Mixed culture of tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) in periphyton-based ponds

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Dedicated to my beloved parents

Contents

Chapter 1

General Introduction

The present world population is expected to grow from 6.1 billion people to 9 billion by 2050 (UN, 2000). As a result, the demand for food including fish is increasing. The demand of aquatic products for human consumption will grow to 121.1 million metric tons by 2010 (Wijkstrӧm, 2003) from its present production level of 101 million metric tons (FAO, 2004). This goes beyond total capture fisheries supply. The shortfall in supply will largely filled in through aquaculture. Since 1970, aquaculture is the fastest growing animal production sector in the world expanding at an average 9.2% per year compared to only 1.4% for capture fisheries (FAO, 2004).

Status and potentials of aquaculture in Bangladesh

Bangladesh is uniquely rich and diverse in water resources. It has innumerable water bodies including ponds, lakes, rivers, haors, baors, beels, tanks, estuaries and inundated paddy fields. Due to favorable climatic condition, the water bodies of Bangladesh are highly productive, and aquaculture is an important commercially viable activity (DoF, 2003). Inland water resources cover an area of 4,047,316 ha of which only 34.8% contributes to capture fisheries (Table 1). Bangladesh inland waters are third after China and India in terms of fish biomass production (FAO, 2000). The floodplains and the beels covering 2,946,953 ha also offer great scope and potential for augmenting fish production by adopting culture based fisheries techniques (DoF, 2005).

The fisheries sub-sector in Bangladesh contributes significantly to nutrition, employment, household income and foreign exchange earnings. Fish provides 63% of the animal protein intake in Bangladesh and the annual per capita fish consumption is 14 kg (DoF, 2005). About 1.2 million people are engaged full-time and 12 million people part-time in the aquatic production sector. Aquatic products are the country’s second largest export commodity contributing 10% of annual export earning, 5.2% of national GDP and 20% of the agriculture GDP (DoF, 2003; Shah, 2003).

During the last ten years, the average annual growth in aquatic production in Bangladesh through capture fisheries and aquaculture was 5 and 15%, respectively, (Figure 1). There are indications that the production from capture fisheries decreased recently which increased pressure on aquaculture to fill the gap. Causes for decreasing capture fisheries production include habitat destruction, construction of flood control barrages, water abstraction for irrigation, over-fishing and reclamation of land for agriculture. Concurrently, aquaculture production increased due to the development and implementation of improved culture techniques and expansion of the pond culture area (Gupta et al., 1999; Alam and Thomson, 2001).

Table 1. Inland water resources in Bangladesh.

Sector of Fisheries	Water Area (Hectare)	Total Catch (Metric Ton)	% of total
A. Inland Fisheries			
(i) Capture			
1. River & Estuaries	1,031,563	137,337	6.5
2. Sundarbans	-	15,242	0.7
3. Beel	114,161	74,328	3.5
4. Kaptai Lake	68,800	7,238	0.3
5. Flood Land	2,832,792	497,922	23.7
Capture Total	4,047,316	732,067	34.8
(ii) Culture			
1. Pond & Ditch	290,500	795,810	37.9
2. Baor	5,488	4,282	0.2
3. Coastal Shrimp & Fish Farm	203,071	114,660	5.5
Culture Total	513,584	914,752	43.5
Inland Total	4,560,900	1,646,819	78.3
B. Marine Fisheries			
Industrial Fisheries			
(i) Trawl		32,606	1.6
(ii) Artisanal Fisheries		422,601	20.1
Marine Total		455,207	21.7
COUNTRY TOTAL		2,102,026	100.0

Source: DoF (2005).

Aquaculture development is recognized as a way to improve the livelihoods of poor people (Lewis, 1997; Gupta et al., 1999; Hussain, 1999) and a tool to increase food security (FAO, 2000).

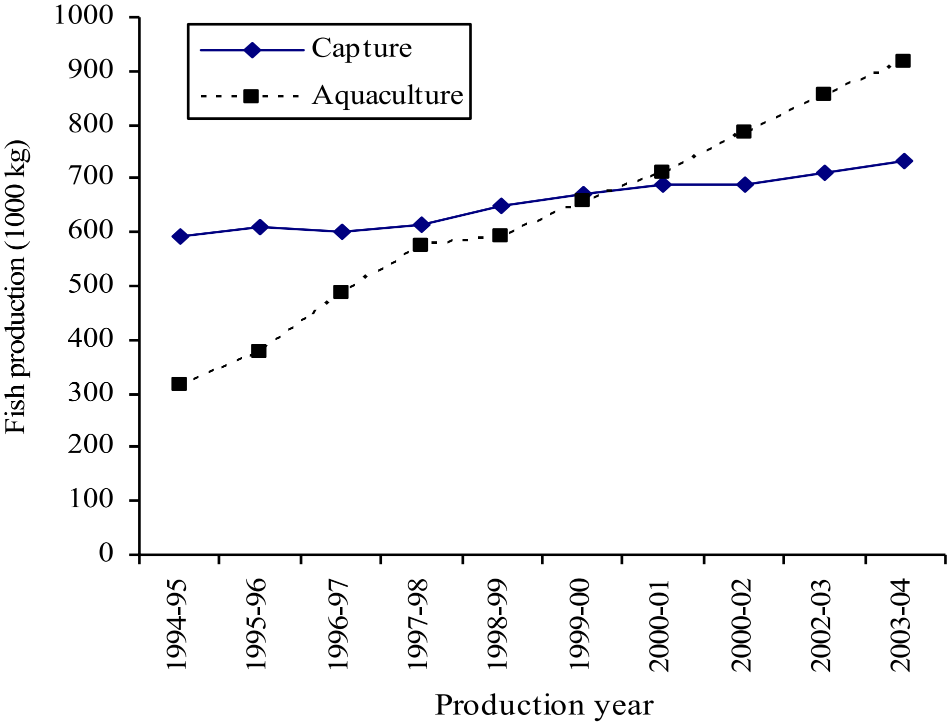

Figure 1. Trends of aquaculture and inland capture fishery production in Bangladesh (Source: DoF, 2005).

Many people are employed in primary, secondary, and tertiary fishery-related activities, including aquaculture, fish trading, fish processing, and fishing gear manufacturing. In Bangladesh, where most of the fish farmers are poor and using old or simple technologies, there is considerable potential for improvement. Novel and simple upgraded technologies, accessible and applicable by poor farmers are needed to improve livelihoods, including nutrition, food security and income (ADB, 2004).

Tilapia and prawn farming in Bangladesh

Nile tilapia (*Oreochromis niloticus*) was one of the first fish species cultured (Popma and Masser, 1999). During the last decades, Nile tilapia, endemic to Africa, became an important culture species in many Asian countries, including China, Indonesia, Bangladesh, Malaysia, the Philippines, Thailand, Vietnam, Myanmar and Sri Lanka.

UNICEF introduced the current stock of Nile tilapia into Bangladesh in 1974. In 1987, the Bangladesh Fisheries Research Institute (BFRI) introduced another stock of Nile tilapia from Thailand (Gupta et al., 1992). Nile tilapia is a preferred culture species because of desirable features like (1) adaptation to a wide range of environments, including shallow or seasonal water bodies and ditches, (2) good taste, (3) fast growth, (4) easy reproduction and (5) versatile feeding behavior. It grows well on a vegetarian diet in part due to the possession of a long intestine and a stomach pH below 2.0 (Moriaty and Moriarty, 1973; Bowen, 1982; Getachew, 1989). Tilapia filter algae from the water column but need additional nutrient sources to fulfill its basic nutrient requirements (Turker et al., 2003).

Shrimps are an important export commodity in Bangladesh. Tiger shrimp, Bagda (*Penaeus monodon*) and giant freshwater prawn, Golda (*Macrobrachium rosenbergii*) are the export species, with Golda accounting for 25-30% of total fishery exports (DoF, 2005). Giant freshwater prawn is suitable for aquaculture due to its (1) favorable harvesting size, (2) growth rate, (3) flexible feeding habits, (4) acceptance of a wide range of culture environments, (5) controlled reproduction and hatchery technology and (6) good survival.

Periphyton based aquaculture

The term ‘periphyton’ was first used by Behning (1924) for the plants grown on buoys, ships and mooring in the River Volga. Periphyton may be defined as the complex of sessile aquatic biota with associated detritus, attached to submerged substrate. It includes sessile algae, microfauna and other bottom organism in combination with microbial bio-films (van Dam et al., 2002). In periphyton, autotrophic and heterotrophic communities are closely linked, providing a fast turn-over of nutrients and a fast regeneration potential after grazing. Nile tilapia grows better grazing on periphyton than filtering suspended algae from the water column (Hem and Avit, 1994; Guiral et al., 1995; Huchette et al., 2000; Azim et al., 2002a).

The idea of periphyton-based aquaculture was originally derived from traditional brush park fishery systems, such as the “Acadjas” of Ivory Coast, West Africa (Welcomme, 1972), the “Kathas” of Bangladesh (Wahab and Kibria, 1994) and the “Samarahs” of Cambodia (Shankar et al., 1998). Dense masses of tree branches or scrubs are established in lakes, lagoons or rivers and the fishes are attracted by the provision of shelter from predators, a suitable breeding habitat and the availability of natural foods, including periphyton. A simplified version of brush parks has led to the development of periphyton-based aquaculture systems in ponds.

Interest on using substrates in fish (Ramesh et al., 1999; Wahab et al., 1999; Azim et al., 2001b; Azim et al., 2002b; van Dam et al., 2002; Keshavanath et al., 2004) and freshwater prawn culture (Tidwell et al., 2000; Tidwell and Bratvold, 2005) has been growing during the last decade. Nearly all studies indicate that the production in substrate-based ponds is higher than in substrate-free ponds (Table 2). Recently, there has been a growing interest in polyculture of freshwater prawn with tilapia (dos Santos and Valenti, 2002; New, 2005). Since both tilapia and freshwater prawn exhibit strong hierarchies, the welfare of the co-inhabiting species should be carefully considered. There are indications that hierarchies can be minimized in substrate-based ponds (Tidwell and Bratvold, 2005).

Tilapia-prawn polyculture in periphyton-based systems has not been tested before. Important factors to consider include food availability, water quality and inter or intra species competition for food or space.

Table 2. An overview of the studies on the effect of substrate addition for periphyton development on growth, survival and production in ponds. Studies are classified by species.

Species		References
Indian and Chinese carps	Rohu (*Labeo rohita*)	Ramesh et al., 1999; Azim et al., 2001b,c; Azim et al., 2004a,b; Ramanath et al., 2005.
	Calbaush (*L. calbasu*)	Wahab et al., 1999; Azim et al., 2004a.
	Gonia (*L. gonius*)	Azim et al., 2001a.
	Catla (*Catla catla*)	Azim et al., 2001b; Azim et al., 2004a,b;
	Mohaser (*Tor khudree*)	Keshavanath et el., 2001; Keshavanath et el., 2002.
	Fringe-lipped carp (*Labeo fimbriatus*)	Keshavanath et el., 2002.
	Mrigel (*Cirrhina mrigala*)	Azim et al., 2004b.
	Common carp (*Cyprinus carpio*)	Sankar et al., 1998; Ramesh et al., 1999.
Mullet	Mugil (*Mugil cephalus*)	Jana et al., 2004.
Tilapia	Nile Tilapia (*Oreochromis niloticus*)	Phillips et al., 1994; Shrestha and Knud-Hansen, 1994; Azam, 1996; Huchette et al., 2000; Azim et al., 2003a,b.
	Mozambique tilapia (*O. mossambicus*)	Sankar et al., 1998.
	Blackchin tilapia (*Sarotherodon melanotheron*)	Legendre et al., 1989; Hem and Avit 1994.
	O. mossambicus x *O. niloticus*	Keshavanath et al., 2004.
Prawn and shrimp	Prawn (*Macrobrachium rosenbergii*)	Sandifer and Smith, 1977; Cohen et al., 1981; Cohen et al., 1983; Ra'anan et al., 1984; Tidwell et al., 1998; Tidwell et al., 1999; Tidwell et al., 2000.
	Shrimp (*Lipopenaeus vannamei*)	Bratvold and Browdy, 2001; Otoshi et al., 2006.

Roles of periphyton in ponds

Improved water quality

Drenner et al. (1997) attempted to use fish and periphyton for removing nutrients from the water column. Suspended solids were trapped in the periphyton mat, which also took up ammonia and nitrate, produced oxygen, broke down organic matter and increased nitrification (Azim, 2001). In traditional aquaculture ponds, nitrification occurs mostly at the sediment surface and is limited not only by surface area but also by oxygen availability. In addition, fast growing heterotrophic bacteria might limit the space needed by the slow growing chemo-autotrophic nitrifying bacteria. If insufficient nitrification takes place, ammonia toxicity can develop which is still one of the major constraints to intensifying pond aquaculture (Hargreaves, 1998).

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. Therefore, periphytic biofilms enhance nitrification (Langis et al., 1988), keeping ammonia levels low. Periphyton can also act as an antibiotic against a variety of fouling bacteria (Boyd et al., 1998) or as a probiotic/vaccine (Azad et al., 1999).

Nutrient efficiency

Periphyton is a complex mixture of autotrophic and heterotrophic organisms 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 biofilms (Verdegem et al., 2005), reducing the accumulation of organic matter on the bottom in periphyton-based ponds.

For many fish species, the food intake rate is higher when it is available as periphyton compared to phytoplankton. Filter feeding of planktonic algae is unlikely to fully cover the energy demands of most herbivorous carp and tilapia species (Dempster et al., 1995). Besides phytoplankton, these fishes generally require in addition other food sources such as benthic algae, algal detritus or plant fodder, which can be eaten more efficiently (Dempster et al., 1993; Yakupitiyage, 1993). In traditional fishponds often benthic algal mats do not develop due to light limitation caused by dense phytoplankton blooms. Thus, in periphyton-based ponds, periphyton communities provide an extra nutrient cycling loop to the pond.

Periphyton as additional food

In extensive and semi intensive aquaculture ponds, in situ produced algae and imported organic matter are the primary food and nutrient sources. Algae produce organic matter by using solar energy, carbon dioxide and inorganic nutrients that can be eaten directly by culture animals, zooplankton, benthos, invertebrates, etc. or decomposed by bacteria, fungi and other micro-organisms. Indirectly, all the organisms contributing to the heterotrophic food web and bits and pieces of decomposing organic matter, also contribute to the nutrition of culture animals (Colman and Edwards, 1987; Moriarty, 1997). It is a common assumption, particularly in aquaculture, that the phytoplankton community is the most important in terms of energy fixation for fuelling the food web. However, macrophytes and periphyton are a significant and often the dominant contributor to primary production, especially in shallow waters (Moss, 1998). Considering that grazing on biofilms is more efficient than filtering planktonic algae, a more efficient transfer of energy will be achieved in periphyton-based ponds compared to traditional ponds with the culture of herbivorous or omnivorous species. Farming species feeding low in the food chain is a priority for developing sustainable aquaculture (Naylor et al., 2000). In addition, periphyton communities tend to be more stable than phytoplankton communities which easily collapse in highly eutrophic ponds causing oxygen depletion. In consequence, periphyton-based ponds are easier to manage than traditional ponds.

Development of periphyton communities

Development of a periphyton layer on a clean surface generally starts with the deposition of a coating of dissolved organic substances to which bacteria are attracted by hydrophobic reactions (Hoagland et al., 1982; Cowling et al., 2000). The presence of free-floating organic microparticles in eutrophic waters stimulates this process. Periphyton assemblages can reach high biomasses (up to 2350 mg m^{-2} of chlorophyll-*a*) (Westlake et al., 1980).

Substrate type has a strong effect on the density of periphyton, as shown by the differences between different substrate types in experiments in Bangladesh and India (Azim et al., 2002a; Keshavanath et al., 2001). Benthic periphyton has an advantage over phytoplankton because it is closer to the nutrient-rich sediment and the interstitial water. It was shown that periphyton on sediments utilized the nutrients in the sediments pore water and therefore responded much less to nutrient enrichment than periphyton growing on wood in the same lake (Blumenshine et al., 1997).

Periphyton lowered the phosphorus of the overlying water (Hansson, 1989; Bratvold and Browdy, 2001) and sediment (Hansson, 1989). By lowering the nutrient concentration, periphyton can affect the growth of phytoplankton, as was shown in a study in Swedish Lakes (Hansson, 1990). There is a tight coupling between autotrophs and heterotrophs in the periphyton mat. The algae supply organic matter to the heterotrophs, the latter inorganic nutrients to the autotrophs. The quality of the input nutrients to the periphytic biofilm affects the turnover rate in the mat as shown in two rivers with different sources of organic matter input (Romaní, 2000). The quality and quantity of dissolved organic matter affects the structure and productivity of periphyton.

