were higher in treatments CN20 and CN15 compared to treatment CN10. Increasing C/N ratio from 10 to 20 increased the biovolume of phytoplankton by 15% and zooplankton by 8.5%. The addition of substrates also influenced the biovolume of all the major groups of plankton (except Chlorophyceae). It decreased the biovolume of phytoplankton by 11.2% and zooplankton by 14.4%. There was an interaction effect of C/N ratio control and periphyton substrates on biovolume of all of the major groups of plankton (except Chlorophyceae), total phytoplankton, total zooplankton and total plankton (Table 2; Figure 1). The ponds provided with periphyton substrates had similar biovolume in treatment CN10, much lower biovolume in CN15 and lower biovolume in CN20 than in ponds without periphyton substrates (interaction effects, Figure 1). However, plankton biomass was always higher in substrates free ponds compared to substrates added ponds (Figure 2) indicating that periphyton systems affect plankton production to some extent. However, although plankton biomass was always lower in substrate added ponds, combined biomass (plankton + periphyton) was significantly higher (95.7%) in these ponds compared to the substrate free ponds (Table 2). The mean biomass of different groups of plankton, total phytoplankton, total zooplankton and total plankton were tending to increase from the second month and continued until the end of the experiment (Table 3). There was an interaction effect of experimental period (months) and substrates addition on biovolume of all of the major groups of plankton (except Bacillariophyceae and Chlorophyceae), total phytoplankton, total

zooplankton and total plankton. However, there was no interaction effect of C/N ratio control and experimental periods on the biovolume of any groups of water column plankton (Table 3).
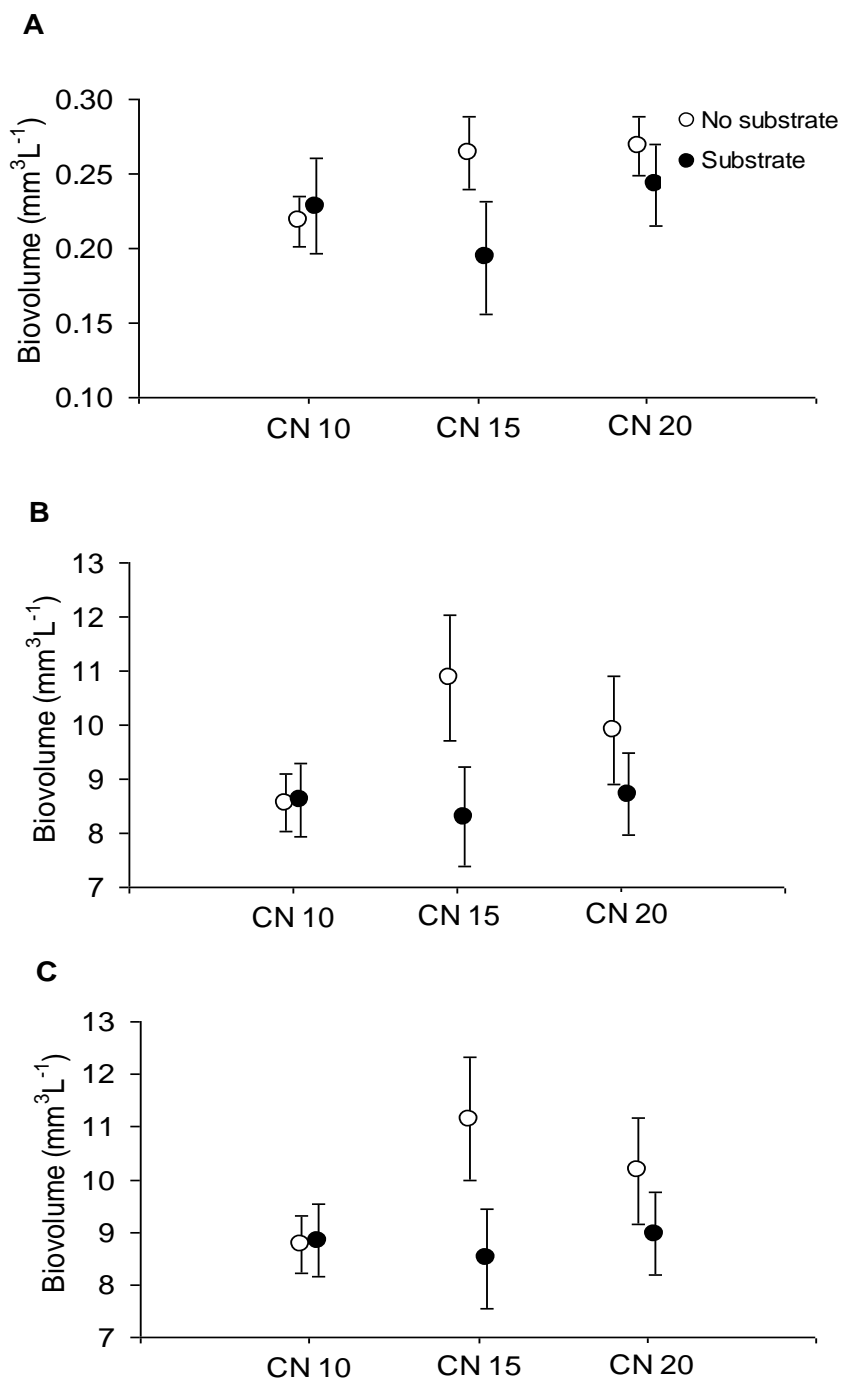
A

B

C

Figure 1. Interaction effects of C/N ratio control and periphyton substrates on the biovolume (mean ±95% CI) of total phytoplankton (A), total zooplankton (B), and total plankton (C). CN 10 = treatment with C/N ratio 10; CN 15 = treatment with C/N ratio 15; CN 20 = treatment with C/N ratio 20.

Table 2. Effects of different C/N ratio and addition of periphyton substrates on the abundance (based on total volume, mm^3 L^{-1}) of different groups of plankton in ponds based on two-way repeated measures ANOVA.

Variable	Means (Tukey test)					Significance (P value)		
	C/N ratio			Substrate				
	CN10	CN15	CN20	Yes	No	C/N	P	C/N×P
Bacillariophyceae	0.021[b]	0.023[ab]	0.024[a]	0.021[b]	0.024[a]	***	***	***
Chlorophyceae	0.044	0.043	0.048	0.044	0.047	NS	NS	NS
Cyanophyceae	0.149	0.152	0.171	0.148[b]	0.166[a]	NS	*	**
Euglenophyceae	0.009[b]	0.011[ab]	0.012[a]	0.009[b]	0.013[a]	**	***	**
Total phytoplankton	0.223[b]	0.229[ab]	0.256[a]	0.222[b]	0.250[a]	*	**	**
Crustacea	4.590[b]	5.262[a]	4.868[ab]	4.520[b]	5.294[a]	**	***	***
Rotifera	3.990[b]	4.332[ab]	4.442[a]	4.027[b]	4.483[a]	*	**	*
Total zooplankton	8.580[b]	9.594[a]	9.311[a]	8.546[b]	9.777[a]	**	***	***
Total plankton	8.804[b]	9.823[a]	9.566[a]	8.768[b]	10.027[a]	**	***	***

C/N ratio = Carbon/Nitrogen ratio; CN10 = treatment with C/N ratio 10; CN15 = treatment with C/N ratio 15; CN20 = treatment with C/N ratio 20; Yes = treatment with addition of periphyton substrates; No = treatment without addition of periphyton substrates; P = Periphyton substrates; C/N×P = interaction of different C/N ratio and periphyton substrates. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; ** $P<0.01$; *** $P<0.001$; NS, Not significant.

Table 3. Effects of experimental period and its interactions with addition of substrates and different C/N ratio on the abundance (based on total volume, $mm^3 L^{-1}$) of different groups of plankton in ponds based on two-way repeated measures ANOVA.

Variable	Means (Tukey test)					Significance (P value)		
	Month					Month	Month×Subs	Month×C/N
	March	April	May	June	July			
Bacillariophyceae	0.025[ab]	0.019[d]	0.020[cd]	0.023[cd]	0.026[a]	***	NS	NS
Chlorophyceae	0.056[a]	0.036[b]	0.042[ab]	0.047[ab]	0.045[ab]	**	NS	NS
Cyanophyceae	0.173[a]	0.143[ab]	0.0131[b]	0.164[ab]	0.176[a]	**	**	NS
Euglenophyceae	0.011[b]	0.010[b]	0.010[b]	0.010[b]	0.041[a]	***	**	NS
Total phytoplankton	0.265[a]	0.207[b]	0.204[b]	0.273[ab]	0.261[a]	***	**	NS
Crustacea	5.089[ab]	4.088[c]	4.747[bc]	4.796[bc]	5.814[a]	***	*	NS
Rotifera	4.570[b]	3.770[c]	3.557[c]	4.079[bc]	5.296[a]	***	*	NS
Total zooplankton	9.659[b]	7.859[c]	8.035[c]	8.875[bc]	11.110[a]	***	**	NS
Total plankton	9.925[b]	8.066[c]	8.508[c]	9.118[bc]	11.371[a]	***	**	NS
Plankton + periphyton (cm^3 $pond^{-1}$)	533.90[b]	456.12[c]	464.52[c]	505.96[bc]	632.50[a]	***	**	NS

The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; ** $P<0.01$; *** $P<0.001$; NS, Not significant.
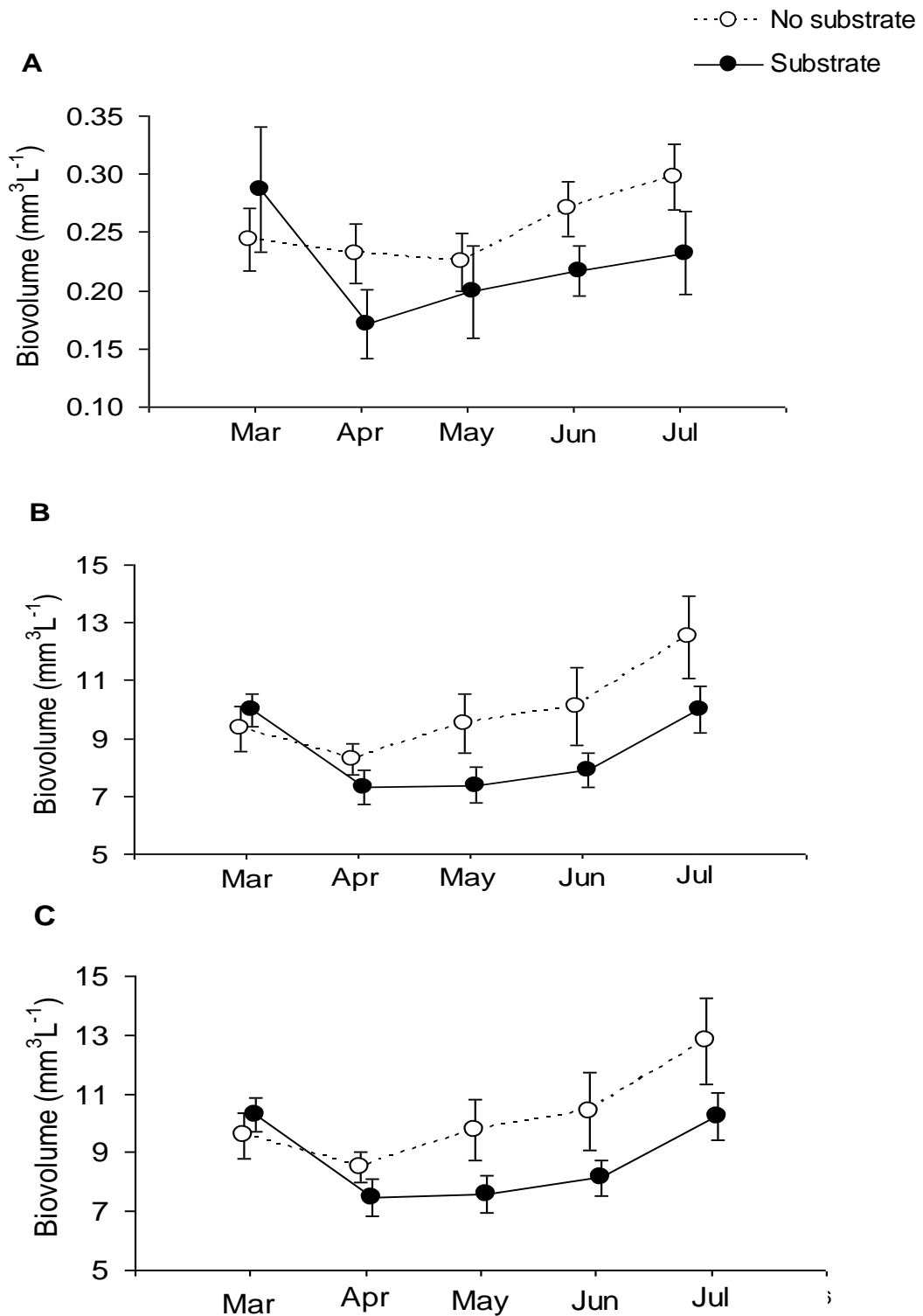
Figure 2. Interaction effects of experimental period and addition of substrates for periphyton development on the biovolume (mean ±95% CI) of total phytoplankton (A), total zooplankton (B), and total plankton (C) in C/N controlled freshwater prawn monoculture ponds.

3.2 Effects on periphytic plankton biovolume

The list of the identified genera under the different groups of periphytic plankton is summarized in Table 1. Most of the identified algal periphytic genera were common in water column phytoplankton (except 5 genera). About 40 genera of algae belonging to Bacillariophyceae (10 genera), Chlorophyceae (21), Cyanophyceae (7) and Euglenophyceae (2) and 6 genera of attached zooplankton belonging to Rotifer (5) and Crustacea (1) were identified as periphyton communities in substrate added ponds. Among autotrophic periphyton communities, *Synedra, Tabellaria, Navicula, Fragillaria, Cyclotella* and *Diatoma* (Bacillariophyceae), *Chlorella, Sphaerocystes, Palmella, Pediastrum, Microspora, Oocystis, Ulothrix* and *Scenedesmus* (Chlorophyceae), *Microcystis, Anabaena, Aphanizomenon, Aphanocapsa and Gomphosphaeria* (Cyanophyceae), *Euglena* and *Phacus* (Euglenophyceae), and among zoobenthic periphyton crustacean nauplii, and *Asplanchna, Brachionus* and *Filinia* (Rotifera) were the dominating genera.

The results of the ANOVA of major groups of periphytic plankton biovolume are shown in Table 4. C/N ratio control influenced the biovolume of all the major groups of periphytic plankton except Crustaceans. The mean total biomass of all the major groups of algal periphyton and zoobenthic periphyton (except Crustaceans) were significantly higher in treatment CN20 than in treatment CN10. Increasing C/N ratio from 10 to 20 increased the biovolume of algal periphyton by 64%, zoobenthic periphyton by 48% and total periphyton by 50%. The biovolume of all the major groups of periphytic plankton (except Euglenophyceae and Crustaceans) also varied with the culture period and the mean total biomass was higher at the end of the culture periods (Table 4). However, there was no interaction effect of C/N ratio control and experimental periods on the biovolume of all groups of periphytic plankton.

Table 4. Effects of C/N ratio control and experimental period on the abundance (based on total volume, mm^3cm^{-2}) of different groups of periphytic plankton in ponds based on two-way repeated measures ANOVA.

	Means (Tukey test)								Significance (P value)		
Variable	C/N ratio			Month							
	CN10	CN15	CN20	March	April	May	June	July	C/N	Month	C/N×Month
Bacillariophyceae	0.015^c	0.020^b	0.026^a	0.014^b	0.023^a	0.019^{ab}	0.023^a	0.023^a	***	***	NS
Chlorophyceae	0.027^b	0.037^a	0.043^a	0.023^c	0.034^{bc}	0.033^{bc}	0.040^{ab}	0.048^a	***	***	NS
Cyanophyceae	0.039^b	0.050^{ab}	0.062^a	0.031^b	0.060^a	0.058^a	0.057^a	0.044^{ab}	**	**	NS
Euglenophyceae	0.001^b	0.001^b	0.002^a	0.001	0.002	0.001	0.001	0.001	*	NS	NS
Total phytoplankton	0.081^c	0.108^b	0.133^a	0.069^b	0.119^a	0.110^a	0.120^a	0.116^a	***	***	NS
Rotifera	0.336^b	0.399^{ab}	0.498^a	0.411^{ab}	0.344^b	0.321^b	0.411^{ab}	0.568^a	*	*	NS
Crustacea	0.149	0.218	0.219	0.204	0.204	0.189	0.175	0.204	NS	NS	NS
Total zooplankton	0.485^b	0.618^{ab}	0.716^a	0.615^{ab}	0.548^b	0.511^b	0.586^{ab}	0.772^a	**	*	NS
Total plankton	0.566^b	0.726^a	0.849^a	0.685^{ab}	0.667^b	0.621^b	0.706^{ab}	0.888^a	***	*	NS

C/N ratio = Carbon/Nitrogen ratio; CN10 = treatment with C/N ratio 10; CN15 = treatment with C/N ratio 15; CN20 = treatment with C/N ratio 20; C/N × Month = interaction of different C/N ratio and months. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; ** $P<0.01$; *** $P<0.001$; NS, Not significant.

3.3 Effects on total heterotrophic bacterial biovolume (THB)

C/N ratio control influenced the THB biovolume of water column, sediment and periphyton whereas the addition of substrates had no effects on them (Table 5). Increasing C/N ratio from 10 to 20 increased the biovolume of water THB by 70%, sediment THB by 36% and periphyton THB by 40%. The biovolume of THB in the water column, sediment and periphyton increased during the culture period and the rate of increase was the highest in treatment CN20, intermediate in treatment CN15 and the lowest in treatment CN10 (Figure 3; Table 6). There was no interaction effect of experimental periods (month) and substrates addition on the biovolume of THB load in water column, sediment and periphyton. However, an interaction effect of experimental period (month) and C/N ratio control was observed on the biovolume of THB load in water column, sediment and periphyton (Table 6).

3.4 Effects on benthic macroinvertebrates biovolume

The results of the ANOVA of major groups of benthic macroinvertebrates biovolume are shown in Table 7. The benthic macroinvertebrates were divided into Chironomidae, Oligochaeta, Mollusca and un-identified groups. Chironomidae was the most dominant groups among benthos contributing 65 to 70% to the total biomass followed by Oligochaeta. C/N ratio control influenced the biovolume of Chironomidae only among all the major groups of benthic macroinvertebrates. Increasing C/N ratio from 10 to 20 increased the biovolume of total benthic macroinvertebrates by 21%. Addition of substrates had no effect on the biovolume of any groups of benthic macroinvertebrates. The biovolume of Chironomidae and total benthic macroinvertebrates was similar during the initial sampling and the first month of culture and then decreased continuously until the end of the culture period (Table 8). However, there was no interaction effect of C/N ratio control and experimental periods and substrates addition and experimental periods on the biovolume of all of the major groups of benthic macroinvertebrates (Table 8).

Table 5. Effects of different C/N ratio and addition of periphyton substrates on the abundance (based on total volume) of total heterotrophic bacterial load in water, sediment and periphyton based on two-way repeated measures ANOVA

| Variable | Means (Tukey test) | | | | | Significance (P value) | | |
| | C/N ratio | | | Periphyton substrate | | | | |
	CN10	CN15	CN20	Yes	No	C/N	P	C/N$\times$P
Water THB ($\times 10^3$ $\mu m^3 ml^{-1}$)	38.33^c	52.49^b	65.29^a	52.20	51.88	***	NS	NS
Sediment THB ($\times 10^5$ $\mu m^3 g^{-1}$)	56.58^c	66.39^b	76.99^a	66.43	66.88	***	NS	NS
Periphyton THB ($\times 10^5$ $\mu m^3 g^{-1}$)	33.45^a	38.97^b	46.74^c	-	-	***	-	-

C/N ratio = Carbon/Nitrogen ratio; CN10 = treatment with C/N ratio 10; CN15 = treatment with C/N ratio 15; CN20 = treatment with C/N ratio 20; C/N$\times$Month = interaction of different C/N ratio and months. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; ** $P<0.01$; *** $P<0.001$; NS, Not significant.

Table 6. Effects of experimental period and its interaction with addition of substrates and different C/N ratio on the abundance (based on total volume) to total heterotrophic bacterial load in water, sediment and periphyton based on two-way repeated measures ANOVA.

| Variable | Means (Tukey test) | | | | | Significance (P value) | | |
| | Month | | | | | | | |
	March	April	May	June	July	Month	Month$\times$Subs	Month$\times$C/N
Water THB ($\times 10^3$ $\mu m^3 ml^{-1}$)	32.97^e	49.05^d	55.38^c	59.36^b	63.44^a	***	NS	***
Sediment THB ($\times 10^5 \mu m^3 g^{-1}$)	49.29^e	60.63^d	67.67^c	74.46^b	81.33^a	***	NS	***
Periphyton THB ($\times 10^5 \mu m^3 g^{-1}$)	24.26^e	34.91^d	42.35^c	46.43^b	50.65^a	***	-	***

The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *** $P<0.001$; NS, Not significant.

Table 7. Effects of different C/N ratio and addition of periphyton substrates on the abundance (based on total volume, $cm^3 m^{-2}$) of different groups of benthic macroinvertebrates in ponds based on two-way repeated measures ANOVA

Variable	Means (Tukey test)					Significance (P value)		
	C/N ratio			Substrate				
	CN10	CN15	CN20	Yes	No	C/N	P	C/N×P
Chironomidae	7.837[b]	7.794[b]	10.058[a]	8.192	8.934	**	NS	NS
Oligochaeta	2.088	2.389	2.345	2.150	2.397	NS	NS	NS
Mollusca	1.037	1.137	1.062	1.144	1.014	NS	NS	NS
Un-identified groups	0.823	0.754	0.841	0.854	0.758	NS	NS	NS
Total benthos	11.787[b]	12.077[ab]	14.309[a]	12.343	13.105	*	NS	NS

C/N ratio = Carbon/Nitrogen ratio; CN10 = treatment with C/N ratio 10; CN15 = treatment with C/N ratio 15; CN20 = treatment with C/N ratio 20; C/N×Month = interaction of different C/N ratio and months. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; ** $P<0.01$; *** $P<0.001$; NS, Not significant.

Table 8. Effects of experimental period and its interactions with addition of substrates and different C/N ratio on the abundance (based on total volume, $cm^3 m^{-2}$) of different groups of benthic macroinvertebrates in ponds based on two-way repeated measures ANOVA

Variable	Means (Tukey test)					Significance (P value)		
	Month							
	March	April	May	June	July	Month	Month×Subs	Month×C/N
Chironomidae	14.321[a]	14.468[a]	7.816[a]	3.143[c]	3.069c	***	NS	NS
Oligochaeta	2.923	2.410	1.939	2.190	1.907	NS	NS	NS
Mollusca	1.445[a]	1.320[ab]	1.205[abc]	0.734[bc]	0.691[c]	**	NS	NS
Un-identified groups	0.817	0.838	0.911	0.796	0.670	NS	NS	NS
Total benthos	19.508[a]	19.037[a]	11.871[b]	6.791[c]	6.413[c]	***	NS	NS

The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *** $P<0.001$; NS, Not significant.
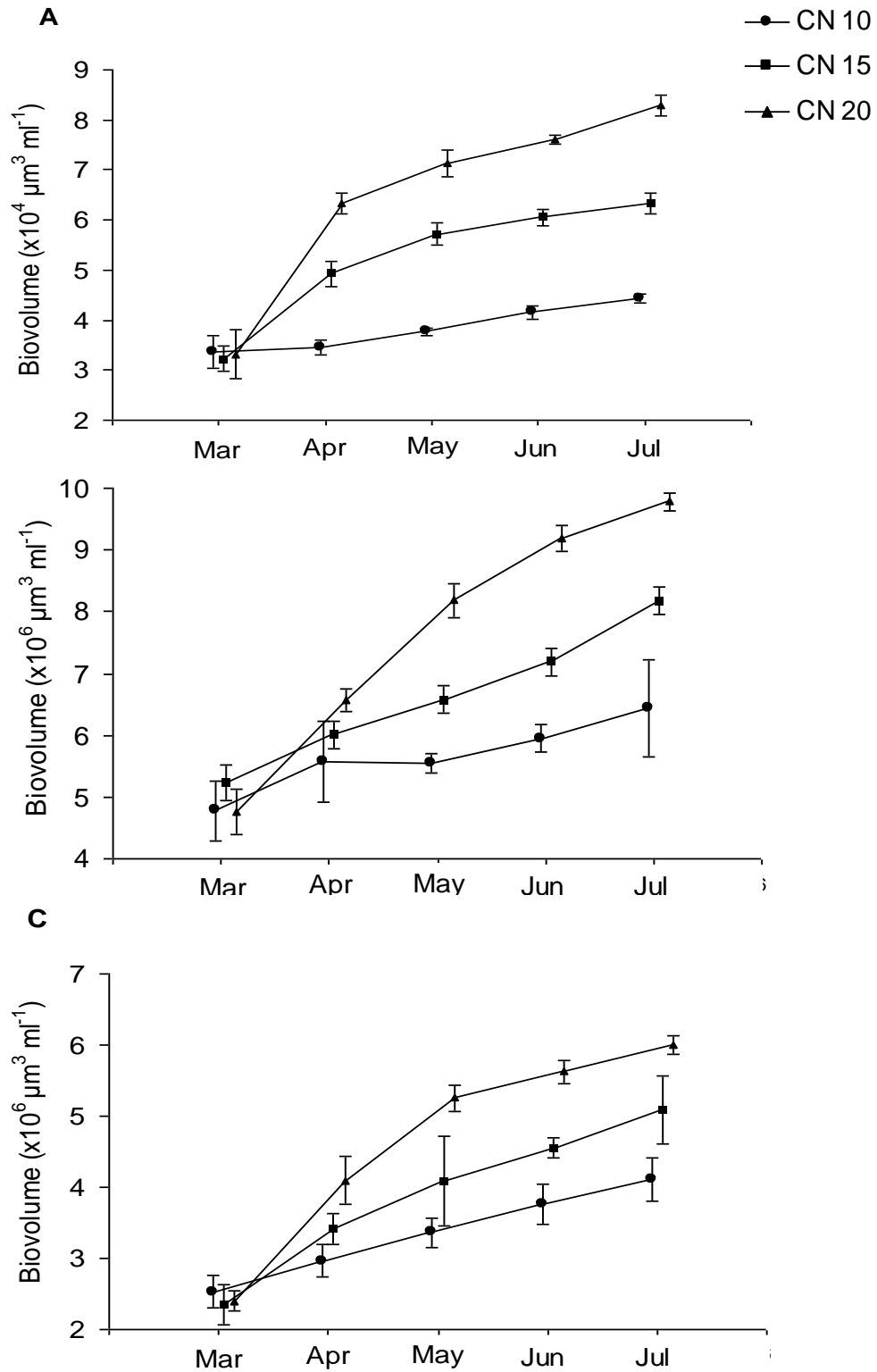
Figure 3. Interaction effects of different C/N ratio and experimental time on the biovolume (mean ±95% CI)) of water heterotrophic bacteria (A), sediment heterotrophic bacteria (B) and periphyton heterotrophic bacteria (C) in C/N controlled freshwater prawn monoculture pond.

4 Discussions

In aquaculture ponds, complex interrelated physical, chemical and biological processes contribute to the formation and the stability of the ecosystem. In C/N-CP ponds, the major pond communities are phytoplankton, periphyton (attached biota), zooplankton, microbial floc, and benthic macroinvertebrates. Among these pond communities, phytoplankton and algal periphyton are considered as autotrophic organisms, forming the base of the aquatic food web and the others are considered as heterotrophic organisms, contributing as consumer or decomposer to the pond ecosystem. The biomass of each of these communities in aquaculture ponds and lakes is influenced by management factors, such as species used in culture system, fish stocking density and ratio, and nutrient input quality and quantity (Milstein, 1993; Diana et al., 1997). Fish feeding habits also have an important influence on the quantity of these freshwater communities both directly by consumption and indirectly through influencing the food web and nutrients availability (Rahman et al., 2006).