Synergism

The provision of substrates in ponds sometimes enhances synergism in polyculture. In periphyton-based aquaculture ponds with a column and surface feeder a 50-300 percent higher production was achieved than in monoculture of either species, depending on the stocking ratio of both species (Azim et al., 2001b; Azim et al., 2002b). In these experiments, the column feeder relied mainly on periphyton, while the surface feeder utilized plankton, with little or no dietary competition. By adding a bottom dwelling species the synergism was further increased. The bottom feeder stimulated the transfer of nutrients from the bottom to the water column, enhancing plankton production and grazing by the column feeder. More sunlight penetrated into the water column which further enhanced both phytoplankton and periphyton production. Similar synergistic effects in polyculture have been reported for various species combinations (Yashouv, 1971; Hepher, 1988; Milstein, 1992). Synergistic effects between tilapia and freshwater prawn have not been reported, but considering the importance of both species, is interesting to explore.

Objectives, hypothesis and overview of the thesis

In a resource constrained country like Bangladesh, poor farmers cannot afford to buy commercial feeds or provide aeration to increase income from aquaculture. The advantage of periphyton-based production systems is that it is a low cost production technology. The goal of this PhD study was to develop a tilapia (*Oreochromis niloticus*) - freshwater prawn (*Macrobrachium rosenbergii*) polyculture production system, affordable to poor farmers. A first experiment (Chapter 2), using individual farmer ponds, evaluated if stocking of prawns in combination with substrate addition to tilapia ponds can enhance overall pond productivity. The results were encouraging, but benefits were still small compared to tilapia monoculture. Therefore, it was decided to try to optimize the stocking densities and ratios of tilapia and prawns. In a second experiment (Chapter 3) different stocking ratios of tilapia and freshwater prawn were tested in periphyton-based ponds, and evaluated against monoculture of each species. In all treatments the combined stocking density was 20,000 individuals ha^{-1}. The conclusion was that a stocking ratio of 3 tilapia:1 freshwater prawn is the best in terms of total production and income. In the subsequent experiment (Chapter 4) the 3 tilapia: 1 freshwater prawn stocking ratio was tested for different combined stocking densities of tilapia and prawns, showing that a combined stocking density of 30,000 individuals ha^{-1} is more profitable. Results of the first 3 experiments suggested a positive interaction between substrate and freshwater prawn on tilapia production, but left questions if supplemental feeding is effective in periphyton-based tilapia-prawn polyculture. In a 4th experiment, the combined effects of feed and substrate on production in tilapia-prawn periphyton-based production ponds were tested (Chapter 5). A comparison was made between a traditional non-fed, a traditional fed, a substrate-based non-fed and a substrate-based fed tilapia-freshwater prawn production system, and the effects of substrate addition and supplemental feeding on tilapia and freshwater prawn production evaluated.

The previous studies mainly looked at production related parameters. To get a better grasp on the principal processes driving production in tilapia-freshwater prawn polyculture ponds, in chapter 6, the data from the 4 experiments were combined in a multi-factorial analysis. The principal sources of variability in water quality and nutrient cycling in the ponds were identified. In the general discussion (Chapter 7) strengths and weaknesses of the followed approach were outlined, and the applicability of the research findings is discussed, reviewing options for further research.

References

ADB (Asian Development Bank), 2004. A report on Small-scale freshwater rural aquaculture development: country case study in Bangladesh, Philippines and Thailand. 69pp.

Alam, F.M., Thomson, K.J., 2001. Current constraints and future possibilities for Bangladesh fisheries. Food Policy 26, 297-313.

Azad, I.S., Shankar, K.M., Mohan, C.V., Kalita, B., 1999. Biofilm vaccine of *Aeromonas hydrophila* –standardization of dose and duration for oral vaccination of carps. Fish Shellfish Immunol. 9, 519-528.

Azam, M.R., 1996. Effects of periphyton on Tilapia production. Department of Fisheries Biology and Limnology, Bangladesh Agricultural University, Mymensingh, Bangladesh. M.S. Thesis. 97 pp.

Azim, M.E., 2001. The potentials of periphyton-based Aquaculture production systems. Ph.D. Thesis. 219 pp.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Milstein, A., Verdegem, M.C.J., 2001a. Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-based aquaculture. Aquac. Res. 32, 749-760.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Huisman E.A., Verdegem, M.C.J., 2001b. Optimization of stocking ratios of two Indian major carps, rohu (*Labeo rohita* Ham.) and catla (*Catla catla* Ham.) in a periphyton-based aquaculture system. Aquaculture 203, 33-49.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2001c. The potential of periphyton-based culture of two Indian major carps, rohu *Labeo rohita* (Hamilton) and gonia *Labeo gonius* (Linnaeus). Aquac. Res. 32, 209-216.

Azim, M.E., Wahab, M.A., Verdegem, M.C.J., van Dam, A.A., van Rooij, J.M., Beveridge M.C.M., 2002a. The effects of artificial substrates on freshwater pond productivity and water quality and the implications for periphyton-based aquaculture. Aquat. Living Resour. 15, 231-241.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., 2002b. Evaluation of polyculture with Indian major carps in periphyton-based pond. Aquaculture 213, 131-149.

Azim, M.E., Rahaman, M.M., Wahab, M.A., Asaeda, T., Little, D.C., Verdegem, M.C.J., 2004a. Periphyton-based pond polyculture system: a bioeconomic comparison of on-farm and on-station trials. Aquaculture 242, 381-396.

Azim, M.E., Verdegem, M.C.J., Singh, M., van Dam, A.A., Beveridge, M.C.M., 2003a. The effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. Aquac. Res. 34, 685-695.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003b. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia, *Oreochromis niloticus* L. Aquac. Res. 34, 685-695.

Azim, M.E., Wahab, M.A., Biswas, P.K., Asaeda, T., Fujino, T., Verdegem, M.C.J., 2004b. The effect of periphyton substrate density on production in freshwater polyculture ponds. Aquaculture 232, 441-453.

Behning, A., 1924. Studien über die Malakostraken des Wolga bassins. I. Systematische Übersicht der im Wolga bassin bis jetzt aufgefundenen Malacostraca. II. über den Ursprung und die Verbreitung der Malakostraken Fauna der Wolga. Int. Rev. ges Hydrobiol. Hydrogr. 12, 228-247.

Blumenshine, S.C., Vadeboncoeur, Y., Lodge, D.M., Cottingham, K.L., Knight, S.E., 1997. Benthic-pelagic links: response of benthos to water-column nutrient enrichment. J. N. Am. Benthol. Soc. 16, 466-479.

Bowen, S.H., 1982. Feeding digestion and growth qualitative considerations. In: Pullin, R.S.V., Lowe-McConnel, R.H. (Eds.), The Biology and Culture of Tilapias. ICLARM Conf. Proc. 7, Manila. pp. 141-156.

Boyd, K.G., Mearns-Spragg, A., Brindley, G., Hatzidimitriou, K., Rennie, A., Bregy, M., Hubbla, M.O., Burgese, J.G., 1998. Antifouling potential of epiphytic marine bacteria from the surfaces of marine algae. In: Le-Gal, Y., Muller-Feuga, A. (Eds.), Proceedings on Marine microorganisms for industry. Plouzane, France. pp. 128-136.

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

Cohen, D., Ra'anan, Z., Brody, T., 1981. Population profile development and morphotypic differentiation in the giant freshwater prawn, *Macrobrachium rosenbergii*. J. World Maricult. Soc. 12, 213-234.

Cohen, D., Ra'anan, Z., Rappaport, U., Arieli, Y., 1983. The production of freshwater prawn *Macrobrachium rosenbergii* in Israel: improved conditions for intensive monoculture. Bamidgeh 35, 31-37.

Colman, J.A., Edwards, P., 1987. Feeding pathway and environmental constraints in in waste-fed aquaculture: balance and optimization. In: Moriarty, D.J.W., Pullin, R.S.V. (Eds.), Detritus and microbial ecology in aquaculture. ICLARM Conf. Proc. 14. pp. 240-281.

Cowling, M.J., Hodgkiess, T., Parr, A.C.S., Smith, M.J., Marrs, S.J., 2000. An alternative approach to antifouling based on analogues of natural processes. The Science of the Total Environment 258, 129-137.

Dempster, P.W., Beveridge, M.C.M., Baird, D.J., 1993. Herbivory in the tilapia *Oreochromis niloticus*: A comparison of feeding rates of phytoplankton and periphyton. J. Fish Biol. 43, 385-392.

Dempster, P.W., Baird, D.J., Beveridge, M.C.M., 1995. Can fish survive by filter feeding on microparticles? Energy balance in tilapia grazing on algal suspensions. J. Fish Biol. 47, 7-17.

DoF, 2003. Sharonika, Matshya Pakha 2003 (in Bengali), Department of Fisheries, Ministry of Fisheries and Livestock, Government of People Republic of Bangladesh, 134 pp.

DoF, 2005. Sharonika, Jatio Matshya Pakha-2005 (in Bengali), Department of Fisheries, Ministry of Fisheries and Livestock, Government of People Republic of Bangladesh, 154 pp.

dos Santos, M.J.M., Valenti, W.C., 2002. Production of Nile tilapia *Oreochromis niloticus* and freshwater prawn *Macrobrachium rosenbergii* stocked at different densities in polyculture systems in Brazil. J. World Aqua. Soc. 33(3), 369-376.

Drenner, R.W., Day, D.J., Basham, S.J., Smith, J.D., Jensen, S.I., 1997. Ecological water treatment system for removal of phosphorous and nitrogen from polluted water. Ecol. Appl. 7, 381-390.

FAO, 2000. Small ponds make a big difference. Integrated fish with crop and livestock farming. Food and Agriculture Organization of the United Nations, Rome. 142 pp.

FAO, 2004. The State of the World Fisheries and Aquaculture. (SOFIA). Food and Agriculture Organization, Rome, 153 pp.

Getachew, T., 1989. Stomach pH, feeding and ingestion rate in *Oreochromis niloticus* L. (Pisces: Cichlidae) in Lake Awasa, Ethiopia. J.Fish Biol. 42, 195-200.

Guiral, D., Gourbault, N., Helleouet, M.N., 1995. Sediment nature and meiobenthos of an artificial reef (Acadja) used for extensive aquaculture. Oceanol. Acta. 18, 543-555.

Gupta, M.V., Ahmed, M., Bimbao, M.A.P., Lightfoot, C., 1992. Socio-economic Impact and Farmers' Assessment of Nile Tilapia (*Oreochromis niloticus*) Culture in Bangladesh. ICLARM Tech. Rep. 35, Manila.

Gupta, M.V., Mazid, M.A., Islam, M.S., Rahman, M., Hussain, M.G., 1999. Integration of aquaculture into the farming system of the floodprone ecosystem of Bangladesh: an evaluation of adoption and impact. ICLARM Tech. Rep. 56. Manila.

Hansson, L.A., 1989. The influence of a periphytic biolayer on phosphorous exchange between substrate and water. Archiv. Hydrobiol. 115, 21-26.

Hansson, L.A., 1990. Quantifying the impact of periphytic algae on nutrient availability for phytoplankton. Freshwat. Biol. 24, 265-273.

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

Hem, S., Avit, J.L.B., 1994. First results on "acadja-enclos" as an extensive aquaculture system (West Africa). Bull. Mar. Sci. 55, 1038-1049.

Hepher, B., 1988. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, 388 pp.

Hoagland, K.D., Roemer, S.C., Rosowski, J.R., 1982. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). Am. J. Bot. 69, 188-213.

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M., 2000. The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture 186, 45-60.

Hussain, M.G., 1999. Recent advancement in rural aquaculture technology. Proceedings of the workshop "Rural aquaculture and poverty alleviation". Bangladesh Fisheries Research Institute, Mymensingh.

Jana, S.N., Garg, S.K. Patra, B.C., 2004. Effect of periphyton on growth performance of grey mullet, *Mugil cephalus* (Linn.), in inland saline groundwater ponds. J. Appl. Ichthyol. 20, 110-117.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquaculture 235, 303-314.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2002. The effect of periphyton and supplemental feeding on the production of the indigenous carps *Tor khudree* and *Labeo fimbriatus*. Aquaculture 213, 207-218.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Rooij, J.M., Beveridge, M.C.M., Baird, V., Verdegem, M.C.J., van Dam, A.A., 2001. Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. Aquac. Res. 32, 189-197.

Langis, R., Proulx, D., de la Noue, J., Couture, P., 1988. Effects of bacterial biofilms on intensive *Daphnia* culture. Aquacult. Eng. 7, 21-38.

Legendre, M., Hem, S.,Cisse, A., 1989. Suitability of brackish water tilapia species from the Ivory Coast for lagoon aquaculture. II. Growth and rearing methods. Aquat. Living Resour. 2, 81-89.

Lewis, D., 1997. Rethinking aquaculture for resource-poor farmers: perspective from Bangladesh. Food Policy 22, 533-546.

Milstein, A., 1992. Ecological aspects of fish species interactions in polyculture ponds. Hydrobiol. 231, 177-186.

Moriarty, C.M. Moriarty, D.J.W., 1973. Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis nigripinnis* in Lake George, Uganda. J. Zool. (London), 171, 209-255.

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333-349.

Moss, B., 1998. Ecology of freshwaters, man and medium, past to future. 3rd edition. Blackwell Science Ltd, Oxford.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017-1024.

New, M.B., 2005. Freshwater prawn farming: global status, recent research and a glance at the future. Aquac. Res. 36, 210-230.

Otoshi, C.A., Montgomery, A.D., Matsuda, E.M., Moss, S.M., 2006. Effects of artificial substrate and water source on growth of juvenial Pacific white shrimp, *Lipopenaeus vannamei*. J. World Aquac. Soc. 37(2), 210-213.

Phillips, P., Russell, A., Bender, J., Munoz, R., 1994. Management plan for utilization of a floating microbial mat with its associated detrital gelatinous layer as a complete tilapia *Oreochromis niloticus* feed system. Bioresour. Technol. 47, 239-257.

Pompa, T., Masser, M., 1999. Tilapia life history and biology. Southern Regional Aquaculture Centre, SAARC Publication No. 283 p.

Ra'anan, Z., Cohen, D., Rappaport, U., Zohar, G., 1984. The production of the freshwater prawn *Macrobrachium rosenbergii* in Israel. The effect of added substrates on yields in a monoculture situation. Bamidgeh 36, 35-40.

Ramanath, M.M., Manissery, J.K., Keshavanath, P., Shankar, K.M., Nandeesha, M.C., Rajesh, K.M., 2005. Effects of paddy straw and sugarcane bagasse on water quality, bacterial biofilm production and growth and survival of rohu, *Labeo rohita* (Hamilton). Aquac. Res. 36, 635-642.

Ramesh, M.R., Shankar, K.M., Mohan, C.V., Varghese, T.J., 1999. Composition of three plants substrates for enhancing carp growth through bacterial biofilm. Aquac. Eng. 19, 119-131.

Romaní, A.M., Sabater, S., 2000. Influence of algal biomass on extracellular enzyme activity in river biofilms. Microb. Ecol. 40, 16-24.

Sandifer, P.A., Smith, T.I.J., 1977. Intensive culture of post larval Malaysian prawns (*Macrobrachium rosenbergii*) in a closed cycle nursery system. Proceedings of the Eighth Annual Meeting of the World Mariculture Society. Louisiana State University, pp. 225-235.

Shah, M.S., 2003. Human resource development activities in fisheries sector. In: Fish Fortnight Compendium 2003. Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh, 57-59 pp.

Shankar, K.M., Mohan, C.V., Nandeesha, M.C., 1998. Promotion of substrate based microbial biofilm in ponds – a low cost technology to boost fish production. Naga 21, 18-22.

Shrestha, M.K., Knud-Hansen, C.F., 1994. Increasing attached microorganism biomass as a management strategy for Nile tilapia (*Oreochromis niloticus*) production. Aquacult. Eng. 13, 101-108.

Tidwell, J.H., Bratvold, D., 2005. Utility of added substrates in shrimp culture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 247-268.

Tidwell J.H., Cycle, S., Van Arnum, A., Weibel, C., 2000. Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. J. World Aquac. Soc. 31, 452-458.