In the present research, the observed increase in the biovolume of water column plankton (9%) and periphytic plankton (50%) in higher C:N ratio treatment might be due to the higher amount of added organic matter in such ponds. The higher C:N ratio treatment ponds (CN20) received additional 0.9 kg tapioca starch for each kg of applied feed to maintain a high C/N ratio compared to the CN10 treatment ponds. Azim and Little (2006) reported that the formation of autotrophic organisms in aquaculture ponds can be supplemented by the addition of organic matter. It has been reported that increased amounts of organic matter indirectly supplies inorganic nutrients through decomposition by bacteria (Moriarty, 1986; Milstein, 1992; Moriarty, 1997). In our previous study (Asaduzzaman et al., 2008), this resulted overall higher inorganic nutrient (except nitrogenous compounds) concentration in treatment CN20 compared to the other treatments. In turn, increased nutrients availability resulted in increased phytoplankton and periphyton production as indicated by a greater biovolume of them in treatment CN20. Another cause might be due to the stimulatory effects between autotrophic and heterotrophic organisms. The experiment was conducted in earthen aquaculture ponds where both autotrophic and heterotrophic organisms interact. Algae and bacteria have a range of stimulatory or inhibitory effects on each other (Cole, 1982). Along with the added carbohydrate, senescent algae or algal detritus are a major source of organic substrate for

heterotrophic bacterial growth whereas living algae provide oxygen for decomposition. In return, bacteria regenerate inorganic nutrients and vitamins that stimulate algal productivity (Cole, 1982). At the same time, higher amount of phytoplankton, periphytic algae and THB might have quickly utilized the nutrients components mainly ammonia and nitrate from the water column resulting in a significant reduction of it in treatment CN20 (Asaduzzaman et al., 2008). The Pearson correlation analysis showed that there was a significant relationship among the nitrogenous compounds concentration, plankton and heterotrophic bacteria biovolume (Figure 4). It showed that observed higher biomass of bacteria and phytoplankton reduced the concentration of TAN and NO_3-N. In general, phytoplanktons take up inorganic N and bacteria release inorganic N (through decomposition). In C/N-controlled system, increased heterotrophic bacteria utilize N to synthesize bacterial protein and new cells thereby, reduced toxic nitrogenous compounds from the aquaculture ponds (Hari et al., 2004).
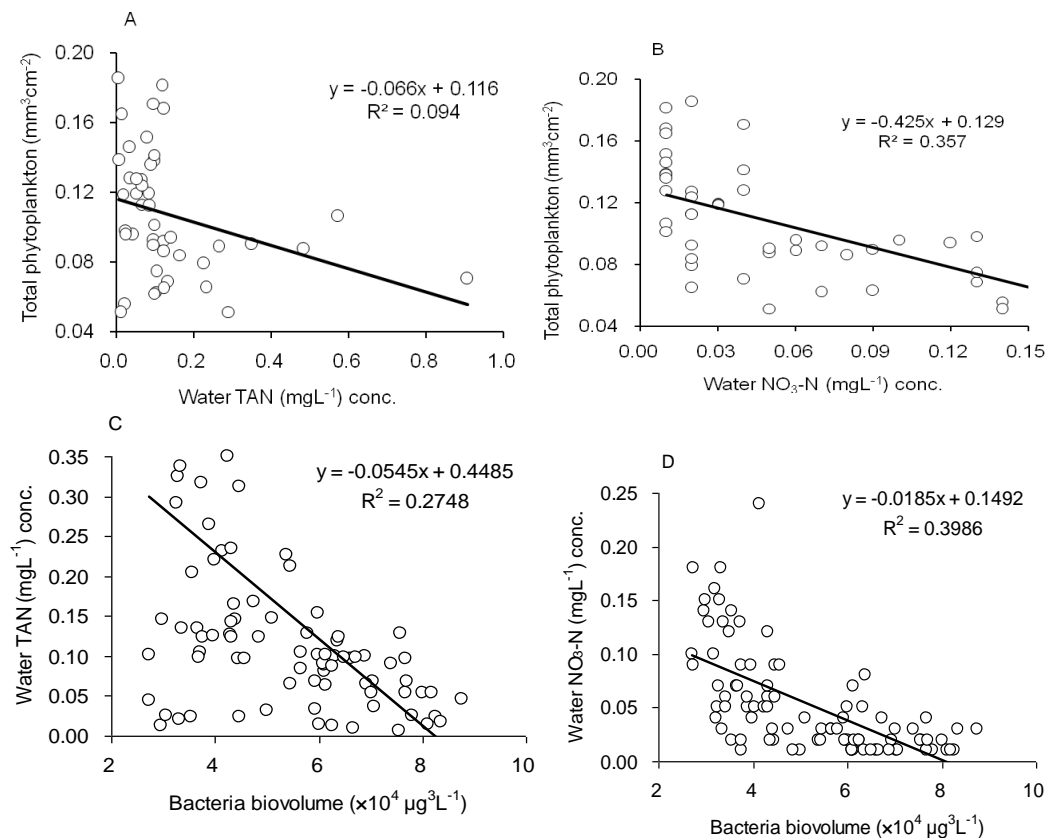

Figure 4. Relationship among water TAN and biovolume of total phytoplankton (A), water NO_3-N and biovolume of total phytoplankton (B), water TAN and biovolume of total heterotrophic bacteria (C), water NO_3-N and biovolume of total heterotrophic bacteria (D).

Periphyton is a complex mixture of autotrophic and heterotrophic organisms (Azim et al., 2005) and hence, there is an intense exchange of inorganic and organic solutes between autotrophic and heterotrophic components within the periphyton assemblage (Verdegem et al., 2005). Again in ponds with substrates, organic matter and nutrients derived from feed and carbohydrates are partly trapped by periphyton (van Dam et al., 2002) which had a fertilization effect on autotrophic periphyton in higher C/N ratio treatments. Hence, a better growth and turnover of bacteria in the periphyton, also means more inorganic nutrients for the algae in higher C/N ratio treatments. The biovolume of all of the major groups of autotrophic periphyton increased steadily during the experimental period (Table 3), indicating the low grazing pressure on periphyton by the overall low biomass of prawns. The reported stocking densities of freshwater prawn were as high as 120,000 ha^{-1} in substrate based systems (Tidwell and Bratvold, 2005) which was much higher than the density maintained in the present study. Freshwater prawn selectively feeds (animal portion and detrital aggregates rather than picking up mixed biomass) on periphyton (Uddin et al., 2006) thereby, allowing them to grow continuously in low stocking density monoculture ponds.

The observed higher biovolume of autotrophic organisms due to increased C:N ratio also influenced zooplankton and zoobenthic periphyton, resulting in higher biomass in treatment CN20 compared to treatment CN10. Substrates addition decreased the phytoplankton biovolume in the water column, which might be due to the competition between periphytic algae and water column algae for light and bioavailable nutrients in overlying water. Secondly, periphyton substrates might have shading effects which reduce sunlight availability for phytoplankton. Thirdly, some algal species might prefer to be colonized on hard substrates and therefore move from planktonic state to the periphytic state if substrate were available. The observed higher biovolume (96%) of combined production of water column plankton and periphytic plankton in substrate based ponds indicated that periphyton substrates compensated the adverse effects on water column plankton. In addition, substrates based system provided additional natural food source (periphyton) compared to substrates free ponds, providing an extra source of natural food item for the cultured species. The observed higher mean biomass of water column phytoplankton, zooplankton and total plankton

in initial month was mainly due to fertilization effects during the pond preparation. Following a decrease in 1st months, the biovolume of all of them increased steadily during the culture period (Figure 3). This might be due to the relatively low grazing pressure of water column plankton by prawn allowing them to grow continuously.

The observed higher biovolume of bacteria in the water column, sediment and periphyton in treatment CN20 revealed that heterotrophic bacteria utilized the added carbon source resulting in higher productivity (Hari et al., 2004). The reported increase of THB count in the water column, sediment and periphyton during the culture period (Figure 3) was mainly because of increased amount of feed and carbohydrate application due to the increased biomass of prawn over the time. The higher autotrophic biomass and lower concentrations of toxic nitrogenous compounds also influenced benthic macroinvertebrate, resulting in higher biomass in treatments CN20 compared to treatment CN10. Despite the fact that the bottom dissolved oxygen concentration were within the suitable range (4.64-6.95 mg l^{-1}) and the ponds became rich in nutrients over the time, the observed decrease in biovolume of total benthos during the culture period could have been caused by grazing by freshwater prawn. Freshwater prawn prefers to forage on animals like oligochates, chironomids, nematodes, gastropods and zooplankton in the natural habitat (Coyle et al., 1996; Tidwell et al., 1997).

A conceptual model of nitrogenous compounds, freshwater prawn and food organisms interaction, as influenced by the increasing C/N ratio from 10 to 20 and addition of substrates for periphyton development using the data from Asaduzzaman et al., (2008) and Tables 2-8 is given in Figure 5. The uneaten feed and feces contributed to the organic mater load of the system. The microbial decomposition of organic matter in the system led to increased levels of TAN and nitrite, both harmful to freshwater prawns even at low concentrations (Jiménez-Montealegre et al., 2002; Torres-Beristain et al., 2006). The process of nitrogenous compounds utilization and transformations take place in water, sediment and periphyton mat as indicated by block arrows (Figure 5). In C/N controlled periphyton based ponds (CN20+P) the added carbon source, together with the waste nitrogen was converted into microbial floc, which in turn can be eaten by the cultured freshwater prawn (Crab et al., 2007). Nitrifying bacteria process the ammonia into nitrite, which is also toxic, and then

nitrite into nitrate, which is much less harmful. Both TAN and nitrate were assimilated by the phytoplankton, periphyton and microbial floc present in the ponds. Increasing C/N ratio increased the biovolume of plankton, periphyton, heterotrophic bacteria and benthos, and finally increased the freshwater prawn production as indicated by upwards block arrows (Figure 5). Among these natural food items, freshwater prawn effectively graze on benthos indicated by solid arrow resulting in decrease of abundance over the time as indicated by downwards black block arrow, whereas, other natural food items were under-utilized by freshwater prawn as indicated by the dotted arrows.
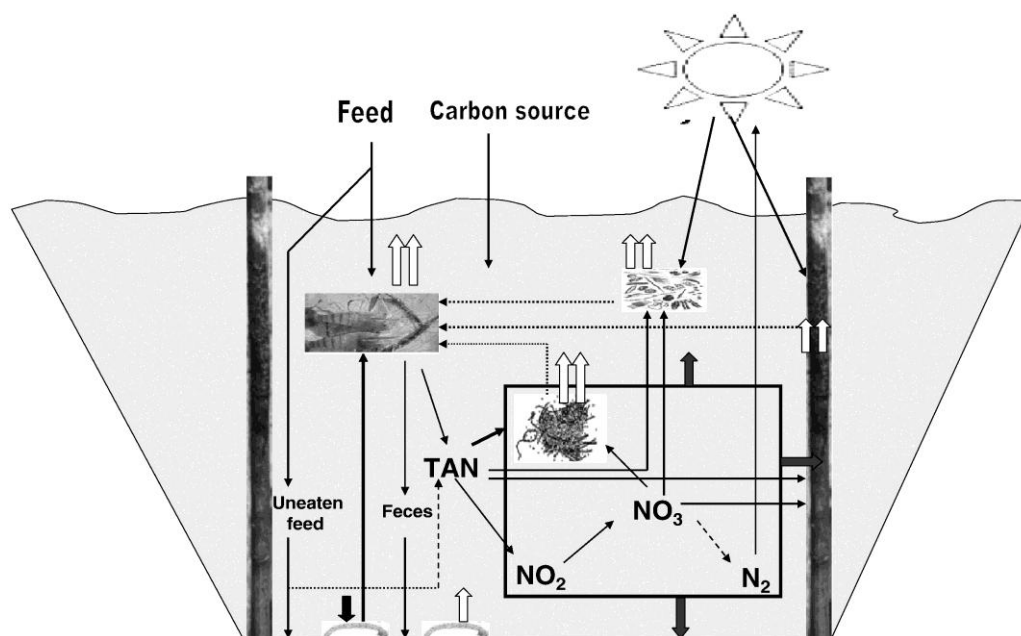
Figure 5. A conceptual model of nitrogenous compounds, freshwater communities and cultured prawn interaction, as influenced by the increasing C/N ratio from 10 to 20 and addition of substrates for periphyton development.

In conclusion, increasing C/N ratio increased the biovolume of plankton, periphyton, heterotrophic bacteria and benthic macroinvertebrate. However, the availability of pond communities in the present research seemed to be underutilized by the freshwater prawn. This suggests further investigation on the possibility of decreasing artificial feeding rate or increasing in stocking density of prawn. In this system, the biomass of plankton and periphyton seemed to be totally unutilized by the freshwater prawn. Therefore, inclusion of both plankton and periphyton grazing fish species like tilapia (Dempster et al., 1993; Huchette et al., 2000; Azim et al., 2003a, Uddin 2007)

can further increase the production, system environment and overall sustainability in C/N-CP ponds and is subject of further research.

Acknowledgement

4

Effects of stocking density of freshwater prawn *Macrobrachium rosenbergii* and addition of different levels of tilapia *Oreochromis niloticus* on production in C/N-controlled periphyton-based system

Chapter 4

Effects of stocking density of freshwater prawn *Macrobrachium rosenbergii* and addition of different levels of tilapia *Oreochromis niloticus* on production in C/N-controlled periphyton-based system

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Abstract

An on-station trial was conducted to evaluate the effect of stocking density of freshwater prawn and addition of different levels of tilapia on production in carbon/nitrogen (C/N) controlled periphyton based system. The experiment had a 2×3 factorial design, in which two levels of prawn stocking density (2 and 3 juveniles m^{-2}) were investigated in 40 m^2 earthen ponds with three levels of tilapia density (0, 0.5 and 1 juveniles m^{-2}). A locally formulated and prepared feed containing 30% crude protein with C/N ratio close to 10 was applied considering the body weight of prawn only. Additionally, tapioca starch was applied to the water column in all ponds to increase C/N ratio from 10 (as in feed) to 20. Increasing stocking density of tilapia decreased the chlorophyll *a* concentration in water and total nitrogen in sediment, and increased the bottom dissolved oxygen. The concentrations of inorganic nitrogenous species (NH$_3$–N, NO$_2$–N and NO$_3$–N) were low due to maintaining a high C/N ratio (20) in all treatment ponds. Increasing prawn density decreased periphyton biomass (dry matter, ash free dry matter, chlorophyll *a*) by 3–6% whereas tilapia produced a much stronger effect. Increasing stocking density of freshwater prawn increased the total heterotrophic bacterial (THB) load of water and sediment whereas tilapia addition decreased the THB load of periphyton. Both increasing densities of prawn and tilapia increased the value of FCR. Increasing prawn density increased gross and net prawn production (independent of tilapia density). Adding 0.5 tilapia m^{-2} on average reduced prawn production by 12–13%, and tilapia addition at 1 individual m^{-2} produced a further 5% reduction (independent of prawn density). The net yield of tilapia was similar between 0.5 and 1 tilapia m^{-2} treatments and increased by 8.5% with increasing stocking density of prawn. The combined net yield increased significantly with increasing stocking density of prawn and tilapia addition. The significantly highest benefit cost ratio (BCR) was observed in 0.5 tilapia m^{-2} treatment but freshwater prawn density had no effect on it. Therefore, both stocking densities (2 and 3 juveniles m^{-2}) of prawn with the addition of 0.5 tilapia m^{-2} resulted in higher fish production, good environmental condition and economic return and hence, polyculture of prawn and tilapia in C/N-controlled periphyton-based system is a promising option for ecological and sustainable aquaculture.

Keywords: Tilapia, Freshwater prawn, Polyculture, Stocking density, C/N ratio, Periphyton, Heterotrophic bacteria

1 Introduction

The use of periphyton substrates and manipulation of C:N ratio in freshwater finfish and prawn production in extensive ponds have been found promising (see reviews of van Dam et al., 2002; Hargreaves, 2006; Azim and Little, 2006). Asaduzzaman et al. (2008) showed that a feed input along with an additional carbohydrate application to maintain a C/N ratio of 20 in combination with substrate addition for periphyton development improved the net yield of freshwater prawn by 75%. Compared to control ponds (C/N ratio 10 and no substrates), these higher yield concurred with reduced levels of toxic inorganic nitrogenous compounds, increased periphyton productivity and higher concentrations of total heterotrophic bacteria in the water column and sediment. In these monoculture ponds, at a stocking density of 2 prawns per m^2, the algal and periphyton biomasses seemed to be underutilized. Therefore, it was hypothesized that higher net yields and benefits can be obtained by increasing the prawn stocking density as well as by addition of tilapia as a predominant periphyton grazer. The key characteristic of this system is the reliance on the combination of natural and artificial feed. Recently, there has been a growing interest in polyculture of freshwater prawn with tilapia (Uddin, 2007; New, 2005; dos Santos and Valenti, 2002). Uddin (2007) showed that in mixed culture the feeding niches of tilapia and prawn only partially overlap, and recommended this duo-culture as an alternative to polyculture of Chinese and Indian carps.

In fed ponds, roughly 3 times the amount of organic matter that is retained in fish production settles to the pond bottom, creating an anoxic zone where nutrients remain trapped (Avnimelech and Zohar, 1986). By tilapia driven re-suspension the bottom nutrients are exposed to aerobic conditions in the water column and better mineralized, stimulating the natural food web (Jiménez-Montealegre et al., 2002). Ritvo et al. (2004) demonstrated that fish driven re-suspension leads to an appreciable mixing and oxidation of the sediment. It can be hypothesized that in ponds with substrates for periphyton development, part of the re-suspended matter will be trapped by the periphytic communities, and hence stay more time in the oxygen rich water column than in substrate free ponds.

In brief, providing substrates for periphyton development, increasing the C/N ratio and stimulating fish driven re-suspension of nutrient rich sediments improve pond

production. These approaches are simple and cheap, making them also socially and economically sustainable, even for small-scale or poor farmers. The novelty of this research is to combine the three approaches, with the goal of rising pond productivity and hence the nutrient use efficiency and farming sustainability. The present research looked at the effects of different prawn and tilapia densities on the water, periphyton and sediment quality and (for periphyton only) the quantity. Attention was also given to the heterotrophic bacterial counts in the water column, sediment and periphytic biofilms.

2 Materials and methods

2.1 Experimental design

An on-station trial was conducted with a $2{\times}3$ factorial design with two levels of stocking density of freshwater prawn (2 and 3 individual m^{-2}) as first factor and three tilapia densities (0, 0.5 and 1 individual m^{-2}) as second factor. The treatments with lower stocking density of prawn (2 juveniles m^{-2}) are referred to as 'P2T0', 'P2T0.5' and 'P2T1', while the treatments with higher stocking density (3 juveniles m^{-2}) are referred to as 'P3T0', 'P3T0.5' and 'P3T1'. P2 and P3 refer to the different stocking densities per m^2 of prawn and T0, T0.5 and T1 refer to the different stocking densities per m^2 of tilapia. Treatments were executed in triplicate and assigned randomly among ponds.

2.2 Experimental site and pond preparation

The experiment was carried out at the Fisheries Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh for a period of 120 days during 20th August to 20th December, 2007. A $81{\times}8.9$ m earthen pond with an average depth of 1 m was drained completely and partitioned by galvanized iron sheets into 18 small ponds of 40 m^2 each. The ponds were rain-fed and fully exposed to prevailing sunlight and were previously used for research. Ponds were manually cleaned of aquatic vegetation before starting the experiment. All unwanted fishes were eradicated by rotenone application at the rate of 50 g $pond^{-1}$. Lime ($CaCO_3$) was applied to all ponds at the rate of 250 kg ha^{-1} on Day 1. On Day 4, ponds were filled with water from a deep tube-well. On Day 6, 15 side shoots of bamboo (locally known as *kanchi*) per m^2 water surface area, with a mean diameter of

2.8 cm were posted vertically into the bottom mud in all ponds, excluding a 0.5 m wide perimeter. This resulted in an additional substrates surface area of 40 m^2 for periphyton development equaling 100% of the pond surface area. On Day 8, all ponds were fertilized with semi-decomposed cattle manure, urea and triple super phosphate (TSP) at the rates of 3,000, 100 and 100 kg ha^{-1}, respectively. The ponds were left for 10 days post-fertilization to allow plankton development in the water column and periphyton growth on substrates, and subsequently stocked.

2.3 Stocking and pond management

Juveniles of *Macrobrachium rosenbergii* (2 ± 0.02 g) and *Oreochromis niloticus* (24 ± 0.24 g) procured from a nearby commercial hatchery were stocked in the ponds according to the experimental design. A locally formulated and prepared pellet feed (2 mm) containing 30% protein with C/N ratio close to 10 was used. The feed was applied considering the body weight of prawn only at a daily feeding rate of 5% body weight at the start of experiment, and gradually declining to 3% body weight at the end of the culture period. Feed was distributed evenly over the pond's surface, twice daily at 07:00 and 18:00 h. Individual weights of minimum 10% of initially stocked prawn and tilapia were sampled monthly to estimate the biomass and adjust the feeding rate. The prawn and tilapia were sampled using a cast net after removing some bamboo *kanchi*, which were re-positioned after sampling.

Locally purchased tapioca starch was used as carbohydrate source for manipulating the C/N ratio. In order to raise the C/N ratio to 20 in all the ponds, 0.9 kg tapioca starch was applied for each kg of formulated feed. The pre-weighed tapioca starch was mixed in a beaker with pond water and uniformly distributed over the ponds' surface directly after the feed application at 07:00 h. The actual proximate composition of the diet and tapioca starch is given in Table 1.

Table 1

Proximate composition of the prepared feed and tapioca starch

Component	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	NFE[*]
Prepared feed	11.6	29.9	8.1	4.8	13.1	32.5
Tapioca starch	12.9	1.6	0.9	5.4	5.2	74.0

The percentages are given on a wet weight basis.

* NFE=nitrogen free extracts

2.4 Prawn/tilapia harvesting and estimation of yield parameters

Prawns and tilapia were harvested after draining the ponds. Individual length (wooden measuring board; precision 0.1 cm) and weight (Denver-xp-3000; precision=0.1 g) were recorded. Specific growth rate (SGR), feed conversion ratio (FCR), and net yields were calculated as follows:

SGR = [(ln final weight − ln initial weight) × 100]/days of experiment

FCR (prawn only) = feed applied (dry weight) / live weight gain

Net yield = total biomass at harvest − total biomass at stocking

2.5 Determination of water quality parameters

Water samples were collected using a horizontal water sampler from three locations of each pond and pooled before analysis. Water quality parameters, temperature (Celsius thermometer), surface and bottom dissolved oxygen (YSI digital DO meter, model 58), pH (CORNING445 pH meter) and transparency (Secchi disc) were monitored *in situ* at 09:00 h on a weekly basis. Before nutrient analysis, water samples were filtered through microfibre glass filter paper (Whatman GF/C), using a vacuum pressure air pump. Total alkalinity (titrimetric method) and NH_3–N, NO_2–N, NO_3–N and PO_4–P concentrations (HACH kit model DR 2010) in the filtrate were measured on a monthly basis (APHA, 1992). The filter paper was kept in a test tube containing 10 ml of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 h. Later, chlorophyll *a* was determined using a spectrophotometer (Milton Roy Spectronic, model 1001 plus) at 750- and 664-nm wave length, following Boyd (1979). Total heterotrophic bacterial (THB) load of water was determined as described in Asaduzzaman et al. (2008).

2.6 Determination of sediment quality parameters

Sediment samples were collected from three locations in each pond using PVC pipes (having 4 cm diameter and sampling depth 10 cm) on a monthly basis between 09:00 and 10:00 h. The samples were dried, ground and sieved with a 2 mm sieve (Soil and Plant Analysis Council Inc., 1999). Soil pH was determined by a direct reading digital pH meter (CORNING 445 pH meter) with soil water ratio 1:2.5 (McLean, 1982). Organic matter of sediment was determined by ignition method (Page et al., 1989). Total nitrogen of sediment was determined by the common Micro-Kjeldahl digestion

method following Page et al. (1989). Total phosphorus of sediment samples were determined by the acid digestion method (Jones and Case, 1990; Watson and Isaac, 1990). Sediment THB load was determined as described in Asaduzzaman et al. (2008).

2.7 Determination of periphyton biomass

From each pond, three poles were selected randomly and two 2×2 cm^2 samples of periphyton were taken at each of three depths (25, 50 and 75 cm below from the water surface) per pole on a monthly basis starting after 7 days of substrate installation. One of the two samples from three poles and three depths were pooled for dry matter and ash free dry matter analysis. Another pooled sample from three poles and three depths were used for chlorophyll *a* determination. Dry matter, ash free dry matter, chlorophyll *a* and THB load of periphyton were analyzed as described in Asaduzzaman et al. (2008). The autotrophic index (AI) was calculated using the following formula (APHA, 1992):

AI=AFDM in µg cm^{-2} /Chlorophyll *a* in µg cm^{-2}.

2.8 Economic analysis

An economic analysis was performed to estimate the net return and benefit cost ratio in the different treatments. The following equation was used:

R = I − (FC + VC + I$_i$)

Where, R=net return, I=income from tilapia and prawn sale, FC=fixed/common costs, VC=variable costs and I$_i$=interest on inputs. The benefit cost ratio was determined with the following equation:

Benefit cost ratio (BCR) = Total net return/Total input cost

The wholesale price per kg of prawn was 400 taka. The wholesale price per kg of tilapia was 70 and 90 taka depending on size. The prices of inputs, fish and prawn correspond to the Mymensingh whole sale market prices in 2007 and are expressed in Bangladeshi taka (1US$=68.5 BDT).

2.9 Statistical analysis

Growth and yield parameters (growth, yield, FCR, SGR, and survival) and economic performance were analyzed by a two-way ANOVA with freshwater prawn stocking density and different levels of tilapia addition as main factors. Sediment and water

quality were compared by repeated measures ANOVA with freshwater prawn stocking density and different level of tilapia addition as main factors and time as the sub-factor (Gomez and Gomez, 1984). The assumptions of normal distributions and homogeneity of variances was checked before analysis. The percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA were performed using SPSS (Statistical Package for Social Science) version 12. 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 Effects on water and sediment quality parameters

Water and sediment quality parameters and outcomes of ANOVA are presented in Table 2. For water quality parameters, both stocking density of prawn and addition of different levels of tilapia influenced the surface and bottom DO. The addition of tilapia at 1 individual m^{-2} increased the bottom DO by 9% compared to the treatments without tilapia. Increasing prawn stocking density decreased the transparency by 8% whereas increasing stocking density of tilapia increased the transparency by 70%. Total alkalinity, NH_3–N, NO_2–N, NO_3–N and PO_4–P were not influenced by the stocking density of prawn and tilapia.

All inorganic nitrogenous compounds (NH_3–N, NO_2–N and NO_3–N) and PO_4–P decreased significantly with the time whereas the total alkalinity was stable over the time (Table 3). The chlorophyll *a* concentration was not influenced by the stocking density of prawn, but addition of tilapia significantly reduced it with no difference between densities of 0.5 and 1 tilapia m^{-2}. Chlorophyll *a* concentration decreased only during the first month with no significant variation during the rest of the experiment (Table 3). The addition of tilapia to the ponds facilitated a significant reduction of total nitrogen in the sediment. Total phosphorus in the sediment increased with increasing prawn density. Increasing prawn density increased the THB load of water and sediment by 7–8%. The THB count in the water column and sediment increased gradually during the culture period (Table 3), the final amounts more than doubling the initial values.

Table 2

Effects of freshwater prawn density and tilapia addition on water and sediment quality parameters based on two-way ANOVA

| Variables | Means (Tukey test) | | | | | P×T |
| | Prawn density | | Tilapia density | | | |
	P2	P3	T0	T0.5	T1	
Water quality parameters						
Temperature (°C)	27.1	26.9	27.0	27.1	27.0	NS
Surface dissolved oxygen (mg l^{-1})	6.47^b	6.51^a	6.50^a	6.46^b	6.51^a	NS
Bottom dissolved oxygen (mg l^{-1})	3.81^b	3.87^a	3.65^c	3.90^b	3.98^a	NS
pH	7.27	7.31	7.30	7.29	7.28	–
Transparency (cm)	40^a	37^b	27^b	42^a	46^a	0.01
Total Alkalinity (mg l^{-1})	127	123	125	123	127	NS
Chlorophyll *a* (µg l^{-1})	146	151	196^a	127^b	122^b	NS
Ammonia-N (mg l^{-1})	0.07	0.06	0.07	0.07	0.06	NS
Nitrite-N (mg l^{-1})	0.007	0.006	0.007	0.007	0.006	NS
Nitrate-N (mg l^{-1})	0.040	0.038	0.041	0.035	0.040	NS
Phosphate-P (mg l^{-1})	0.64	0.72	0.62	0.67	0.74	NS
THB ($\times 10^5$ cfu ml^{-1})	6.81^b	7.39^a	7.04	7.11	7.15	NS
Sediment quality parameters						
pH	7.36	7.13	7.34	7.20	7.19	-
Organic matter (%)	2.08	2.12	2.06	2.17	10.6	NS
Total nitrogen (%)	0.13	0.13	0.14^a	0.13^{ab}	0.12^b	NS
Total phosphorus (mg l^{-1})	9.8^b	10.8^a	10.2	10.2	10.6	NS
THB ($\times 10^7$ cfu g^{-1})	7.18^b	7.67^a	7.38	7.45	7.43	NS

P2=treatment with 2 prawn m^{-2}; P3=treatment with 3 prawn m^{-2}; T0=treatment without addition of tilapia; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T1=treatment with addition of 1 tilapia m^{-2}, P×T=interaction of freshwater prawn density and addition of different levels of tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. NS=not significant.

3.2 Effects on periphyton biomass

Periphyton biomass (dry matter, ash free dry matter and chlorophyll *a*) per unit substrate surface area and THB load are given in Table 4. Increasing prawn density decreased these parameters by 3–6%. Tilapia produced a much stronger effect. Adding 0.5 tilapia m^{-2} decreased these parameters by 25–50%, and at 1 tilapia m^{-2} these parameters decreased by a further 7–24%. Increasing tilapia density also decreased the mean values of all these parameters. The DM, AFDM and chlorophyll *a* contents in the treatments with tilapia increased during the first half of the experimental period after which they constantly decreased, in contrast to treatments without tilapia in which they increased steadily during the experiment (Figure 1). Tilapia addition at 1 individual m^{-2} decreased the THB load of periphyton by 7% compared to ponds without or with 0.5 tilapia m^{-2}.

Table 3

Water and sediment quality parameters over the sampling periods$^\Psi$

Variables	Sampling periods					Significance Φ
						P value
	Initial	Period 1	Period 2	Period 3	Period 4	
Water quality parameters						
Total Alkalinity (mg l^{-1})	122	131	135	119	119	NS
Chlorophyll *a* (µg l^{-1})	192^a	143^b	137^b	135^b	135^b	**
Ammonia-N (mg l^{-1})	0.12^a	0.07^b	0.06^c	0.04^d	0.03^d	***
Nitrite-N (mg l^{-1})	0.014^a	0.007^b	0.005^{bc}	0.004^c	0.003^c	***
Nitrate-N (mg l^{-1})	0.096^a	0.045^b	0.022^c	0.017^c	0.015^c	***
Phosphate-P (mg l^{-1})	1.25^a	0.81^b	0.46^c	0.42^c	0.45^c	***
THB ($\times10^5$ cfu ml^{-1})	4.02^e	5.99^d	7.46^c	8.42^b	9.59^a	***
Sediment quality parameters						
pH	7.60	6.84	7.64	7.28	6.84	-
Organic matter (%)	2.10^{ab}	1.96^c	2.00^c	2.24^a	2.08^{bc}	***
Total nitrogen (%)	0.188^a	0.108^d	0.112^{cd}	0.123^{bc}	0.123^{bc}	***
Total phosphorus (mg L^{-1})	9.9^{bc}	9.7^c	9.1^c	11.6^a	11.3^{ab}	***
THB ($\times10^7$ cfu g^{-1})	4.76^e	6.45^d	7.49^c	8.55^b	9.86^a	***

Mean values in the same row with no superscript letter in common differ significantly (*P*<0.05).
Ψ One sampling period is 30 days. Φ Results from repeated measures 2-way ANOVA. **P< 0.01;
***P<0.001.