Tidwell, J.H., Coyle, S.D., Schulmeister, G., 1998. Effects of added substrate on the production and population characteristics of freshwater prawns *Macrobrachium rosenbergii* in ponds. J. World Aquac. Soc. 29(1), 17-21.

Tidwell, J.H., Coyle, S.D., Weibel, C., Evans, J., 1999. Effects of interactions of stocking density and added substrate on production and population structure of freshwater prawns *Macrobrachium rosenbergii*. J. World Aquac. Soc. 30(2), 174-179.

Turker, H., Eversole, A.G., Brune, D.E., 2003. Filtration of green algae and cyanobacteria by Nile tilapia, *Oreochromis niloticus*, in the Partitioned Aquaculture System. Aquaculture 215: 93-101.

UN, 2000. World population prospects, the 1998 revision; and estimates. United Nations Population Division. http// www.prb.org. as retrieved on 11.02.2006.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1-31.

Verdegem, M.C.J., Eding, E.H., Sereti. V., Munubi, R.N., Satacruz-Reyes, R.A., van Dam, A.A., 2005. Similarities between microbial and periphytic biofilms in Aquaculture systems. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 191-205.

Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S., 1999. The potential of periphyton-based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton). Aquac. Res. 30, 409-419.

Wahab, M.A., Kibria, M.G., 1994. Katha and Kua fisheries- unusual fishing methods in Bangladesh. Aquaculture News 18-24.

Welcomme, R.L., 1972. An evaluation of the acadja method of fishing as practiced in the coastal lagoons of Dahomey (West Africa). J. Fish Biol. 4, 39-55.

Westlake, D.F., Adams, M.S., Bindloss, M.E., Ganf, G.G., Gerloff, G.C., Hammer, U.T., Javornicky, P., Koonce, J.F., Marker, A.F.H., McCracken, M.D., Moss, B., Nauwerck, A., Pyrina, I.L., Steel, J.A.P., Tilzer, M., Walters, C.J., 1980. Primary production. In: LeCren, E.D., Lowe-McConnell, R.H. (Eds.), The Functioning of Freshwater Ecosystems (International Biological Programme 22). Cambridge University Press, Cambridge, 588 pp.

Wijkström, U.N., 2003. Short and long term prospects for consumption of fish. Vet. Res. comm., 27 supplement 1, 461-468.

Yakupitiyage, A., 1993. Constraints to the use of plant fodder as fish feed in tropical small scale tilapia culture systems: an overview. In: Kaushik, S.J., Luquet, P. (Eds.), Fish nutrition in practice. Institut National de la Recherche Agronomique, Les Colloques, no. 61, Paris, pp. 681-689.

Yashouv, A., 1971. Interaction between the common carp (*Cyprinus carpio*) and the silver carp (*Hypophthalmichthys molitrix*) in fishponds. Bamidgeh 23, 85-92.

Chapter 2

Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia-prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development

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Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia-prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development

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Abstract

The effects of periphyton grown on bamboo substrate, on growth and production of Nile tilapia, *Oreochromis niloticus* (Genetically Improved Farmed Tilapia strain) in monoculture and polyculture with the freshwater prawn (*Macrobrachium rosenbergii*) were studied. The experiment had 2 x 2 factorial design: the first factor was presence or absence of substrate for periphyton development, the second factor was related to culture system. The first system was monoculture of the GIFT strain of Nile tilapia stocked at 20,000 fingerlings ha^{-1}, the second system was tilapia-prawn polyculture with each stocked at 20,000 fingerlings or postlarvae ha^{-1}. Bamboo poles were posted vertically in ponds under substrate treatments. Feed and inorganic fertilizers were applied to all ponds.

There were no differences in phytoplankton biomass and primary productivity between the treatments ($P > 0.05$). The electivity indices indicated that there were lower dietary overlaps between tilapia and prawn. Survivals of tilapia and prawn were higher in ponds with bamboo substrate (60% and 35%, respectively) than in the control ponds without substrates (55% and 20%, respectively). Addition of substrate significantly ($P < 0.05$) increased growth and production of both species. In monoculture, substrate contributed 40% to tilapia production, whereas, in polyculture, it contributed 46%. Prawn production was increased by 127%. Highest total yield (2445 kg ha^{-1} tilapia and 141 kg ha^{-1} prawn) over a 145 days culture period was recorded in substrate-based polyculture ponds. However, there was conclusive evidence that addition of periphyton substrates resulted in higher fish production and hence, polyculture of tilapia and prawn in periphyton ponds is a promising option for low-input ecological aquaculture.

Keywords: Periphyton, tilapia, freshwater prawn, monoculture, polyculture, substrates

Introduction

The use of periphyton substrates in freshwater finfish and prawn production has been found potentially promising (van Dam et al., 2002), and thus it has created awareness among the scientific communities and the farmers to explore further how to make the technology more robust and sustainable. To this end, polyculture of Indian major carps in non-fed periphyton systems has been developed and found technologically and economically sound (Ramesh et al., 1999; Wahab et al., 1999; Azim et al., 2002, 2004). Preliminary trials on tilapia monoculture in periphyton-based systems have given mixed results: periphyton contributed very marginal (Shrestha and Knud-Hansen, 1994) to several folds increase in production (Hem and Avit, 1994; Keshavanath et al., 2004). While the culture potential of finfish species (especially Indian major carps) in substrate-based system was found promising, more efforts are warranted to try this technology with other high valued aquaculture species like freshwater prawn and penaeid shrimp. The culture of high valued species on a commercial basis is mostly intensive and beyond the reach of most resource poor people in Asia, because it involves high technology and big investment. On the other hand, periphyton-based aquaculture resulted in higher fish production and profit, and profitability can still be increased by stocking a high value species like prawn without increasing other inputs even at low density. However, it would be advantageous if the low cost substrate-based system was found to work well for culture of these high valued species. Experiments conducted in USA have led to the conclusion that substrate-based systems can increase freshwater prawn production to a significantly higher level when compared to traditional production systems (Tidwell and Bratvold, 2005). In these trials, synthetic substrates were used mainly to increase the available surface area to minimize territorialism among the individuals while growth mostly depended on artificial pelleted feed. Nevertheless, the use of substrates in freshwater prawn culture has created new dimension that much deserved for testing the technology in Asian region preferably using locally available substrates in finfish-freshwater prawn polyculture ponds. There has been an enormous interest in polyculture of freshwater prawn with fish, especially with tilapia (New, 2005). Interest on using substrates in carp ponds (Wahab et al., 1999; Azim et al., 2002) and freshwater prawn ponds (Tidwell et al., 2000; Tidwell and Bratvold, 2005) have been growing since last decade. However, there has been hardly any efforts so far made to explore the possibility of polyculture of prawn and tilapia in the substrate based ponds. Therefore, it looks promising to add prawns in low density to fed tilapia periphyton-based ponds. Tilapias and prawns have different food and feeding habits, but for both species, the addition of substrates resulted in extra growth (Hem and Avit, 1994; Tidwell et al., 1998; Tidwell et al., 2000; Keshavanath et al., 2004). As a part of an overall research objective towards the development of a low-cost polyculture system of tilapia and prawn, this piece of research was carried out a) to quantify the contribution of substrates to both tilapia and prawn production; b) to determine whether prawn addition affects tilapia production, and c) to investigate the effects of substrates and prawn on pond productivity.

Materials and methods

Experimental design

The experiment had 2 x 2 factorial design: the first factor was presence or absence of substrate for periphyton development, the second factor was related to culture system. The first system was monoculture of the GIFT strain of Nile tilapia stocked at 20,000 fingerlings ha^{-1}, the second system was polyculture with tilapia and prawn each stocked at 20,000 fingerlings or postlarvae ha^{-1}. The experiment was carried out in 12 75-m^2, 1.2 m deep, earthen ponds at the Fisheries Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh for a period of 145 days between May and October, 2003. Ponds were assigned randomly to the four treatments in triplicate.

Pond preparation and management

The ponds were rain-fed and fully exposed to prevailing sunlight. Pond embankments were covered with grass. Water levels were maintained by supplying deep tube-well water whenever needed. Before starting the experiment, ponds were reshaped and manually cleaned of aquatic vegetation. All unwanted fishes were eradicated by rotenone application at the rate of 100 g pond^{-1}. The dead fishes and other aquatic organisms were removed by repeated netting. Ponds were limed with 250 kg ha^{-1} $CaCO_3$ on day 1. On day 4, 5 bamboo poles m^{-2} with a mean diameter of 5.5 cm were posted vertically into the bottom mud in substrate treatment ponds, excluding a one meter wide perimeter water surface from the dike (i.e. 44 m^{-2} area was provided with substrate). The total submerged substrate area per pond was calculated as: S=2π radius of a pole (0.0275 m) x length of each pole (1.2 m) x number of poles per m^2 (5) x area of ponds provided with substrate. This resulted in an additional area for periphyton development equaling about 60% (i.e. 45 m^2) of the pond surface area. On day 5, 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. Subsequently, all ponds were fertilized fortnightly with urea and TSP at the rate of 50 kg ha^{-1} each until harvesting.

Eleven days after installing the bamboo poles, 30 day-old post-larvae (PL-30) of the freshwater prawn (*Macrobrachium rosenbergii)* were released into the ponds under polyculture treatments. On day 30, two weeks after stocking the PLs, Nile tilapia fingerlings of the GIFT strain (*Oreochromis niloticus*) with an average weight of 1.82 g were stocked into all ponds. All ponds were subjected to the same regime of feeding and fertilization. A low protein (25% crude protein) pelleted fish feed procured from the market was applied daily to the ponds at the rate of 3% of the tilapia biomass for the entire experimental period. The tilapias and prawns were sampled at monthly intervals using a cast net after removing some bamboo poles. After sampling, poles were put back to their original positions. Weight of approximately 10% of total number of fish and prawn were measured individually to estimate the tilapia biomass and to adjust the feeding rate.

At the end of the experiment, bamboo poles were removed, water was pumped out of the ponds and all fish and freshwater prawns were collected, counted and weighed.

Measurement of chlorophyll a and primary productivity

Chlorophyll *a* concentrations of pond water were determined monthly. A known amount of water sample were filtered through micro-fibre glass filter paper (Whatman GF/C) using a vacuum pressure air pump. The filter paper was kept in a test tube containing 10 ml 90%

acetone, ground with a glass rod and preserved in a refrigerator for 24 hours. Later, Chlorophyll *a* was determined using a spectrophotometer (Milton Roy Spectronic, Model 1001 plus) at 664 and 750 nm wave length following Boyd (1979).

Primary productivity was measured on days 50th and 130th of the experiment. Water samples from surface (5 cm below from water surface), middle ((50 cm below from water surface) and bottom (100 cm bellow from water surface) were taken using water sampler and 250 ml light and dark BOD bottles. Three light and two dark bottles were filled with water from each depth. One of the light bottles was immediately used to measure the initial oxygen concentration by using DO meter (YSI model 85-10 FT), while the remaining bottles were hanged at the depths from where the water samples were collected with the help of stake and rope. The samples were incubated in situ for 6 h during 0900 h to 1500 h, then removed to measure the oxygen concentration. Gross and net photosynthesis and respiration were calculated in mg C l^{-1} h^{-1} (Wetzel and Likens, 1991).

Periphyton sampling and analysis

Each month, starting from day 34, the periphyton biomass on the bamboo poles was sampled. Three poles were randomly selected from each pond. From each pole, two 2x2-cm^2 of periphyton samples were collected at 25, 50 and 75 cm depths. The periphyton samples were collected with a sharp blade from the surface area of the substrate, care being taken not to remove any of the substrate itself, and the material transferred to pre-weighed and labeled pieces of aluminum foil. One sample was used to determine dry matter and ash contents using oven and muffle furnace, respectively. Samples were placed in a drying oven (Memmert, Model UM/BM 100-800) and dried at 105^0c until constant weight (24 h), before being transferred to a desiccator until weighed (BDH, Model 100A; precision 0.0001 g). Dry samples from depth, poles and ponds per treatment were pooled, transferred to a muffle furnace and ashed at 450^0C for 6 h and re-weighed. Ash and ash free dry matter (AFDM) were calculated from weight differences. Another sample was used to determine chlorophyll *a* and pheophytin *a* following standard methods (APHA, 1992).

Gut content analysis and electivity indices

The gut content of the stocked tilapia and prawn were examined on the 30th and 60th day of the experiment. On the sampling dates, feed was applied to the ponds in the early morning. Three individuals of each species were collected from each pond by cast net at 1600-1700 h after removing some bamboo poles. After each sampling, an equal number of similar sized tilapias and prawns were released into the respective ponds. The entire intestine of the tilapia was dissected out, while the intestine from the thorax region was removed from prawns, immediately after catching. The gut contents of each individual were carefully excised and preserved in 10% buffered formalin until analysis. The gut contents of three individuals from each pond were then carefully washed out with distilled water, mixed together in a petri dish, and diluted to 50 ml. A 1-ml subsample was transferred to a Sedgewick Rafter Counting Cell (S-R cell), and all plankton in 10 randomly selected squares were identified up to genus level and counted using a binocular microscope (Olympus BH-2 with phase contrast facilities; magnification 40x). For each gut sample, three subsamples were examined in the similar way. Plankton genera were expressed as the percentage of the total gut contents.

A 20-l composite water sample, taken from different randomly selected locations in each pond was passed through a 45-µm mesh plankton net. The filtered samples were then carefully transferred to a measuring cylinder and made up to a standard volume (50ml) with distilled water. Buffered formalin of 10% was added as a preservative and the samples were stored in small, sealed plastic bottles until examination. Plankton density was estimated using the same procedure mentioned in Azim et al. (2001) and using the following formula:

$$N = (P \text{x} C \text{X} 100)/\text{L}$$

Where N = Numbers of plankton cells or units per liter of pond water; P = Total number of plankton counted in 10 fields; C = volume of final concentrate of the sample in ml. Plankton was identified using the classification keys of Bellinger (1992), Prescott (1962) and Needham and Needham (1962).

Electivity (E) indices were calculated following Ivlev (1961) as:

$$E = (Pg - Pw)/(Pg + Pw)$$

Where, Pg = the relative content of any ingredient in the ration, expressed as percentage of total ration; Pw = the percentage of the same item in the pond water. The resultant value of E ranges from +1 to -1, where positive values indicate active selection of particular food item and the negative values indicate avoidance.

Statistical analysis

Yield parameters were compared by two-way ANOVA with addition of substrate (with and without) and freshwater prawn (with and without) as main factors. A repeated measures 2-way ANOVA was performed for chlorophyll *a*, primary productivity and electivity indices data (Gomez and Gomez, 1984). The arcsine transformation was used for comparing percentage data before statistical analysis but percent values are reported. The effects were tested at 5% level of significance using statistical package, Stat View (SAS Institute, Cary, NC 27513, USA).

Results

Effects of substrates and freshwater prawn and their interactions on tilapia

Effects of addition of substrates and freshwater prawn, and their interactions on yield parameters of tilapia are given in Table 1. Survival, individual weight gain and net yield were significantly higher ($P < 0.05$) in ponds provided with substrates than in ponds without substrates. On average, substrates contributed 40% higher net yield of tilapia in monoculture and 56% in polyculture. Individual weight gain of tilapia increased by 30% due to addition of substrates in both mono- and polyculture ponds. There were no significant effects of addition of freshwater prawn on survival, individual weight gain and net yield of tilapia (Table 1). Both substrate and freshwater prawn had significant effects on FCR of tilapia: substrates improved food conversion ratio by 32% whereas, FCR increased about 12% due to addition of freshwater prawn (Table 1).

Substrate also had significant effects ($P < 0.01$) on survival and net yield of freshwater prawn. There were 75% higher survival and 127% increased net yield of prawn in periphyton-based ponds than in control ponds (Table 2). However, substrate had only a marginal effect on individual weight gain of prawn ($P = 0.107$).

Primary productivity and phytoplankton and periphyton biomass

Autotrophic carbon productions and respirations are given in Table 3. There were no significant differences ($P > 0.05$) in mean values of gross and net carbon productivity and respiration among the treatments and between the two sampling dates. Whereas, gross and net C productions decreased with increasing water depth, respirations were more or less similar at all three depths. Phytoplankton biomass in terms of chlorophyll *a* concentrations did not show any differences among the treatments indicating that neither substrates nor prawns had a significant effect on the standing phytoplankton biomass of the water column (Figure 1). However, the concentrations decreased during last three sampling dates except in substrate ponds with only tilapia. The periphyton biomass in terms of dry matter (DM) and chlorophyll *a* per unit surface area of substrates did not show any significant differences between monoculture and polyculture treatments (Figure 2). However, the biomass increased sharply during the first half of the experiment and decreased during the second half of the experiment indicating high grazing pressure during the second half period.