Table 4

Effects of freshwater prawn density and tilapia addition on periphyton biomass scraped from bamboo *kanchi* by factor based on two-way ANOVA

Variables	Means (Tukey test)					P×T
	Prawn density		Tilapia density			
	P2	P3	T0	T0.5	T1	
Dry matter (mg cm^{-2})	2.27^a	2.16^b	3.14^a	1.92^b	1.48^c	NS
Ash free dry matter (mg cm^{-2})	1.55^a	1.45^b	2.43^a	1.18^b	0.90^c	NS
Chlorophyll-a (µg cm^{-2})	9.68^a	9.42^b	13.0^a	8.62^b	7.04^c	0.048
Autotrophic index (AI)	151^a	144^b	181^a	136^b	126^c	NS
THB ($\times10^7$ cfu g^{-1})	3.90	3.99	4.15^a	3.94^a	3.76^b	NS

P2=treatment with 2 prawn m^{-2}; P3=treatment with 3 prawn m^{-2}; T0=treatment without addition of tilapia; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T1=treatment with addition of 1 tilapia m^{-2}, P×T=interaction of freshwater prawn density and addition of different levels of tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. NS=not significant.
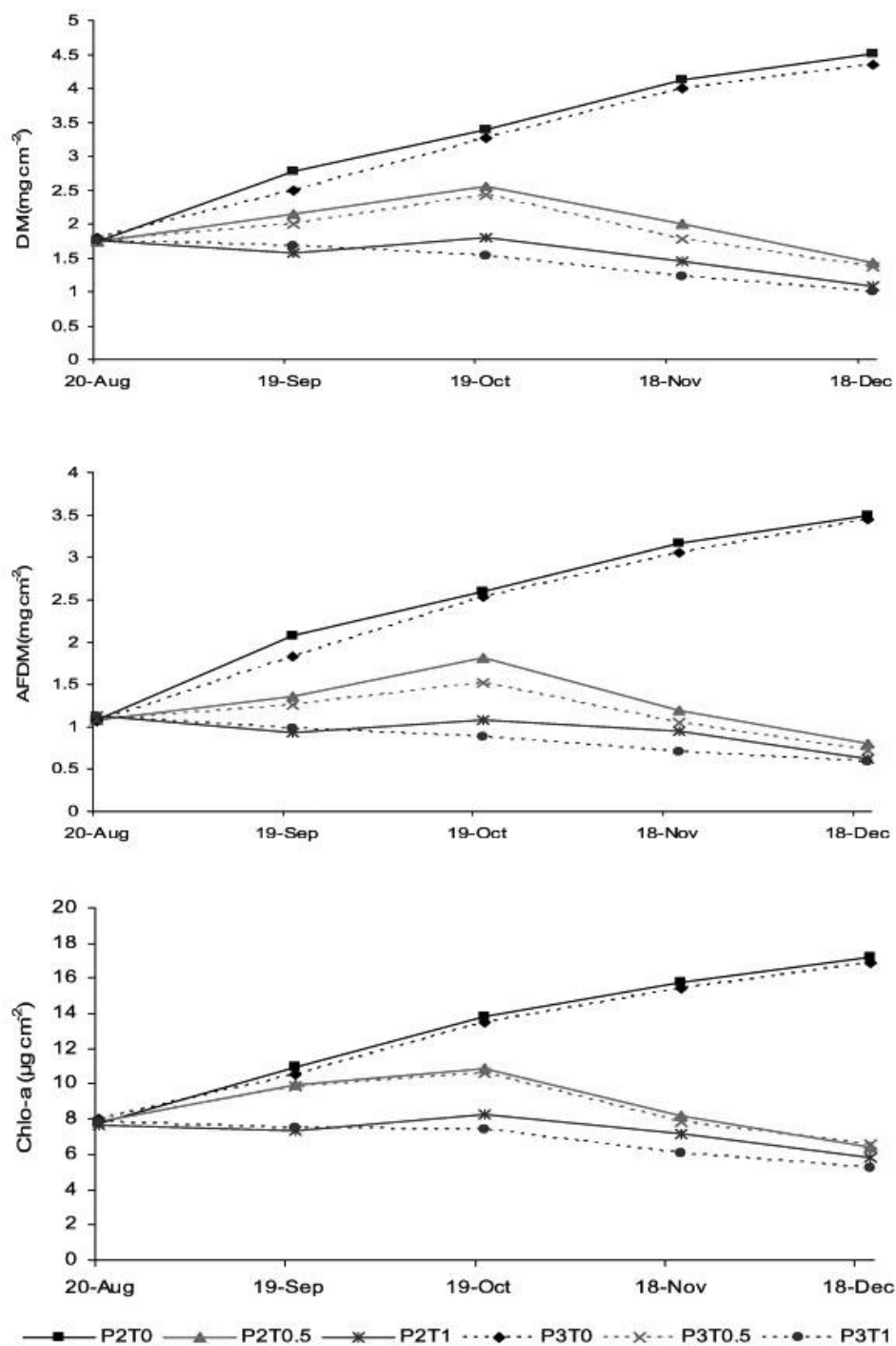
Figure 1. Quantity of periphyton biomass per unit surface area during the experimental period. Values are means of three replicates (each replicates contain three poles and three depth samples) per sampling dates in each treatment. P2T0=treatment with 2 prawn and no tilapia m^{-2}; P2T0.5=treatment with 2 prawn and 0.5 tilapia m^{-2}; P2T1=treatment with 2 prawn and 1 tilapia m^{-2}; P3T0=treatment with 3 prawn and no tilapia m^{-2}; P3T0.5=treatment with 3 prawn and 0.5 tilapia m^{-2}; P3T1=treatment with 3 prawn and 1 tilapia m^{-2}.

3.3 Fish/prawn growth and yield parameters

Growth and yield parameters of freshwater prawn, tilapia and their combined performances are shown in Table 5. Individual harvesting weight and individual weight gain of prawn decreased with increasing stocking density of prawn and with tilapia addition. Increasing prawn density did not influence the specific growth rate of prawn but the addition of tilapia decreased the specific growth rate with no significant difference between 0.5 and 1 tilapia m^{-2}. Increasing stocking density of prawn and tilapia addition increased the FCR.
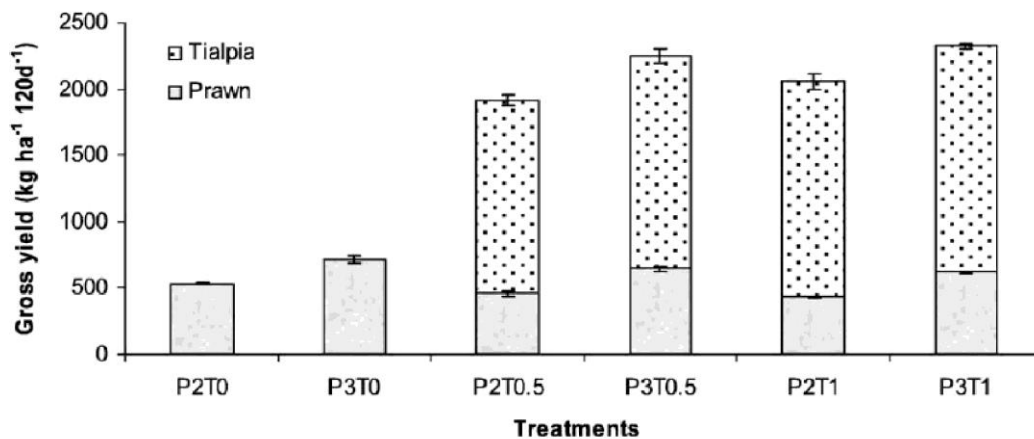

Figure 2. Gross yield of fish and relative contribution of freshwater prawn and tilapia in the six treatments. P2T0=treatment with 2 prawn and no tilapia m^{-2}; P2T0.5=treatment with 2 prawn and 0.5 tilapia m^{-2}; P2T1=treatment with 2 prawn and 1 tilapia m^{-2}; P3T0=treatment with 3 prawn and no tilapia m^{-2}; P3T0.5=treatment with 3 prawn and 0.5 tilapia m^{-2}; P3T1=treatment with 3 prawn and 1 tilapia m^{-2}. Error bars represent standard deviations for prawn and tilapia production, respectively.

The survival of prawn was not influenced by the stocking densities of prawn and tilapia addition. On average, increasing prawn density increased gross and net prawn production by almost 40% (independent of tilapia density). Adding 0.5 tilapia m^{-2} on average reduced prawn production by 12–13%, and tilapia addition at 1 individual m^{-2} produced a further 5% reduction (independent of prawn density). For tilapia, increasing stocking density of prawn did not influence the individual harvesting weight and individual weight gain. The highest individual weight at harvest (85% higher for T0.5 compared to T1) and individual weight gain (98.7%) were observed in treatments with 0.5 tilapia m^{-2}. Increasing stocking density of prawn did not influence the specific growth rate but increasing tilapia density decreased their own specific

growth rate. Tilapia survival was not influenced by the stocking density of any of the species. The gross yield increased by 8.5% and 8.4% with increasing stocking density of prawn and tilapia, respectively. The net yield of tilapia also increased by 8.5% with increasing prawn stocking density but tilapia density had no effect on it. For combined production, increasing prawn density increased the combined FCR by 17% whereas the combined FCR decreased 3.72 and 3.95 times with the addition of 0.5 and 1 tilapia m^{-2}, respectively (with no significant differences between them). With increasing prawn density, the combined gross and net yield of prawn and tilapia increased by 14.9% and 16.5%, respectively. The combined gross yield of prawn and tilapia also increased with increasing prawn and tilapia density (Figure 2).

Table 5

Effects of freshwater prawn density and tilapia addition on growth and yield parameters of prawn and tilapia per factor based on 2-way ANOVA

| Variables | Means (Tukey test) | | | | | P×T |
| | Prawn density | | Tilapia density | | | |
	P2	P3	T0	T0.5	T1	
Macrobrachium rosenbergii						
Individual Stocking weight (g)	2	2	2	2	2	NS
Individual harvesting weight (g)	32[a]	29[b]	34[a]	30[b]	28[b]	NS
Individual weight gain (g)	30[a]	27[b]	32[a]	28[b]	26[b]	NS
Specific growth rate (% bw d^{-1})	2.21	2.16	2.28[a]	2.15[b]	2.12[b]	NS
Food conversion ratio	2.50[b]	2.72[a]	2.42[b]	2.66[a]	2.74[a]	NS
Survival (%)	75	75	75	75	76	NS
Gross yield (kg ha^{-1} 120 d^{-1})	478[b]	663[a]	627[a]	554[b]	529[b]	NS
Net yield (kg ha^{-1} 120 d^{-1})	433[b]	597[a]	573[a]	498[b]	474[b]	NS
Oreochromis niloticus						
Individual Stocking weight (g)	24	24	-	24	24	NS
Individual harvesting weight (g)	241	252	-	320[a]	173[b]	NS
Individual weight gain (g)	217	228	-	296[a]	149[b]	NS
Specific growth rate (% bw d^{-1})	1.87	1.91	-	2.14[a]	1.63[b]	NS
Survival (%)	95	97	-	96	96	NS
Gross yield (kg ha^{-1} 120 d^{-1})	1537[b]	1653[a]	-	1530[b]	1659[a]	NS
Net yield (kg ha^{-1} 120 d^{-1})	1355[b]	1470[a]	-	1409	1416	NS
Combined						
Food conversion ratio	1.13[b]	1.32[a]	2.42[a]	0.65[b]	0.61[b]	NS
Gross yield (kg ha^{-1} 120 d^{-1})	2015[b]	2316[a]	627[c]	2084[b]	2188[a]	NS
Net yield (kg ha^{-1} 120 d^{-1})	1458[b]	1699[a]	573[c]	2028[b]	2134[a]	NS

P2=treatment with 2 prawn m^{-2}; P3=treatment with 3 prawn m^{-2}; T0=treatment without addition of tilapia; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T1=treatment with addition of 1 tilapia m^{-2}, P×T=interaction of freshwater prawn density and addition of different levels of tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. NS=not significant.

3.4 Economic comparison

The cost-benefit analysis of different treatments is shown in Table 6. Freshwater prawn juveniles, feed, tapioca starch (carbohydrate) and the substrates were the most expensive inputs. The extrapolated cost of all variable inputs were higher in high prawn stocking density (3 juveniles m^{-2}) treatments due to the higher prawn juveniles cost, increased feed and carbohydrate cost, as feed was applied based on the body weight of prawn. The significantly highest benefit cost ratio (BCR) was observed in 0.5 tilapia m^{-2} treatments but freshwater prawn density had no effect on it.

4 Discussion

4.1 Effects on water and sediment quality parameters

Water quality is strongly influenced by pond management including culture species combinations, stocking densities, and the quality and quantity of the nutrient inputs (Milstein, 1993; Diana et al., 1997). Decomposition and accumulation of organic matter in the sediment and water column affect water quality parameters in traditional earthen ponds without substrates. In ponds with substrates decomposition and accumulation also occur in the periphyton mats, resulting in synergistic and competitive relationships among them (Azim et al., 2003b). The oxygen budget in ponds is strongly affected by the balance/dominance of autotrophic and heterotrophic processes. The observed higher DO concentration in increased prawn density treatment might be attributed to the increased autotrophic activity. Higher prawn density ponds received higher amount of nutrients in the form of feed and carbohydrate, which facilitated the growth of phytoplankton thereby increasing DO by autotrophic activity. The addition of tilapia also increased the surface and bottom DO. Tilapia kept the phytoplankton population in a fast growing stage thereby increasing DO due to higher photosynthetic rate. This stimulating tilapia effect on phytoplankton has already been reported (Milstein and Svirsky, 1996). In addition, tilapia activity on the pond bottom and water column brings some oxygen to the bottom layers (Jiménez-Montealegre et al., 2002). Chlorophyll *a* concentration decreased and transparency of water increased with increased tilapia density due to the grazing on phytoplankton by tilapia.

Table 6. Effects of freshwater prawn density and tilapia addition on economic parameters per factor based on two-way ANOVA

Variables	Amount	Price rate	Means (Tukey test)					P×T
			Prawn density		Tilapia density			
			P2	P3	T0	T0.5	T1	
Fixed/common cost								
Land rental cost	1 ha	21,000 ha^{-1} y^{-1}	7000	7000	7000	7000	7000	-
Labor (Stocking to harvesting)	50 man-day	120 man-day^{-1}	6000	6000	6000	6000	6000	-
Rotenone	12.5 kg	220 kg^{-1}	2750	2750	2750	2750	2750	-
Lime	250 kg	10 kg^{-1}	2500	2500	2500	2500	2500	-
Cowdung	3000 kg	0.5 kg^{-1}	1500	1500	1500	1500	1500	-
Urea	100 kg	10 kg^{-1}	1000	1000	1000	1000	1000	-
TSP	100 kg	25 kg^{-1}	2500	2500	2500	2500	2500	-
Bamboo *kanchi* (reuse-5 times)	150,000 pieces	1 piece^{-1}	30,000	30,000	30,000	30,000	30,000	
Fuel cost	500 units	4 unit^{-1}	2000	2000	2000	2000	2000	-
Subtotal			55,250	55,250	55,250	55,250	55,250	-
Variable cost								
Prawn juveniles	20,000 ha	4 juvenile^{-1}	80,000	120,000	100,000	100,000	100,000	-
Tilapia juveniles		2 juvenile^{-1}	10,000	10,000	-	10,000	20,000	-
Feed		25 kg^{-1}	30,347[b]	46,625[a]	39,375	37,604	36,979	NS
Tapioca starch (Carbohydrate)		20 kg^{-1}	21,850[b]	32,850[a]	28,350	27,075	26,625	NS
Subtotal			142,197[b]	208,475[a]	167,725	174,679	183,604	NS
Total			197,447[b]	263,725[a]	222,975	229,929	238,854	NS
Interest on inputs (4 months)		10% anually	6581[b]	8790[a]	7432	7664	7961	NS
Total inputs			204,029[b]	272,516[a]	230,407	237,593	246,815	NS
Financial returns								
Prawn sale		400 kg^{-1}	191,044	265,200[a]	250,900	221,700	211,766	NS
Tilapia sale (depend on size)		70 & 90 kg^{-1}	81,429	87,834	-	137,733	116,162	NS
Total returns			272,473	353,034[a]	250,900	359,433	327,928	NS
Total net returns			68,444[b]	80,518[a]	20,492	121,840	81,112	NS
Benefit cost ratio (BCR)			0.332	0.293	0.089	0.517	0.332	NS

Calculation was based on 1 ha pond and 120 days experimental period. P2=treatment with 2 prawn m^{-2}; P3=treatment with 3 prawn m^{-2}; T0=treatment without addition of tilapia; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T1=treatment with addition of 1 tilapia m^{-2}, P×T=interaction of freshwater prawn density and addition of different levels of tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. NS=not significant.

The very low nitrogenous compounds in all treatments compared to other studies of freshwater prawn farming (Wahab et al., 2008; Kunda et al., 2008) might be due to maintaining a high C/N ratio (20) during the experimental period (Asaduzzaman et al., 2008). The decreasing trend of nitrogenous compounds (NH_3–N, NO_2–N, NO_3–N) over the time could be attributed to the addition of carbonaceous substrates that lead to increased microbial biomass, which immobilized TAN (Asaduzzaman et al., 2008; Hari et al., 2004) and uptake of the nitrogenous compounds by phytoplankton and periphyton. Increasing prawn stocking density increased the total phosphorus in the sediment in response to the increase in feeding. Increasing the stocking density of tilapia decreased the total nitrogen in the sediment possibly due to increased denitrification in response to fish driven oxygenation events (Torres-Beristain et al., 2006). The reported increase of THB count in the water column and sediment during the culture period (Table 3) is mainly because of increased amount of feed and carbohydrate application due to the increased biomass of prawn over the time. The increased bacterial load again led to higher decomposition rates releasing inorganic nutrients that in turn further stimulate bacterial development (Avnimelech et al., 1989).

4.2 Effects on periphyton biomass

In tilapia added treatments, the steady periphyton biomass increase during the first two months followed by a continuous decrease until the end of the experiment (Figure 1) may be accounted for by changes in the tilapia grazing pressure on periphyton. The low biomass of fish initially exerted low grazing pressure allowing periphyton to grow. As fish grew its increased grazing pressure led to reduced periphyton biomass. The observed lower level of periphytic algae and biomass (DM, Ash, AFDM and chlorophyll *a*) per unit surface area in tilapia added ponds indicated the preference of tilapia for periphyton as food. Tilapias are omnivores capable of feeding on benthic and attached (periphyton) algal and detrital aggregates (Dempster et al., 1993; Azim et al., 2003a). There is also evidence that Nile tilapia grows better grazing on periphyton than filtering suspended algae from water column (Hem and Avit, 1994; Guiral et al., 1995; Huchette et al., 2000; Azim et al., 2003b). Freshwater prawns were reported to selectively feed on periphyton (Uddin et al., 2006). It may have picked preferentially on animal portion and detritus aggregates rather than picking up the mixed biomass. The autotrophic index (AI) reported in the present experiment (85–210) indicates more algal component in the periphyton mass than AI values of 190–350 reported by Azim (2001) under un-grazed conditions. With grazing the algal

biomass increased as shown by lower AI values of 130–225 (Azim, 2001). 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). The observed decrease of THB load in periphyton when tilapia density increases might be due to the increased tilapia grazing reducing periphyton biomass and the associated THB count.

4.3 Effects on growth and yield parameters of prawn/tilapia

For freshwater prawn, survival was not influenced by prawn density and tilapia addition. In tilapia-prawn polyculture system, Cohen and Ra'anan (1983) reported that survival rate did not correlate with either prawn or tilapia stocking rates. According to the Uddin (2007), tilapia density might affect prawn survival during molting. But the observed similar survival (75–76%) of prawn with different tilapia densities revealed that addition of substrates might have minimized the territoriality and different water quality parameters fell in the favorable limits of *M. rosenbergii* due to maintaining a high C:N ratio in all treatments. A limited level of cannibalism during the molting is normal and may be responsible for a mortality of 4% monthly (AQUACOP, 1990). Although survival of freshwater prawn was not affected by its own stocking density, its individual weight gain and specific growth rate were significantly lower in ponds stocked with the higher number of prawn or tilapia possibly due to the intra-specific and inter-specific competition for food and space (Uddin, 2007). The FCR calculated based on prawn biomass increased significantly with the addition of tilapia because part of the feed was eaten by the tilapia. This is also reflected in the gross and net yields of freshwater prawn, in which that the resulting inter-specific competition for food (and probably other things) between tilapia and prawn decreased the net yield of prawn when tilapia was present.

The observed growth parameters of tilapia were influenced by the stocking density of tilapia and were higher in lower density (0.5 tilapia m^{-2}) treatments, possibly due to the inter-specific prawn–tilapia competition for food and space (Uddin, 2007). In this experiment, growth and production performances of tilapia was higher compared to the Uddin et al. (2006), who observed 574 kg ha^{-1} 125 d^{-1} production with 180 g average harvesting weight and 64% survival rate, while stocked with 20,000 fish ha^{-1} (75% prawn plus 25% tilapia) in tilapia-prawn polyculture. The higher production of tilapia in the present research mainly was due to maintaining a high C:N ratio leading to better environmental conditions.

The combined net yield of prawn and tilapia was satisfactory. This indicates that natural food in the form of periphyton biomass, plankton and microbial bio-floc compensated the demand of supplementary feed by tilapia. The tilapias were regularly observed grazing on substrates for periphyton. Uddin et al. (2006) suggested that artificial feed can only be provided to freshwater prawn, whereas tilapia can depend on natural food. Therefore, it can be concluded that the C/N-controlled periphyton-based system could replace the supplemental feed for tilapia through supplying adequate natural foods (periphyton, plankton and microbial bio-floc) in prawn-tilapia polyculture.

5 Conclusion

This study demonstrated that addition of tilapia at 0.5 individual m^{-2} with freshwater prawn in C/N-controlled periphyton-based ponds provided adequate natural food in the form of periphyton, plankton and microbial bio-floc that offers a good alternative to supplemental feeding for tilapia. Additionally, prawn polyculture with tilapia has a potentially higher net return than prawn monoculture. Generally, small scale farmers use their own resources as completely as possible including land, labor, substrates and manures. Therefore, the input costs in reality would be very low and net benefit would be higher in this system compared to the analyzed value of this research. Some ecological advantages of C/N-controlled periphyton-based system of prawn-tilapia polyculture, such as improved water and sediment quality and proper utilization of natural food, further increase the sustainability of this form of aquaculture. The future challenge is to identify the cheap carbohydrate source for manipulating the C/N ratio and adoption of this technology at on-farm levels through direct participation of farmers.

5

Effects of addition of tilapia *Oreochromis niloticus* and substrates for periphyton developments on pond ecology and production in C/N-controlled freshwater prawn *Macrobrachium rosenbergii* farming systems

Chapter 5

Effects of addition of tilapia *Oreochromis niloticus* and substrates for periphyton developments on pond ecology and production in C/N-controlled freshwater prawn *Macrobrachium rosenbergii* farming systems

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Abstract

The present research investigated the effect of addition of tilapia and substrates for periphyton development on pond ecology, production and economic performances in C/N controlled freshwater prawn farming system. The absence and presence (0 and 0.5 individual m^{-2}) of tilapia were investigated in 40 m^2 ponds stocked with 3 prawn juveniles (individual weight 5 g) m^{-2} with or without added substrates for periphyton development. A locally formulated and prepared feed containing 30% crude protein (C/N ratio 10) was applied daily, initially at 10% of the prawn stocked biomass and was gradually reduced to 3% of prawn biomass. Tapioca starch was used as carbohydrate source for increasing the C/N ratio from 10 (as in feed) to 20 and was applied to the water column separately from the feed. Addition of periphyton substrates significantly reduced the inorganic N-species (NH_3–N, NO_2–N and NO_3–N) in the water column. It decreased the abundance of plankton in the overlying water and increased the abundance of benthic macroinvertebrates. The abundance of periphytic algae and periphyton biomass (dry matter, ash free dry matter and chlorophyll *a*) were significantly higher in tilapia free ponds compared to tilapia added ponds. Both substrates and tilapia had significant effects on feed conversion ratio (FCR) of freshwater prawn: substrates decreased FCR by 14% while tilapia addition increased it by 16%. The addition of substrates did not influence prawn and tilapia size at harvest but improved the survival of prawn from 54 to 77%. Substrates contributed 44% and 19% higher net yield of prawn and tilapia, respectively whereas tilapia addition decreased the net yield of prawn by 14%. The economic analysis showed that addition of tilapia and periphyton substrates jointly improved the benefit–cost ratio. Addition of tilapia and periphyton substrates in C/N controlled system benefited the freshwater prawn culture practices through (1) reducing toxic inorganic nitrogenous compounds in water (2) enhancing the utilization of natural foods (3) improving survival, production and economic benefit.

1 Introduction

Freshwater prawn farming is an important aquaculture industry in many Asian countries, which contributes over 98% of the global freshwater prawn production. The increasing demand and steadily rising price in the international market have caused a silent revolution in the development of freshwater prawn farming in Bangladesh (Asaduzzaman et al., 2005). At present, the prawn culture area has increased to an estimated 50,000 ha (Khondaker, 2007). This figure is expected to rise with the increasing expansion of prawn cultivation in ponds and extensive low lying agricultural lands throughout the country (Kunda et al., 2008). On average, the annual production of freshwater prawn has been recorded at 412 kg ha^{-1} in monoculture and 390 kg ha^{-1} in polyculture with finfish species (Asaduzzaman et al., 2006a), which is very low compared to other neighboring prawn producing countries. As a resource poor country, efforts are needed to intensify prawn farming systems by using the resources derived from other agricultural systems and enhancing natural food production and utilization, thereby maximizing overall nutrient retention (Azim and Little, 2006).

Introducing substrates for periphyton development (Uddin, 2007; Tidwell and Bratvold, 2005; van Dam et al., 2002; Tidwell et al., 2000), manipulation of C:N ratio (Hargreaves, 2006; Azim and Little, 2006; Crab et al., 2007; Avnimelech, 2007) and the combination of both C:N ratio and periphyton substrates in freshwater prawn ponds (Asaduzzaman et al., 2008) were found promising. These techniques require installation of hard substrates and application of cheap carbohydrates, resources which are available within the farmers' traditional agricultural systems. Besides substrate and carbohydrate addition in freshwater prawn culture system, stocking tilapia was suggested to reduce underutilized natural foods (plankton, periphyton and microbial floc) observed in monoculture ponds (Asaduzzaman et al., 2008). In such system, tilapia depends on natural foods in the form of plankton (Perschbacher and Lorio, 1993), periphyton (Uddin, 2007; Azim et al., 2003a; Dempster et al., 1993) and microbial flocs (Azim and Little, 2008; Avnimelech, 2007; Beveridge et al., 1989). In addition, tilapia driven movements and re-suspension increase the bottom dissolved oxygen availability leading to better mineralization and stimulating the natural food web (Jiménez-Montealegre et al., 2002). Tilapias and prawns have different food and

feeding habits, but for both species, the addition of substrates resulted in extra growth and production (Uddin et al., 2006; Tidwell et al., 2000; Hem and Avit, 1994). Substrates increased production of freshwater prawn by providing shelter rather than growing periphyton as food (Asaduzzaman et al., 2008). Preliminary trials also showed that the addition of tilapia did not influence the survival of prawn in periphyton-based systems (Asaduzzaman et al., 2009a). This study monitored the effect of tilapia addition on prawn survival and production, pond ecology, and economic performance in presence and absence of substrates for periphyton development in C/N controlled ponds. Special attention was given to the effects of tilapia and substrates addition on (1) water and sediment quality; (2) abundance of plankton, periphyton and benthic macroinvertebrates; (3) heterotrophic bacterial counts in water, sediment and periphyton; and (4) production and economic performances of such system.

2 Materials and methods

2.1 Experimental design

An on-station trial was conducted with a 2×2 factorial design with the absence and presence (0 or 0.5 individual m^{-2}) of tilapia in monoculture of freshwater prawn (3 juveniles m^{-2}) as first factor, and with and without substrates addition for periphyton development as second factor. Treatments were executed in triplicate and assigned randomly between ponds.

2.2 Experimental site and pond preparation

The experiment was carried out at the Fisheries Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh for a period of 120 days during 20th February to 20th June, 2008. A 81×8.9 m earthen pond with an average depth of 1 m was drained completely and partitioned by galvanized iron sheets into 18 small ponds of 40 m^2 each. Among the 18 ponds, 12 ponds were used for this research. The ponds were rain-fed and fully exposed to prevailing sunlight and used before for research. Ponds were manually cleaned of aquatic vegetation before starting the experiments. All unwanted fishes were eradicated by rotenone application at the rate of 60 g $pond^{-1}$. Lime ($CaCO_3$) was applied to all

ponds at the rate of 250 kg ha^{-1} on Day 1. On Day 5, ponds were filled with groundwater from a deep tube-well. On Day 7, 15 side shoots of bamboo (locally known as *kanchi*) per m^2 water surface area, with a mean diameter of 2.8 cm were posted vertically into the bottom mud in substrate treatment ponds, excluding a 0.5 m wide perimeter. This resulted in an additional substrates surface area of 40 m^2 for periphyton development equaling 100% of the pond surface area. On Day 10, all ponds were fertilized with semi-decomposed cattle manure, urea and triple super phosphate (TSP) at the rates of 3000, 100 and 100 kg ha^{-1}, respectively. Ponds were left for 7 days post-fertilization to allow plankton development in the water column and periphyton growth on substrates, and subsequently stocked.

2.3 Stocking and pond management

Juveniles of *Macrobrachium rosenbergii* (5 ± 0.04 g) procured from a nearby commercial hatchery were stocked at 3 juveniles m^{-2} in the ponds and nursed juveniles of all-male *Oreochromis niloticus* (24.3 ± 0.24 g) were stocked according to the experimental design. A locally formulated and prepared pellet feed (2 mm size) containing 30% protein with C/N ratio close to 10 was used. The feed was applied considering the body weight of prawn only at a daily feeding rate of 10% body weight at the start of the experiment, and was gradually reduced (first two months at 1.5% and last two months at 2%) to 3% body weight at the end of the culture period. Feed was distributed evenly over the ponds' surface twice daily at 07:00 and 18:00 h.

Individual weights of minimum 10% of initially stocked prawn in numbers were recorded monthly to estimate the biomass and adjust the feeding rate. The prawns were sampled using a cast net after removing some bamboo *kanchi*, which were re-positioned after the sampling. Locally purchased tapioca starch was used as carbohydrate source for manipulating the C/N ratio. In order to raise the C/N ratio to 20 in all the ponds, 0.9 kg tapioca starch was applied for each kg of formulated feed. The pre-weighed tapioca starch was mixed in a beaker with pond water and uniformly distributed over the ponds' surface directly after the feed application at 07:00 h.

2.4 Prawn/tilapia harvesting and estimation of yield parameters

Prawns and tilapia were harvested after draining the ponds. Individual length (wooden measuring board; precision 0.1 cm) and weight (Denver-xp-3000; precision=0.1 g) were recorded. Specific growth rate (SGR), feed conversion ratio (FCR), and net yields were calculated as follows:

SGR = [(ln final weight−ln initial weight) × 100]/days of experiment

FCR (prawn only) = feed applied (dry weight) / live weight gain

Net yield = total biomass at harvest − total biomass at stocking

2.5 Determination of water and sediment quality parameters

Water samples were collected using a horizontal water sampler from three locations of each pond and pooled before analysis. Water quality parameters, temperature (Celsius thermometer), surface and bottom dissolved oxygen (YSI digital DO meter, model 58), pH (CORNING 445 pH meter) and transparency (Secchi disc) were monitored *in situ* at 09:00 h on a weekly basis. Before nutrient analysis, water samples were filtered through microfibre glass filter paper (Whatman GF/C), using a vacuum pressure air pump. Total alkalinity (titrimetric method) and NO_2–N, NO_3–N, TAN and PO_4–P concentrations (HACH kit model DR 2010) in the filtrate were measured on a monthly basis (APHA, 1992). The filter paper was kept in a test tube containing 10 ml of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 h. Later, chlorophyll *a* was determined using a spectrophotometer (Milton Roy Spectronic, model 1001 plus) at 750- and 664-nm wave length, following Boyd (1979).

Sediment samples were collected from three locations and pooled together in each pond using PVC pipes (having 4 cm diameter and sampling depth 10 cm) on a monthly basis between 09:00 and 10:00 h. The samples were dried, ground and sieved with a 2 mm sieve (Soil and Plant Analysis Council, 1999). Soil pH was determined by a direct reading digital pH meter (CORNING 445 pH meter) with soil water ratio 1:2.5 (McLean, 1982). Organic matter of sediment was determined by the ignition method (Page et al., 1989). Total nitrogen of sediment was determined by the common Micro-Kjeldahl digestion method following Page et al. (1989). Total phosphorus of sediment samples were determined by the acid digestion method (Jones and Case, 1990; Watson and Isaac, 1990).

2.6 Assessment of plankton, heterotrophic bacterial load and benthic macroinvertebrates

Plankton samples were collected monthly by passing 10 L of water at five locations of each pond through plankton net (mesh size 45 μm). The concentrated samples were preserved in small plastic bottles with 5% buffered formalin. The preserved samples were enumerated as described in Azim (2001) using a binocular microscope (Swift,M-4000). Samples to measure the total heterotrophic bacterial load (THB) of pond water, sediment and periphyton were collected monthly between 09:00 and 10:00 h. In each pond, samples were collected at 5 different locations, mixed homogenously and taken in sterile glass bottles. Total heterotrophic bacterial load of water, sediment and periphyton was determined as described in Asaduzzaman et al. (2008). The benthic macroinvertebrates samples were collected monthly with an Ekman dredge (area 225 cm^2). In each pond, bottom mud samples were collected from 3 different locations and washed through a 250 μm mesh size sieve. Benthic macroinvertebrates remaining on the sieve were preserved in a plastic vial containing a 10% buffered formalin solution. Identification keys used for benthic macroinvertebrates were Brinkhurst (1971) and Pinder and Reiss (1983). Benthic macroinvertebrates density was calculated using the formula,

$$N = Y \times 10\ 000/3A$$

with N=the number of benthic organisms (number m^{-2}); Y=total number of benthic organisms counted in 3 samples; A=area of Ekman dredge (cm^2).

2.7 Study of the taxonomic composition and biomass of periphyton

From each pond, three poles were selected randomly and three 2×2 cm^2 samples of periphyton were taken at each of three depths (25, 50 and 75 cm below from the water surface) per pole on a monthly basis starting after 7 days of substrate installation. One of the three samples from three poles and three depths were pooled for dry matter and ash free dry matter analysis. The other two pooled samples from three poles and three depths were used for chlorophyll *a* and taxonomic study. Periphyton biomass and autotrophic index were analyzed as described in Asaduzzaman et al. (2009a). Periphytic algae were enumerated as described in Azim (2001) using a binocular microscope (Swift, M-4000).

2.8 Economic analysis

An economic analysis was performed to estimate the net return and benefit–cost ratio in the different treatments. The following equation was used:

$R = I - (FC + VC + I_i)$

where, R=net return, I=income from tilapia and prawn sale, FC=fixed/common costs, VC=variable costs and I_i=interest on inputs. The benefit cost ratio was determined with the following equation:

Benefit cost ratio (BCR) = Total net return/Total input cost

The prices of inputs, fish and prawn correspond to the Mymensingh wholesale market prices in January to June 2008 and are expressed in Bangladeshi taka (1US\$=69 BDT). The wholesale price per kg of prawn was 400 taka. The wholesale price per kg of tilapia was 100 taka.

2.9 Statistical analysis

Growth and yield parameters and economic performance (growth, yield, FCR, SGR, and survival) were analyzed using a 2-way ANOVA with tilapia (0 and 0.5 tilapia m^{-2}) and periphyton substrates (with and without) addition as main factors. Sediment, water quality, THB counts, plankton, periphyton, benthos data were compared by repeated measures ANOVA with the addition of tilapia (0 and 0.5 tilapia m^{-2}) and periphyton substrates (with and without) as main factors and time as the sub-factor (Gomez and Gomez, 1984). The assumptions of normal distributions and homogeneity of variances were checked before analysis. The percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA were tested at 5% level of significance using SPSS (Statistical Package for Social Science) version 14.

3 Results

3.1 Effects on water and sediment quality parameters

Water and sediment quality parameters and outcomes of ANOVA are presented in Table 1. Water temperature and pH were similar among the treatments. The addition of tilapia increased the bottom DO by 7%. Both the addition of tilapia and periphyton substrates significantly increased transparency and decreased the chlorophyll *a* concentration of water. The chlorophyll *a* concentration was always significantly lower in tilapia and periphyton substrates added ponds compared to tilapia and substrates free ponds during the culture periods (Figure 1).The mean values of NH_3–N, NO_2–N, NO_3–N and PO_4–P decreased with the addition of periphyton substrates whereas the addition of tilapia increased only PO_4–P concentration in water. The concentrations of all inorganic nitrogenous species decreased continuously during the culture periods in all treatments except for NO_3–N in the treatment without tilapia and periphyton substrates (Figure 2). Among the sediment quality parameters, the addition of tilapia decreased the total nitrogen by 30% as compared to the treatment without tilapia.
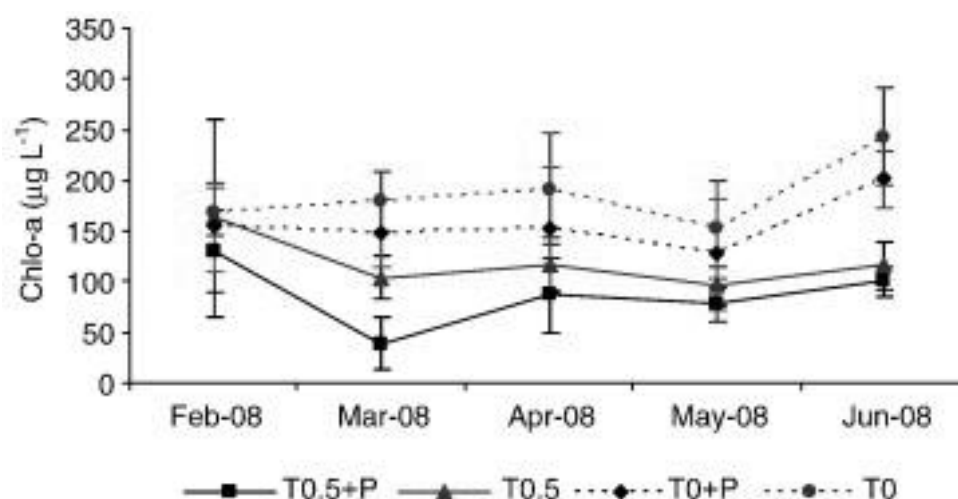

Figure 1. Mean concentrations (±SD) of chlorophyll *a* of water in different treatment ponds during the experimental period. T0.5+P=tilapia 0.5 m^{-2}+ substrates, T0.5=tilapia 0.5 m^{-2} + no substrates, T0+P=no tilapia + substrates, T0=no tilapia + no substrates.

Table 1. Effects of addition of periphyton substrates and tilapia on water and sediment quality parameters per factor based on 2-way ANOVA

Variables	Means (Tukey test)				ANOVA Significance (*P* value)		
	Periphyton substrate		Tilapia				
	Yes	No	T0.5	T0	P	T	P×T
Water quality parameters							
Temperature (°C)	30.7	30.7	30.7	30.7	NS	NS	NS
Surface dissolved oxygen (mg l^{-1})	5.3	5.4	5.4	5.3	NS	NS	NS
Bottom dissolved oxygen (mg l^{-1})	3.0	3.0	3.1^a	2.9^b	NS	**	NS
pH range	7.7-9.8	6.9-9.1	7.7-9.9	6.8-9.0	-	-	-
Transparency (cm)	38.7^a	35.2^b	44.2^a	29.7^b	**	***	NS
Total Alkalinity (mg l^{-1})	141.5	146.7	145.9	142.2	NS	NS	NS
Chlorophyll *a* (µg l^{-1})	121.6^b	153.0^a	102.9^b	171.8^a	*	***	NS
Ammonia-N (mg l^{-1})	0.038^b	0.059^a	0.047	0.050	*	NS	NS
Nitrite-N (mg l^{-1})	0.006^b	0.010^a	0.008	0.009	*	NS	NS
Nitrate-N (mg l^{-1})	0.044^b	0.075^a	0.051	0.068	**	NS	NS
Phosphate-P (mg l^{-1})	1.27^b	1.87^a	1.84^a	1.30^b	**	*	NS
Sediment quality parameters							
pH	6.7-7.3	6.8-7.3	6.7-7.1	6.7-7.3	-	-	-
Organic matter (%)	2.06	2.10	2.08	2.08	NS	NS	NS
Total nitrogen (%)	0.167	0.168	0.138^b	0.197^a	NS	***	NS
Total phosphorus (mg l^{-1})	11.5	10.8	11.4	10.9	NS	NS	NS

Yes=treatment with addition of periphyton substrates; No=treatment without periphyton substrates; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T0=treatments without addition of tilapia; P=periphyton substrates; T=tilapia addition; P×T=interaction of addition of periphyton substrates and tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.
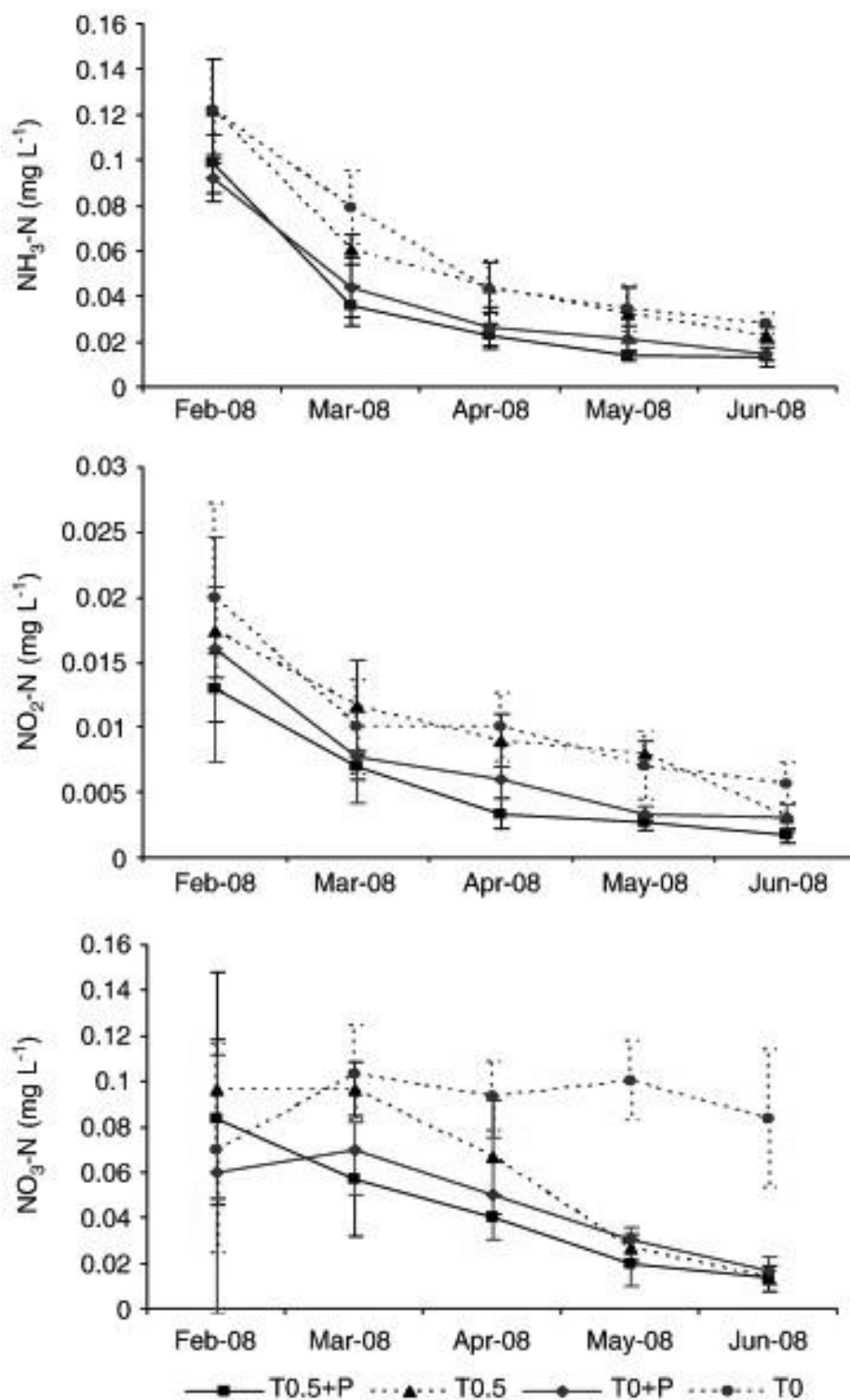
Figure 2. Mean concentrations (±SD) of inorganic nitrogenous species of water in different treatment ponds during the experimental period. T0.5+P=tilapia 0.5 m^{-2}+ substrates, T0.5=tilapia 0.5 m^{-2} + no substrates, T0+P=no tilapia + substrates, T0=no tilapia + no substrates.

3.2 Effects on the abundance of plankton, bacteria and benthos

The abundance of plankton, total heterotrophic bacterial (THB) load and benthos and outcomes of the ANOVAs are presented in Table 2. The plankton communities in pond water consisted of four groups of phytoplankton and two groups of zooplankton in all treatments. Forty five genera of phytoplankton belonging to Bacillariophyceae (11 genera), Chlorophyceae (23), Cyanophyceae (7) and Euglenophyceae (4) were found. Chlorophyceae followed by the Bacillariophyceae were the most dominant groups in terms of number of genera among phytoplankton in each treatment. The dominant genera were *Synedra, Tabellaria, Navicula, Fragillaria, Cyclotella* and *Nitzschia* (Bacillariophyceae), *Ankistrodesmus, Chlorella, Sphaerocystes, Palmella, Pediastrum* and *Scenedesmus* (Chlorophyceae), *Microcystis, Merismopedia, Gleocapsa* and *Gomphosphaeria* (Cyanophyceae), *Euglena* and *Phacus* (Euglenophyceae). The addition of tilapia significantly reduced the abundance of all phytoplankton groups. The addition of periphyton substrates also reduced the abundance of all phytoplankton groups except Bacillariophyceae. Ten genera of zooplankton, including five of Rotifera and five of Crustaceae were also identified. *Cyclops, Diaphanosoma* and Nauplius larvae (Crustaceae), and *Brachionus* and *Filinia* (Rotifera) were the dominant genera. The abundance of zooplankton did not vary significantly among the treatments. Both the addition of periphyton substrates and tilapia significantly reduced the number of total plankton. The abundance of all groups of phytoplankton decreased in the first months and then steadily increased during the rest of the period (Table 3).

3.3 Effects on periphyton composition and biomass

The periphyton composition per unit substrate surface area and the outcomes of ANOVA are presented in Table 4. About 40 genera of algae belonging to Bacillariophyceae (10 genera), Chlorophyceae (21), Cyanophyceae (7) and Euglenophyceae (2) and 6 genera of attached zooplankton belonging to Rotifer (5) and Crustacea (1) were also identified as periphytic communities in the substrate treatments. Chlorophyceae were the most abundant and Euglenophyceae were the least abundant groups of periphytic algae in each treatment. The addition of tilapia significantly reduced the number of all periphyton communities except Euglenophyceae and Rotifera.

Table 2. Effects of addition of periphyton substrates and tilapia on the abundance of plankton, THB load and benthos per factor based on 2-way ANOVA

Variables	Means (Tukey test)				ANOVA Significance (P value)		
	Periphyton substrates		Tilapia				
	Yes	No	T0.5	T0	P	T	P×T
Plankton (×10³ cells or colonies L⁻¹)							
Bacillariophyceae	28.85	37.65	23.35^b	43.15^a	NS	**	NS
Chlorophyceae	44.50^b	66.10^a	36.13^b	74.47^a	***	***	NS
Cyanophyceae	9.85^b	13.18^a	9.53^b	13.5^a	*	*	NS
Euglenophyce	8.05^b	11.50^a	7.48^b	12.07^a	*	**	NS
Total phytoplankton	91.25^b	128.43^a	76.50^b	143.18^a	***	***	NS
Rotifera	4.27	4.98	4.72	4.53	NS	NS	NS
Crustacea	1.82	1.73	1.75	1.80	NS	NS	NS
Total zooplankton	6.08	6.72	6.46	6.33	NS	NS	NS
Total plankton	97.33^b	135.15^a	82.97^b	149.52^a	***	***	NS
Total heterotrophic bacterial load							
Water (×10⁵ cfu ml⁻¹)	5.18	5.12	5.10	5.21	NS	NS	NS
Sediment (×10⁷ cfu g⁻¹)	6.1	6.1	6.0	6.1	NS	NS	NS
Periphyton (×10⁷ cfu g⁻¹)	-	-	3.06^b	5.02^a	-	**	-
Benthic macroinvertebrate (individual m⁻²)							
Chironomidae	908^a	594^b	712	790	*	NS	NS
Olligochaeta	80^a	50^b	56	75	*	NS	NS
Mollusca	241	171	169^b	243^a	NS	*	*
Un-identified groups	85	88	89	85	NS	NS	NS
Total benthos	1314^a	903^b	1026	1193	**	NS	NS

Yes=treatment with addition of periphyton substrates; No=treatment without periphyton substrates; T0.5=treatment with addition of 0.5 tilapia m⁻²; T0=treatments without addition of tilapia; P=periphyton substrates; T=tilapia addition; P×T=interaction of addition of periphyton substrates and tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. $*P<0.05$; $**P<0.01$; $***P<0.001$; NS, not significant.

Table 3. Abundance of plankton, total heterotrophic bacteria and benthos over the sampling periods[a]

Variables	Sampling periods					Significance [b]
						P value
	Initial	Period 1	Period 2	Period 3	Period 4	
Plankton (×10³ cells or colonies L⁻¹)						
Bacillariophyceae	72.9^a	17.5^b	20.1^b	21.8^b	33.9^b	***
Chlorophyceae	54.3	45.9	48.8	58.3	69.2	NS
Cyanophyceae	8.5^{bc}	7.1^c	8.0^{bc}	12.8^b	21.1^a	***
Euglenophyce	10.0^{ab}	4.7^b	10.9^a	10.4^{ab}	12.9^a	**
Total phytoplankton	145.8^a	75.2^c	87.8^{bc}	103.3^{abc}	137.1^{ab}	**
Rotifera	6.6^a	3.2^b	6.6^a	3.9^{ab}	2.8^b	***
Crustacea	1.3	2.6	1.9	1.4	1.7	NS
Total zooplankton	7.9^{ab}	5.8^{abc}	8.5^a	5.3^{bc}	4.5^c	**
Total plankton	153.7^a	80.9^c	96.3^{bc}	108.6^{abc}	141.6^{ab}	**
Total heterotrophic bacterial load						
Water (×10⁵ cfu ml⁻¹)	2.5^a	3.0^{bc}	3.9^{abc}	4.6^{ab}	5.2^a	**
Sediment (×10⁷ cfu g⁻¹)	3.3^e	3.8^d	5.1^c	6.3^b	7.2^a	***
Periphyton (×10⁷ cfu g⁻¹)	4.2^e	5.0^d	6.0^c	7.5^b	8.1^a	***
Benthic macroinvertebrate (individual m⁻²)						
Chironomidae	1262^a	1235^a	623^b	319^b	315^b	***
Olligochaeta	123^a	64^b	14^c	48^{bc}	77^b	***
Mollusca	243	153	190	251	194	NS
Un-identified groups	193^a	78^b	79^b	74^b	12^c	***
Total benthos	1820^a	1530^a	906^b	691^b	598^b	***

Mean values in the same row with no superscript letter in common differ significantly (*P*<0.05).
** *P*<0.01; *** *P*<0.001. [a] One sampling period is 30 days.
[b] Results from repeated measures 2- way ANOVA.

The numbers of animals in the periphyton communities were not influenced by the addition of tilapia. *Synedra, Tabellaria, Navicula, Fragillaria, Cyclotella, Diatoma* and *Coscinodiscus* (Bacillariophyceae), *Chlorella, Sphaerocystes, Palmella, Pediastrum, Microspora, Oedogonium, Oocystis, Ulothrix* and *Scenedesmus* (Chlorophyceae), *Microcystis, Anabaena, Aphanizomenon, Aphanocapsa* and *Gomphosphaeria* (Cyanophyceae), *Euglena* and *Phacus* (Euglenophyceae), Nauplius larvae (Crustaceae), and *Asplanchna, Brachionus* and *Filinia* (Rotifera) were the dominant genera.The addition of tilapia decreased the number of total periphyton by 52% compared to the treatment without tilapia. Periphyton dry matter (DM), ash, ash free dry matter (AFDM), chlorophyll *a*, and autotrophic index per unit substrate surface area are given in Table 4. Mean values of all of these parameters were significantly higher in ponds without tilapia. The DM, ash, AFDM and chlorophyll *a* contents increased during the first month after which they constantly decreased in the treatment with tilapia, in contrast to the treatment without tilapia (Figure 3). In the treatments without tilapia, DM, ash, AFDM and chlorophyll *a* contents increased steadily during the experiment (Figure 3).

Table 4. Effects of addition of tilapia on the abundance of periphyton and biomass scraped from bamboo *kanchi* in different treatments

Variables	Tilapia		Significance
	T0.5	T0	*P* value
Periphytic abundance ($\times 10^3$ cells or colonies cm^{-2})			
Bacillariophyceae	8.99[b]	19.90[a]	***
Chlorophyceae	15.51[b]	36.90[a]	***
Cyanophyceae	9.75[b]	15.75[a]	***
Euglenophyce	0.44	0.56	NS
Total algae	34.70[b]	73.11[a]	***
Rotifera	0.72	0.70	NS
Crustacea	0.15[b]	0.25[a]	*
Total zooperiphyton	0.87	0.95	NS
Total periphyton	35.57[b]	74.07[a]	***
Quantitative biomass			
Dry matter (mg cm^{-2})	1.92[b]	3.58[a]	***
Ash free dry matter (mg cm^{-2})	1.17[b]	2.33[a]	***
Ash (mg cm^{-2})	0.75[b]	1.25[a]	***
Chlorophyll *a* (μg cm^{-2})	9.11[b]	13.56[a]	***
Autotrophic index (AI)	120[b]	170[a]	***

T0.5=treatment with addition of 0.5 tilapia m^{-2}; T0=treatments without addition of tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. *$P<0.05$; *** $P<0.001$; NS, not significant.
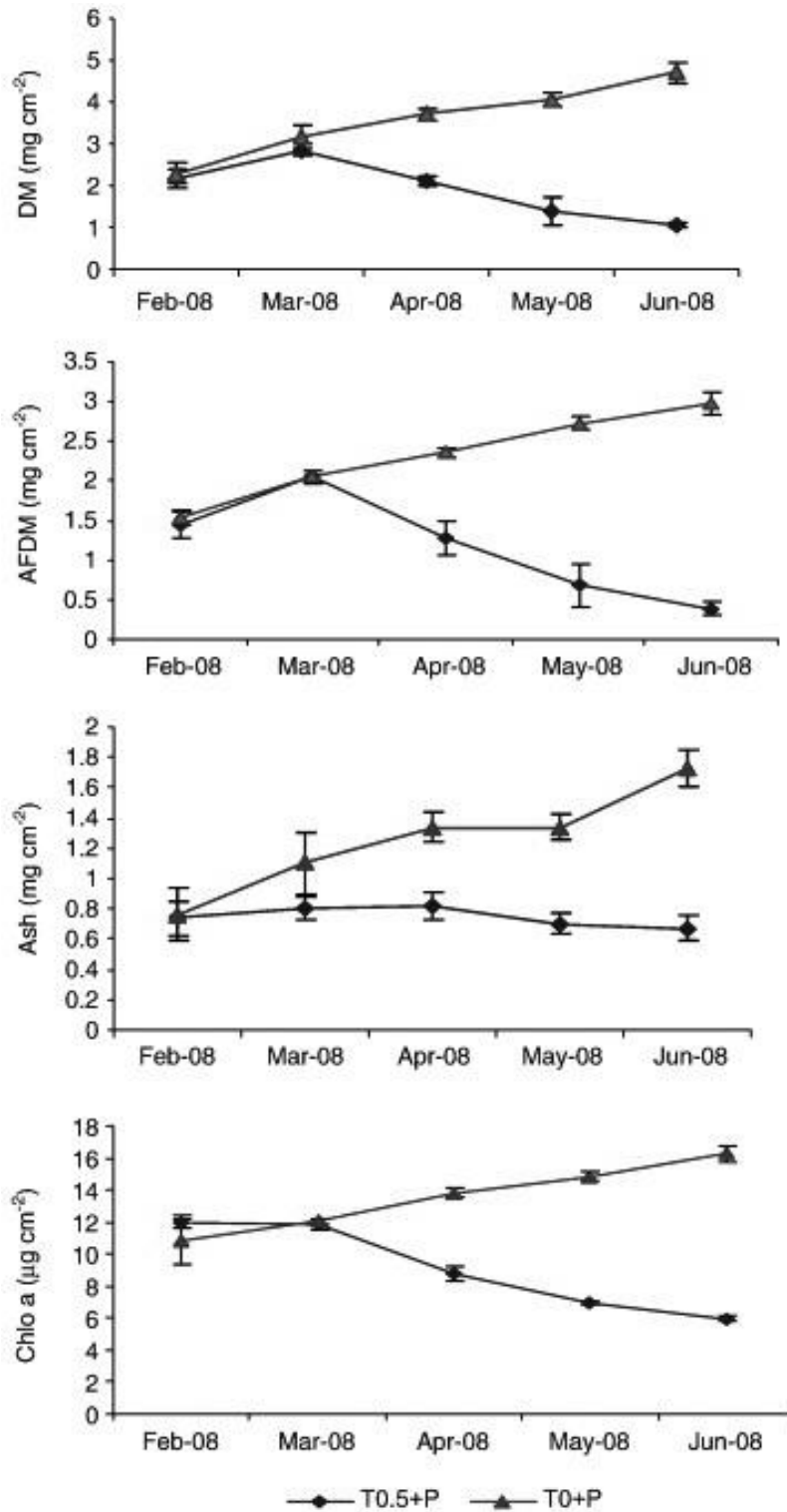
Figure 3. Quantity of periphyton biomass per unit surface area during the experimental period. Values are means (±SD) of three replicates (each replicate was composed by three poles and three depth samples) per sampling date in each treatment. T0.5 + P=tilapia 0.5 m^{-2}+substrates, T0+P=no tilapia + substrates.

3.4 Effects on growth and yield parameters of freshwater prawn and tilapia

Effects of addition of periphyton substrates and tilapia, and their interactions on yield parameters of freshwater prawn are given in Table 5. The addition of periphyton substrates increased survival of prawn by 41% compared to the treatment without substrates. Both substrates and tilapia had significant effects on FCR of freshwater prawn: substrates decreased FCR by 14% while tilapia addition increased it by 16%. Gross and net yields of prawn were higher in ponds provided with substrates than in ponds without substrates. On average, substrates contributed 33% higher gross yield and 43% higher net yield of freshwater prawn. The addition of tilapia decreased the gross and net yield of prawn by 11% and 14%, respectively.

Growth and yield parameters of tilapia with and without periphyton are presented in Table 5. Substrates had no significant effect on individual weight gain and survival of tilapia. The SGR value of tilapia was increased by 5% due to addition of periphyton substrates. Gross and net yield of tilapia was significantly higher in ponds provided with substrates than in ponds without substrates. On average, substrates contributed 16% higher gross yield and 19% higher net yield of tilapia. The contribution of freshwater prawn and tilapia to the gross yield in each treatment are shown in Figure 4.
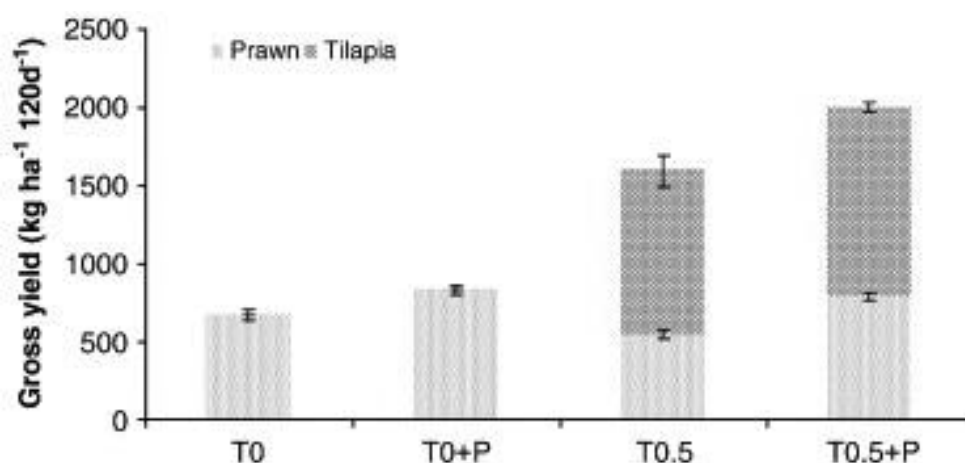

Figure 4. Gross yield of fish and prawn and contribution of freshwater prawn and tilapia in each treatment. T0.5+P=tilapia 0.5 m^{-2} + substrates, T0.5=tilapia 0.5 m^{-2} + no substrates, T0+P=no tilapia + substrates, T0=no tilapia + no substrates.