Electivity indices

There were differences in electivity indices for different plankton groups among the different systems. Sampling dates had also an effect on electivity indices for different groups of food organisms. The unidentified portion of the gut content for tilapia and prawn were about 20% and 35%, respectively, which was excluded during calculation. The average electivity indices of tilapia and prawn for different major groups of plankton at two sampling dates are shown in Table 4.

Among 60 genera of plankton available in pond water, tilapia preferred *Cyclotella* (average electivity index +0.77) followed by *Surirella* (+0.42) and *Melosira* (+0.35) in the Bacillariophyceae group; *Tetraedron* (+0.81) followed by *Botryococcus* (+0.55), *Volvox* (+0.35), *Closterium* (+0.29) and *Ulothrix* (+0.20), in the Chlorophyceae group; *Oscillatoria* (+0.58) followed by *Anabaena* (+0.18) and *Microcystis* (+0.18) in the Cyanophyceae group; and *Phacu*s (+0.24) followed by *Euglena* (+0.07*)* in Euglenophyceae group. *Lecane* (+0.08) was the preferred zooplankton followed by Nauplius (+0.01).

The electivity indices showed that freshwater prawn selectively feed on organisms belonging to the Bacillariophyceae and Chlorophyceae, than on other groups of phytoplankton. The electivity index for Chlorophyceae was higher in the second sampling date and the opposite was observed in the case of the Euglenophyceae. *Rhizosolenia* (+ 0.92) was the most preferred genus of phytoplankton followed by *Diatoma* (+0.85), *Cyclotella* (+0.78) and *Navicula* (+0.58) in the Bacillariophyceae group; *Gonatozygon* (+0.77) followed by *Tetraedron* (+0.66), *Closterium* (+0.65) and *Chlorella* (+0.52) in the Chlorophyceae group;

and *Aphanocapsa* (+0.52) followed by *Aphanizomenon* (+0.22) in the Cyanophyceae group. For zooplankton, *Keratella* was the preferred genus (+0.02) for the first sampling date and *Trichocerca* was the preferred genus (+0.24) for the second sampling date. For prawn, Nauplius was the preferred one that was observed during the first and second sampling dates in the substrate free ponds (+0.47 and 0.06, respectively). The tilapias and prawns both preferred diatoms (Bacillariophyceae), whereas tilapias showed preference for Cyanophyceae and prawns strongly avoided them.

Discussion

Addition of substrates enhanced survival and production of both tilapia and freshwater prawn in mono- and polyculture system and improved FCR for tilapia. This is mainly because of additional shelter and natural food in the form of periphyton colonized on bamboo substrates 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). Since the phytoplankton biomass and primary productivity of pond water were similar in all treatment ponds (Figure 1 and Table 2), periphyton in substrate ponds served as an additional food without reducing the pelagic productivity. For finfish, the reported increase in production due to substrates ranged from 30-115% in carp monoculture (Wahab et al., 1999; Keshavanath and Gangadhar, 2005) and 30-210% in carp polyculture (Azim and Wahab, 2005), depending on several factors. Addition of similar number of freshwater prawn seed stock to the tilapia ponds did not affect survival, individual weight gain and net yield of tilapia in the presence of substrates. For the polyculture of freshwater prawns (*Macrobrachium rosenbergii*) with tilapia (*Oreochromis niloticus*), it is reported that prawn did not affect the production of tilapia (Dos Santos and Valenti, 2002). Tilapia is regarded as an omnivorous species and capable of feeding on benthic and attached algal and detrital aggregates (Bowen, 1982; Dempster et al., 1993; Azim et al., 2003). Indeed, tilapias were regularly observed grazing on the substrates for periphyton in the experimental ponds. The amount of periphyton ingested by tilapia can be estimated using the contribution of substrate to net fish yield in this experiment (744 kg ha^{-1} 135 d^{-1}; from Table 1) and using a reported periphyton FCR value of 1.34 on ash free dry matter basis (Azim et al., 2003). To produce 744 kg ha^{-1} fish, about 997 kg AFDM periphyton per ha pond (6000 m^2 substrate surface area in this experiment) was needed which is equivalent to about 1.23 g AFDM m^{-2} d^{-1} (or 1.76 g dry matter, 30% ash). In general, high values for periphyton productivity are found on coral reef systems, ranging from 1 to 3 g C m^{-2} d^{-1} (Carpenter, 1986; Polunin, 1988; Klumpp and Polunin, 1989; Van Rooij et al., 1998). The periphyton productivity in aquaculture ponds ranged 2.2-2.8 g AFDM m^{-2} d^{-1} depending on substrate types (Azim et al., 2002). Periphyton biomass increased steadily during the first two months and then decreased continuously until the end of the experiment (Figure 2). This might be because of low grazing pressure on periphyton by the low biomass of fish at the earlier stage of fish stocking and then increased grazing pressure by increased fish biomass due to growth at the later stage of the experiment. However, there were no differences in periphyton biomass due to addition of freshwater prawn (Figure 2) indicating that this species either did not eat periphyton or may have picked up animal portion and detrital aggregates rather than picking up the mixed biomass or their grazing may be replaced by greater turnover of nutrients by phytoplankton rather than periphyton as is often the case in ecological production (Hwang et al., 1998). However, visual observation was not possible to confirm whether freshwater prawn grazed on periphyton since they mainly inhabit the deeper region either on the substrates or simply crawling over on the bottom, or both. There are evidences that prawns in their natural habitats prefer to forage on animals like trichopterans, chironomids, oligochates, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996; Tidwell et al., 1997). There is also evidence that substrate based systems enhanced the production of benthos in the culture systems (Tidwell et al., 2005; Azim, 2001).

Although it was impossible to separate periphytic and planktonic portions of gut contents, efforts had been made to check whether there were significant dietary competitions for natural food between tilapia and freshwater prawn by gut content analysis. A considerable portion of gut contents were already semi-digested/unidentifiable. The electivity indices suggest that there were rarely food competitions between the two species. From the electivity indices, it was also found that tilapias showed a considerable positive selection for the Cyanophyceae group whereas, prawns had a good number of positive selections for Bacillariophyceae and Chlorophyceae and they avoided Cyanophyceae in most cases. From microscopic examination of Nile tilapia stomach contents, *Microcystis* was the most abundant genus in the Cyanophyceae group in diet of fish sampled from two Ethiopian Rift Valley lakes, Awasa and Zwai (Getachew, 1987). Abdelghany (1993) found green algae (i.e. *Ankistrodesmus, Pediastrum* and *Closterium*) and cyanobacteria (i.e. *Anabaena, Oscillatoria* and *Microcystis*) in Nile tilapia stomach from the Nile River, Egypt. The cell counts of phytoplankton in water filtered by tilapias indicated significant reduction in green algae and cyanobacteria (Turker et al., 2003). The most reliable method of determining food preferences of fish is to use a combination of food item availability and stomach sampling. But in crustaceans, such as prawns, food habits are difficult to study and their results are not reliable, due to incidental ingestion of nutritionally unimportant items, small stomach size, small prey size, and mastication of food items at consumption and in the stomach (Brown et al., 1992). However, the fish were fed pelleted diet, which might interfere or reduce the extent of natural feed consumption by the tilapias and freshwater prawns.

In polyculture, tilapia might have affected prawn survival during molting as reported by Uddin et al. (2006). Therefore, further trials with different stocking combinations of these two species provided with different amount of substrates are recommended for developing a substrate-based finfish-crustacean polyculture for earthen tropical ponds.

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References

Abdelghany, A.E., 1993. Food and feeding habits of Nile tilapia from the Nile River at Cairo, Egypt. In: Reinertsen, L., Dahle, A., Jorgensen, C., Twinnereim, R. (Eds.), Fish Farming Technology. Rotterdam, Netherlands, pp. 447-453.

APHA, 1992. Standard Methods for the Examination of the Water and Wastewater. American Public Health Association, Washington, DC.

Azim, M.E., 2001. The potential of periphyton-based aquaculture production systems. Ph.D. Thesis. Fish Culture and Fisheries Group, Wageningen University, The Netherlands, 219 pp.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Milstein, A., Verdegem, M.C.J., 2001. Optimization of fertilization rates for maximizing periphyton growth on artificial substrates and implications for periphyton-based aquaculture. Aquac. Res. 32, 749-760.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., 2002. Evaluation of polyculture of Indian major carps in periphyton-based ponds. Aquaculture 213, 131-149.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia, *Oreochromis niloticus*. L. Aquac. Res. 34, 85-92.

Azim, M.E., Rahaman, M.M., Wahab, M.A., Asaeda, T., Little, D.C., Verdegem, M.C.J., 2004. Periphyton-based pond polyculture systems: a bio-economic comparison of on-farm and on-station trials. Aquaculture 242, 381-396.

Azim, M.E., Wahab, M.A., 2005. Periphyton-based pond polyculture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 207-222.

Bellinger, E.G., 1992. A key to common algae. The Institute of Water and Environmental Management , London, UK, 138 pp.

Bowen, S.H., 1982. Feeding, digestion and growth-qualitative considerations. In: Pullin, R.S.V., Lowe-McConnell, R.H. (Eds.), The Biology and Culture of Tilapias. ICLARM Conference Proceedings 7, International Center for Living Aquatic Resources Management, Manila, Philippins, pp. 141-156.

Boyd, C.E., 1979. Water Quality in Warmwater Fish Ponds. Auburn University, Craftmaster Printers, Opelika, Alabama, 359 pp.

Brown, P.B., Wetzel, J.E., Spacie, A., Konopka, A., 1992. Evaluation of nutritionally occurring organisms as food for juvenile crayfish, *Procambarus clarkii*. J. World Aqua. Soc. 23, 211-216.

Carpenter, R.C., 1986. Partitioning herbiviry and its effect on coral reef algal communities. Ecological monographs 56, 345-363.

Corbin, J.S., Fujimoto, M.M., Iwai, T.U., Jr., 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P. (Ed), CRC Handbook of Mariculture. CRC Press, Boca Raton, FL, pp. 391-423.

Coyle, S.D., Najeeullah, T., Tidwell, J.H., 1996. Evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawns *Macrobrachium rosenbergii*. J. Appl. Aqua. 6, 57-66.

Dempster, P.W., Beveridge, M.C.M., Baird, D.J., 1993. Herbivory in the tilapia *Oreochromis niloticus*: A comparison of feeding rates of phytoplankton and periphyton. J. Fish Biol. 43, 385-392.

Dos Santos, M.J.M., Valenti, W.C., 2002. Production of Nile tilapia *Oreochromis niloticus* and freshwater prawn *Macrobrachium rosenbergii* stocked at different densities in polyculture system in Brazil. J. World Aqua. Soc. 33, 369-376.

Getachew, T., 1987. A study on an herbivores fish, *Oreochromis niloticus*, diet and its quality in two Ethiopian Rift Valley lakes, Awasa and Zwai. J. Fish Biol. 30, 439-449.

Gomez, K.A., Gomez A.A., 1984. Statistical procedures for agricultural research, 2nd edition. John Wiley and Sons, New York, 680 pp.

Hem, S., Avit, J.L.B., 1994. First results on "acadjas enclos" as an extensive aquaculture system (West Africa). Bull. Mar. Sci. 55 , 1040-1051.

Hwang, S.J., Havens, K.E. Steinman, A.D., 1998. Phosphorus kinetics of planktonic and benthic assemblages in a shallow subtropical lake. Freshwat. Biol. 40, 729-745.

Ivlev, V.S., 1961. Experimental ecology of the feeding of fish. Yale University Press, New Haven, Connecticut, USA, 302 pp.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquaculture 235, 303-314.

Keshavanath, P., Gangadhar, B., 2005. Research on periphyton-based aquaculture in India. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 223-236.

Klumpp, D.W., Polunin, N.V.C., 1989. Partitioning among grazers of food resources within damselfish territories on a coral reef. J. Exp. Mar. Ecol. 125, 145-169.

Milstein, A., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2003. The effects of periphyton, fish and fertilizer dose on biological processes affecting water quality in earthen fish ponds. Env. Biol. Fish. 68, 247-260.

Needham, J.G., Needham, P.R., 1962. A guide to the study of freshwater biology, fifth edition. Holden Day Inc., San Francisco, California, USA, 108pp.

New, M.B., 2005. Freshwater prawn farming: global status, recent research and a glance at the future. Aquac. Res. 36, 210-230.

Polunin, N.V.C., 1988. Efficient uptake of algal production by single residence herbivorous fish on the reef. J. Mar. Biol. Ecol. 123, 61-76.

Prescott, G.W., 1962. Algae of the Western Great Lakes Area. Wm. C. Brown Co., Inc., Dubuque, Iowa, 977 pp.

Ramesh, M.R., Shankar, K.M., Mohan, C.V., Varghese, T.J., 1999. Composition of three plants substrates for enhancing carp growth through bacterial biofilm. Aquac. Eng. 19, 119-131.

Shrestha, M.K., Knud-Hansen, C.F., 1994. Increasing attached microorganism biomass as a management strategy for Nile tilapia (*Oreochromis niloticus*) production. Aquac. Eng. 13, 101-108.

Tidwell, J.H., Coyle, S.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, S.J., D'Abramo, L.R., Fuller, M.J., 1997. Relative prawn production and benthic macroinvertebrate densities in unfed, organically fertilized, and fed pond systems. Aquaculture 149, 227-242.

Tidwell, J.H., Coyle, S.D., Schulmeister, G., 1998. Effects of added substrate on the production and population characteristics of freshwater prawns *Macrobrachium rosenbergii* in ponds. J. World Aqua. Soc. 29(1), 17-21.

Tidwell, J.H., Cycle, S., Van Arnum, A., Weibel, C., 2000. Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. J. World Aqua. Soc. 31. 452-458.

Tidwell, J.H., Coyle, S.D., VanArnum, A., Weibel, C., 2002. Effects of substrate amount and orientation on production and population structure of freshwater prawns *Macrobrachium rosenbergii* in ponds. J. World Aqua. Soc. 33 (1), 63-69.

Tidwell, J.H., Bratvold, D., 2005. Utility of added substrates in shrimp culture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 247-268.

Turker, H., Eversole, A.G., Brune, D.E., 2003. Filtration of green algae and cyanobacteria by Nile tilapia, *Oreochromis niloticus*, Lower Case in the Partitioned Aquaculture System. Aquaculture 215, 93-101.

Uddin, S., Azim, M.E., Wahab, A., Verdegem, M.C.J., 2006. The potential of mixed culture of genetically improved farmed tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. Aquac. Res. 37, 241-247.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1-31.

Van Rooij, J.M., Videler, J.J., Bruggemann, J.H., 1998. High biomass and production but low energy transfer effiency of Caribbean parrotfish: implications for tropic models of coral reefs. J. Fish Biol. 53, 154-178.

Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S., 1999. The potential of periphyton based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton). Aquac. Res. 30, 409-419.

Wetzel, R.G., Likens, G.E., 1991. Limnological analysis. 2nd edn., Verlag, New York, 391 pp.

Table 1

2-way ANOVA comparing yield parameters of tilapia between substrate addition (with or without) and prawn addition (with and without). The mean values (±SD) followed by the different superscript letter in each factor (Substrate and Prawn) indicate significant difference at 0.05 level.

Parameters	Substrate (S)		Prawn (P)		S x P
	Without	With	Without	With	
Survival (%)	55±4^{a}	60±3^{b}	55±4	60±4	NS
Individual weight gain (g)	155±6^{a}	202±16^{b}	183±14	173±15	NS
Total yield (kg ha^{-1})	1702±160^{a}	2445±240^{b}	2044±14	2107±123	NS
Net yield (kg ha^{-1})	1666±158^{a}	2410±243^{b}	2007±11	2010±112	NS
FCR	1.88±0.19^{a}	1.42±0.14^{b}	1.53±0.29^{a}	1.77±0.24^{b}	NS

NS, not significant.

Table 2

Outcomes of 2-way ANOVA comparing yield parameters of prawn with or without addition substrates in polyculture systems. The mean values (±SD) followed by the different superscript letter in same row indicate significant difference at 0.05 level.

Parameter	Prawn	
	without substrate	with substrate
Survival (%)	20±3^{a}	35±3^{b}
Individual weight gain (g)	16±2	20±3
Net yield (kg ha^{-1})	62±9^{a}	141±10^{b}

Table 3

Mean (±SD) primary productivity and respiration (in mg C l^{-1} h^{-1}) in different depths of water column under different treatments. Values are means of three replicated ponds and two sampling dates (n=6).