Table 5. Effects of addition of periphyton substrates and tilapia on growth and yield parameters of freshwater prawn and tilapia per factor based on 2-way ANOVA

Variables	Means (Tukey test)				ANOVA Significance (P value)		
	Periphyton substrates		Tilapia				
	Yes	No	T0.5	T0	P	T	P×T
M. rosenbergii							
In. Stocking weight (g)	5.0	4.9	5.0	4.9	NS	NS	NS
In. harvesting weight (g)	35.2	37.2	35.2	37.2	NS	NS	NS
In. weight gain (g)	30.2	32.3	30.2	32.3	NS	NS	NS
Specific growth rate (% bw d^{-1})	1.63^b	1.70^a	1.63	1.68	*	NS	NS
Food conversion ratio	2.05^b	2.37^a	2.38^a	2.05^b	**	**	NS
Survival (%)	76.9^a	54.4^b	63.6	67.8	***	NS	NS
Gross yield (kg ha^{-1} 120 d^{-1})	810^a	609^b	668^b	751^a	***	**	*
Net yield (kg ha^{-1} 120 d^{-1})	660^a	463^b	519^b	604^a	***	**	*
O. niloticus							
In. Stocking weight (g)	23.8	24.8	-	-	NS	-	-
In. harvesting weight (g)	253.1	233.3	-	-	NS	-	-
In. weight gain (g)	229.1	208.5	-	-	NS	-	-
Specific growth rate (% bw d^{-1})	1.97^a	1.87^b	-	-	*	-	-
Survival (%)	96.7	90.1	-	-	NS	-	-
Gross yield (kg ha^{-1} 120 d^{-1})	1222^a	1051^b	-	-	*	-	-
Net yield (kg ha^{-1} 120 d^{-1})	1103^a	927^b	-	-	*	-	-

Yes=treatment with addition of periphyton substrates; No=treatment without periphyton substrates; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T0=treatments without addition of tilapia; P=periphyton substrates; T=tilapia addition; P×T=interaction of addition of periphyton substrates and tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.

Table 6. Effects of freshwater prawn density and tilapia addition on economic parameters per factor based on 2-way ANOVA

| Variables | Amount | Price rate | Means (Tukey test) | | | | ANOVA Significance (P value) | | |
| | | | Tilapia | | Periphyton substrates | | | | |
			T0.5	T0	Yes	No	T	P	T×P
Fixed/common cost									
Land rental cost	1 ha	24,000 ha^{-1} y^{-1}	8000	8000	8000	8000	-	-	-
Labor (Stocking to harvesting)	50 man-day	140 man-day^{-1}	7000	7000	7000	7000	-	-	-
Rotenone	12.5 kg	220 kg^{-1}	2750	2750	2750	2750	-	-	-
Lime	250 kg	10 kg^{-1}	2500	2500	2500	2500	-	-	-
Cowdung	3000 kg	0.5 kg^{-1}	1500	1500	1500	1500	-	-	-
Urea	100 kg	12 kg^{-1}	1200	1200	1200	1200	-	-	-
TSP	100 kg	40 kg^{-1}	4000	4000	4000	4000	-	-	-
Fuel cost	500 units	4 unit^{-1}	2000	2000	2000	2000	-	-	-
Prawn juveniles		4 juvenile^{-1}	120,000	120,000	120,000	120,000	-	-	-
Subtotal			148,950	148,950	148,950	148,950	-	-	-
Variable cost									
Tilapia juveniles		2 juvenile^{-1}	10,000	0	5000	5,000	-	-	-
Bamboo *kanchi* (reuse 5 times)		1 piece^{-1}	15,000	15,000	30,000	0	-	-	-
Feed		25 kg^{-1}	33,980	34,145	37,754^a	30,371^b	NS	**	*
Tapioca starch (Carbohydrate)		20 kg^{-1}	24,465	24,584	27,183^a	21,867^b	NS	**	*
Subtotal			83,445^a	73,728^b	99,936^a	57,238^b	**	***	*
Total			232,396^a	222,678^b	248,886^a	206,188^b	**	***	*
Interest on inputs (4 months)		10% anually	7746^a	7422^b	8295^a	6873^b	**	***	*
Total inputs			240,141^a	230,100^b	257,182^a	213,060^b	**	***	*
Financial returns									
Prawn sale		400 kg^{-1}	267,133^b	300,533^a	323,983^a	243,683^b	**	***	*
Tilapia sale		100 kg^{-1}	113,670^a	0^b	61,104^a	52,567^b	***	*	*
Total returns			380,804^a	300,533^b	385,088^a	296,250^b	***	***	**
Total net returns			140,663^a	70,432^b	127,905^a	83,189^c	***	***	*
Benefit cost ratio (BCR)			0.579^a	0.303^b	0.492^a	0.390^c	***	**	NS

Calculation was based on 1 ha pond and 120 days experimental period. Yes=treatment with addition of periphyton substrates; No=treatment without periphyton substrates; T0.5=treatment with addition of 0.5 tilapia m^{-2}; T0=treatments without addition of tilapia; P=periphyton substrates; T=tilapia addition; P×T=interaction of addition of periphyton substrates and tilapia. The mean values with no superscript letter in common per factor indicate significant difference at 0.05. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.

3.5 Economic comparison

The benefit–cost analysis of different treatments is shown in Table 6. Freshwater prawn juveniles, feed, tapioca starch (carbohydrate) and the substrates were the most expensive cost inputs. The extrapolated costs of all variable inputs were higher in substrates and tilapia added treatments. The economic analysis showed that addition of tilapia and periphyton substrates jointly improved the benefit–cost ratio. Therefore, it is concluded that the addition of tilapia and substrates for periphyton development is economically profitable compared to the substrates and tilapia free ponds in C/N controlled freshwater prawn farming system.

4 Discussion

4.1 Effects on water and sediment quality parameters

Water quality in lentic natural water bodies is strongly dependent on the autotrophic and heterotrophic organisms developing within the systems. In periphyton-based system, the close linkage between autotrophic and heterotrophic processes in periphyton mats speed up nutrient cycling and positively influences water quality (Azim et al., 2003b; Milstein et al., 2003). The observed water temperature and pH were within the suitable range for freshwater prawn and tilapia culture (Zimmermann and Boyd, 2000; New, 2002). The observed DO concentrations were also suitable for prawn culture, although very low bottom DO values were recorded on a few occasions in tilapia free ponds. The addition of tilapia brings some oxygen to the bottom layers by their movements (Jiménez-Montealegre et al., 2002), thus increasing the bottom dissolved oxygen. Periphyton lowered the PO_4–P of the overlying water which was also reported by Hansson (1990) and Bratvold and Browdy (2001). By lowering the nutrients concentration, periphyton reduced the phytoplankton biomass increasing water transparency. The observed lower level of nitrogenous compounds in substrates based ponds was due to enhanced nitrification. According to Langis et al. (1988) and Ramesh et al. (1999) bacterial biofilm (periphyton), including nitrifying bacteria, develop on the substrates which are located in the water column where more oxygen is available than at the water-sediment interface. In addition, the periphytic algal community contributes to the processing of the nitrogenous wastes in ponds (Shilo and Rimon, 1982; Diab and Shilo, 1988). The very low nitrogenous

compounds in all treatments compared to other studies of freshwater prawn farming (e.g.:Wahab et al., 2008; Kunda et al., 2008) could be attributed to the addition of carbonaceous substrates to maintain a C/N ratio of 20 during the experimental period. This led to increased microbial biomass, which immobilized TAN (Asaduzzaman et al., 2008; Asaduzzaman et al., 2006b; Hari et al., 2004) and uptake of the nitrogenous compounds by phytoplankton and periphyton. Addition of tilapia decreased the total nitrogen in the sediment possibly due to increased denitrification in response to fish driven oxygenation events (Torres-Beristain et al., 2006).

4.2 Effects on the abundance of plankton, THB load and benthos

The major natural foods in C/N controlled ponds are phytoplankton, zooplankton, microbial flocs, periphyton and benthic macroinvertebrates. The amounts of these natural foods in ponds are influenced by management factors such as species combination, stocking density and ratio, and nutrient input quality and quantity (Milstein, 1993; Diana et al., 1997). The phytoplankton species composition was representative of that found in Bangladesh prawn farming in rice fields and ponds (Wahab et al., 2008; Kunda et al., 2008; Uddin, 2007). The addition of tilapia affected phytoplankton directly by grazing and indirectly by nutrient re-suspension. The direct effect was more pronounced than the indirect effect, indicating that tilapia addition resulted in a higher grazing pressure on phytoplankton. Perschbacher and Lorio (1993) reported that tilapia stocked at densities higher than 5000 ha^{-1} promoted a very effective biological control over phytoplankton. However, the addition of tilapia did not have any significant effect on the abundance of zooplankton possibly due to escaping predation and less preference for zooplankton by tilapia (Uddin, 2007). Substrate addition decreased plankton abundance by lowering the nutrients concentration of the overlying water. The observed decrease in abundance of phytoplankton during the first month might be attributed to grazing by tilapia. The steadily increase in abundance of phytoplankton after the first month might be due to increased nutrient re-suspension by tilapia of increasing body size. Avnimelech et al. (1999) reported that tilapias do appreciably re-suspend sediment, and such activity is more pronounced in large fish.

The lower level of THB load of periphyton in the tilapia added treatment might be attributed to the increased tilapia grazing reducing periphyton biomass and the associated THB. The observed THB increase in the water column, sediment and periphyton during the culture period is mainly due to increased feed and carbohydrate application concurring with the increasing prawn biomass over time. The increased bacterial load again led to higher decomposition rates releasing inorganic nutrients that in turn further stimulated bacterial development (Avnimelech et al., 1989).

Substrates addition enhanced the production of benthos in the culture systems. Similar findings were reported by Azim (2001). The observed decrease in number of total benthos during the culture period might be due to grazing by prawn. There are evidence that prawns in their natural habitats prefer to forage on animals like trochopterans, chironomids, oligochaetes, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996; Tidwell et al., 1997).

4.3 Effects on the periphyton composition and biomass

The observed lower level of periphytic algae and biomass (DM, Ash, AFDM and chlorophyll *a*) per unit surface area in tilapia added ponds indicate the preference of tilapia for periphyton as food. Tilapias are omnivores capable of feeding on benthic and attached (periphyton) algal and detrital aggregates (Dempster et al., 1993; Azim et al., 2003a). There is also evidence that Nile tilapia grows better grazing on periphyton than filtering suspended algae from the water column (Hem and Avit, 1994; Guirat et al., 1995; Huchette et al., 2000; Azim et al., 2003b). The similar abundance of periphytic zooplankton in all treatments indicates that the zooplankton communities were less preferable for the tilapias or escaped predation. The higher ash contents of periphyton in ponds stocked with freshwater prawn alone might also be related to low grazing pressure (Makrevich et al., 1993; Huchette et al., 2000). Generally, the ash content increase when the periphyton communities grow older under low grazing pressure (Makarevich et al., 1993). In tilapia added treatments, periphyton biomass increased steadily during the first months and then decreased continuously until the end of the experiment. Initially tilapia predation was lower than periphyton development, but after one month tilapia grazing reduced the periphyton biomass. The autotrophic index (AI) value was lower (120) in tilapia added ponds

compared to the tilapia free ponds (170), indicating more algal component in the periphyton mass in tilapia added ponds. With grazing the algal component in the periphytic biofilms increased as shown by lower AI values (Azim, 2001). It is evident that periphytic algae that are grazed constantly maintain productivity (Hatcher, 1983; Hay, 1991; Huchette et al., 2000).

4.4 Effects on the growth and yield parameters of prawn and tilapia

The increase in gross and net yield of prawn in substrate added ponds was mainly due to the increased survival, not to faster individual growth. Addition of substrates minimized territoriality of freshwater prawn, provided additional shelter and natural food along with improvements of environmental conditions through a range of ecological and biological processes (Tidwell et al., 2000; Tidwell et al., 2002; van Dam et al., 2002; Milstein et al., 2003; Asaduzzaman et al., 2008). The net yield of freshwater prawn was significantly higher with no tilapia than with tilapia, indicating that inter-specific competition between tilapia and prawn occurs. The FCR calculated based on prawn biomass increased significantly with the addition of tilapia because part of the feed was eaten by the tilapia, whereas substrates decreased the FCR value by 13% contributing periphyton as additional food. Also Uddin (2007) reported that FCR was 13% lower in fed-periphyton based ponds compared to fed-substrate-free-ponds. In case of tilapia, substrate addition increased the gross and net yield, indicating that substrates provide additional food (Uddin, 2007). The economic analysis revealed that prawn–tilapia polyculture with a stocking density of 3 prawns and 0.5 tilapia m^{-2} in C/N-controlled periphyton-based system would be a very profitable business.
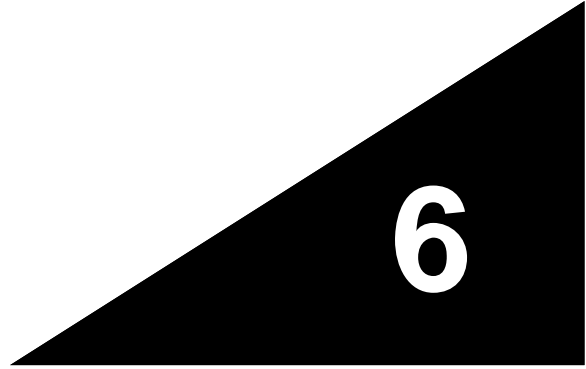
5 Conclusion

In summary, addition of tilapia (0.5 individual m^{-2}) and periphyton substrates in C/N controlled ponds (C/N ratio 20) benefited freshwater prawn production (3 juveniles m^{-2}) through (1) reducing toxic inorganic nitrogenous compounds in water (2) enhancing the availability of plankton, periphyton, microbial floc and benthic macroinvertebrates thus reducing the demand by tilapia for supplemental feed (3) improving survival, production and economic benefit. The result of the present study could be useful in improving the sustainability of freshwater prawn farming in terms

of ecological, social and financial benefits. Economic sustainability could still be further enhanced by identifying cheaper on-farm carbohydrate sources and periphyton substrates, and is subject of further research.

Acknowledgements

6

Effects of carbohydrate source for maintaining a high C:N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems

Chapter 6

Effects of carbohydrate source for maintaining a high C:N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems

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Abstract

The present research investigated the effect of carbohydrate (CH) source for maintaining a high C:N ratio, and tilapia driven bioturbation on pond ecology, production and economical performances in C/N-controlled periphyton-based (C/N-CP) freshwater prawn ponds. Two carbohydrate sources (high-cost tapioca starch and low-cost maize flour) were compared in 40 m^2 ponds stocked with 80 freshwater prawn (*Macrobrachium rosenbergii*) juveniles (individual weight 0.81±0.03 g) and 20 finfish fingerlings (Nile tilapia, *Oreochromis niloticus* and Indian major carp rohu, *Labeo rohita*) in three different combinations: 100% tilapia, 50% tilapia+50% rohu, and 100% rohu (individual weight 27.7±0.6 g). The CH sources for increasing C:N ratio from 10 (as in feed) to 20 had no significant effect ($P>0.05$) on water quality parameters, abundance of natural food (plankton, periphyton and benthos) and production of prawn and finfish. However, different fish combination had significant effects on pond ecology. The highest PO$_4$–P ($P<0.001$) and the lowest chlo-*a* ($P<0.01$) concentrations in water were observed in ponds with 100% tilapia as compared to ponds stocked with 100% rohu. The abundance of phytoplankton, periphyton biomass (dry matter, ash, ash free dry matter and chlo-*a*) and benthos was significantly higher ($P<0.05$) in 100% rohu ponds than in 100% tilapia ponds indicating the more efficient utilization of natural food items by tilapia than by rohu. The freshwater prawn production was not affected ($P>0.05$) by the different stocking combinations of finfish. The net yield and survival of finfish were significantly higher in 100% tilapia ponds and lower in 100% rohu ponds resulting in 58% higher combined net yield (both prawn and finfish) in the former treatment during a 120-d culture period. This treatment gave the best economic return in terms of benefit–cost ratio while maize flour was used as CH source. In conclusion, maize flour can be used as an alternative cheap on-farm CH source for maintaining a high C:N ratio and tilapia driven re-suspension in C/N-CP system improves culture environment, natural food utilization, production and economic return, further enhancing economic sustainability of C/N-CP freshwater prawn farming system.

1 Introduction

Pond aquaculture contributes the bulk of the world aquaculture production and research efforts have been made to improve the productivity and sustainability of pond production. To this end, several recent developments seem to be promising: (1) C/N ratio control (Avnimelech, 1999, 2007; Hari et al., 2004); (2) providing substrates for periphyton development (van Dam et al., 2002; Tidwell et al., 2000, 2002; Tidwell and Bratvold, 2005; Azim et al., 2003a,b; Keshavanath et al., 2001; Milstein et al., 2009); and (3) fish driven re-suspension (Riise and Roos, 1997; Jiménez-Montealegre et al., 2002; Ritvo et al., 2004; Milstein et al., 2002). Recently, Asaduzzaman et al. (2008, 2009a,b) combined these techniques, using freshwater prawn as a key species, with the goal to raise pond productivity above levels obtained with each one of these techniques separately, and to increase the nutrient use efficiency in ponds above levels presently achieved, further enhancing environmental and economical sustainability. This combined technology has been referred to as C/N-controlled periphyton-based (C/N-CP) system.

Operation of intensive aquaculture of freshwater prawn demands high investment and technical expertise, which are not affordable by resource-poor farmers in developing countries like Bangladesh. Therefore, efforts are needed to intensify aquaculture by using the resources derived from other agricultural systems and manipulating natural food thereby maximizing overall nutrient retention (Azim and Little, 2006). In C/N-CP system, the added carbon source together with the waste nitrogen is converted into microbial bio-flocs, which in turn can be eaten by the cultured organisms. This technique provides an additional inexpensive protein source and improves the overall nutrient efficiency of the pond. Tapioca starch was used as CH source for maintaining a high C:N ratio in all our previous research on C/N-CP system (Asaduzzaman et al., 2008, 2009a,b). The major problem of using tapioca starch as CH source in Bangladesh is its poor acceptance by the farmers due to very high cost (0.44 US$kg^{-1}) and irregular availability due to an import product. Asaduzzaman et al. (2009b) recommended that identification of an alternative cheap on-farm CH source, which could potentially be produced within the farmers' traditional agricultural systems, is essential for economic sustainability of C/N-CP technology. In the present study, maize (*Zea mays*) flour is considered as a potential carbohydrate source due to its low

cost (0.18 US$kg^{-1}), easy availability and wide acceptance by the farmers as one of the potential feed ingredients, and compared with tapioca starch in C/N-CP system.

In our previous experiment, it has been shown that addition of omnivorous tilapia (0.5 individual m^{-2}) in C/N-CP based freshwater prawn culture system improved natural food utilization, production and economic benefit (Asaduzzaman et al., 2009b). The periphyton community took up both TAN and nitrate and edible biomass was formed. The added tilapia can effectively graze on the periphyton (Uddin, 2007; Azim et al., 2003a; Dempster et al., 1993; Milstein et al., 2009) as well as phytoplankton community (Perschbacher and Lorio, 1993). Therefore, this technique improves the overall conversion efficiency of the feed. In addition, tilapia driven movements and re-suspension increase the bottom dissolved oxygen availability leading to better mineralization and stimulating the natural food web (Jiménez-Montealegre et al., 2002). Of all species stocked in polyculture, fish farmers in south Asia like to stock a native major carp, commonly known as rohu, because it fetches the highest market price and has the highest consumer preference (Dey et al., 2005). This species is a column feeder mainly living on plankton (Jhingran and Pullin, 1985) and periphyton (Azim et al., 2003c) but it has no reported sediment re-suspension activity (Costa-Pierce and Pullin, 1989; Riise and Roos, 1997; Avnimelech et al., 1999; Jiménez-Montealegre et al., 2002). Considering the importance of rohu as an indispensable species in south Asian aquaculture, both tilapia and rohu were considered in C/N-controlled freshwater prawn ponds to determine the suitability of either species by comparing how tilapia and rohu interact in the exploitation of natural foods in ponds or how they influence natural food availability. Therefore, the present study investigated three different stocking combinations of tilapia and rohu, and evaluated the effect of tilapia driven re-suspension on pond ecology, production and economic returns in C/N-CP system ponds. Special attention was given to the effects of different CH sources and tilapia driven re-suspension on (1)water quality parameters; (2) abundance of plankton, periphyton and benthic macroinvertebrate; and (3) production and economic performances of such system.

2 Materials and Methods

2.1 Experimental design

An on-station trial was conducted with a 2×3 factorial design with two different carbohydrate (CH) sources (high-cost tapioca starch and low-cost maize flour) for

maintaining the C:N ratio at 20 as first factor, and 20 tilapia and/or rohu (27.7±0.6 g) in 40 m^2 pond under three different stocking combinations (100% tilapia, 50% tilapia+50% rohu, and 100% rohu) as second factor. All ponds were stocked with 80 prawn juveniles (0.81±0.03 g). The various treatment combinations of different CH sources are abbreviated as MF (maize flour) and TS (tapioca starch) whereas, stocking ratios of tilapia and rohu are abbreviated as 100T, 50T/50R and 100R, T representing tilapia and R rohu.

2.2 Experimental site and pond preparation

The experiment was carried out at the Fisheries Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh for a period of 120 days during 1st August to 30 November, 2008. An 81×8.9 m earthen pond with an average depth of 1 m was drained completely and partitioned by galvanized iron sheets into 18 small ponds of 40 m^2 each. The ponds were rainfed and fully exposed to prevailing sunlight and used before for research. Ponds were manually cleaned of aquatic vegetation before starting the experiments. All unwanted fishes were eradicated by rotenone application at the rate of 100 g pond^{-1}. Lime (CaCO$_3$) was applied to all ponds at the rate of 250 kg ha^{-1} on Day 1. On Day 4, ponds were filled with groundwater from a deep tube-well. On Day 6, 15 side shoots of bamboo (locally known as *kanchi*) per m^2 water surface area, with a mean diameter of 2.8 cm were posted vertically into the bottom mud in all ponds, excluding a 0.5 m wide perimeter. This resulted in an additional substrates surface area of 40 m^2 for periphyton development equaling 100% of the pond surface area. On Day 9, all ponds were fertilized with semi-decomposed cattle manure, urea and triple super phosphate (TSP) at the rates of 3000, 100 and 100 kg ha^{-1}, respectively. Ponds were left for 10 days post-fertilization to allow plankton development in the water column and periphyton growth on substrates, and subsequently stocked.

2.3 Stocking and pond management

In all ponds, juveniles of *Macrobrachium rosenbergii* (individual weight 0.81±0.03 g) procured from a nearby commercial hatchery were stocked at 2 prawns m^{-2}. Nursed juveniles of all-male *Oreochromis niloticus* and *Labeo rohita* (individual weight 27.7±0.6 g) were stocked according to the experimental design. A locally formulated

and prepared pellet feed (2mm) containing 24.3% protein with C/N ratio close to 10 was used. The feed was applied considering the body weight of prawn at a daily feeding rate of 10% body weight at the start of experiment, and gradually reduced (2% reduction in each month) to 4% body weight at the end of the culture period. Feed was distributed evenly over the ponds' surface twice daily at 07:00 and 18:00 h. Individual weights of minimum 10% of initially stocked prawn in numbers were recorded monthly to estimate the biomass and adjust the feeding rate. The prawns were sampled using a cast net after removing some bamboo *kanchi*, which were re-positioned after the sampling.

Locally purchased tapioca starch and maize flour were used as carbohydrate source for manipulating the C/N ratio. The analyzed proximate composition of feed, maize flour and tapioca starch is given in Table 1. In order to raise the C/N ratio to 20 in all the ponds, 0.82 kg tapioca starch was applied for each kg of formulated feed for TS treatment ponds, whereas 1.3 kg maize flour was applied for each kg of formulated feed in MF treatment ponds. The pre-weighed tapioca starch and maize flour were mixed in a beaker with pond water and uniformly distributed over the ponds' surface directly after the feed application at 07:00 h.

Table 1

Proximate composition of the prepared feed, tapioca starch and maize flour. The percentages are given on a wet weight basis.

Component	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	NFE (%)
Prepared feed	8.69	24.27	10.0	6.15	20.61	30.28
Tapioca starch	12.9	1.6	0.9	5.4	5.2	74.0
Maize flour	11.08	7.72	4.64	5.40	1.14	70.02

NFE = Nitrogen free extract = 100 − (moisture + protein + lipid + crude fiber + ash)

2.4 Prawn/finfish harvesting and estimation of yield parameters

Prawns and finfish were harvested after draining the ponds. Individual length (wooden measuring board; precision 0.1cm) and weight (Denver-xp-3000;

precision=0.1g) were recorded. Feed conversion ratio (FCR), and net yields were calculated as follows:

FCR (prawn only) = feed applied (dry weight)/live weight gain

Net yield = total biomass at harvest − total biomass at stocking.

2.5 Determination of water quality parameters

Water samples were collected using a horizontal water sampler from three locations of each pond and pooled before analysis. Water quality parameters, surface and bottom temperature (Celsius thermometer), surface and bottom dissolved oxygen (YSI digital DO meter, model 58) and pH (CORNING 445 pH meter) were monitored *in situ* at sunrise (07:00 h) and sunset (18:00 h) on a weekly basis. Transparency (Secchi disc) was recorded weekly at 10:00 h. Before nutrient analysis, water samples were filtered through microfibre glass filter paper (Whatman GF/C), using a vacuum pressure air pump. Total alkalinity (titrimetric method) and NO_2–N, NO_3–N, NH_3–N and PO_4–P concentrations (HACH kit model DR 2010) in the filtrate were measured on a monthly basis (APHA, 1992). The filter paper was kept in a test tube containing 10 mL of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 h. Later, chlo-*a* was determined using a spectrophotometer (Milton Roy Spectronic, model 1001 plus) at 750- and 664-nm wave length, following Boyd (1979).

2.6 Assessment of plankton and benthic macroinvertebrate

Plankton samples were collected monthly by pooling 10 l of water from five locations in each pond and passing it through a 45 µm mesh plankton net. The concentrated samples were preserved in small plastic bottles with 5% buffered formalin. Plankton numbers were estimated using a Sedgewick–Rafter (S–R) cell and was left to stand for 15 min to allow plankton to settle. Then, the plankters on 10 randomly selected fields of the chamber were counted under a binocular microscope (Swift, M-4000). Taxa were identified to genus level using keys from Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976), and Bellinger (1992). Plankton abundance was calculated using the following formula:

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

Where N is the number of plankton cells or units per liter of original water; P, the number of plankton counted in 10 fields; C, the volume of final concentrate of the sample (mL); L, the volume (L) of the pond water sample.

The benthic macroinvertebrate samples were collected monthly with an Ekman dredge (covering an area of lower month 225 cm^2). In each pond, bottom mud samples were collected from 3 different locations and washed through a 250 μm mesh size sieve. Benthic macroinvertebrate remaining on the sieve was preserved in a plastic vial containing a 10% buffered formalin solution and pooled together. Identification keys used for benthic macroinvertebrate were Brinkhurst (1971), and Pinder and Reiss (1983). Benthic macroinvertebrate density was calculated using the formula,

$$N = Y \times 10000/ 3A$$

with N=the number of benthic organisms (number m^{-2}); Y=total number of benthic organisms counted in 3 samples; A=area of Ekman dredge (cm^2).

2.7 Study of the biomass of periphyton

From each pond, three poles were selected randomly and two 2×2 cm samples of periphyton were taken at three depths (25, 50 and 75 cm below the water surface) per pole on a monthly basis starting after 7 days of substrate installation. Half of the 2×2 cm samples from three poles per pond per sampling day were pooled for dry matter (DM), ash and ash free dry matter (AFDM) analysis. The scraped samples from each pond were collected on pre-weighed and labeled pieces of aluminum foil, dried at 105 °C until constant weight (24 h in a Memmert stove, Model UM/BM 100–800), and kept in a desiccator until weighed (BDH 100A; precision 0.0001 g). Dry samples from different depth and poles per pond were pooled, transferred to a muffle furnace and ashed at 450 °C for 6 h and weighed. The dry matter (DM), ash and ash free dry matter (AFDM) were determined by weight differences (APHA, 1992).

The other half of the 2x2 cm samples per pond per sampling day were pooled and used to determine chlo-*a* concentrations following standard methods (APHA, 1992). Collected materials were immediately transferred to labeled tubes containing 10 mL

of 90% acetone, sealed and stored overnight in a refrigerator. The following morning, samples were homogenized for 30 s with a tissue grinder, refrigerated for 4 h, and then centrifuged for 10 min at 2000–3000 rpm. The supernatant was carefully transferred to a 1 cm glass cuvette and absorption measured at 750 and 664 nm using a spectrophotometer (Milton Roy Spectronic, model 1001 plus). Chlo-*a* concentration was calculated using the equation given in APHA (1992). The autotrophic index (AI) was calculated using the following formula (APHA, 1992):

$$AI = AFDM \text{ in } \mu g \text{ } cm^{-2}/Chlorophyll \text{ } a \text{ in } \mu g \text{ } cm^{-2}$$

2.8 Economical analysis

An economical analysis was performed to estimate the net return and benefit-cost ratio in the different treatments. The following equation was used:

$$R = I - (FC + VC + I_i)$$

Where, R = net return, I = income from prawn, tilapia and rohu sale, FC = fixed/common costs, VC = variable costs and I_i = interest on inputs. The benefit cost ratio was determined by following equation:

Benefit cost ratio (BCR) = Total net return/Total input cost.

The wholesale price per kg of prawn was 400 taka. The wholesale price per kg of tilapia and rohu was 100 taka. The prices of inputs, fish and prawn correspond to the Mymensingh wholesale market prices in July to December 2008 and are expressed in Bangladeshi taka (1US\$ = 69 BDT).

2.9 Statistical analysis

Yield parameters (prawn and finfish growth, yield, FCR and survival) and economic parameters were analyzed by a 2-way ANOVA with CH source (maize flour and tapioca starch) and different stocking ratios of tilapia and rohu (100% tilapia, 50% tilapia+50% rohu, and 100% rohu) as main factors. Water quality, plankton, periphyton and benthic macroinvertebrates data were compared by repeated measures ANOVA with CH source (maize flour and tapioca starch) and different stocking ratios

of tilapia and rohu (100% tilapia, 50% tilapia+50% rohu, and 100% rohu) as main factors and time as the sub-factor (Gomez and Gomez, 1984). The assumptions of normal distributions and homogeneity of variances were checked before analysis. The percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA were tested at 5% level of significance using SPSS (Statistical Package for Social Science) version 14.