Parameters	Without substrate		With substrate	
	Mono	Poly	Mono	Poly
Gross Carbon productivity				
Surface	0.398±0.025	0.367±0.048	0.369±0.027	0.372±0.057
Middle	0.161±0.025	0.158±0.024	0.154±0.030	0.149±0.019
Bottom	0.030±0.016	0.056±0.008	0.023±0.027	0.041±0.016
Average	0.196±0.019	0.194±0.014	0.182±0.012	0.188±0.018
Net Carbon productivity				
Surface	0.368±0.029	0.334±0.040	0.338±0.033	0.325±0.090
Middle	0.143±0.025	0.128±0.027	0.113±0.031	0.119±0.020
Bottom	0.001±0.009	0.018±0.022	-0.008±0.022	0.003±0.005
Average	0.171±0.005	0.160±0.011	0.148±0.014	0.149±0.036
Respiration				
Surface	0.036±0.023	0.040±0.023	0.038±0.010	0.057±0.043
Middle	0.021±0.016	0.035±0.008	0.050±0.013	0.036±0.005
Bottom	0.035±0.011	0.045±0.017	0.038±0.021	0.045±0.019
Average	0.031±0.007	0.040±0.010	0.042±0.006	0.046±0.021

Table 4

Electivity indices of tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) for different plankton groups during two sampling dates.

	Day-30				Day-60			
	Without		With		Without		With	
	Mono	Poly	Mono	Poly	Mono	Poly	Mono	Poly
Tilapia								
Bacillariophyceae	0.25	0.25	0.16	0.07	-0.15	-0.02	0.33	0.10
Chlorophyceae	-0.12	-0.12	-0.06	-0.26	0.06	0.13	-0.07	0.08
Cyanophyceae	0.30	0.30	0.10	0.37	0.08	-0.04	0.16	0.08
Euglenophyceae	0.31	0.31	0.22	0.50	-0.07	-0.32	-0.13	-0.30
Dinophyceae	-0.21	-0.21	0.12	-0.08	0.01	-0.53	-0.52	-0.90
Rotifera	0.06	0.06	-0.07	-0.51	-0.40	-0.56	-0.49	-0.58
Prawn								
Bacillariophyceae		0.27		0.43		0.38		0.44
Chlorophyceae		0.05		0.08		0.22		0.14
Cyanophyceae		-0.49		-0.62		-0.63		-0.58
Euglenophyceae		0.29		0.36		-0.30		-0.33
Dinophyceae		-0.09		-0.51		-0.67		-0.72
Rotifera		-0.44		-0.69		-0.62		-0.69
Crustacea		-0.33		-0.63		-0.67		-0.88

Figure Captions

Figure 1. Average chlorophyll *a* concentrations of water column in different treatments during the experimental period. Values are means (±S.E.) of three replicated ponds per sampling date (*N*=3) in each treatment

Figure 2. Quantity of periphyton dry matter (a) and chlorophyll *a* (b) per unit surface area for two culture systems during the experimental period. Values are means (±S.E.) of three depth and three ponds (*N*=9) per sampling dates in each treatment

Figure 1

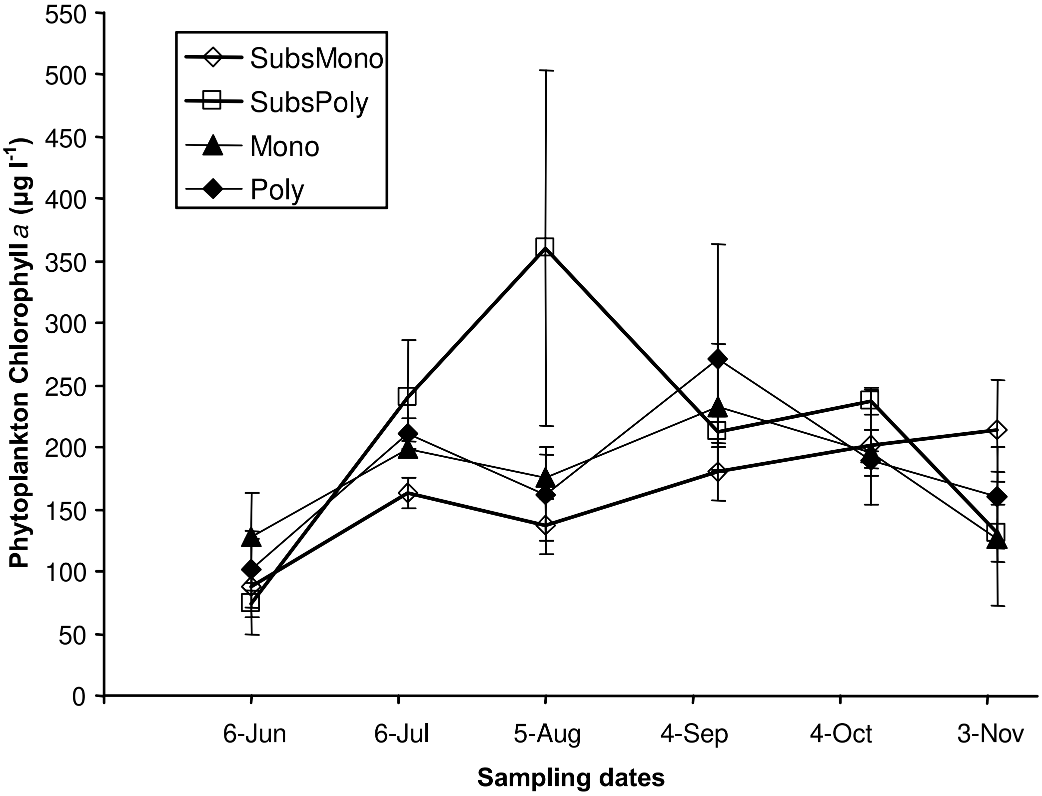
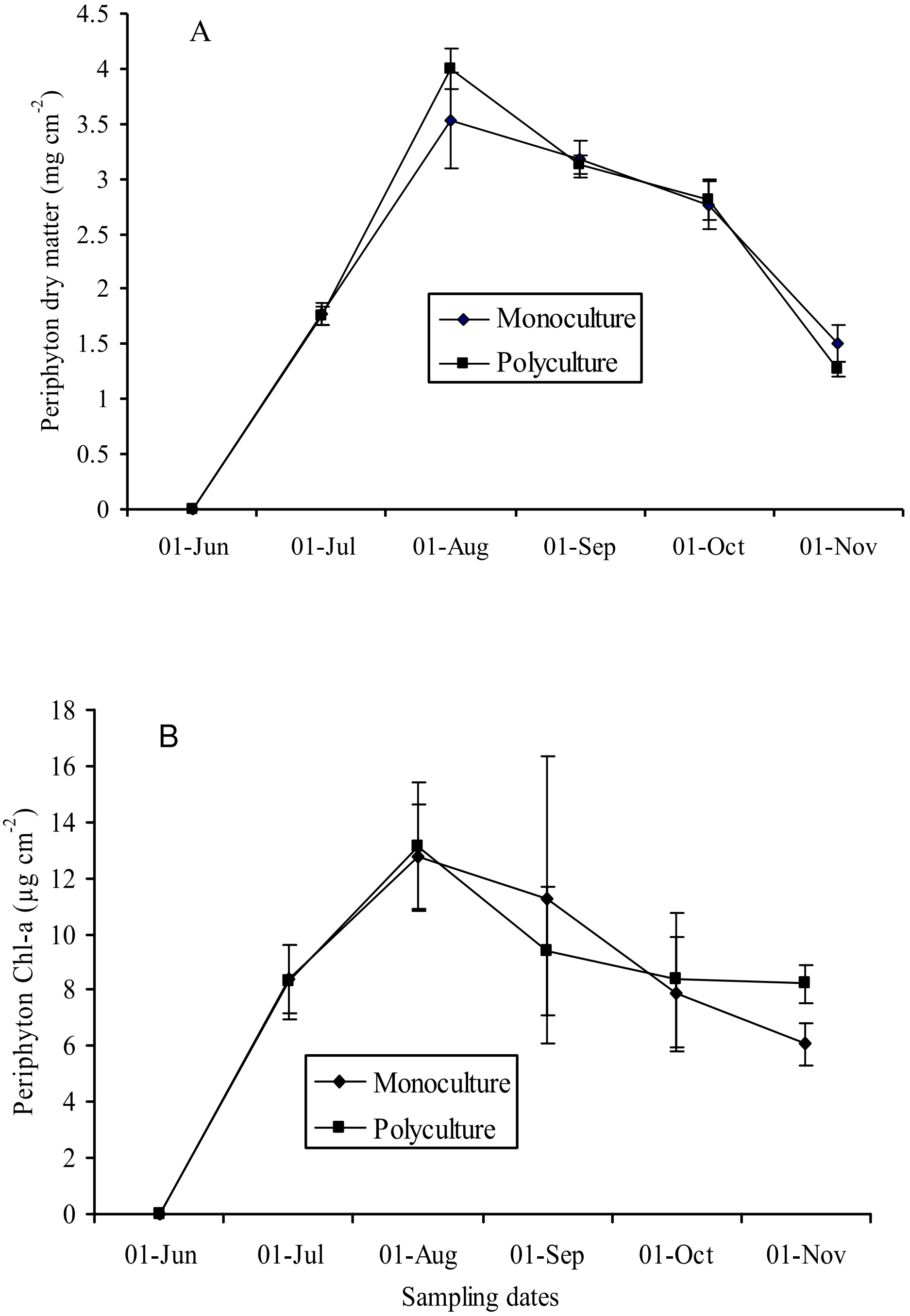

550
500
450
400
350
300
250
200
150
100
50
0
Phytoplankton Chlorophyll *a* (µg l-1)
SubsMono
SubsPoly
Mono
Poly
6-Jun
6-Jul
5-Aug
4-Sep
4-Oct
3-Nov
Sampling dates

Figure 2


A
Periphyton dry matter (mg cm^{-2})
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
01-Jun
01-Jul
01-Aug
01-Sep
01-Oct
01-Nov
Monoculture
Polyculture
B
Periphyton Chl-a (µg cm^{-2})
18
16
14
12
10
8
6
4
2
0
01-Jun
01-Jul
01-Aug
01-Sep
01-Oct
01-Nov
Sampling dates
Monoculture
Polyculture

Chapter 3

The potential of mixed culture of genetically improved tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems

Chapter 3

The potential of mixed culture of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems

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Abstract

The production performance of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) in periphyton-based systems were studied in farmers' ponds at Mymensingh, Bangladesh. Fifteen ponds (200-300 m^2 area and 1.0-1.5 m in depth) were used to compare five stocking ratios in triplicate: 100% GIFT, 75% GIFT plus 25% prawn, 50% GIFT plus 50% prawn, 25% GIFT plus 75% prawn and 100% prawn. Ponds were stocked at a total density of 20,000 GIFT and/or prawn ha^{-1}. Bamboo poles (mean diameter 6.2 cm and 5.5 pole m^{-2}) were posted in pond bottoms vertically as periphyton substrate. Periphyton biomass in terms of dry matter, ash free dry matter and chlorophyll *a* were significantly higher in ponds stocked with prawn alone than in ponds with different combinations of GIFT and prawn. Survival of GIFT was significantly lower in ponds stocked with 100% GIFT (monoculture) whereas, that of prawn was significantly higher in its monoculture ponds indicating detrimental effects of GIFT on prawn's survival. Individual weight gains for both species were significantly higher in polyculture than in monoculture. The highest total fish and prawn yield (1623 kg GIFT and 30 kg prawn ha^{-1}) over 125-140 days culture period was recorded in ponds with 75% GIFT and 25% prawn followed by 100% GIFT alone (1549 kg ha^{-1}), 50% GIFT plus 50% prawn (1114 kg GIFT and 68 kg prawn ha^{-1}), 25% GIFT plus 75% prawn (574 kg GIFT and 129 kg prawn ha^{-1}) and 100% prawn alone (157 kg ha^{-1}). This combination also gave the highest economic return. Therefore, a stocking ratio of 75% GIFT plus 25% prawn at a total density of 20 000 ha^{-1} appeared to be the best stocking ratio in terms of fish production as well as economics for a periphyton-based polyculture system.

Keywords: Periphyton, polyculture, *Oreochromis niloticus*, *Macrobrachium rosenbergii*, benefit-cost ratio

Introduction

Periphyton-based aquaculture is a recent concept and eco-friendly approach in the pond aquaculture. Periphyton is a preferable natural food for herbivorous and omnivorous fish species especially for Indian major carps (Azim et al., 2002; Keshavanathet al., 2002) and tilapias (Legendre et al., 1989; Hem and Avit, 1994; Azim et al., 2003). Preliminary trials on tilapia monoculture in periphyton-based systems have given mixed results: periphyton contributed very marginal (Shrestha and Knud-hunsen, 1994) to several folds increase in production (Hem and Avit, 1994; Keshavanath et al., 2004). While the culture potential of finfish species (especially Indian major carps) in substrate-based system was found promising, more efforts are warranted to try this technology with other high valued aquaculture species like freshwater prawn and penaeid shrimp. In pond fish culture, substrates are commonly used vertically to enhance natural production associated with periphyton. In freshwater prawn culture, layers of horizontal surfaces are used to increase available territory per volume of water thereby reducing the territorialism (Tidwell and Bratvold, 2005). In both the cases, it was reported that fish production from ponds provided with substrate is higher than that from substrate free ponds (van Dam et al., 2002; Tidwell and Bratvold, 2005). However, although tilapia is known to be a periphyton grazer (Dempster et al., 1993), its productions in periphyton-based systems were highly variable, ranging from no contribution of periphyton (Shrestha and Knud-Hansen, 1994) to several folds increase in production (Hem and Avit, 1994; Keshavanath et al., 2004). Since the purpose of using substrates in prawn culture was mainly to provide shelter rather than growing periphyton as food, it was also uncertain whether freshwater prawn ate periphyton. However, monoculture ponds with freshwater prawn might have been experienced with excessive algal blooms leading to environmental deterioration. The omnivorous and periphyton feeding tilapia species might be able to potentially utilize remaining food resources in freshwater prawn culture ponds. Therefore, it might be advantageous to culture tilapia and freshwater prawn together in periphyton-based ponds.

The objective of this experiment was to test the technical viability of a periphyton based polyculture with genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*). The yield parameters were compared from different stocking ratios of these two candidate species in polyculture as well as monoculture of either species in substrate-based systems. A subsequent paper will deal with differences in environmental parameters due to different stocking combinations.

Materials and Methods

Study area and experimental design

The experiment was carried out in 15 earthen farmer's ponds of Montola-Goneshampur village in Mymensingh district, Bangladesh, during July-October, 2003. The ponds were rectangular in shape with area ranging from 200 to 300 m^2 and water depth ranging from 1.0 to 1.5 m. Ponds were rain fed, well exposed to sunlight and without inlet and outlet. The experiment consisted of five stocking ratios in triplicate: 100% tilapia (herein called treatment 100T), 75% tilapia plus 25% prawn (treatment 75T/25P), 50% tilapia plus 50% prawn (treatment 50T/50P), 25% tilapia plus 75% prawn (treatment 25T/75P) and 100% prawn (100P). Ponds were stocked at a fixed total stocking density of 20,000 juveniles/post-larvae ha^{-1}. Ponds were randomly assigned to the treatments.

Pond preparation and fish stocking

Since the experiment was started in the rainy season, ponds were already filled up by precipitations before commencement of the experiment. Aquatic vegetation was cleaned manually from the ponds and predators and fishes were eradicated using rotenone. Bamboo poles (mean length 2.0 m and mean diameter 6.2 cm) were planted vertically into the bottom mud extending upper portions of the poles above the water surface. Substrates were installed in an area, leaving a 2-m perimeter free of poles in each pond. The bamboo substrate (on average 5.5 poles /m^2) added an effective surface area of about 60% of pond surface area in each pond. It took three days to complete substrates installation in all ponds, and the last day of substrate installation is named Day 1 of the experiment. Powdered lime ($CaCO_3$) was broadcasted over the water surface at a rate of 250 kg ha^{-1} on Day 2. On Day 6, ponds were fertilized with semi-decomposed cow manure, urea and triple super phosphate (TSP) at 3,000, 100 and 100 kg ha^{-1}, respectively. On Day 14, postlarvae (PL-28) of *Macrobrachium rosenbergii* were released into the ponds in numbers according to the design. After 15 days of PL stocking (Day 29), juveniles of GIFT (*Oreochromis niloticus*, 1.97 g) were stocked according to the design. The GIFT juveniles and prawn PL were collected from Bangladesh Fisheries Research Institute, Mymensingh and stocking was done in the morning, care being taken for temperature acclimatization to the pond conditions. The prawn was cultured for 140 days, the tilapia for 125 days.

Post stocking management and fish sampling

All ponds maintained the same regime of feeding and fertilization. Commercial pelleted fish feed (25% protein) procured from the market was applied to the ponds at a rate of 5% of total stocked biomass for the first two months and 3% of the total biomass for the rest of the experimental period. GIFT and prawn were sampled at monthly intervals using lift net after removing some bamboo poles. After sampling, poles were put back to their original positions. Length and weight of approximately 10% of total number of fish and prawn were measured individually to check their growth and to calculate feed requirement. All ponds were fertilized with urea and TSP at a rate 50 kg ha^{-1} each at fortnightly intervals.

At the end of the experiment, bamboo poles were removed, water was pumped out of the ponds and all fish and prawn were collected, weighed (Denver-xp-3000; precision=0.1 g), and measured (measuring board; precision=1 mm). Weight gain per fish was calculated by deducting the average initial weight from the average final weight. Specific Growth Rate (SGR; % body weight gain day^{-1}) was estimated as:
SGR =[Ln(final weight) - Ln(initial weight) x 100]/ cultured period (days).

Periphyton sampling and analysis

The periphyton biomass, in terms of dry mater (DM) content and pigment concentration (chlorophyll *a* and pheophytin *a*), growing on bamboo substrates were determined fortnightly 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 by random number tables and two 3x2 cm^2 samples of periphyton were taken at each of four depth (25, 50, 75 and 100 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 105^0C until constant weight (24 h in a Memmert stove, Model UM/BM 100-800), and kept in a dessicator until weighed (BDH 100A; precision 0.0001g). Dry samples from depth and poles per pond were pooled, transferred to a muffle furnace and ashed at 450^0C for 6 h and weighed. The DM, ash free mater (AFDM) and ash content were determined by weight differences (APHA, 1992).

Another sample was used to determine chlorophyll *a* and pheophytin *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 sec with a tissue grinder, after refrigerated for 4 h, and then centrifuged for 10 min at 2000-3000 rpm. The supernatant was carefully transferred to 1 cm glass cuvettes and absorption measured at 750 and 664 nm using a spectrophotometer (Milton Roy Spectronic, model 1001 plus). Samples were then acidified by addition of three drops of 0.1 *N* HCl and absorbance measured again at 750 and 665 nm after 90-sec acidification. Chlorophyll *a* and pheophytin *a* concentrations were calculated using the equation given in APHA (1992).

Statistical and economical analyses

For yield parameters, net returns and benefit-cost ratio, a one-way ANOVA was used to compare the treatment means. Periphyton biomass was compared in a repeated measures

ANOVA. Percentage/ratio data were arcsine transformed before analysis. Significance was assigned at the 5% level.

A simple cost and return analysis was done to determine the benefit-cost ratio of fish/prawn culture in different treatments. The following algebraic equation was used to quantify the profitability of tilapia-prawn culture in periphyton-based pond culture systems:

$R = P_{bi}B_i - P_{xj}X_j - TFC$

where, R = profit or net return, P_{bi} = per unit price of ith products (BDT/kg), B_i = quantity of ith products sold (kg), P_{xj} = per unit price of jth inputs, X_j = quantity of jth inputs, i = 1, 2, 3, - - - n, TFC = total fixed cost.

Results

Fish/prawn yield parameters

Yield parameters of GIFT are given in Table 1. Survival was significantly lower in monoculture ponds than in all combinations of polyculture ponds with prawn. Although same length and weight of fish were stocked, their length and weight at harvest varied significantly with higher mean values with lower densities, and vice versa. Individual weight gains also increased with lowering their own stocking density. However, specific growth rates (SGR) were significantly higher in polyculture treatments than in monoculture one. Net yields were significantly different among different treatments, with the highest mean value in treatment 75T/25P followed by treatments 100T, 50T/50P and 25T/75P, respectively.

Yield parameters of freshwater prawn are given in Table 2. As opposite to GIFT, survival of prawn was significantly higher in its monoculture ponds than in polyculture ponds with GIFT. However, though same length and weight of prawn PL were stocked, individual harvesting weight, individual weight gains and SGR were significantly lower in monoculture ponds than in polyculture ponds. Prawn yields in different treatments corresponded to total number of prawn stocked in different treatments, respectively.

The combined net yields of the two species were 1549, 1653, 1183, 703 and 157 kg ha^{-1} in treatments 100T, 75T/25P, 50T/50P, 25T/75P and 100P, respectively.

Economics

The economical analysis summarized in Table 3 indicates that monoculture of GIFT as well as all combinations of GIFT-prawn polyculture were profitable with the highest net returns and benefit-cost ratio from ponds stocked with 75% tilapia and 25% prawn. Monoculture of freshwater prawn, however, was found marginally profitable.

Periphyton biomass

Periphyton dry matter (DM), ash free dry matter (AFDM) and ash contents, and pigment concentrations per unit substrate surface area are given in Table 4. Mean values of all these parameters were significantly higher in ponds stocked with freshwater prawn alone. The DM contents increased until the first half of the experimental period after which they constantly reduced in all treatments with tilapia, in contrast to treatment 100P in which only freshwater prawn was stocked (Figure 1). In this treatment, DM content was more or less stable until the end of the experiment. The more or less same trends were reflected for the pigment concentrations with higher mean values in ponds with freshwater prawn only as compared to ponds with GIFT tilapia. Ash contents of periphyton dry matter ranged from 29 to 37% and increased as the stocking ratio of prawn increased.

The relationship between periphyton biomass and fish/prawn biomass at harvest is shown in Figure 2. The regression lines indicate that periphyton biomass decreased with increasing biomass of tilapia, and increased with increasing biomass of freshwater prawn. However, the relationships were not linear rather quadratic.

Discussion

Periphyton biomass increased with decreasing GIFT:prawn biomass and was the highest in ponds stocked with freshwater prawn alone (Figure 2). It indicates the preference of tilapia for periphyton as food. Tilapias are known to be omnivorous species and capable of feeding on benthic and attached (periphyton) algal and detrital aggregates (Bowen, 1982; Dempster et al., 1993; Azim et al., 2003). However, it is not sure whether freshwater prawn utilized periphyton as food. It might be assumed that the freshwater prawn had selectivity in taking food from periphyton matrix and the grazing pressure was insufficient to potentially reduce the periphyton biomass especially in ponds stocked with only freshwater prawn. In ponds, freshwater prawn preferred forage animals like trichopterans, chironomids, oligochates, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996; Tidwell et al., 1997). Azim et al. (2002) identified some of these macroinvertebrates in periphyton composition from aquaculture ponds. The low grazing pressure might also be attributed to the low stocking biomass in monoculture ponds of freshwater prawn as compared to monoculture of tilapias (20,000 ha^{-1} prawn vs 20,000 ha^{-1} tilapia) and higher mortality of freshwater prawn as compared to tilapias. The reported stocking densities of freshwater prawn ranged 40,000 - 120,000 ha^{-1} in substrate-based systems (Tidwell and Bratvold, 2005) which were much higher than the density maintained in the present study. 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).

The survivals of tilapia and freshwater prawn in the present experiment (57-66% and 28-48%, respectively) in the present experiment were much lower than those in substrate-based ponds reported by Keshavanath et al. (2004) for tilapia (88-96%) and Tidwell and Bratvold (2005) for freshwater prawn (more than 80%). In addition to the differences in pond management in those experiments, one of the most important reasons for the low survival is that the present experiment was carried out in farmer's ponds which were completely dependent on natural rainfall and not well-managed as compared to on-station experimental or commercial ponds. The natural productivity of those ponds was much lower because of their multipurpose uses (like fish culture, watering gardens, bathing, household washing etc.) as compared to well-managed on-station research ponds (Azim et al., 2004). However, similar lower survival of freshwater prawn with a range from 23% to 37% was reported from farmer's ponds of Bangladesh, while cultured with tiger shrimp (*Penaeus monodon*) (Azim et al., 2001).

It is interesting to note that the survival of tilapia was significantly higher in polyculture whereas that of prawn was significantly higher in monoculture. This clearly indicates the existence of intra- and inter-specific antagonistic behaviors of the former for food and space. Especially, tilapia might have affected prawn survival during molting in polyculture ponds. On the other hand, addition of substrates might have minimized territoriality of freshwater prawn especially in monoculture ponds. Although survival of freshwater prawn was not affected by their own stocking density, individual weight gain was significantly lower in ponds stocked with the highest number of freshwater prawns possibly due to intra-specific competition.

The highest combined production was recorded in ponds stocked with 75% tilapia and 25% freshwater prawn. This indicates that the synergistic benefits compensate for any inter-specific or intra-specific dietary competition in this combination. Nevertheless, an alternative approach should be developed to minimize the antagonistic behaviour of tilapia on its co-inhabiting species. Caged tilapia in freshwater prawn culture or caged freshwater prawn in tilapia culture might be an option. In the latter case, artificial feed can only be provided to freshwater prawn, whereas tilapia can depend on natural food. The contribution of periphyton substrates to fish production in this polyculture system was not determined which is worth looking at in future experiments using this optimum species combination in ponds with and without substrates.

The cost benefit analysis revealed that the monoculture of tilapia and addition of prawn at any ratio to the tilapia ponds were profitable indicating the economic viability of this technology. However, since the market prize of fish and prawns is strongly size dependent, and farmers need year-round income, further economic optimization should be based on annual production.

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References

APHA, 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC.

Azim, M.E., Rahaman, M.M., Wahab, M.A., Asaeda, T., Little, D.C., Verdegem, M.C.J., 2004. Periphyton-based pond polyculture systems: a bio-economic comparison of on-farm and on-station trials. Aquaculture 242, 381-396.

Azim, M.E., Mazid, M.A., Alam, M.J., Nurullah, M., 2001. The potential of mixed culture of freshwater giant prawn *Macrobrachium rosenbergii* de Man and tiger shrimp *Penaeus monodon* Fab. at Khulna region. Bangladesh Journal of Fisheries Research 5(1), 67-74.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia *Oreochromis niloticus* L. Aquac. Res. 34, 85-92.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., 2002. Evaluation of polyculture of Indian major carps in periphyton-based ponds. Aquaculture 213, 131-149.

Bowen, S.H., 1982. Feeding, digestion and growth-qualitative considerations. In: Pullin, R.S.V., Lowe-McConneell, R.H. (Eds.), The Biology and Culture of Tilapias. ICLARM Conf. Proc., vol. 7. Manila. pp. 141-156.

Corbin, J.S., Fujimoto, M.M., Iwai, T.U.Jr., 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P. (Ed.), CRC Handbook of Mariculture. CRC Press, Boca Raton, FL. pp. 391-423.

Coyle, S.D., Najeeullah, T., Tidwell, J.H., 1996. Evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawns *Macrobrachium rosenbergii*. Journal of Applied Aquaculture 6, 57-66.

Dempster, P.W., Beveridge, M.C.M., Baird, D.J., 1993. Herbivory in tilapia *Oreochromis niloticus*: a comparison of feeding rates on phytoplankton and periphyton. J. Fish Biol. 43, 385-392.

Hem, S., Avit, J.L.B., 1994. First results on 'acadjas enclos' as an extensive aquaculture system (West Africa). Bulletin of Marine Science 55, 1038-1049.

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M., 2000. The impacts by grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrates in cages. Aquaculture 186, 45-60.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A. A., Beveridge, M.C.M., Verdegem, M.C.J., 2002. The effect of periphyton and supplemental feeding on the production of the indigenous carps, *Tor khudree* and *Labeo fimbriatus.* Aquaculture 213, 207-218.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A. A., Beveridge, M.C.M., Verdegem, M.C.J., 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquaculture 235, 303-314.

Legendre, M., Hem, S., Cisse, A., 1989. Suitability of brackish water tilapia species from the Ivory Coast for lagoon aquaculture. II.- growth and rearing methods. Aquat. Living Resour. 2, 81-89.

Makarevich, T.A., Zhukova, T.V., Ostapenya, A.P., 1993. Chemical composition and energy value of periphyton in a mesotrophic lake. Hydrobiol. 29, 34-38.

Shrestha, M.K., Knud-Hansen, C.F., 1994. Increasing attached microorganism biomass as a management strategy for Nile tilapia (*Oreochromis niloticus*) production. Aquacult. Eng. 13, 101-108.

Tidwell, J.H., Bratvold, D., 2005. Utility of added substrates in shrimp culture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton:

Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK. pp. 247-268.

Tidwell, J.H., Coyle, S.D., Webster, C.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, S.J., D'Abramo, L.R. Dainels, H.W., Fuller, M.J., 1997. Relative prawn production and benthic macroinvertebrate densities in unfed, organically fertilized, and fed pond systems. Aquaculture 149, 227-242.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1-31.

Table 1 Comparisons of means (and pooled ± SE) of yield parameters of GIFT in different stocking ratios by ANOVA. If main effects are significant, then means followed by the different superscript letter in the same row indicate significant difference at 0.05 level based on Tukey test

Yield parameters		Treatments				±S.E.M.
		100T	75T/25P	50T/50P	25T/75P	
Survival (%)	Mean	57^{b}	66^{a}	65^{a}	64^{a}	±7
Harvesting length (cm)	Mean	19.01^{c}	19.94^{b}	20.20^{b}	20.85^{a}	±0.10
Harvesting weight (g)	Mean	135.95^{c}	164.92^{b}	172.66^{ab}	179.67^{a}	±2.26
Individual weight gain (g)	Mean	133.79^{d}	163.11^{c}	170.61^{b}	177.91^{a}	±17.71
SGR (% bw d^{-1})	Mean	3.32^{b}	3.61^{a}	3.55^{a}	3.70^{a}	±0.37
Net y-ield (kg ha^{-1}125 d^{-1})	Mean	1549^{b}	1623^{a}	1114^{c}	574^{d}	±164

SGR: % body weight gain day^{-1}, S.E.M.: Standard Error Mean

Table 2 Comparisons of means (and pooled ± S.E.) of yield parameters of prawn in different stocking ratios by ANOVA. If main effects are significant, then means followed by the different superscript letter in the same row indicate significant difference at 0.05 level based on Tukey test.

Yield parameters		Treatments				±S.E.M.
		75T/25P	50T/50P	25T/75P	100P	
Survival (%)	Mean	28^c	30^c	40^b	48^a	±3
Individual weight gain (g)	Mean	21.14^a	22.14^a	21.58^a	16.41^b	±1.17
SGR (% bw d^{-1})	Mean	5.46^a	5.50^a	5.48^a	5.28^b	±0.63
Yield (kg ha^{-1}140 d^{-1})	Mean	30^d	68^c	129^b	157^a	±16

SGR: % body weight gain day-1, S.E.M.: Standard Error Mean

Table 3 Comparative economical analysis of fish/prawn culture in different treatment ponds based on 1 ha pond and about four months culture period. Currency is given in Bangladesh Taka, BDT (1 USD = 60 BDT).

Items	100T	75T/25P	50T/50P	25T/75P	100P
Gross return	116 208	135 086	114 261	101 158	70 592
Gross cost	85 527	81 540	75 624	70 732	63 921
Net return	30 681	53 546	38 637	30 426	6671
Benefit cost ratio (BCR)	0.36	0.66	0.51	0.43	0.10

Table 4 Means (and pooled ±S.E.) of periphyton biomass and pigment parameters scraped from bamboo substrates in different treatments. Values are means of three sampling dates, four depths, three poles and three ponds (*N= 108*)

Parameters		Treatments					±S.E.M.
		100T	75T/25P	50T/50P	25T/75P	100P	
DM ($mg\ cm^{-2}$)	Mean	2.06^b	2.19^b	2.65^b	2.48^b	3.55^a	±0.12
AFDM ($\mu g\ cm^{-2}$)	Mean	1.43^b	1.53^b	1.68^b	1.56^b	2.14^a	±0.07
Chlorophyll *a* ($\mu g\ cm^{-2}$)	Mean	8.90^b	9.43^b	10.51^b	11.47^{ab}	15.37^a	±0.48
Pheophytin *a* ($\mu g\ cm^{-2}$)	Mean	3.08^b	3.07^b	3.20^b	3.18^b	4.03^a	±0.11

DM: Dry matter, AFDM: Ash free dry matter, Chlorophyll *a* and Pheophytin a: Pigment concentration, S.E.M.: Standard Error Mean

Figure captions

Fig. 1 Periphyton dry matter (DM) contents per unit substrate surface area for different tilapia-prawn combinations during the experimental period.