3 Results

3.1 Effects on water quality parameters

Mean values of water quality parameters and outcomes of ANOVA are presented in Table 2. The CH source for maintaining a high C:N ratio had no significant effect on any water quality parameters. There was no interaction effect of carbohydrate sources and finfish stocking ratio on water quality parameters as well. The finfish stocking ratio had also no significant effects on any of the measured water quality parameters at sunrise (7:00 h) and sunset (18:00 h) except bottom DO at sunset. The bottom dissolved oxygen was significantly higher in both treatments with tilapia as compared to ponds stocked with 100% rohu. Among other parameters, Secchi disc transparency was lower in treatment 50T/50R than in treatments 100R and 100T. The highest chlo-*a* concentration was observed in treatment 100R, intermediate in treatment 50T/50R and the lowest in 100T ponds. The concentration was found to decrease gradually during the experimental periods with the higher rate in treatment 100T followed by treatments 50T/50R and 100R, respectively (Figure 1). The NO_2–N concentration was always very low in all treatments compared to NO_3–N and NH_3–N concentration. All of the nitrogenous compounds were not affected by the stocking ratio of tilapia and rohu. Higher concentration of PO_4–P was observed in treatments with 100T and 50T/50R compared to the treatment 100R. The PO_4–P concentration was more or less similar during the experimental periods in 100R ponds but, it tends to increase gradually after the 1 month of experimental period in 100T and 50T/50R ponds and the rate of increase was higher in 100T ponds compared to 50T/50R ponds (Figure 1).
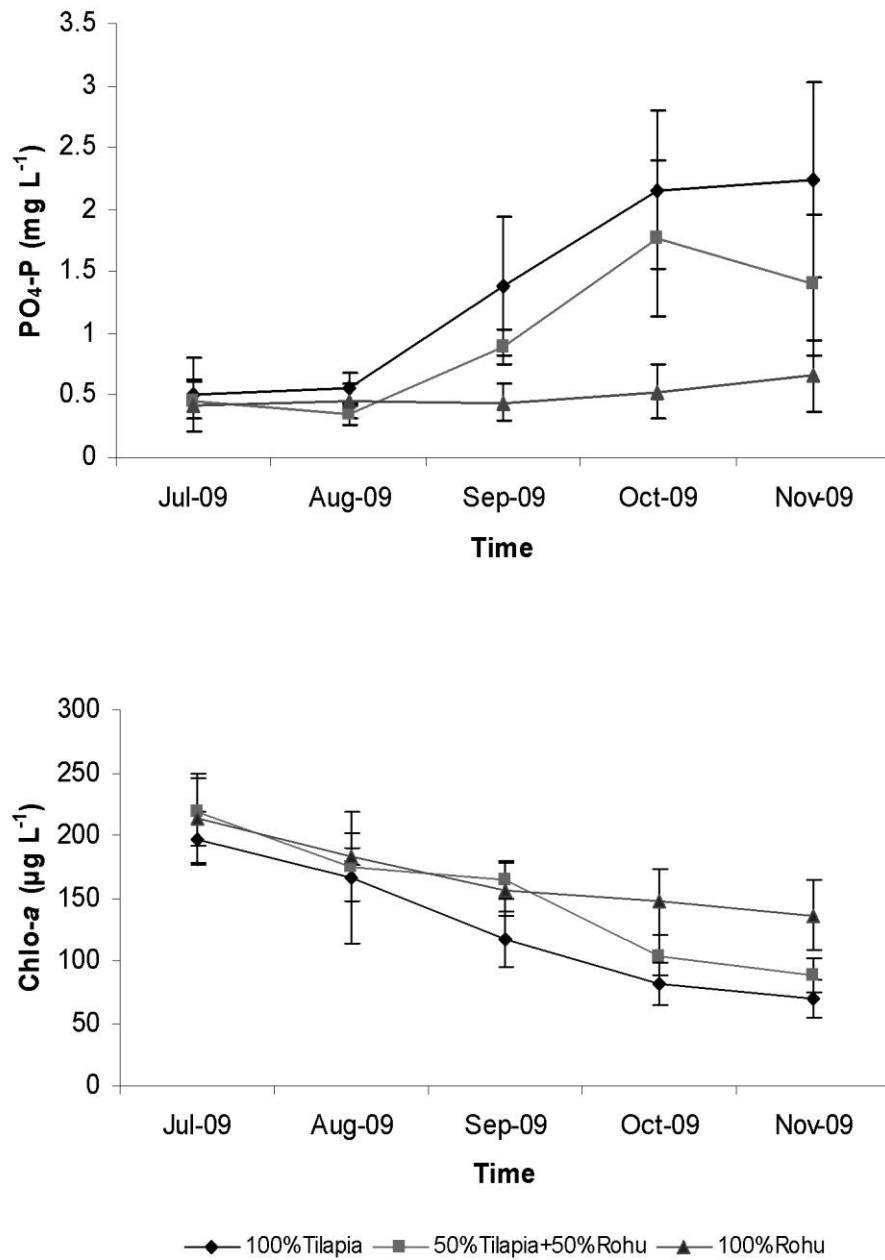
Figure 1. Mean (±SD) concentration of PO_4-P and Chlorophyll-*a* in different stocking ratio of tilapia and rohu (0.5 finfish m^{-2}) treatment ponds during the experimental periods in C/N-CP based freshwater prawn (2 individual m^{-2}) farming system.

Table 2. Effects of carbohydrate source and finfish stocking combinations on water quality parameters based on 2-way ANOVA

| Variable | Means (Tukey test)); Prawn 2 m^{-2} in all treatments | | | | | Significance (P value) | | |
| | CH source | | Finfish stocking ratio (0.5 individual m^{-2}) | | | | | |
	TS	MF	100T	50T/50R	100R	CH	F	CH×F
At Sunrise (7 am)								
Surface Temp. (°C)	27.3	27.2	27.3	27.3	27.4	NS	NS	NS
Bottom Temp. (°C)	27.1	27.0	26.9	27.0	27.1	NS	NS	NS
Surface DO (mg L^{-1})	4.99	5.01	5.03	5.01	4.97	NS	NS	NS
Bottom DO (mg L^{-1})	3.43	3.44	3.44	3.45	3.42	NS	NS	NS
Mean pH range	7.5-7.6	7.5-7.6	7.5-7.6	7.5-7.6	7.4-7.6	-	-	-
At sunset (6 pm)								
Surface Temp. (°C)	29.2	29.2	29.4	29.4	29.2	NS	NS	NS
Bottom Temp. (°C)	29.0	28.9	29.1	29.2	28.9	NS	NS	NS
Surface DO (mg L^{-1})	8.05	8.03	8.14	8.04	7.96	NS	NS	NS
Bottom DO (mg L^{-1})	5.22	5.29	5.48[a]	5.42[a]	4.85[b]	NS	***	NS
Mean pH range	7.8-7.9	7.7-7.8	7.7-7.8	7.8-8.0	7.7-7.8	-	-	-
At morning (10 am)								
Secchi depth (cm)	43.2	43.7	45.9[a]	39.9[b]	44.5[a]		**	NS
T. Alkalinity (mg L^{-1})	94.1	100.7	95.9	92.4	104.0	NS	NS	NS
Chlorophyll *a* (µg L^{-1})	150.1	145.7	126.3[b]	150.2[ab]	167.1[a]	NS	**	NS
NH$_3$-N (mg L^{-1})	0.203	0.195	0.189	0.198	0.209	NS	NS	NS
NO$_2$-N (mg L^{-1})	0.009	0.008	0.008	0.009	0.009	NS	NS	NS
NO$_3$-N (mg L^{-1})	0.056	0.059	0.065	0.057	0.051	NS	NS	NS
PO$_4$-P (mg L^{-1})	0.983	0.913	1.37[a]	0.97[a]	0.51[b]	NS	***	NS

CH = Carbohydrate source to increase C/N ratio from 10 to 20; TS = treatments with tapioca starch as CH source; MF= treatments with maize flour as CH source; 100T = treatment with 100% tilapia; 50T/50R = treatments with 50%tilapia + 50% rohu; 100R= treatments with 100% rohu; F = Finfish (tilapia+rohu) stocking ratio; CH×F = Interaction of carbohydrate source and finfish stocking ratio. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, Not significant.

3.2 Effects on the abundance of plankton and benthic macroinvertebrates

The abundance of plankton and benthos and outcomes of ANOVA are presented in Table 3. The plankton communities in pond water consisted of four groups of phytoplankton and two groups of zooplankton in all treatments. Forty two genera of phytoplankton belonging to Bacillariophyceae (11 genera), Chlorophyceae (21 genera), Cyanophyceae (7 genera) and Euglenophyceae (3 genera) were found. Chlorophyceae was the most dominant group in terms of number of genera and abundance (cells or colonies L^{-1}) among phytoplankton in each treatment. Seventeen genera of zooplankton, including eight genera of Rotifera and nine genera of Crustaceae were also identified. Among phytoplankton *Synedra, Tabellaria, Diatoma, Fragillaria, Cyclotella* and *Nitzschia* (Bacillariophyceae), *Chlorella, Sphaerocystes, Palmella, Pediastrum, Stigeoclonium, Ulothrix* and *Scenedesmus* (Chlorophyceae), *Microcystis, Anabaena* and *Gomphosphaeria* (Cyanophyceae), *Euglena* and *Phacus* (Euglenophyceae), and among zooplankton *Cyclops, Diaphanosoma* and Nauplius larvae (Crustaceae), and *Brachionus, Asplanchna, Trichocerca, Polyarthra* and *Filinia* (Rotifera) were the dominating genera.

CH sources for maintaining a high C:N ratio had no significant effect on the abundance of any major group of phytoplankton and zooplankton. However, stocking combination of finfish affected the abundance of all the groups of phytoplankton and zooplankton. The mean abundance of all groups of phytoplankton was higher in treatment 100R except Bacillariophyceae and lower in treatment 100T. The opposite trend was observed with zooplankton. The mean abundance of all groups of zooplankton was higher in treatment 100T and lower in treatment 100R. The interaction between carbohydrate source and finfish stocking ratio had significant effect on zooplankton abundance only indicating that the carbohydrate source affected zooplankton abundance differently in different stocking combinations. All major groups of phytoplankton were tending to decrease for the first two months then increased gradually during the experimental periods (Table 4). In the case of zooplankton, the trend was not similar as phytoplankton during the culture period. The variations in abundance of phytoplankton and zooplankton during experimental periods are shown in Figure 2.

The benthic macroinvertebrate were divided into Chironomidae, Oligochaeta, Mollusca and un-identified groups. Chironomidae followed by Oligochaeta was the most dominant groups among benthos in each treatment. CH sources for maintaining a high C:N ratio had no effect on the abundance of any major group of benthic macroinvertebrate. Stocking ratio of finfish affected the abundance of Chironomidae

and Oligochaeta among all identified groups of benthic macroinvertebrates and the mean values were higher in treatment 100R and lower in treatment 100T. As a result, total benthos was 41% higher in number in treatment 100R than in treatment 100T. The number of all major groups increased during the first month then decreased gradually during the culture period except Mollusca (Table 4). The abundance of total benthos was found to decrease gradually during the experimental periods with the higher rate in treatment 100T followed by treatments 50T/50R and 100R, respectively (Figure 2).
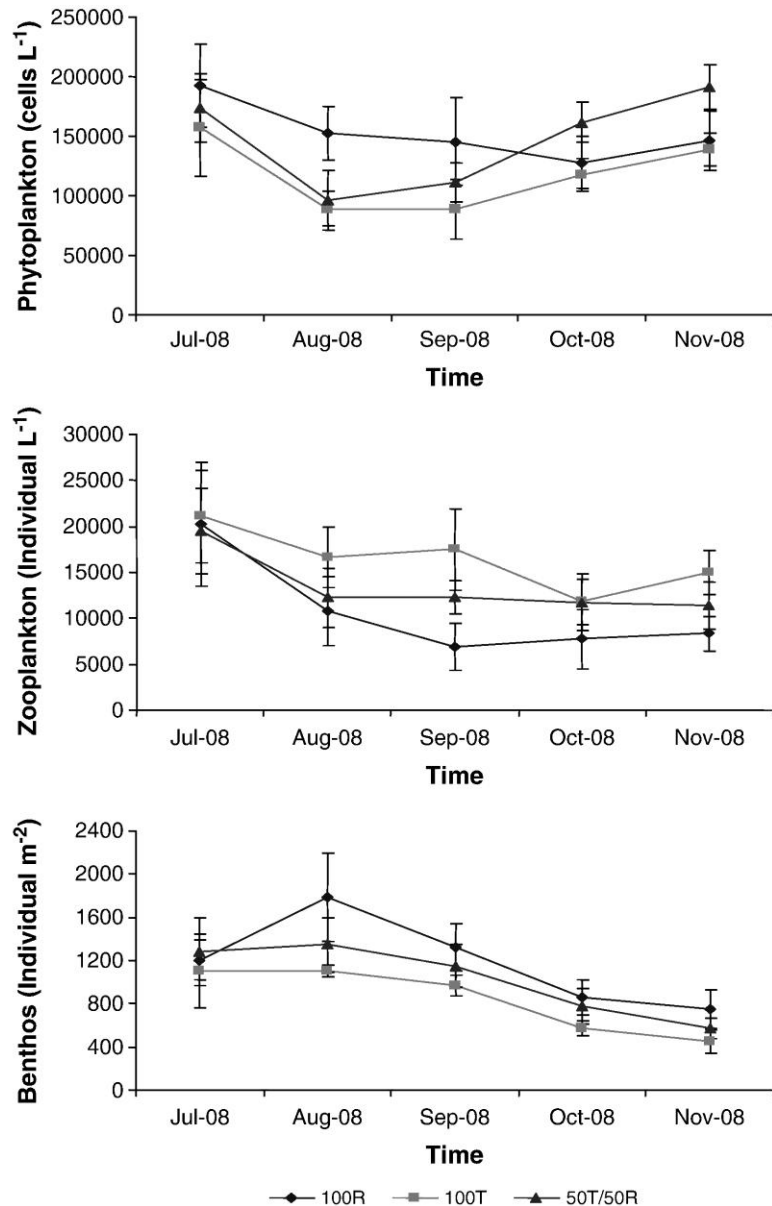
Figure 2. Mean ($\pm$SD) abundance of phytoplankton, zooplankton and benthos in different stocking combination of tilapia and rohu (0.5 finfish m^{-2}) treatment ponds during the experimental periods in C/N-CP based freshwater prawn (2 individual m^{-2}) farming system

Table 3

Effects of carbohydrate sources and finfish stocking combinations on abundance of plankton and benthos based on 2-way ANOVA

	Means (Tukey test)); Prawn 2 m^{-2} in all treatments					Significance (*P* value)		
Variables	CH source		Finfish stocking ratio (0.5 individual m^{-2})					
	TS	MF	100T	50T/50R	100R	CH	F	CH×F
Plankton (×10³ cells or colonies L^{-1})								
Bacillariophyceae	17.18	16.49	16.00^{b}	18.87^{a}	15.63^{b}	NS	*	NS
Chlorophyceae	89.21	90.43	76.12^{b}	95.82^{a}	97.53^{a}	NS	***	NS
Cyanophyceae	28.08	31.80	24.05^{b}	29.43^{ab}	36.33^{a}	NS	***	NS
Euglenophyce	2.61	2.76	2.03^{b}	2.78^{a}	3.23^{a}	NS	***	NS
Total phytoplankton	137.1	141.5	118.2^{b}	146.9^{a}	152.7^{a}	NS	***	NS
Crustacea	6.91	6.31	8.20^{a}	6.40^{b}	5.23^{b}	NS	***	NS
Rotifera	6.61	6.46	8.21^{a}	5.82^{b}	5.57^{b}	NS	***	*
Total zooplankton	13.52	12.77	16.41^{a}	12.22^{b}	10.80^{b}	NS	***	*
Total plankton	137.07	141.48	118.2^{b}	146.9^{a}	152.7^{a}	NS	***	NS
Benthos (individual m^{-2})								
Chironomidae	621	573	473^{c}	586^{b}	731^{a}	NS	***	*
Olligochaeta	253	266	211^{b}	270^{ab}	298^{a}	NS	***	NS
Mollusca	137	133	133	145	129	NS	NS	NS
Un-identified groups	25	25	24	25	26	NS	NS	NS
Total benthos	1036	997	841^{c}	1026^{b}	1184^{a}	NS	***	NS

CH = Carbohydrate source to increase C/N ratio from 10 to 20; TS = treatments with tapioca starch as CH source; MF= treatments with maize flour as CH source; 100T = treatment with 100% tilapia; 50T/50R = treatments with 50%tilapia + 50% rohu; 100R= treatments with 100% rohu; F = Finfish (tilapia+rohu) stocking ratio; CH×F = Interaction of carbohydrate source and finfish stocking ratio. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *P<0.05; **P<0.01; ***P<0.001; NS, Not significant

Table 4

Abundance of plankton and benthos over the sampling periods.[ψ]

Variables	Sampling periods					Significance[Φ] P value
	Initial	Period 1	Period 2	Period 3	Period 4	
Plankton (×10³ cells or colonies L⁻¹)						
Bacillariophyceae	20.86[a]	14.19[b]	14.89[b]	16.31[b]	17.92[ab]	***
Chlorophyceae	117.13[a]	69.11[c]	76.39[c]	85.44[bc]	101.03[ab]	***
Cyanophyceae	33.36[a]	26.81[b]	22.06[b]	30.86[ab]	36.61[a]	*
Euglenophyce	2.97[a]	2.47[ab]	1.92[b]	3.03[a]	3.03[a]	*
Total phytoplankton	174.33[a]	112.58[c]	115.25[c]	135.64[bc]	158.58[ab]	***
Crustacea	9.53[a]	7.53[ab]	6.39[bc]	4.69[c]	4.92[c]	***
Rotifera	8.69[a]	5.69[b]	5.86[b]	5.75[b]	6.66[ab]	***
Total zooplankton	18.22[a]	13.22[b]	12.25[b]	10.44[b]	11.58[b]	***
Total plankton	174.33[a]	112.58[c]	115.25[c]	135.64[bc]	158.58[ab]	***
Benthos (individual m⁻²)						
Chironomidae	849[a]	857[a]	657[b]	363[c]	260[c]	***
Olligochaeta	188[b]	392[a]	323[a]	225[b]	169[b]	***
Mollusca	134	129	135	131	149	NS
Un-identified groups	28[ab]	33[a]	32[a]	21[bc]	12[c]	***
Total benthos	1198[b]	1410[a]	1147[b]	739[c]	589[c]	***

Mean values in the same row with no superscript letter in common differ significantly ($P<0.05$).
[ψ] One sampling period is 30 days
[Φ] Results from repeated measures 2- way ANOVA
*$P<0.05$ ** $P<0.01$; *** $P<0.001$

3.3 Effects on periphyton biomass

Periphyton DM, ash, AFDM, Chlo-*a*, and autotrophic index per unit substrate surface area are given in Table 5. CH sources for maintaining a high C:N ratio had no effect on any of the parameters of periphyton biomass. Mean values of all of these parameters were significantly higher in treatment 100R than in treatment 100T except autotrophic index but treatment 50T/50R had no significant difference with either treatment. The DM, ash, AFDM and chlo-a contents increased during the first month after which they constantly reduced in all treatments during the experiment (Figure 3).

Table 5

Effects of carbohydrate sources and finfish stocking combinations on periphyton biomass scraped from bamboo *kanchi* based on 2-way ANOVA

Variables	Means (Tukey test)); Prawn 2 m^{-2} in all treatments					ANOVA Significance *P*-value		
	CH source		Finfish stocking ratio (0.5 individual m^{-2})					
	TS	MF	100T	50T/50R	100R	CH	F	CH×F
Dry matter (mg cm^{-2})	2.27	2.30	2.02^b	2.27^{ab}	2.56^a	NS	*	NS
Ash (mg cm^{-2})	0.76	0.76	0.82^b	0.77^{ab}	0.88^a	NS	**	NS
Ash free DM (mg cm^{-2})	1.51	1.54	1.40^b	1.50^{ab}	1.68^a	NS	*	NS
Chlo. *a* (µg cm^{-2})	9.68	9.62	8.91^b	9.47^{ab}	10.57^a	NS	*	NS
Autotrophic index	156.3	158.3	155.2	158.6	158.1	NS	NS	NS

CH = Carbohydrate source to increase C/N ratio from 10 to 20; TS = treatments with tapioca starch as CH source; MF= treatments with maize flour as CH source; 100T = treatment with 100% tilapia; 50T/50R = treatments with 50%tilapia + 50% rohu; 100R= treatments with 100% rohu; F = Finfish (tilapia+rohu) stocking ratio; CH×F = Interaction of carbohydrate source and finfish stocking ratio. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, Not significant.

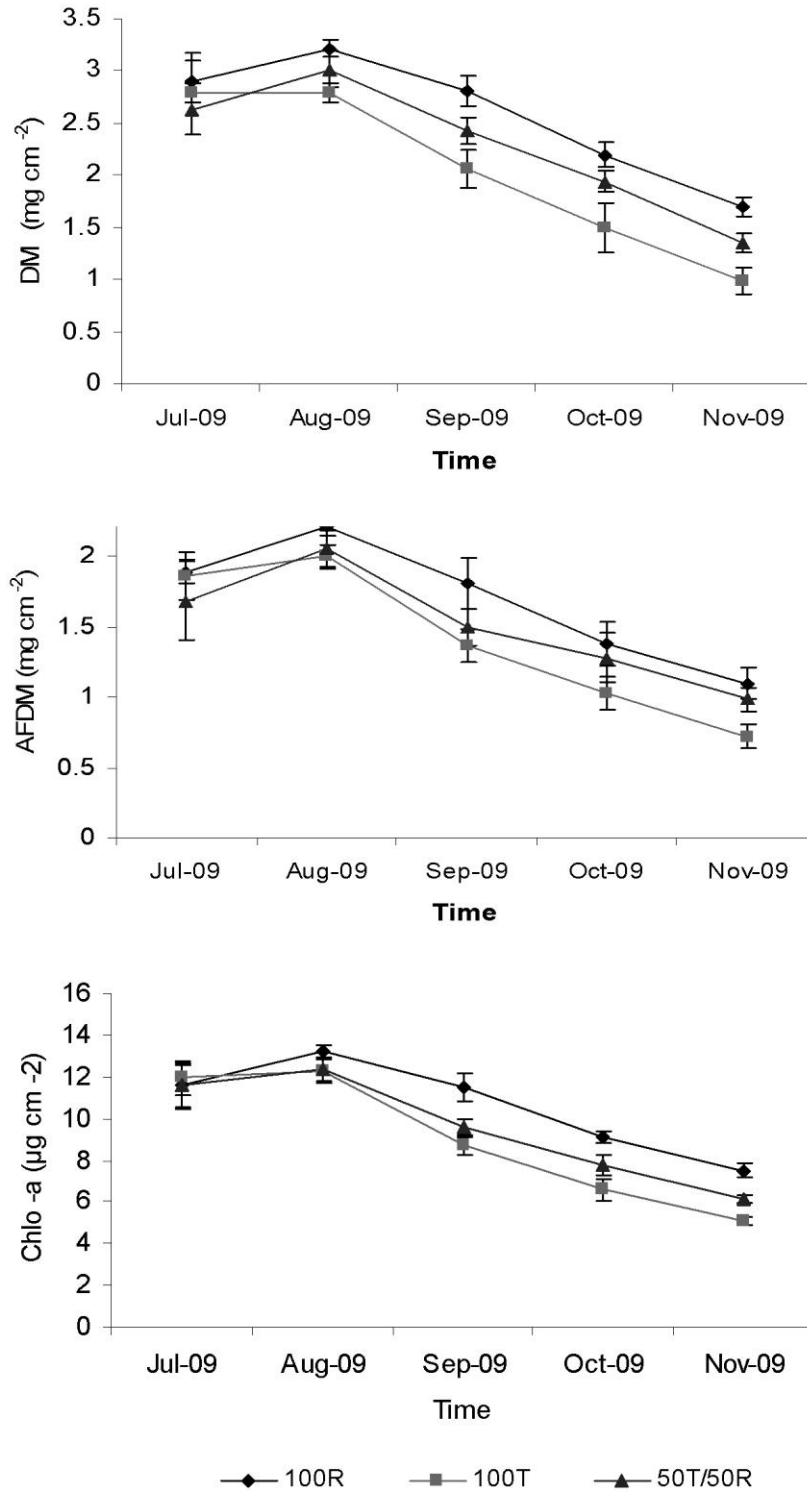**Figure 3.** Quantity of periphyton biomass per unit surface area in different stocking ratio of tilapia and rohu (0.5 finfish m^{-2}) treatment ponds during the experimental periods in C/N-CP based freshwater prawn (2 individuals m^{-2}) farming system. Values are means ($\pm$SD) of three replicates (each replicate was composed by three poles and three depth samples) per sampling date in each treatment.

3.4 Effects on growth and yield parameters of freshwater prawn and finfish

Effects of carbohydrate sources and finfish stocking ratio and their interactions on yield parameters of freshwater prawn and finfish are given in Table 6. The carbohydrate sources had no effect on the growth and yield parameters of freshwater prawn, tilapia, rohu and their combination. Although stocking ratio of finfish did not affect freshwater prawn, the ratio had significant effects on the growth and yields of the finfish themselves. Individual harvesting weight of tilapia was 23% higher in treatment 50T/50R compared to treatment 100T. On the other hand, the survival of rohu was significantly higher in treatment R100 compared to treatment T50/R50 but individual harvesting weight did not vary significantly among the treatments. The net yield of finfish in treatment 100T was 17% and 113% higher as compared to treatments 50T/50R and 100R, respectively.

3.5 Economic comparison

The benefit–cost analysis of different treatments is shown in Table 7. Freshwater prawn juveniles, feed, tapioca starch or maize flour and the substrates were the most expensive cost inputs. The extrapolated costs of all variable inputs were significantly higher in TS treatment than in MF treatment ponds. The total input cost was similar among different stocking ratio of tilapia and rohu ponds. The total return was significantly higher in treatments 100T and 50T/50R than in treatment 100R but the CH sources had no effect on it. The economic analysis showed that the benefit–cost ratio was 35% higher in MF treatment than in TS treatment. Again, the benefit–cost ratio in treatment T100 was 31% and 137% higher when compared with treatments T50/R50 and R100, respectively.

Table 6 Effects of carbohydrate sources and finfish stocking combinations on production of freshwater prawn and finfish based on 2-way ANOVA

| Variables | Means (Tukey test)); Prawn 2 m^{-2} in all treatments | | | | | Significance (*P* value) | | |
| | CH source | | Finfish stocking ratio (0.5 individual m^{-2}) | | | | | |
	TS	MF	100T	50T/50R	100R	CH	F	CH×F
M. rosenbergii								
In. Stocking wt. (g)	0.81	0.82	0.82	0.81	0.82	NS	NS	NS
In. harvesting wt. (g)	37.8	36.9	38.1	37.3	36.6	NS	NS	NS
Food conversion ratio	2.06	2.19	2.08	2.19	2.09	NS	NS	NS
Survival (%)	77.9	77.2	77.7	77.0	77.9	NS	NS	NS
Gr. Yield (kg ha^{-1} 120 day^{-1})	586	568	592	571	567	NS	NS	NS
Net yield (kg ha^{-1} 120 day^{-1})	570	552	575	555	551	NS	NS	NS
O. niloticus								
In. Stocking wt. (g)	27.8	27.9	27.7	28.0	-	NS	NS	NS
In. harvesting wt. (g)	289.2	293.5	261.1^{b}	321.6^{a}	-	NS	**	NS
Survival (%)	96.7	97.5	97.5	96.7	-	NS	NS	NS
Gr. Yield (kg ha^{-1} 120 day^{-1})	1014	1035	1272^{a}	777^{b}	-	NS	***	NS
Net yield (kg ha^{-1} 120 day^{-1})	909	930	1133^{a}	707^{b}	-	NS	***	NS
L. rohita								
In. Stocking wt. (g)	27.6	27.6	-	27.7	27.5	NS	NS	NS
In. harvesting wt. (g)	171.1	161.2	-	177.0	155.1	NS	NS	NS
Survival (%)	81.7	80.0	-	75.0^{b}	86.7^{a}	NS	***	NS
Gr. Yield (kg ha^{-1} 120 day^{-1})	523	480	-	333^{b}	670^{a}	NS	***	NS
Net yield (kg ha^{-1} 120 day^{-1})	419	376	-	264^{b}	531^{a}	NS	***	NS
Combined finfish								
Survival (%)	90.0	90.0	97.5^{a}	85.8^{b}	86.7^{b}	NS	***	NS
Gr. Yield (kg ha^{-1} 120 day^{-1})	1024	1010	1272^{a}	1110^{b}	670^{c}	NS	***	NS
Net yield (kg ha^{-1} 120 day^{-1})	886	871	1133^{a}	970^{b}	532^{c}	NS	***	NS
Prawn & finfish								
Gr. Yield (kg ha^{-1} 120 day^{-1})	1611	1578	1864^{a}	1681^{b}	1237^{c}	NS	***	NS
Net yield (kg ha^{-1} 120 day^{-1})	1456	1423	1709^{a}	1525^{b}	1083^{c}	NS	***	NS

CH = Carbohydrate source to increase C/N ratio from 10 to 20; TS = treatments with tapioca starch as CH source; MF= treatments with maize flour as CH source; 100T = treatment with 100% tilapia; 50T/50R = treatments with 50%tilapia + 50% rohu; 100R= treatments with 100% rohu; F = Finfish (tilapia+rohu) stocking ratio; CH×F = Interaction of carbohydrate source and finfish stocking ratio. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *P<0.05; **P<0.01; ***P<0.001; NS, Not significant.

Table 7. Effects of carbohydrate sources for maintaining a high C:N ratio and tilapia driven re-suspension on economic parameters per factor based on 2-way ANOVA. Calculation was based on 1 ha pond and 120 days experimental period. Currencies are given in Bangladeshi Taka, BDT (1 US$ = 69 BDT).