Fig. 2 Relationships between periphyton dry matter content and net tilapia biomass (A) or freshwater prawn biomass (B).

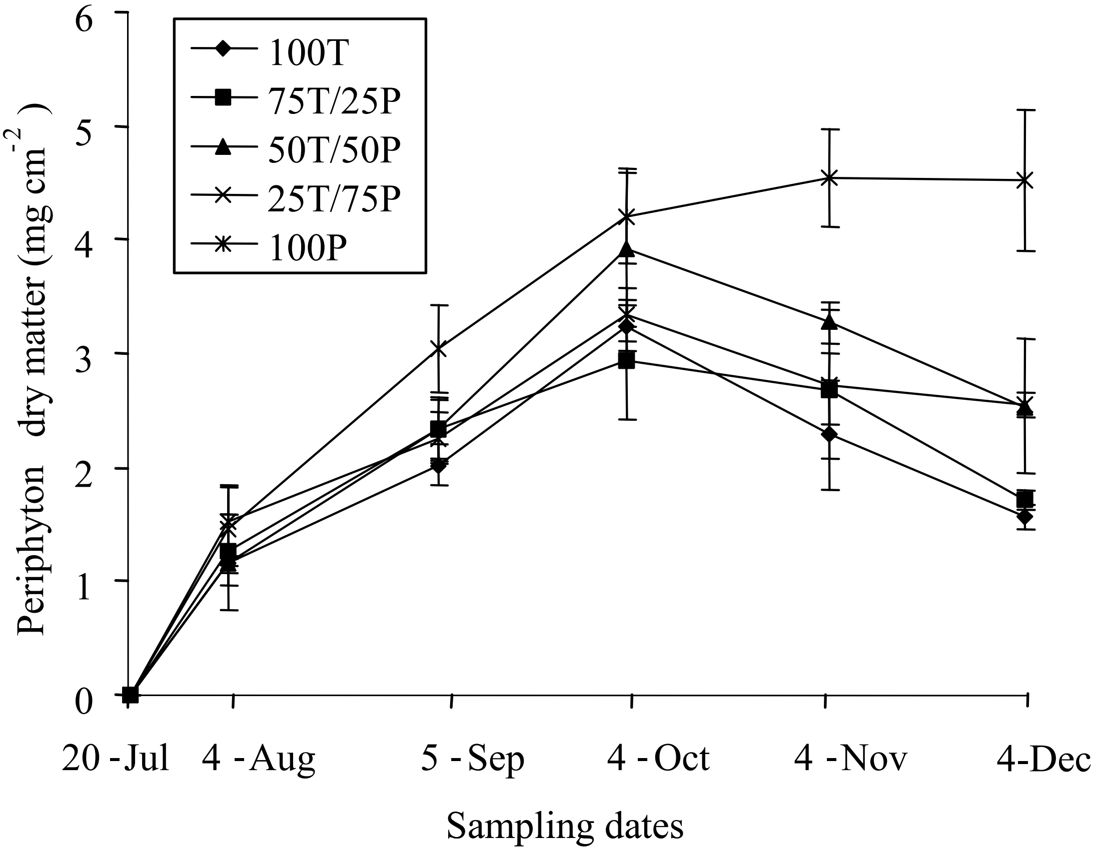
Periphyton dry matter (mg cm^{-2})
6
5
4
3
2
1
0
100T
75T/25P
50T/50P
25T/75P
100P
20 -Jul
4 -Aug
5 - Sep
4 - Oct
4 -Nov
4-Dec
Sampling dates

Figure 1

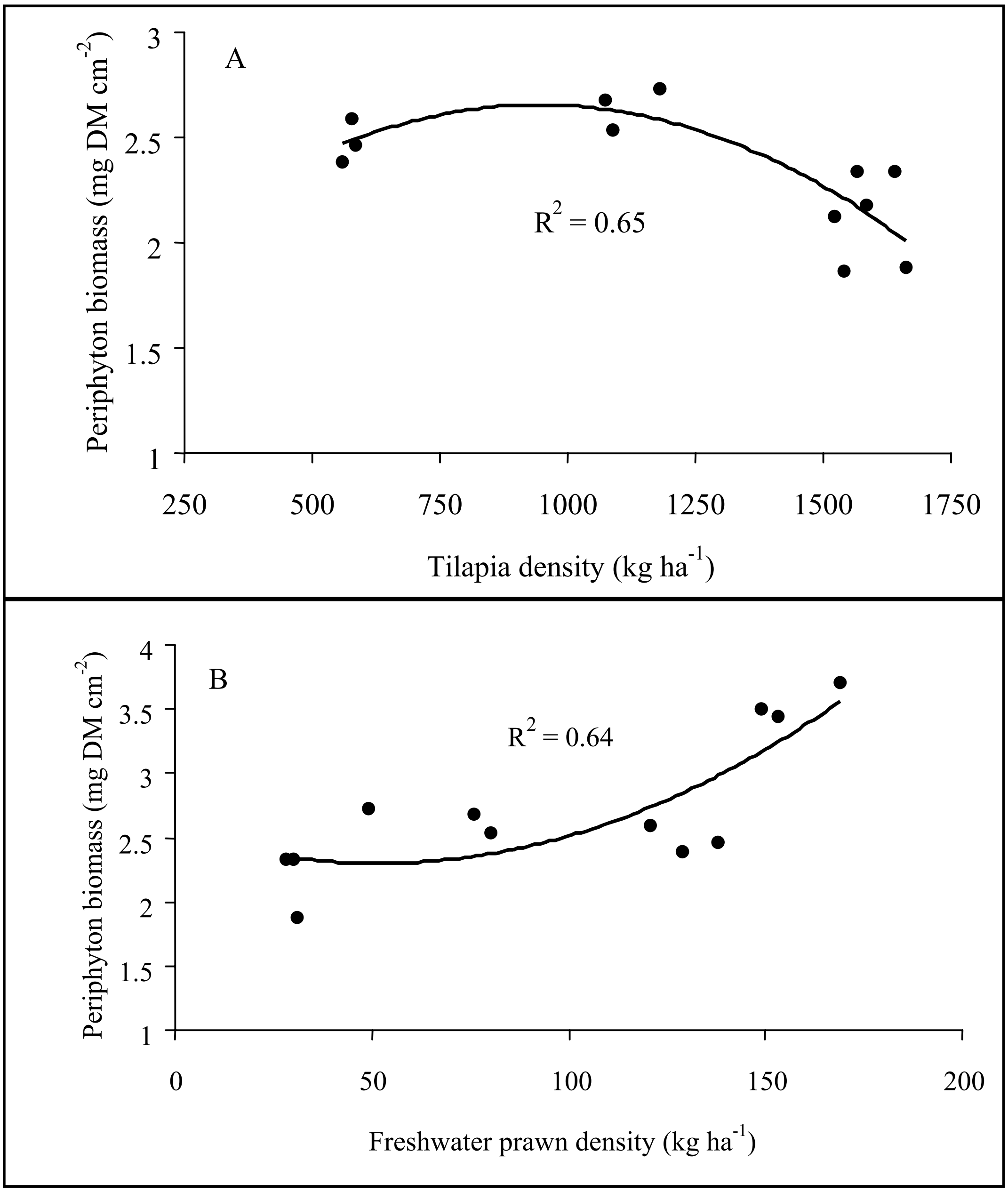
A
Periphyton biomass (mg DM cm^{-2})
3
2.5
2
1.5
1
250
500
750
1000
1250
1500
1750
Tilapia density (kg ha^{-1})
$R^2 = 0.65$
B
Periphyton biomass (mg DM cm^{-2})
4
3.5
3
2.5
2
1.5
1
0
50
100
150
200
Freshwater prawn density (kg ha^{-1})
$R^2 = 0.64$

Figure 2

Chapter 4

The effects of stocking density on production and economics of Nile tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems

Chapter 4

The effects of stocking density on production and economics of Nile tilapia *(Oreochromis niloticus)* and freshwater prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems

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Abstract

The present research investigated the effect of stocking density on pond (75 m^2, depth 1.2 m) production of Nile tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) stocked at a fixed 3:1 tilapia: prawn ratio. Three stocking densities were tried in triplicate: 20,000 (treatment TP-20), 30,000 (TP-30) and 40,000 ha^{-1} (TP-40). The ponds were provided with bamboo as substrate for periphyton development. Bamboo poles (mean diameter 5.5 cm and 5.0 poles m^{-2}) were posted vertically into pond bottoms resulting in 60% additional surface area in each pond. On average, 43 genera of algae and 17 genera of zooplankton were identified from pond water, whereas 42 genera of algae and 6 genera of microfauna were attached to bamboo substrates. No differences were observed between treatments in ash free dry matter (AFDM), chlorophyll *a* and pheophytin *a* content of periphyton ($P > 0.05$). Survival of tilapia and prawn and individual weight gain of tilapia were lower ($P < 0.05$) in treatment TP-40. The net yields were higher ($P < 0.05$) in treatments TP-30 (2,209 and 163 kg ha^{-1} 105 day^{-1} of tilapia and prawn, respectively) and TP-40 (2,162 and 141 kg ha^{-1} tilapia and prawn, respectively) than in treatment TP-20 (1,505 and 136 kg ha^{-1} tilapia and prawn, respectively). The net tilapia yields were quadratic correlated ($R^2 = 0.92$) with fish stocking density. The cost-benefit analysis shows that the net profit margin was highest in treatment TP-30 (69%) followed by TP-20 (50%) and TP-40 (44%).

Key words: Periphyton; polyculture; stocking density; tilapia; freshwater prawn; net margin

Introduction

Interests in providing substrates in finfish ponds (Ramesh et al., 1999; Wahab et al., 1999; Azim et al., 2002; van Dam et al., 2002; Keshavanath et al., 2004) and freshwater prawn ponds (Tidwell et al., 2000; Tidwell and Bratvold, 2005) have been growing during the last decade. In both the cases, it has been evident that production in substrate ponds is higher than in substrate free ponds. Recently, there has been an enormous interest in polyculture of freshwater prawn with finfish, especially with tilapia (New, 2005). Since tilapia exhibit strong hierarchies, polyculture with other finfish or crustacean species needs to be evaluated carefully considering the growth and welfare of the co-inhabiting species. Also prawn exhibits strong hierarchies, but it seems that hierarchies are reduced in substrate-based ponds (Tidwell and Bratvold, 2005). Therefore, it looks promising to add low densities of prawns to fed tilapia periphyton-based ponds considering the high economic value of the prawn.

Preliminary results indicated that a stocking ratio of 75% genetically improved farmed tilapia (GIFT) plus 25% prawn at a fixed total density of 20 000 ha^{-1} was the best stocking ratio in terms of production and economics (Uddin et al., 2006). In the next experiment, effects of tilapia and addition of freshwater prawn on production were quantified (Uddin et al., in press). In both experiments, addition of high valued prawn to periphyton-based tilapia-prawn polyculture resulted in higher fish production. However, the first experiment was based on a total stocking density of 20,000 ha^{-1}. Higher densities can be stocked relying on supplemental feeding and periphyton development, but will this also generate more income? Considering the low survival rate of prawn post larvae (PL) described in Uddin et al., 2006, in the present experiment the ponds were stocked with comparative larger size freshwater prawn juveniles.

The objective of this experiment was to optimize the stocking density of tilapia and prawn in periphyton-based polyculture system. A previously adjusted ratio of tilapia: prawn, 3:1 was used (Uddin et al., 2006).

Materials and methods

Study area and experimental design

The 120-days experiment was conducted between April to July 2004 at the Fisheries Field Laboratory, Bangladesh Agricultural University, Mymensingh. Nine rectangular earthen ponds with an area of 75 m^2 and an average depth of 1.2 m each were used. The ponds were well exposed to sunlight, not interconnected by inlet and outlet and the main sources of water were rainfall and water supply from a deep tube-well using a flexible plastic pipe whenever needed. The embankment was well protected and covered with grass.

The design was based on a previous experiment (Uddin et al., 2006), that resulted in an optimal stocking ratio of 75% tilapia and 25% freshwater prawn with a total stocking density

20,000 ha^{-1} in a periphyton based system. In the present experiment, three stocking densities were tested in triplicate: 20,000 (herein called treatment TP-20), 30,000 (treatment TP-30) and 40,000 ha^{-1} (treatment TP-40).

Pond preparation and fish stocking

Prior to the trial, all unwanted fishes were eradicated by rotenone application at the rate of 100 g pond^{-1}. The dead fishes and other aquatic organisms were removed by repeated netting. Ponds were treated with lime ($CaCO_3$, 250 kg ha^{-1}) and filled with water on day 1. On day-3 bamboo poles (5 per m^2 with a mean diameter of 5.5 cm) were posted vertically into the bottom mud in all ponds, excluding a one meter wide perimeter water surface from the dike (i.e. 44 m^{-2} area was provided with substrate). This resulted in an additional area for periphyton development equaling about 60% (i.e. 45 m^2) of the pond surface area. On the same day the 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. On day 15, when the periphyton biomass had developed well on the substrates, ponds were stocked with juveniles of freshwater prawn (*Macrobrachium rosenbergii*) and GIFT tilapia (*Oreochromis niloticus*) with a mean weight of 2.08 and 1.89 g, respectively. Juveniles of freshwater prawn and tilapia were collected from Bangladesh Fisheries Research Institute (BFRI), Mymensingh and care was taken to minimize the mortality during stocking. Subsequently, all ponds were fertilized fortnightly with urea and TSP at the rate 50 kg ha^{-1} each until harvesting. Commercial pelleted fish feed (25% crude protein) procured from the local market was applied daily to the ponds at the rate of 5% of the tilapia biomass per day for the first month and 2% of the tilapia biomass for the rest of the experimental period.

Plankton and periphyton sampling

For the taxonomic composition study of plankton and periphyton, samples were taken monthly, starting on day 30. For plankton enumeration, 10 l water samples were collected from different locations and depths in each pond and filtered through a 45µm mesh phytoplankton net. The filtered samples were then carefully transferred to a measuring cylinder and made up to a standard volume of 50 ml with distilled water and buffered formalin (5%) and were stored in small sealed plastic bottles until examination. Plankton was counted using a Sedgewick-Rafter counting cell (S-R cell). A 1 ml sub-sample was transferred to the counting chamber of the S-R cell (providing 1000 fields) and all cells or colony forming units occurring in 10 randomly chosen fields were counted using a binocular microscope (Swift M-4000). For periphyton, from each pond three bamboo poles were selected randomly and periphyton samples were taken carefully by a scalpel blade. Three 2x2 cm^2 samples of periphyton were taken at each of three depth, (25, 50 and 75 cm below from the water surface) per pole and pooled together and resuspended in 50 ml of distilled water and preserved with 5% buffered formalin in sealed plastic vials. Periphyton was enumerated using an S-R cell according to the procedure for plankton given above. Identification and enumeration of plankton and periphyton were performed as described by Azim et al. (2001a)

Measurement of phytoplankton biomass

Chlorophyll *a* concentrations of pond water were determined fortnightly. A known amount of water sample were filtered through micro-fiber glass filter paper (Whatman GF/C) using a vacuum pressure air pump. The filter paper was kept in a test tube containing 10 ml 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 hours. Later, Chlorophyll *a* was determined using a spectrophotometer (Milton Roy Spectronic, Model 1001 plus) at 664 and 750 nm wave length following Boyd (1979).

Chlorophyll *a* ($\mu g\ l^{-1}$) = $11.9(E_{665}\text{-}E_{750})V/Lx1000/S$

Where, E_{665} = optical density of sample at 665 nm; E_{750} = optical density of sample at 750 nm; V = acetone volume used (ml); L = volume of sample filtered (ml); S = length of light path in the spectrophotometer (cm).