Variables	Amount	Price rate	Means (Tukey test); Prawn 2 individual m^{-2} in all treatments						ANOVA Significance (P value)		
			CH Source		Finfish stocking ratio (0.5 fish m^{-2})							
			TS	MF	100T	50T/50R	100R		CH	F	CH×F
Fixed/common cost											
Land rental cost	1 ha	21,000 ha^{-1} y^{-1}	7000	7000	7000	7000	7000		-	-	-
Labor	50 man-day	120 man-day^{-1}	6000	6000	6000	6000	6000		-	-	-
Rotenone	12.5 kg	220 kg^{-1}	2750	2750	2750	2750	2750		-	-	-
Lime	250 kg	10 kg^{-1}	2500	2500	2500	2500	2500		-	-	-
Cowdung	3000 kg	0.5 kg^{-1}	1500	1500	1500	1500	1500		-	-	-
Urea	100 kg	10 kg^{-1}	1000	1000	1000	1000	1000		-	-	-
TSP	100 kg	25 kg^{-1}	2500	2500	2500	2500	2500		-	-	-
Fuel cost	500 units	4 $unit^{-1}$	2000	2000	2000	2000	2000		-	-	-
Substrates (reuse-5 times)	150,000 Pieces	1 $piece^{-1}$	30,000	30,000	30,000	30,000	30,000		-	-	-
Subtotal			55,250	55,250	55,250	55,250	55,250		-	-	-
Variable cost											
Prawn juveniles	20,000 ha	4 $juvenile^{-1}$	80,000	80,000	80,000	80,000	80,000		-	-	-
Tilapia juveniles		2.5 $juvenile^{-1}$	12,500	12,500	25,000[a]	12,500[b]	-		NS	***	NS
Rohu juveniles		3 $juvenile^{-1}$	15,000	15,000	-	15,000[b]	30,000[a]		NS	***	NS
Feed		25 kg^{-1}	30,069	30,902	30,729	31,250	29,479		NS	NS	NS
Tapioca starch/maize flour		30/12 kg^{-1}	27,062[a]	19,283[b]	23,401	23,783	22,333		***	NS	NS
Subtotal			164,631[a]	138,402[b]	149,510	152,718	152,322		***	NS	NS
Total			219,881[a]	193,653[b]	204,760	207,968	207,572		***	NS	NS
Interest on inputs (4 months)		10% anually	7329[a]	6455[b]	6825	6932	6919		***	NS	NS
Total inputs			227,211[a]	200,107[b]	211,585	214,901	214,492		***	NS	NS
Financial returns											
Prawn sale		400 kg^{-1}	234,500	227,177	236,950	228,516	227,050		NS	NS	NS
Tilapia sale		100 kg^{-1}	67,566	68,989	127,183[a]	77,650[b]	-		NS	***	NS
Rohu sale		80 kg^{-1}	27,895	25,591	-	26,660[b]	53,575[a]		NS	***	NS
Total returns			329,962	321,757	364,133[a]	332,827[a]	280,620[b]		NS	***	NS
Total net returns			102,751[b]	121,650[a]	152,547[a]	117,925[b]	66,127[c]		**	***	NS
Benefit cost ratio (BCR)			0.453[b]	0.610[a]	0.730[a]	0.556[b]	0.308[c]		**	***	NS

CH = Carbohydrate source to increase C/N ratio from 10 to 20; TS = treatments with tapioca starch as CH source; MF= treatments with maize flour as CH source; 100T = treatment with 100% tilapia; 50T/50R = treatments with 50%tilapia + 50% rohu; 100R= treatments with 100% rohu; F = Finfish (tilapia+rohu) stocking ratio; CH×F = Interaction of carbohydrate source and finfish stocking ratio. The mean values followed by the different superscript letter in each factor indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, Not significant.

4 Discussion

4.1 Effects on water quality parameters

The water temperature, dissolved oxygen and pH were within the suitable range for freshwater prawn and tropical fish culture (Zimmermann and Boyd, 2000; New, 2002). The observed higher bottom dissolved oxygen at sunset in ponds stocked with freshwater prawn and tilapia only was mainly due to the bioturbation effects by tilapia. Previous research reported that bioturbutary activities of tilapia can bring some DO downwards to the lower layers of the water column, improving aerobic conditions on the pond bottom (Phan-Van et al., 2008; Jiménez-Montealegre et al., 2002). That such an effect on bottom dissolved oxygen by tilapia was not found at sunrise might be due to the less availability of surface oxygen and poor bioturbutary effects by tilapia at very early morning. The observed lower concentration and decreasing trend of chlo-a in this treatment (Figure 1) might be due to higher turbidity reducing photosynthesis and hence primary production or higher grazing on phytoplankton by tilapia than rohu. Perschbacher and Lorio (1993) reported that tilapia stocked at densities higher than 5000 ha^{-1} promoted a very effective biological control over phytoplankton. The similar inorganic N-species concentrations and other water quality parameters in ponds supplied with both maize flour and tapioca starch showed the possibility of using low-cost maize flour as cheap CH source for maintaining good water quality in C/N-CP system. The very low nitrogenous compounds in all treatments compared to other studies of freshwater prawn farming (e.g.Wahab et al., 2008; Kunda et al., 2008) could be attributed to the addition of carbohydrate to maintain a C/N ratio of 20 and periphyton substrates during the experimental period (Asaduzzaman et al., 2008, 2009a,b). The observed higher level of PO$_4$–P in ponds stocked with tilapia alone and tilapia–rohu together indicated that tilapia re-suspension induced nutrient release from the accumulated organic matter of the sediment into the water phase through the mud–water exchange mechanism, which enhances the overlying water PO$_4$–P concentration (Jana and Das, 1992; Jana and Sahu, 1993; Saha and Jana, 2003).

4.2 Effects on abundance of plankton and benthic macroinvertebrates

The abundance of plankton and benthic macroinvertebrates in the culture system is influenced by a number of management factors, among them fish species combinations in polyculture, stocking density and ratio, and the nutrient input quality and quantity are most important (Milstein, 1993; Diana et al., 1997). Apart from these

management factors, fish feeding habits have an important influence on the abundance of plankton and benthos, both directly by consumption and indirectly through influencing the food web and nutrient availability. The phytoplankton species composition identified in the present experiment was representative of that found in Bangladesh prawn farming in rice fields and ponds (Asaduzzaman et al., 2009b; Wahab et al., 2008; Kunda et al., 2008; Uddin, 2007). The similar abundance of plankton and benthic macroinvertebrate in MF ponds and TS ponds reflected that both CH sources in C/N-CP system had the same effect. The comparatively lower abundance of phytoplankton in ponds with tilapia as finfish compared to ponds with rohu as finfish indicated that tilapia grazes on phytoplankton more efficiently than rohu does. In periphyton-based freshwater prawn-tilapia polyculture, Uddin (2007) reported that electivity indices of tilapia were negative for all zooplankton and positive for all phytoplankton groups except Bacillariophyceae, indicating that it preferred phytoplankton above zooplankton. In contrast, the abundance of zooplankton was lower in ponds with rohu compared to ponds with tilapia, indicating that rohu had a stronger preference for zooplankton than tilapia. Rohu is a column feeder browsing on zooplankton and decaying organic matter (Das and Moitra, 1955). Rahman (2006) showed that rohu's electivity indices were positive for all zooplankton groups and negative for all phytoplankton groups, confirming that it preferred zooplankton over phytoplankton. They concluded that in fed ponds, rohu ingested 1.3 times more zooplankton than phytoplankton although the abundance of phytoplankton was higher than zooplankton. Again, this result in a way agrees with Miah et al. (1984), who reported that zooplankton is a more preferable food item than phytoplankton for rohu fry. In addition, tilapias re-suspend sediments, thereby influencing nutrient availability in the water column, which in turn affects photosynthesis and subsequently phytoplankton production. The observed decrease and/or similar abundance of phytoplankton during the first two months might be attributed to grazing by tilapia. The steadily increase in abundance of phytoplankton after the second month might be due to increased nutrient (mainly PO_4–P) re-suspension by tilapia of increasing body size. Avnimelech et al. (1999) reported that tilapias do appreciably re-suspend sediment, and such activity is more pronounced in large fish. The observed lowest abundance of Chironomidae, Oligochaeta and total benthic macroinvertebrate in tilapia ponds might indicate that tilapia directly feed on these benthic fauna or indirectly facilitated the feeding by freshwater prawn during sediment burrowing. Zur (1980) reported that tilapia is omnivorous and feeds on

benthic detritus and fauna too. Chironomid and some other benthic larvae dwell from a few millimeters to several centimeters deep in the sediment (Winkel, 1987). Therefore, prawn predation on chironomids may be facilitated due to the digging and sieving of sediments by tilapia. The observed decrease in number of benthos during the culture period might be due to increased grazing pressure by prawn and tilapia (Asaduzzaman et al., 2009b). There is evidence that prawns in their natural habitats prefer to forage on animals like trichopterans, chironomids, oligochaetes, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996; Tidwell et al., 1997).

4.3 Effects on periphyton biomass

The similar effects of maize flour and tapioca starch on periphyton biomass showed the potential of using low-cost maize flour instead of relatively high-cost tapioca starch for improving the periphyton quality in C/N-CP ponds. The observed lower level of periphytic biomass per unit surface area in tilapia ponds compared to rohu ponds indicates that periphyton is more effectively utilized by tilapia. Tilapias are omnivores capable of feeding on benthic and attached (periphyton) algal and detrital aggregates (Dempster et al., 1993; Azim et al., 2003a). Laboratory-based grazing trials also indicated that tilapias can ingest more plant based food per unit time when presented as periphyton than as plankton (Dempster et al., 1993, 1995). There are similar evidences that Nile tilapia shows better grazing on periphyton than filtering suspended algae from water column (Hem and Avit, 1994; Guirat et al., 1995; Huchette et al., 2000; Azim et al., 2003b). Again, rohu is known to be a predominantly column-feeding fish but it also feeds on periphyton in ponds provided with substrates (NFEP, 1997; Ramesh et al., 1999; Azim et al., 2001). Stable isotope analysis confirmed that rohu mostly relied on periphyton for food in periphyton-based system (Azim et al., 2002). Indeed, both species grazed on periphyton as evident by the gradual decrease of periphyton biomass in all treatment throughout the experiment as apparent in Figure 3 but it also indicated that tilapia is more efficient than rohu. The low range of AI values also indicated continuous grazing pressure on periphyton mass (Huchette et al., 2000). AI values between 100 and 200 are considered as algae dominating periphytic matter (APHA, 1992). Algae typically grow on the outer surfaces of substrate where there is sufficient sunlight and are continuously harvested

by fish grazing. The periphyton biomass in all ponds increased during the first month followed by a continuous decrease until the end of the experiment (Figure 3). This might be accounted for by changes in the tilapia and rohu grazing pressure on periphyton. The low biomass of tilapia and rohu initially exerted low grazing pressure allowing periphyton to grow and later, with increased fish biomass, grazing pressure led to reduced periphyton biomass.

4.4 Effects on growth and yield parameters of prawn and finfish

The growth and production performances of prawn and finfish were similar between TS and MF ponds. This may be due to the fact that both CH sources had the similar effects on water quality parameters and abundance of plankton, benthic macroinvertebrate and periphyton biomass. Therefore, it can be considered that maize flour can benefit the freshwater prawn farming like tapioca starch through reducing toxic inorganic nitrogen content, increasing heterotrophic bacteria and algal abundance and improving periphyton productivity (Asaduzzaman et al., 2008). In previous studies, different carbohydrate sources like tapioca starch (Asaduzzaman et al., 2008, 2009a,b), tapioca flour (Hari et al., 2004), mollases (Burford et al., 2004), glucose and cassava meal cellulose powder (Avnimelech and Mokady, 1988; Avnimelech et al., 1989; Avnimelech, 1999) were used in prawn, shrimp and finfish ponds to improve the water quality and productivity of ponds. Pond ecological and growth data revealed that maize flour can be a good source of organic carbon to maintain a high C:N ratio in C/N-controlled periphyton-based freshwater prawn ponds. Freshwater prawn production was not affected by the different stocking combinations of rohu and tilapia indicating that feeding niches of freshwater prawn did not or only partially overlapped with tilapia and/or rohu. In periphyton-based system, tilapia and/or rohu mainly depend on plankton and periphyton (Asaduzzaman et al., 2009a; Uddin, 2007; Ramesh et al., 1999; Azim et al., 2001); whereas previous research reported that plankton and periphyton had very little contribution to a prawn's diet (Asaduzzaman et al., 2008; Uddin, 2007). Uddin (2007) showed that in mixed culture the feeding niches of tilapia and prawn only partially overlap. The observed highest net yield of finfish in tilapia ponds might be due to the fast growth rate, more efficient utilization of natural food and bioturbation effects by tilapia as compared to rohu. The realistic economic analysis revealed that the use of maize flour in C/N-CP system

reduced the carbohydrate cost thereby improving the economic benefits. Economic benefit can be increased further by stocking only tilapia rather than tilapia–rohu or only rohu in C/N-controlled periphyton-based freshwater prawn farming. Market price of tilapia was 100 BDT whereas rohu was 80 BDT. However, if the rohu were grown to 1 kg, it would be 250 BDT. On the other hand, market size of freshwater prawn should be at least 50 g which was not achieved in the present experiment. Therefore, economic analysis based on short term culture period is questionable especially for rohu. However, here is the advantage of culturing tilapia; it reached market size in 3–4 months.

Conclusion

Based on the findings of the present research, maize flour can be considered as an alternative cheap on-farm carbohydrate source due to its low costs, local production and wide utilization by the farmers as a fish and animal feed ingredients. The findings of the present research also confirmed that tilapia (0.5 fish m^{-2}) driven re-suspension in freshwater prawn ponds improved the natural food utilization efficiency, pond productivity and economic benefit. The result of the present study could be useful in improving the economic sustainability of freshwater prawn farming in C/N-CP system. There exists scope for further improvement of economic sustainability of this technology by comparing the potential of other cheap carbohydrate sources such as sugarcane wastes and molasses. In the present study it was not possible to estimate the contribution of artificial feed and different types of natural food to the growth of freshwater prawn, tilapia and rohu. Therefore, studies with labeled ^{13}C or ^{15}N ingredients could help in tracing the utilization of organic carbon and inorganic nitrogen by different flora and elucidating food webs in ponds, and is subject of further research.

Acknowledgement

7

General Discussion

Introduction

In semi-intensive aquaculture, manufactured feed constitutes the main nutrient input (Hargreaves, 1998) and is the most expensive cost factor for production (Sevilleja, 1985). Cultured animals retain on average 30-50% of the feed, the rest contributing directly or indirectly to the nutrient load of water and sediment (Naylor et al., 2000). The microbial decomposition of organic matter in the system leads to an increased levels of TAN and nitrite, both harmful to fish at low concentrations. In stagnant water ponds, TAN tends to accumulate within the system due to insufficient nitrification activity (Grommen et al., 2002). Deteriorated water quality has resulted in disease outbreaks, low productivity and heavy financial losses and in criticism from various environmental organizations as being environmentally irresponsible. Therefore, farmers need new production sustainable management concepts for stagnant ponds, preferentially relying on locally available resources and requiring little investment (see review of Azim and Little, 2006). The major aim of this thesis was to develop a sustainable methodology for stagnant ponds not requiring a massive investment which is common to intensive systems. In order to achieve the goal, we combined C:N ratio control, periphyton technology and fish driven re-suspension into a low cost technology, further referred to as **C/N-controlled periphyton-based (C/N-CP) technology**, applicable by small scale farmers. The underlying principle of enhancing pond productivity and sustainability in this system is based on the stimulation of suspended and attached bacteria and algae development, and by using them to improve water quality, provide additional food and improve nutrient efficiency.

We first observed the effects of C/N ratio control and substrates addition for periphyton development on water quality and production of freshwater prawn. Secondly, we investigated how C/N ratio control and addition of substrates influenced the natural food communities in freshwater monoculture ponds. Thirdly, we studied the effects of increasing stocking density of prawn and addition of different level of tilapia on pond ecology and production in C/N-CP ponds. Fourthly, we studied the effects of addition of periphyton substrates and tilapias on pond ecology and production in C/N controlled system. Finally, we determined the effects of carbohydrate source to identify a cheaper on-farm carbohydrate to maintain a high

C:N ratio and fish driven re-suspension on pond ecology and production in C/N-CP ponds.

In this chapter, the results from the above-mentioned studies are synthesized and cross-checked, while highlighting the major conclusion. We also outline strength and weakness of the followed approach and finally identified areas for further research.

C/N ratio control reduced toxic nitrogenous compounds through immobilization by bacterial biomass

In aquatic systems the values measured in nitrogenous compounds reflect the result of a wide range of biological and chemical processes that occur simultaneously. The uneaten feed and feces contribute to the organic matter load in the sediment of ponds. In stagnant ponds, the oxygen supply to the bottom sediment is limited. Mineralization of accumulated organic matter under anaerobic conditions leads to the formation of toxic metabolites like TAN, spoiling the living environment of the cultured organisms (Fast and Boyd, 1992; Hopkins et al., 1994; Avnimelech and Ritvo, 2003).

Bacterial immobilization of ammonia in aquaculture ponds can be promoted by manipulating the C:N ratio of the nutrient input (Avnimelech et al., 1989; Avnimelech, 1999; Burford et al., 2004; Hari et al., 2004). The C:N ratio of most of the feeds used in semi-intensive aquaculture ponds is around 10:1, but bacteria require about 20 units of carbon per unit of nitrogen assimilated (Avnimelech, 1999). If the C:N ratio is increased by adding a carbohydrate source in addition to the regular feed, the increased availability of carbon allows the heterotrophic bacterial population to grow to a dense mass. We observed increasing C/N ratio from 10 to 20 significantly increased total heterotrophic bacterial abundance in water and sediment by 70% and 36%, respectively **(Chapter 2)**. Under aerobic condition, microbial breakdown of organic matter leads to the production of new bacterial biomass, amounting to 40–60% of the metabolized organic matter (Avnimelech, 1999).

The accumulation of inorganic nitrogen in a stagnant pond can be minimized by: (1) addition of organic carbon sources with a wide C:N ratio and (2) reduction of feed protein content. In our study, we increased C/N ratio from 10 (as in feed) to 15 and 20

by adding 0.45 and 0.9 kg tapioca starch for each kg of formulated feed, respectively. Increasing the C/N ratio from 10 to 20 significantly reduced the TAN concentration by 67.2% and NO_2–N by 36.4% **(Chapter 2)**, with a large fraction of the input N incorporated in new bacteria cells (single cell protein). This promoted nitrogen uptake by bacterial growth decreased the toxic nitrogenous compounds more rapidly than nitrification (Figure 1). The observed significant reduction in NO_2–N concentration in the water column could be attributed to low availability of TAN as substrate for nitrification (Avnimelech, 1999; Hari et al., 2004). The inhibition of nitrification in aquatic environments by organic carbon was reported previously (Hanaki *et al*., 1990; Strauss and Lamberti 2000, 2002). Strauss and Lamberti (2000) added glucose as a carbon source and they observed a decreased nitrification when carbon level increased. These authors stated that at a high C/N ratio typical for nitrogen limited environments, the heterotrophic bacteria are more successful to capture the available nitrogenous compounds since they are more abundant and grow faster than the chemo-autotrophic nitrifying bacteria. Nitrification can also be suppressed due to space competition between nitrifying and heterotrophic bacteria (Wijeyekoon, et al., 2004).
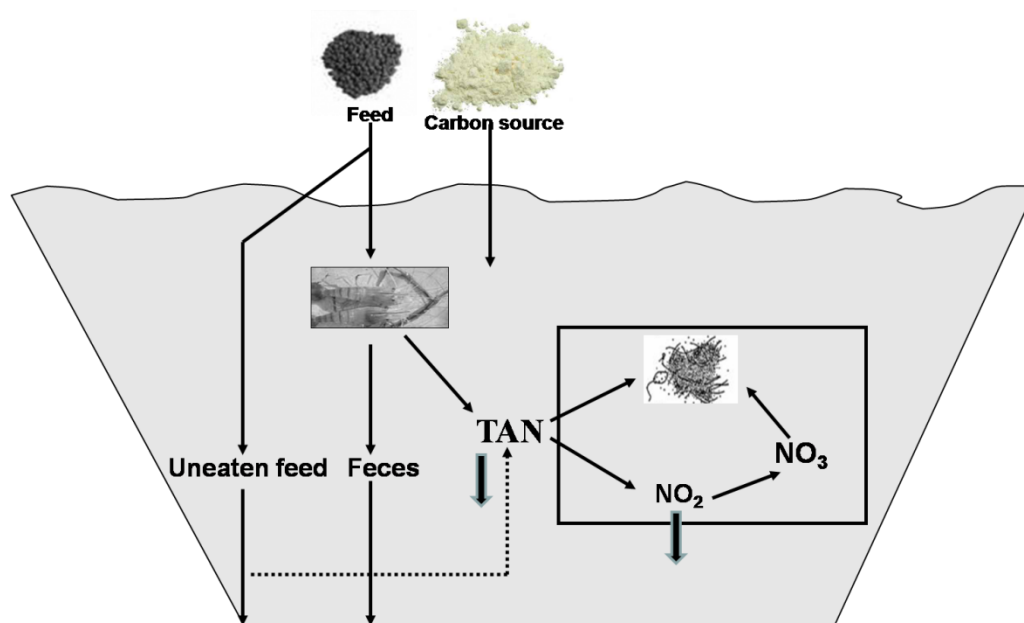
Figure 1. Mechanism of reduction of toxic nitrogenous compounds through immobilization by bacterial biomass. This figure showed that added carbon source for controlling of C/N ratio and TAN was converted into microbial biomass. The concentration of NO_2 was also low due to low availability of TAN as substrate for nitrification. The downwards black block arrows indicated marked reduction of toxic nitrogenous compounds (Source: Modified from Crab et al., 2007)

C/N ratio control improved periphyton productivity

Periphyton communities are comprised by bacteria, fungi, protozoa, phytoplankton, zooplankton, benthic organism and some invertebrates and their larvae (Azim, 2001). The added substrates in ponds water suported a periphyton community. The substrate will first be coated by organic substances, further colonized by bacteria and finally by algae and invertebrates, all of them embedded in a mucopolysaccharide matrix where organic detritus is trapped (van Dam et al., 2002). The presence of free-floating organic microparticles in pond water stimulates this process. Therefore, the quantity and quality of free-floating organic microparticles affects the speed with which the periphytic biofilms develops on the substrates in stagnant ponds. Increasing C/N ratio from 10 to 20 significantly increased the periphytic algal biomass in terms of biovolume by 64.2% (**Chapter 3**) and periphyton biomass in terms of dry matter by 17% (**Chapter 2**). Most of the added carbohydrates remained suspended in the water column for a long time. The suspended organic matter and nutrients derived from feed and carbohydrates are partly trapped by periphyton (van Dam et al., 2002) which had a fertilization effect on autotrophic periphyton in higher C/N ratio treatments. There is an intense exchange of inorganic and organic solutes between autotrophic and heterotrophic components within the periphyton assemblage (Verdegem et al., 2005). The periphytic algae supplied organic matter (trapped OM and dead periphyton) to the heterotrophs, the latter inorganic nutrients to the autotrophs. The heterotrophic microbial community developing on substrates can be manipulated using C/N control in a way equivalent to bio-floc technology ponds. Therefore, increased heterotrophic bacterial activity in the periphyton mat stimulated autotrophic production in high C/N ratio ponds and ultimately improved periphyton production.

Autrophic algal vs heterotrophic bacterial interaction in C/N-controlled ponds

The relations between the autotrophic phytoplankton community and the heterotrophic organisms such as bacteria that depend on it are still not well understood and quantified (Hansson et al., 1998). These interactions include competition, mutualism, inhibition, stimulation and coexistence. Autotrophic algae, which are nutrient limited (mainly N and P), can only use dissolved inorganic nutrients while heterotrophic bacteria, can use both dissolved and particulate nutrients. Therefore, heterotrophic bacteria can compete with algae for dissolved nutrients (Aota

and Nakajima, 2000). The algae are generally ineffective in competing for available organic substrates at substrate concentrations maintained by active bacterial heterotrophic activity (Wetzel, 2001). When there is a strong carbon limitation (low C:N and C:P ratios) bacteria tend to be out-competed. However, under N or P limitation (high C:N and C:P ratio) algae will be less competitive (Torres Beristain, 2005). At intermediate C:N and C:P ratios algae and bacteria will be both active (Thingstad and Pengerud, 1985). In previous research, the number of bacterial cells was found to increases linearly with the chlorophyll *a* concentration (Gasol and Duarte, 2000). In our experiment, increasing C/N ratio from 10 to 20 increased the biovolume of phytoplankton by 15% and heterotrophic bacteria in water column by 70%, indicated mutual interaction between autotrophic algae and heterotrophic bacteria in C/N controlled ponds **(Chapter 3)**. In aquaculture ponds, algae and bacteria have a range of stimulatory or inhibitory effects on each other (Cole, 1982). Along with the added carbohydrate, senescent algae or algal detritus are a major source of organic substrate for heterotrophic bacterial growth whereas living algae provide oxygen for decomposition. In return, bacteria regenerate inorganic nutrients and vitamins that stimulate algal productivity. C/N-controlled ponds received higher amount of nutrients in the form of carbohydrates and feeds. The increased amount of artificial feed and carbohydrates indirectly supplied nutrients to the autotrophic algae through decomposition by heterotrophic bacteria (Moriarty, 1986; Moriarty, 1997).

Substrates addition affected water quality and phytoplankton availability

When substrates are installed in the stagnant pond, the food web is enlarged by the extra periphyton loop (Azim, 2001). In periphyton-based system, the close linkage between autotrophic and heterotrophic processes in periphyton mats speed up nutrient cycling and positively influences water quality (Azim et al., 2003b; Milstein et al., 2003). Periphyton mats improved pond water quality through trapping of suspended solids, oxygen production, organic matter breakdown, ammonium and nitrate uptake and enhancement nitrification (Azim, 2001; Bratvold and Browdy, 2001; Thompson et al*.,* 2002; van Dam et al., 2002). In stagnant aquaculture ponds, nitrification mostly occurs at the sediment and is limited not only by surface area but also by oxygen availability. In C/N-controlled system fast growing heterotrophic bacteria might limit the space needed by the slow growing chemo-autotrophic nitrifying bacteria. In our

research, addition of substrates significantly decreased mean values of TAN, NO_2–N, NO_3–N and PO_4–P in C/N-controlled ponds **(Chapter 5)**. Supplying substrates improved the nitrogen-related processes developing in the water column and the nitrogen flow is mainly linked to autotrophic and heterotrophic activity that takes place in the periphyton (Milstein, 2005). In a substrates based system, Langis et al. (1988) and Ramesh et al. (1999) reported that bacterial biofilm (periphyton), including nitrifying bacteria, develop on the substrates which are located in the water column where more oxygen is available than at the water-sediment interface. In addition, the periphytic algal community contributes to the processing of the nitrogenous wastes in ponds (Shilo and Rimon, 1982; Diab and Shilo,1988). The periphyton community takes up both TAN and nitrate and edible biomass is formed (Crab et al., 2007). In addition to the uptake of TAN and nitrate, periphyton grown on the added substrates lowered the phosphorus of the overlying water (Hansson, 1989; Bratvold and Browdy, 2001).

In a stagnant fish pond, phytoplankton is the most important component for energy fixation and fuelling the food web. While in ponds phytoplankton productivity is positively correlated with nutrient concentrations (Boyd, 1990), in periphyton-based ponds, this relationship is interfered with by competition and interactions between periphyton and phytoplankton. In our research, substrates addition decreased the phytoplankton abundance by 29% **(Chapter 5)** and biomass based on biovolume by 14% (**Chapter 3**). Possible explanations include (1) competition between periphytic algae and water column algae for light and bioavailable nutrients in overlying water, (2) periphyton substrates might have shading effects which reduce sunlight availabity for phytoplankton, (3) some algal species might prefer to be colonized on hard substrates and therefore move from planktonic state to the periphytic state if substrate were available. Although plankton biomass was always lower in substrate added ponds, combined biomass (plankton + periphyton) was significantly higher (95.7%) in these ponds compared to the substrate free ponds **(Chapter 3)**.

Role of tilapia driven re-suspension in C/N-CP ponds

In stagnant pond, organic residues including uneaten feed, fecal pellets and dead algae settle to the pond bottom, creating an anoxic zone where nutrients remain trapped (Avnimelech and Zohar, 1986). The development of anaerobic conditions in stagnant

ponds constrains production and is considered to be a barrier for future intensification. The cycling of organic matter in the pond is influenced by sedimentation and re-suspension processes. Re-suspension brings organic matter and nutrients back into the oxygen rich water column where organic matter decomposition occurs much more efficiently, yielding less toxic components than in the sediment. Bioturbation activity generated by tilapia has the potential to improve bottom soil quality by increasing oxygen supply to a greater depth in aquaculture ponds bottoms. In our research, the addition of tilapia increased the bottom dissolved oxygen by 7-13% **(Chapter 5, 6)**. Previous research reported that bioturbutary activities of tilapia can bring some dissolved oxygen downwards to the lower layers of the water column, improving aerobic conditions on the pond bottom (Phan-Van et al., 2008; Jiménez-Montealegre et al., 2002). Mineralization of organic matter happens faster under aerobic condition (Torres Beristain, 2005). Therefore, favouring aerobic decomposition of organic matter will stimulate nutrient cycling in ponds. The digging and sieving of sediment by tilapia also increased diffusion rates across the sediment-water interface (Hohener and Gachter, 1994), which in turn increased nutrient availability in the overlying water column. The tilapia-driven bioturbation increased PO_4-P concentration in overlying water by 168% compared to rohu in C/N-CP freshwater prawn ponds **(Chapter 6)**. Previous studies reported that tilapia re-suspension induced nutrient release from the accumulated organic matter of the sediment into the water phase through the mud-water exchange mechanism, which enhances the overlying water PO_4-P concentration (Jana and Das, 1992; Jana and Sahu, 1993; Saha and Jana, 2003).

Plankton and periphyton grazing by tilapia

About 42-45 genera of phytoplankton and 10-17 genera of zooplankton were identified in the water column of different experimental trials **(Chapter 3, 5, 6)**. The addition of tilapia affected phytoplankton directly by grazing and indirectly by nutrient re-suspension. It decreased the abundance of phytoplankton by 46.5% indicating that direct effect was more pronounced than the indirect effect **(Chapter 5)**. According to Meade (1988) and Schwartz (1998), tilapia decrease the overall phytoplankton cell age by higher grazing pressure. Therefore, tilapia is important not only for nutrient cycling but also for structuring the plankton community (Diana et al. 1991). According to Dempster et al., (1995) and Lu et al. (2006), tilapia is an active

filter feeder that can grow rapidly by grazing on phytoplankton. Therefore, tilapia is considered as a potentially ideal animal for the control of algal biomass (Turker et al., 2003; Lu et al. 2006). Perschbacher and Lorio (1993) reported that tilapia stocked at densities higher than 5000 ha^{-1} promoted a very effective biological control over phytoplankton. However, the addition of tilapia did not have any significant effect on the abundance of zooplankton. Possible explanations are (1) zooplankton escapes predation during grazing by tilapia and (2) less preference for zooplankton by tilapia.

Natural food availability for tilapia is higher in substrate-based ponds as periphyton serves as an additional food web besides phytoplankton (Azim 2001). Laboratory studies by Dempster et al. (1993) have demonstrated that ingestion rates by tilapias are up to 25 times greater when algae are presented as periphyton than when given as phytoplankton. In our research, tilapia addition at 0.5 individual m^{-2} in freshwater prawn ponds decreased periphyton biomass (dry matter) by 39 to 46% **(Chapter 4, 5)**. There is evidence that Nile tilapia grows better grazing on periphyton (Hem and Avit, 1994; Guirat et al., 1995; Huchette et al., 2000; Azim et al., 2003b). In tilapia added ponds, the periphyton biomass decreased over time due to tilapia grazing **(Chapter 4, 5)**. In these experiments, periphyton biomass in terms of dry matter, ash free dry matter and chlorophyll-*a* concentration increased during first 1-2 months of stocking then decreased steadily subsequently. Possible explantions include (1) the low biomass of fish initially exerted low grazing pressure allowing periphyton to grow for first 2 months and after that as fish grew its increased grazing pressure led to reduced periphyton biomass and (2) selective grazing by tilapia at initial stage and then grazing become less selective at later stage. The periphytic algae must be grazed constantly and kept at low biomass in order to maintain high productivity, because increased standing biomass in the absence of grazers may lead to self-shading and death of algae, with consequent sloughing and dislodgement of the community (Hatcher, 1983; Hay, 1991; Huchette et al., 2000).

Grazing pattern of tilapia and rohu on plankton and periphyton

Fish feeding habits have an important influence on the abundance of plankton and periphyton, both directly by consumption and indirectly through influencing the food web and nutrient availability. Tilapias are omnivores capable of feeding on suspended plankton, and benthic and attached (periphyton) algal and detrital aggregates

(Dempster et al., 1993; Azim et al., 2003a). Rohu is known to be a predominantly column-feeding fish but it also feeds on periphyton in ponds provided with substrates (NFEP, 1997; Ramesh et al., 1999; Azim et al., 2001). In our research, we observed comparatively lower abundance of phytoplankton (22.6%) in ponds with tilapia compared to rohu in C/N-controlled freshwater prawn farming system **(Chapter 6).** This indicated tilapia was more efficient in grazing suspended phytoplankton than rohu. However, it was found that rohu was more efficient in grazing zooplankton. Uddin (2007) reported that electivity indices of tilapia were negative for all zooplankton and positive for all phytoplankton groups except Bacillariophyceae. In contrast, Rahman (2006) reported that rohu's electivity indices were positive for all zooplankton groups and negative for all phytoplankton groups, confirming that it preferred zooplankton over phytoplankton. In fed ponds, rohu ingested 1.3 times more zooplankton than phytoplankton although the abundance of phytoplankton was higher than zooplankton. In the case of periphyton, it was also found that mean values of periphyton biomass was significantly lower in ponds with tilapia compared to rohu in C/N-controlled freshwater prawn farming system **(Chapter 6).** Although both species are known as periphyton grazer as evident by gradual decrease of periphyton biomass **(Chapter 6)** but above result indicated that tilapia was more efficient periphyton grazer than rohu. Therefore, feed utilization efficiency was more when tilapia is added in C/N-CP ponds. However, considering the different grazing pattern of tilapia and rohu on phytoplankton and zooplankton, further research can be carried out on duo-culture of these species in C/N-CP ponds.

Maize flour as an alternative to tapioca starch as carbohydrate source

In C/N-controlled ponds, adding the carbon rich substrate encourages microbial metabolism and growth, immobilizes inorganic nitrogen and serves as a means to control water quality (Avnimelech, 1999). Many carbon sources can be used. In previous studies, several carbohydrate sources added to promote immobilization of inorganic nitrogen include glucose, cassava meal (Avnimelech and Mokady, 1988), cellulose, sorghum meal (Avnimelech et al., 1989), corn flour (Milstein et al., 2001), molasses (Burford et al., 2004), and tapioca flour (Hari et al., 2004). The criteria to select carbonaceous substrates should be its bio-availability and ability to be dispersed in the water. A readily bio-degradable substrate is preferable in C/N-controlled systems. The substrate should be soluble or given in fine powdered form, so as to

slow its sedimentation rate and to keep it suspended in the water as much as possible. Based on these properties, we selected tapioca starch as carbohydrate source for controlling C/N ratio at 20 **(Chapter 2,3,4,5)**. The major problems of using tapioca starch as CH source in Bangladesh were its poor acceptance by the farmers due to very high cost and irregular availability of this imported product. In order to improve economic sustainability, one should select substrates that are not costly. We selected maize flour as a potential carbohydrate source **(Chapter 6)** due to its low cost, easy availability and wide acceptance by the farmer as one of the potential feed ingredients. We observed similar effects of maize flour and tapioca starch on water quality parameters, abundance of natural food and production of prawn and finfish **(Chapter 6)**. In addition, the economic analysis showed that the benefit-cost ratio was 35% higher when maize flour was used as carbohydrate source compared to tapioca starch **(Chapter 6)**.

Additive effects of C/N ratio control and periphyton substrates on prawn production

In addition to water quality control, increasing C/N ratio led to the buildup of microbial protein that contributed to fish nutrition and thereby, improved production. The net yield of freshwater prawn increased by 40% due to increasing C/N ratio from 10 to 20 **(Chapter 2)**. The higher yield in the present study showed that freshwater prawn could well utilize the additional protein derived from the increased bacterial biomass as a result of increasing C/N ratio from 10 to 20. Previous studies reported that microbial dense mass, commonly known as biofloc, might 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). Although not confirmed in our study, we hypothesized that as like other reported species, microbial floc might be utilized by freshwater prawn as food source. Our hypothesis was supported by a 19% reduction of the FCR and 24% increase of protein efficiency ratio in C/N ratio 20 ponds compared to C/N ratio 10 ponds **(Chapter 2)**. C/N ratio control increased freshwater prawn production 40% while the water quality was better.

The substrates addition positively influenced freshwater prawn production in tilapia-prawn polyculture system (Uddin, 2007). We observed that addition of substrates for periphyton development increased net yield of freshwater prawn by 23% **(Chapter 2)**.

This increase in net yield was mainly due to the increased survival since periphyton substrates did not have an effect on individual weight at harvest. We also observed that addition of substrates for periphyton development improved freshwater prawn production by 15% in tilapia free ponds **(Chapter 2)** and 41% in tilapia added ponds **(Chapter 5)**. Addition of substrates might have minimized territoriality effect of freshwater prawn (Uddin, 2007). In addition, substrates addition decreased FCR value by 13% contributing periphyton as additional food. Uddin (2007) reported that FCR was 13% lower in fed-periphyton based ponds compared to fed-substrate-free-ponds. Therefore, the possible explanations for increased freshwater prawn production due to the addition of substrates are (1) increased survival due to minimized territoriality effects, (2) additional natural food in the form of periphyton colonized on bamboo *kanchi*, (3) improvements of water quality due to reduction of toxic nitrogenous compounds through a range of ecological and biological process, or (4) a combination of these factors. The interaction between C/N ratio control and addition of substrates for periphyton development was not significant for the net yield of freshwater prawn **(Chapter 2)**. The effects of C/N ratio control and substrate addition for periphyton development were additive, jointly increased net yield of freshwater prawn by 75%.

Tilapia addition affected freshwater prawn production

The analysis of natural food communities in C/N-controlled periphyton-based ponds **(Chapter 3)** showed that the biomass of plankton and periphyton was totally unutilized in freshwater prawn monoculture. Therefore, we considered that inclusion of plankton and periphyton grazing fish species like tilapia (Dempster et al., 1993; Huchette et al., 2000; Azim et al., 2003a) could further increased the production and improved environmental quality and system stability in C/N-CP ponds. In our experiment, adding 0.5 tilapia m^{-2} on average reduced prawn production by 12–14% **(Chapter 4, 5)**, and tilapia addition at 1 individual m^{-2} produced a further 5% reduction, independent of prawn density. Since tilapia addition did not influence the survival of prawn negatively, the observed decrease of net yield of freshwater prawn might be due to the inter-specific competition for food and space. In our experiments, feed was applied considering only the freshwater prawn biomass, neglecting fish biomass. During feeding, we observed that part of the supplied feed was quickly eaten by tilapia before settling to the pond bottom. This concurred with a 10% and 13% increase in FCR with the addition of 0.5 and 1 tilapia m^{-2}, respectively, compared to

prawn monoculture ponds **(Chapter 4)**. Apart from the supplied artificial feed, tilapia may compete with freshwater prawn for natural food. Uddin (2007) reported that in mixed culture the feeding niches of tilapia and prawn only partially overlapped. In our observation, this was mostly evident for plankton and periphyton only in freshwater prawn-tilapia polyculture. Prawns in their natural habitats prefer to forage on animals like trochopterans, chironomids, oligochaetes, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996; Tidwell et al., 1997). We observed that total benthos was 41% higher in number in prawn-rohu ponds compared to the prawn-tilapia ponds **(Chapter 6),** which suggests that tilapia directly feed on the benthic fauna (Zur, 1980). Although, tilapia addition decreased freshwater prawn production to some extent, the overall combined production was satisfactory. With the addition of 0.5 tilapia m^{-2} to ponds with 2 prawns m^{-2}, the available natural food (plankton, periphyton, benthos, microbial floc) was used much better than before. Tidwell et al., 2000 suggests that addition of tilapia with freshwater prawn may improve overall pond efficiency. Although, addition of 0.5 tilapia m^{-2} in this system reduced production to some extent (12-14%) but the net yield of freshwater prawn in various experiment ranges from 433-660 kg ha^{-1} 120 d^{-1} (1082-1650 kg ha^{-1} yr^{-1} considering 10 month culture periods in a year in Bangladesh). The above level of production of freshwater prawn is around 2.5 to 3.5 times higher than the previously reported production in polyculture system of Bangladesh (Asaduzzaman et al., 2006a). However, actual production of freshwater prawn in C/N-CP at farm level may be low compared to the present study. In addition to the freshwater prawn, tilapia production in various experiment ranges from 1100-1400 kg ha^{-1} 120 d^{-1} (2750-3500 kg ha^{-1} yr^{-1} considering 10 month culture periods in a year in Bangladesh). Significantly higher benefit-cost ratio in C/N-CP system also indicated the economic sustainability of this system.

Conclusion and further perspectives

C/N-CP system benefited freshwater prawn farming by (1) improving water quality through reducing toxic inorganic nitrogen content such as ammonia and nitrite, (2) enhancing natural food availability, (3) improving nutrient utilization efficiency, (4) improving farm productivity and economic returns, and (5) reducing nutrient discharge. The above technology requires installation of hard substrates and

application of cheap carbohydrates, resources which can be produced within the farmers' traditional agricultural systems. The system can be applied in different parts of the world and with different culture species. Therefore, C/N-CP will be able to satisfy future demands for aquatic products, while providing the opportunity to resource poor farmers to participate and benefit significantly from the growth of aquaculture production.

The major strength of this study was that it looked at the combined effects of C/N ratio control, addition of substrates for periphyton development and fish driven re-suspension. Although the effects of C/N control, substrate addition, and fish driven re-suspension on pond ecology and production are well documented by many authors previously, their combined effects on pond ecology and productivity have never been investigated in stagnant ponds. In all experiments, we always monitored water and sediment quality, abundance of natural foods (plankton, periphyton, heterotrophic bacteria, benthic macroinvertebrates), and production and economic performances. The previous research on C/N ratio control by carbohydrate addition mainly focused on the water quality, nutrient budget and production but not on the pond ecology and economic benefits. The approach to look at these aspects in combination proved fruitful. By combining the results from the series of different studies, our understanding of changes in pond ecology, nutrient dynamics, pond productivity and economic benefits improved. A better understanding of the microbiological aspects, particularly bacteria growth patterns, characterization of biofilms and possible manipulation of the microbial community will be helpful to further optimize C/N-CP technology. In the present study, it was not possible to estimate the contribution of artificial feed and different types of natural food to the growth of freshwater prawn, tilapia and rohu. Therefore, studies with labeled ^{13}C or ^{15}N ingredients can help in tracing the utilization of organic carbon and inorganic nitrogen by different flora and elucidating food webs in ponds, and is subject of further research. There exists scope for further improvement of economic sustainability of this technology by comparing the potential of other cheap carbohydrate sources and cheaper on-farm periphyton substrates. In many cases, result from the on-station trial varied from the farm level implementation. Therefore, adoption of this technology at on-farm levels through direct participation of farmers should be carried out to validate the result of on-station trials.

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Summary

C/N-controlled periphyton-based freshwater prawn farming system: a sustainable approach to increase pond productivity

Summary
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Operation of intensive freshwater prawn farming (*Macrobrachium rosenbergii*) demands high investment and technical expertise, things not affordable by resource-poor farmers. In stagnant freshwater ponds, the production capacity is limited because excessive accumulation and anaerobic decomposition of organic matter creates adverse culture conditions. Therefore, new concepts to reduce organic matter accumulation and increase nutrient cycling and retention should be explored. Three technologies showed to improve productivity and sustainability of pond production: (1) C/N ratio control, (2) providing substrates for periphyton development, and (3) fish driven re-suspension. Although the effects of C/N control, substrate addition, and fish driven re-suspension on production are well documented, their combined effects on productivity have never been investigated in extensive and semi-intensive ponds. The novelty of this research is to combine these technologies, with the goal to raise pond productivity above levels obtained with each one of these technologies separately, and to increase the nutrient use efficiency in ponds above levels presently achieved, further enhancing sustainability. This combined technology is further referred to as **C/N controlled periphyton (C/N-CP)** technology. C/N-CP technology relies on hard substrates and carbohydrates, resources available within the traditional agricultural farming systems. The present research explores the hypothesis that combining C/N ratio control, providing substrates for periphyton development and fish driven resuspension, will increase average farm productivity more than with either of these techniques alone. In this thesis, the present status of the 3 technologies was briefly reviewed, followed by a series of experiments testing C/N-CP technology in extensive freshwater prawn ponds in Bangladesh.

Experiment 1 explored the effect of C:N ratio control in ponds with or without substrate addition for periphyton development on production of giant freshwater prawn. Increasing the C/N ratio from 10 to 20 reduced all of the nitrogenous species (TAN, NO_2-N, and NO_3-N) in water column and total Kjeldahl nitrogen (TKN) in sediment. The addition of substrates did not influence size at harvest but improved the survival of prawn by 14.6%. Increasing the C:N ratio from 10 to 20 increased the net yield by 40% and addition of substrate increased the net yield by 23%. The combination of C:N ratio control and substrate addition increased the net yield from 309 to 540 kg ha^{-1} (120 days)$^{-1}$. This 75% higher production concurred with (1) a lower inorganic nitrogen content in the water column, (2) a higher total heterotrophic bacteria (THB) abundance supplying additional single cell protein to augment the prawn production, and (3) an improved periphyton productivity and quality.

Experiment 2 explored how C:N ratio control and presence and absence of added substrates influenced the natural food communities in aquaculture ponds. Increasing the C:N ratio from 10 to 20 significantly increased the biovolume of plankton, periphytic plankton and chironomids by 8.7%, 50% and 28%, respectively. Increasing the C:N ratio from 10 to 20 raised the biovolume of total heterotrophic bacteria (THB) in the water column (70%), sediment (36%) and periphyton (40%). The addition of substrates decreased the biovolume of water column plankton by 14% but the combined biovolume (plankton + periphyton) was almost double in substrate-added ponds. This study demonstrated that plankton, periphyton and microbial biomass were underutilized by the freshwater prawn in treatment with C:N ratio 20. This left room for increasing the stocking density of prawn and/or inclusion of periphyton grazing fish species to improve nutrient utilization efficiency and sustainability.

Experiment 3 explored the effect of increasing stocking density (2 and 3 individuals m^{-2} of freshwater prawn and addition of different levels (0, 0.5 and 1 individual m^{-2}) of tilapia on production in C/N-CP systems. Increasing prawn density increased gross and net prawn production (independent of tilapia density). Adding 0.5 tilapia m^{-2} on average reduced prawn production by 12–13%, and tilapia addition at 1 individual m^{-2} produced a further 5% reduction (independent of prawn density). The net yield of tilapia was similar between 0.5 and 1 tilapia m^{-2} treatments and increased by 8.5% with increasing stocking density of prawn. The significantly highest benefit cost ratio (BCR) was observed in 0.5 tilapia m^{-2} treatment but freshwater prawn density had no effect on it. Therefore, both stocking densities (2 and 3 prawns m^{-2}) of prawn with the addition of 0.5 tilapia m^{-2} resulted in higher fish production, good environmental conditions and economic return and hence, polyculture of prawn and tilapia in C/N-CP system is a promising option for ecological and sustainable aquaculture.

Experiment 4 investigated the effect of addition of tilapia (0.5 individual m^{-2}) and substrates for periphyton development on pond ecology, production and economic performances in a C/N-controlled freshwater prawn farming system. The addition of periphyton substrates significantly reduced inorganic N-species (TAN, NO_2-N, and NO_3-N) in the water column. Tilapia addition decreased the abundance of natural foods (plankton, periphyton and total heterotrophic bacteria), indicating the preferential feeding of tilapia on these items. Substrates addition improved survival of prawn by 42.6% but tilapia addition had no effect on it. Substrates contributed to 44% and 19% higher net yield of prawn and tilapia, respectively, whereas tilapia addition decreased the net yield of prawn by 14%. The economic analysis showed that addition

of tilapia and periphyton substrates jointly improved the benefit-cost ratio. Addition of tilapia and periphyton substrates in C/N controlled system benefited the freshwater prawn culture practices through reducing toxic inorganic nitrogenous compounds in water, enhancing the utilization of natural foods, and improving survival, production and economic benefit.

Experiment 5 investigated the effect of carbohydrate source (high-cost tapioca starch and low-cost maize flour) for maintaining a high C:N ratio, and fish driven bioturbation (0.5 individual m^{-2} in three different combinations: 100% tilapia, 50% tilapia + 50% rohu, and 100% rohu) on pond ecology, production and economical performances in C/N-CP freshwater prawn ponds. Similar effects of tapioca starch and maize flour on water quality, natural foods abundance and production were observed. Bioturbutary activities of tilapia increased dissolved oxygen to the bottom layer, improving aerobic condition of the pond bottom. In addition to the bioturbutary effects, tilapia more effectively utilized the natural food items compared to rohu. The net yield and survival of finfish were significantly higher in 100% tilapia ponds during a 120-d culture period. This treatment gave the best economic return in terms of benefit-cost ratio while maize flour was used as carbohydrate source. In conclusion, maize flour can be used as an alternative cheap on-farm carbohydrate source for maintaining a high C:N ratio and tilapia driven re-suspension in C/N-CP system improves culture environment, natural food utilization, production and economic return, further enhancing economic sustainability of C/N-CP freshwater prawn farming system.

In the general discussion, the major conclusions are integrated and interpreted and suggestions for further studies are given. Both the addition of substrates and tilapia (0.5 individual m^{-2}) were found to be beneficial in C/N-controlled (C:N=20:1) prawn (2 or 3 individual m^{-2}) farming system. The strength of this research is that it looked at the combined effects of (1) C:N ratio control, (2) addition of substrates for periphyton development and (3) tilapia driven bioturbation. How the various combinations affected water and sediment quality, natural food availability, production and economical benefits, was documented. A significant improvement of system environment, productivity and economic benefits was observed due to synergism among C:N ratio control, addition of periphyton substrates and tilapia driven bioturbation. C/N-CP technology is a promising technology, improving the sustainability and productivity of present prawn farming by simple and affordable means.

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Samenvatting

**C/N-gecontroleerde productie in periphyton vijvers
voor zoetwatergarnaal: een productieverhogende en
duurzame technologie**

Intensieve teelt van zoetwatergarnaal (*Macrobrachium rosenbergii*) vereist hoge investeringen en technische kennis. Dit kunnen kleine boeren niet betalen. In zoetwatervijvers zonder doorstroming is de productiecapaciteit beperkt omdat ophoping en anaerobe afbraak van organische stof de vijver ongeschikt maakt voor kweek. Daarom dienen er nieuwe concepten ontwikkeld te worden die de ophoping van organische stof verminderen en nutriëntkringlopen en –retentie versnellen. Van 3 technologieën is bekend dat ze de productiviteit en duurzaamheid in vijvers verbeteren: (1) C/N ratio beheer, (2) het inbrengen van substraat voor periphyton, en (3) het opwarrelen van sediment door vissen. Hoewel elk van deze 3 technieken goed beschreven is in de literatuur is hun combinatie nooit onderzocht in extensieve en semi-intensieve vijvers. Het vernieuwende van dit onderzoek is het combineren van deze technieken met als doel de productiviteit te verhogen tot een niveau niet haalbaar met elk van deze technieken apart, en de gebruikefficiëntie van nutriënten in extensieve vijvers op een hoger niveau te brengen dan momenteel mogelijk. Hierdoor wordt ook de duurzaamheid verbeterd. Deze combinatie van technieken wordt **C/N gecontroleerde periphyton (C/N-CP)** technologie genoemd. C/N-CP technologie maakt gebruik van hard substaat en koolstofbronnen, zaken die voorhanden zijn op traditionele landbouwbedrijven. Het onderzoek test de hypothese dat het combineren van het aansturen van de C/N ratio, het installeren van substraat om periphyton ontwikkeling te stimuleren en het uitzetten van bodem omwoelende vissen, de productiviteit meer zal verhogen dan mogelijk met elk van deze technieken afzonderlijk. De thesis begint met een korte beschrijving van elk van de 3 bovengenoemde technieken, gevolgd door een serie experimenten met C/N-CP technologie in extensieve zoetwatergarnaalvijvers in Bangladesh.

In het eerste experiment werd het effect onderzocht van verschillende C:N verhoudingen in vijvers met of zonder substraat voor periphyton op de productie van zoetwatergarnaal. Door de C/N ratio te herhogen van 10 naar 20 nam de concentratie van stikstofverbindingen (TAN, NO_2-N, and NO_3-N) in het water en Kjeldahl stikstof (TKN) in de bodem, af. Het uitzetten van substaat had geen effect op de individuele grootte van de garnalen bij de oogst, maar de overleving steeg 14.6%. De netto opbrengt steeg 40% door de C:N ratio te verhogen van 10 tot 20. Het inbrengen van substaat verhoogde de productie met 23%. De combinatie van C:N ratio verhoging en het toevoegen van substraat, deed de netto opbrengst stijgen van 309 tot 540 kg ha^{-1} in

120 dagen. Deze 75% toename in productie ging samen met (1) minder anorganische stikstofverbindingen in de waterkolom, (2) meer heterotrofe bacteriën die als 'single cell protein' bijdragen aan het verhogen van de garnaalproductie, en (3) een hogere kwaliteit en productie van periphyton.

In het tweede experiment werd onderzocht hoe C:N verhoudingen en toevoeging van substraat de beschikbaarheid van natuurlijk voedsel in vijvers beïnvloeden. Het verhogen van de C:N ratio van 10 naar 20 leidde tot een 8.7% meer plankton, 50% meer periphyton en 28% meer chirominiden in termen van biovolume. Het verhogen van de C:N ratio van 10 tot 20 deed het biovolume van heterotrofe bacteriën in het water toenemen met 70%, in het sediment met 36% en in het periphyton met 40%. Door het toevoegen van substraat nam het biovolume van plankton in het water met 14% af, maar het gecombineerde plankton en periphyton biovolume was bijna 2 keer zo hoog in vijvers met substraat. Aangetoond werd dat het plankton, periphyton en bacteriën onderbenut zijn in vijvers met zoetwatergarnaal met een C:N verhouding van 20. Er was dus nog ruimte om de bezettingsdichtheid van garnalen te verhogen en vissen uit te zetten die grazen op periphyton. Dit kan leiden tot een verbetering van de nutriëntbenutting en duurzaamheid.

In een derde experiment werd onderzocht hoe de bezettingsdichtheid van zoetwatergarnaal (2 en 3 individuen m^{-2}) en tilapia (0, 0.5 en 1 individu m^{-2}) de productie in C/N-CP vijvers beïnvloed. Verhoging van de bezettingsdichtheid van garnalen leidde tot een hogere bruto en netto productie (onafhankelijk van de tilapia dichtheid). Door 0.5 tilapia m^{-2} uit te zetten nam de productie van zoetwatergarnaal met 12-13% af. Werd de bezettingsdichtheid van tilapia verder verhoogd tot 1 tilapia m^{-2} dan daalde de garnaalproductie nog een extra 5% (onafhankelijk van de garnaal dichtheid). De netto opbrengt van tilapia was vergelijkbaar bij 0.5 en 1 tilapia m^{-2}, en steeg 8.5% door toename van de garnaal bezettingsdichtheid. Het uitkering kostencoëfficiënt was het beste bij 0.5 tilapia m^{-2}, en werd niet beïnvloed door de bezettingsdichtheid van garnalen. Beide garnaal bezettingsdichtheden (2 en 3 garnalen m^{-2}) in combinatie met 0.5 tilapia m^{-2} leidden dus tot een hogere productie, goede omgevingsomstandigheden en aanzienlijke winst. Policultuur van zoetwatergarnaal en tilapia in C/N-CP vijvers is een veelbelovende optie voor een ecologisch verantwoorde en duurzame aquacultuur.

In het vierde experiment werd in C/N ratio gecontroleerde zoetwatergarnaalvijvers onderzocht hoe toevoeging van 0.5 tilapia m^{-2} en substraat voor periphyton, de ecologie, de productie en het economisch bedrijfsresultaat beïnvloeden. Het installeren van substraat verminderde significant de concentratie van anorganische N-verbindingen (TAN, NO_2-N, and NO_3-N) in de waterkolom. In aanwezigheid van tilapia nam de hoeveelheid natuurlijk voedsel (plankton, periphyton en heterotrofe bacteriën) af, wat er op duidt dat tilapia bij voorkeur natuurlijk voedsel eet. Met substraat nam de overleving van zoetwatergarnaal 42.6% toe. Toevoegen van tilapia had geen effect op de overleving van garnaal. Met substraat was de garnaal opbrengst 44% hoger en de tilapia opbrengst 19%. Met tilapia nam de netto opbrengst van garnaal met 14% af. Zowel het toevoegen van substraat als het uitzetten van tilapia leidde tot een verbetering van het uitkering kostencoëfficiënt. Het toevoegen van tilapia en substraat in C/N ratio gecontroleerde zoetwatergarnaalvijvers is voordelig omdat de hoeveelheid anorganische toxische N-verbindingen afneemt, de hoeveelheid beschikbaar natuurlijk voedsel toeneemt, en de overleving, productie en winst stijgen.

In een vijfde experiment werd onderzocht hoe de koolstofbron (duur tapioca zetmeel en goedkope maïsbloem) die gebruikt wordt om de C:N ratio hoog te houden, en het omwoelen van de bodem door vissen (0.5 vis m^{-2} in drie verschillende combinaties: 100% tilapia, 50% tilapia + 50% rohu, en 100% rohu), de vijver ecologie, productie en winst beïnvloeden in C/N-CP vijvers. Er was geen verschil in waterkwaliteit, beschikbaarheid van natuurlijk voedsel en productie tussen tapiocameel en maïsbloem. Het omwoelen van de bodem door tilapia verhoogde de hoeveelheid beschikbare opgeloste zuurstof in de bodem. Daarnaast gebruikte tilapia beter het beschikbare natuurlijke voedsel dan rohu. De netto visopbrengst en -overleving gedurende de 120 dagen kweekperiode was significant beter in de 100% tilapia vijvers. Maïsbloem als koolstofbron in combinatie met 100% tilapia toonde het beste uitkering kostencoëfficiënt. Het onderzoek toonde aan dat maïsbloem een uitstekende goedkope koolstofbron is voor het hoog houden van de C:N ratio, en dat het omwoelen van de bodem door tilapia in C/N-CP vijvers, de kweekomgeving, de productie van natuurlijk voedsel, de vis- en garnaalproductie en de winst verbetert. Hierdoor wordt de economische duurzaamheid van C/N-CP vijvers verder verbeterd.

In de algemene discussie worden de belangrijkste bevindingen geïntegreerd en geïnterpreteerd, en worden er suggesties gegeven voor vervolgonderzoek. Zowel het toevoegen van substraat en van tilapia (0.5 tilapia m^{-2}) beïnvloeden C/N gecontroleerde (C:N ratio = 20:1) zoetwatergarnaal (2 of 3 garnalen m^{-2}) vijvers in positieve zin. De kracht van dit onderzoek was dat het de bestaande technieken (1) C:N ratio beheer, (2) het toevoegen van substaat voor periphyton ontwikkeling, en (3) bodembioturbatie door tilapia, combineerde. Welk effect de verschillende combinaties hadden op water- en bodemkwaliteit, beschikbaarheid van natuurlijk voedsel, vijverproductie en winst werd onderzocht. De productieomgeving, productiviteit en winst werd significant verbeterd dank zij synergie tussen C:N ratio beheer, het toevoegen van substraat voor periphyton, en bioturbatie door tilapia. C/N-CP technologie is veelbelovend. De technologie verbetert de duurzaamheid en productiviteit van bestaande zoetwatergarnaal productievijvers, en steunt daarbij op eenvoudige en betaalbare middelen.

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Md. Asaduzzaman
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Curriculum Vitae (Md. Asaduzzaman)

Md. Asaduzzaman was born on 02 February, 1982 in the village Goaria of the district Pabna, Bangladesh. He is the eldest son of Mr. Md. Mokbul Hossain Biswas and Mrs. Sahida Khatun. He completed elementary education level at the Udaypur Primary School, Pabna, Bangladesh, secondary education level (SSC) at Udaypur High School, Pabna, Bangladesh and higher secondary school education (HSC) at the Govt. Rajbari College, Rajbari, Bangladesh. He obtained his Bachelor degree of science in Fisheries (Honours) in 2004 from the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. He obtained 1st position in B.Sc level with 80.65% marks, which was the record mark of Faculty of Fisheries as well as the Bangladesh Agricultural University. He finalized his Master of Science (MS) degree in Fisheries Management in December 2005 from the same University and obtained 1st position with GPA 4 (Grade A^+) in scale of 4. He also received Japanese Government Monbukagakusho (MEXT) Scholarship and completed a second Master of Science degree in Aquatic Molecular Biology and Biotechnology from The University of Tokyo, Japan. He was awarded four **"Gold Medal Awards"** and one "**Merit Award**" in recognition of excellent results in B.Sc Fisheries (Hons.) and Master level.

Immediately after completion of Master's degree, he was involved as a Research Fellow in a number of internationally (USAID and EU) funded projects in the Department of Fisheries Management at Bangladesh Agricultural University, Mymensingh, Bangladesh. He also worked as a Principal Investigator of International Foundation for Science (IFS), Sweden funded project on "Low Cost Approaches to Raise the Freshwater Prawn *(Macrobrachium rosenbergii)* Productivity of Stagnant Sustainable Ponds in Bangladesh". He attended in a number of international and national conferences and published around 20 peer reviewed articles in international and national journals to date. Md. Asaduzzaman is an active member of different renowned societies such as Bangladesh Fisheries Research Forum (BFRF), Japanese Society for Fisheries Science (JSFS), Asian Fisheries Science (AFS) and World Aquaculture Society (WAS).

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List of Publications

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