Determination of periphyton biomass

The periphyton biomass growing on bamboo substrates, viz. dry matter (DM) and pigment concentration (Chlorophyll *a* and pheophytin *a*) were determined monthly following standard methods (APHA, 1992), starting from day 30. From each pond, three poles were randomly selected and two 2x2 cm^2 of periphyton samples were collected at 25, 50 and 75 cm depth. The periphyton samples were scraped with a sharp blade from the surface area of the substrate, care being taken not to remove any of the substrate itself, and the material was transferred to pre-weighed and labeled pieces of aluminium foil. After sampling, the poles were replaced in their original positions, marked and excluded from subsequent sampling. One sample was used to determine dry matter (DM) and ash contents. Samples were placed in a drying oven (Memmert, Model UM/BM 100-800) and dried at 105^0c until constant weight (24 h), before being transferred to a desiccator until weighed (BDH, Model 100A; precision 0.0001 g). Dry samples were transferred to a muffle furnace and ashed at 450^0 C for 6 h and re-weighed. Ash free dry matter (AFDM) were calculated from weight differences. The autotrophic index (AI) was calculated using the following formula (APHA, 1992):

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

Another sample was used to determine chlorophyll *a* and pheophytin *a* following standard methods (APHA, 1992). Upon removal, the materials was immediately transferred to labeled tubes containing 10 ml 90% acetone, sealed and transferred to the laboratory where they were stored overnight in a refrigerator. The following morning, samples were homogenized for 30 seconds with a tissue grinder, refrigerated for 4 hours and centrifuged for 10 minutes at 3,000 rpm. The supernatant was carefully transferred to a 1 cm glass cuvette and absorption measured at 750 and 664 nm using spectrophotometer (Milton Roy Spectronic, model 1001 plus). Samples were then acidified by addition of three drops of 0.1*N* HCl and absorbance measured again at 750 and 665 nm after 90 seconds acidification. Chlorophyll *a* and pheophytin *a* were calculated using the equations given in APHA (1992).

Fish harvesting

After 105 days, bamboo poles were removed, water was pumped out of the ponds and per pond all fishes and prawns were collected and weighed individually. Individual weight gain was calculated by deducting the average initial weight from the average final weight. Specific growth rate (SGR) was estimated as:

SGR = [Ln (final weight)-Ln (initial weight) x 100]/ cultured period (days).

Economical analysis

An economical analysis was performed to estimate the net return and profit margins in the different treatments. The following simple 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 = interests on inputs. The wholesale price per kg of tilapia and prawn were 60 taka and 350 taka, respectively. The prices of inputs, fish and prawn correspond to the Mymensingh wholesale market prices in 2004 and are expressed in Bangladeshi taka (BDT (1US$ = 67 taka).

Statistical analysis

For the statistical analysis of data, one-way ANOVA was used for yield parameters. Survival of GIFT and prawn was analyzed using arcsine-transformed data but percent values are reported. Plankton and periphyton biomass and composition were compared in a repeated-measure ANOVA. If the main effect was found significant, the ANOVA was followed by a Tukey-HSD test. All statistical tests were done at a 5% probability level using the SPSS (Statistical Package for Social Science) version-12.0.

Results

Plankton and periphyton biomass and composition

The plankton communities in pond water consisted of five groups of phytoplankton and two groups of zooplankton in all the treatments. Forty three genera of phytoplankton belonging to Bacillariophyceae (11), Chlorophyceae (21), Cyanophyceae (7), Euglenophyceae (3) and Dinophyceae (1) were found. Chlorophyceae followed by Bacillariophyceae was the most dominant group among phytoplankton in each treatment. Seventeen genera of zooplankton, including eight genera of Rotifera and nine genera of Crustaceae were also identified. The total number of phytoplankton increased gradually in the first half of the experimental period and then steadily decreased during the rest of the period (Figure 1A). Among phytoplankton *Synedra*, *Tabellaria*, *Navicula*, *Fragillaria* and *Nitzschia* (Bacillariophyceae), *Chlorella*, *Sphaerocystes*, *Stigeoclonium*, *Palmella*, *Pediastrum* and *Scenedesmus* (Chlorophyceae), *Microcystes*, *Anabaena* and *Gomphosphaeria* (Cyanophyceae) *Euglena* and *Phacus* (Euglenophyceae), and among zooplankton *Cyclops, Diaphanosoma* and Nauplius (Crustaceae), and *Brachionus*, *Trichocerca* and *Filinia* (Rotifera) were the dominating genera. The number of zooplankton community showed a static trend and did not vary significantly among the treatments (Figure 1B)

About 41 genera of phytoplankton belonging to Bacillariophyceae (10), Chlorophyceae (21), Cyanophyceae (7), Euglenophyceae (2) and Dinophyceae (1) and 6 genera of zooplankton belonging to Rotifera (5) and Crustacea (1) were also identified in all treatments in the periphytic communities. Although it shows a higher number of periphytic algae on second sampling date (Figure 2A) the common trend was a decrease in the total number of periphytic plankton in all treatments (Figure 2A, B). Phytoplankton biomass in terms of chlorophyll *a* concentrations did not show any significant differences among the treatments indicating that stocking density had no significant effect on the standing phytoplankton biomass of the water column (Figure 3). However, it shows that chlorophyll *a* concentrations decreased continuously and steadily during the entire experimental period in all treatments.

On the first sampling date, periphyton ash free dry matter (AFDM) was more or less equal (1.24±0.03 mg cm^{-2}) in all the treatments. On the following sampling date AFDM increased to a maximum of 1.63 - 2.19 mg cm^{-2} and subsequently declined to 0.53 - 0.99 mg cm^{-2} on the last sampling date (Figure 4A).). No differences were observed between treatments ($P > 0.05$). The same trend was observed for ash, reaching 1.4 mg cm^{-2} (TP-20) on the third sampling date (Figure 4A). Chlorophyll *a* concentration of periphyton was highest with 10.88 µg cm^{-2} on the third sampling date for treatment TP-20 followed by 9.53 µg cm^{-2} on the second sampling date for the same treatment (Figure 4B). Pheophytin *a* concentration was highest on second sampling date, ranging from 6.55 (TP-30) to 4.85 µg cm^{-2} (TP-40) (Figure 4C). However, thereafter chlorophyll *a* and pheophytin *a* concentration decreased. Differences between treatments were not significant ($P > 0.05$). The autotrophic index (AI) values decreased over time in all treatments (Figure 4D).

Fish yield parameters

Fish yield parameters are shown in Table 1. Individual tilapia weights at harvest were higher at the low and medium stocking densities (TP-20 and TP-30) than at high stocking density (TP-40). Individual weight gain of tilapia was significantly ($P < 0.05$) higher in treatment TP-20 and TP-30 than in TP-40, but it showed no differences ($P > 0.05$) for freshwater prawn among the treatments. Survivals of tilapia and freshwater prawn were significantly ($P < 0.05$) higher in treatments TP-20 and TP-30 than in TP-40 but did not differ between TP-20 and T-30 ($P > 0.05$).

The net yield of tilapia was 1.47 and 1.44 times higher ($P < 0.05$) in treatments TP-30 and TP-40, respectively, than in treatment TP-20. However, net yield of freshwater prawn did not vary ($P > 0.05$) among the treatments. The combined net yield of tilapia and freshwater prawn was higher ($P < 0.05$) in TP-30 (2,372) and TP-40 (2,303) than in TP-20 (1,641 kg ha^{-1}). However, the combined yield of treatments TP-30 and TP-40 was not different ($P > 0.05$; ANOVA and Tukey test).

Cost-benefit analysis

The cost-benefit analysis of different treatments is given in Table 2. The substrates, supplemental feed and the fresh water prawn juveniles were the most expensive inputs. Net profit margin was highest in treatment TP-30 (69%) followed by TP-20 (50%) and TP-40 (44%) treatments.

Discussion

The plankton population in fish pond is linked to the productive status of the experimental ponds, representing both direct and indirect sources of food. The phytoplankton species composition was representative of that found in Bangladesh fish ponds (Dewan et al., 1991; Wahab et al., 1999). The total phytoplankton count was decreasing steadily during the last part of the trial due to increased grazing pressure by increased biomass of tilapia. Perschbacher and Lorio (1993) reported that tilapia stocked at densities higher than 5,000 ha^{-1} promoted a very effective biological control over phytoplankton. It is also reported that the filtration rate by tilapia for both green algae and cyanobacteria increased linearly when water temperature increased (Turker et al., 2003).

The periphyton communities were observed to evolve over time, with marked changes in abundance. Initially, the periphytic algae on the substrates were increasing in the first half of the trial and then decreased steadily. Wahab et al. (1999) and Azim et al. (2004) found similar patterns among the phytoplankton community. Indeed, tilapias were regularly observed grazing on the periphytic substrates. Prawns were not seen grazing on periphyton. In ponds, freshwater prawn preferred forage animals like trichopterans, chironomids, oligochates, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996 and Tidwell et al., 1997), organisms associated with the sediment. The abundance of periphytic zooplankton was also similar to that found by Azim et al. (2004) in a periphyton based carp culture trial in Bangladesh, suggesting that the zooplankton communities were less preferable for the tilapias in their adult stage.

The significant decrease of periphyton biomass (AFDM) with time was the result of increased tilapia grazing pressure in all treatments. The autotrophic index (AI) ranged from 85-708 in the present experiment. Azim (2001) reported AI values ranged from 190-350 and 130-225 in ungrazed and grazed conditions, respectively, depending on substrate types. On the last two sampling days, the periphyton AFDM was lowest ($P < 0.05$) at the highest stocking density indicating that periphyton was grazed more heavily at higher stocking densities. In some experiments with other organisms such as snails and insect larvae (Jacoby, 1987; Swamikannu and Hoagland, 1989), grazing resulted in lowering periphyton biomass. This mechanism did not show in the experiment by Keshavanath et al. (2004), possibly because of the relatively small size fishes were present in that experiment. Fish grazing on attached algal mat in coral reefs or on low-quality detritus are known to be selective in order to maximize the dietary protein:energy ratio (Montgomery and Gerkling, 1980; Bowen et al., 1995). 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 ash content of the periphytic mats was highly variable and ranged from 16-42% of the dry matter content. Azim et al. (2001b) reported more or less similar periphyton ash contents in carp polyculture ponds. The result differ with the findings of Keshavanath et al. (2001) who suggested that the ash content was to a large extent derived from suspended particles entrapped in the periphytic community. In addition, the substrate type may also influence periphyton composition. The ash content increased when the periphyton communities grow older (Makarevich et al., 1992).

Net yields of tilapia were different between treatments due to differences in survival and growth rate. The low survival rates observed in higher stocking density may be due to the predation on the comparatively smaller sized fishes in high stocking density ponds by birds, snakes etc. Rouse and Kahn (1998) observed that the predation stopped when fishes attained 50 g in a tilapia and red claw crayfish (*Cherax quadricarinatus*) polyculture. They reported 84% survival rate and a production of 3,623 kg ha^{-1} over a 135 day culture period. In tilapia-prawn polyculture system Cohen and Ra'anan (1983) reported that survival rate did not correlate with either prawn or tilapia stocking rates. Wohlforth et al. (1985) observed that prawns with a stocking rate of 5,000-40,000 ha^{-1} did not affect fish production in earthen ponds when studying polyculture of prawn with carp and tilapia species in Israel. In that experiment fish stocking varied between 8,500-17,000 ha^{-1} and growth of fish and prawns were independent. In the present experiment the net yields of tilapia were 44-47% higher in medium and high stocking densities as compared to low density. García-Pérez et al. (2000) observed 2,769 kg ha^{-1}145 d^{-1} production with 331 g average weight and 84% survival rate, while stocked with 10,000 tilapia ha^{-1} in tilapia-prawn polyculture. However, a comparison of the result suggested that the lower production with lower stocking density obtained in this present experiment may be compensated by applying a longer culture period resulting in a higher average fish weight at harvest.

The amount of supplemental feed was calculated initially at 5% of tilapia body weight, and later decreased to 2% following practices as outlined by Rakocy and McGinty (1989). They calculated the FCR only for tilapia presuming the prawns were not able to utilize much of the supplemental feed, since prawns are not that quick on the feed as the fast swimming tilapias.

Net yield and individual harvesting weight of prawn were not significantly different among the treatments. Survival of prawn was inversely proportional to density. This is a common trend in monoculture (Valenti and New, 2000). It is known that prawns feed on benthic organisms (Tidwell et al., 1995), detritus (Valenti, 1996), and feces of other fishes (Zimmermann and New, 2000). Therefore, energy and nutrients for prawn development might be supplied by benthos, formulated feed residues, periphytic mats and tilapia feces. In the present study the tilapia density was equally proportionate with the prawn density and also supplemental feed was applied with a same ratio but the survival rate was inversely proportional to the density (Table 1). Martino and Wilson (1986) observed most interactions were intraspecies and cannibalism, which was detected among prawns, was not influenced by the presence of tilapia in polyculture. There are several causes that affect the survival rate of prawn, such as environmental tress, cannibalism and bird predation (García-Pérez et al., 2000). Karplus et al. (1990), and Willis and Berrigan (1977) reported prawn predation by birds. Cannibalism during the molting period is normal and may be responsible for a mortality of 4% monthly (AQUACOP, 1990). The management practices like size grading of prawn juveniles prior to pond stocking had been shown to increase overall production (Tidwell and Bratvold, 2005)

The combined net yield from tilapia and prawn was significantly higher in higher stocking densities (Figure 5). In an earlier experiment, total fish biomass was lower (Uddin et al., 2006) than in the current experiment, even at the same stocking density and stocking ratio. However, in the experiment reported here 2-g juvenile prawns were stocked compared to PLs in the previous experiment. In the earlier reported experiment the average survival rate of prawn was (28-48%) lower than this one (39-57%). It is interesting that in the present experiment the survival rate of prawn was significantly lower at the highest stocking density. This indicates the existence of intra-and inter-specific competition for food and space. Especially, tilapia might have affected prawn survival during molting in polyculture ponds. Uddin et al., 2006 reported that the survival of freshwater prawn was not affected by their own stocking density. A second difference between the 2 experiments was that the previous experiment was carried out in farmer's ponds which were not as strictly managed as in the present on-station experiment. Management related differences like fluctuating water levels and shading effects from surrounding canopy, combined with less strict feeding regimes influence periphyton and overall pond productivity (Azim et al., 2004).

The substrate was one of the major input costs. The dependency on costly bamboo used as substrate could be replaced if cheaper and available alternative substrate can be found. The economical analysis revealed that the periphyton-based tilapia-prawn polyculture can bring profit to the small scale farmers who own household ponds. Although all input costs are considered in this analysis, in reality, most of the small scale fish farmers in Asia use on-farm available resources including land, labor, substrate, manure or household waste. Therefore, the input costs in reality would be lower and net benefit higher. Nevertheless, the results obtained at a research station, in ideal conditions, may greatly differ from the conditions in the farmer's pond.

It might be concluded that from an economical point of view a stocking density of tilapia and freshwater prawn of 30,000 ha^{-1} with a ratio of 3:1 shows the best result. To find an optimum stocking density, a modeling approach of periphyton-based systems might be useful to explore all possible combinations of substrate densities and fish densities and ratios. Additional trials focusing on the evaluation of the combined effect of fertilization and supplemental feeding on periphyton based tilapia and prawn polyculture production, can result in a further increased of profits.

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References

APHA, 1992. Standard Methods for the Examination of the Water and Wastewater 18th ed. American Public Health Association, Washington, DC.

AQUACOP, 1990. Freshwater prawns. In: Norton, M. (Ed.), Aquaculture. Bookcraft Limited, London, Great Britain, 501-528.

Azim, M.E., 2001. The potential of periphyton-based aquaculture production systems. Ph.D. Thesis. Fish Culture and Fisheries Group, Wageningen University, The Netherlands, 219 pp.

Azim, M.E., Rahaman, M.M., Wahab, M.A., Asaeda, T., Little, D.C., Verdegem, M.C.J., 2004. Periphyton-based pond polyculture systems: a bio-economic comparison of on-farm and on-station trials. Aquaculture 242, 381-396.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., 2002. Evaluation of polyculture of Indian major carps in periphyton-based ponds. Aquaculture 213, 131-149.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Milstein, A., Verdegem, M.C.J., 2001a. Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-based aquaculture. Aquac. Res. 32, 749–760.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Huisman, E.A., Verdegem M.C.J., 2001b. Optimization of stocking ratios of two Indian major carps, rohu (*Labeo rohita* Ham.) and catla (*Catla catla* Ham.) in a periphyton-based aquaculture system. Aquaculture 203, 33–49.

Bowen, S.H., Lutz, E.V., Ahlgren, M.O., 1995. Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76, 899-907.

Boyd, C.E., 1979. Water Quality in Warmwater Fish Ponds. Auburn University, Craftmaster Printers, Opelika, Alabama, 359 pp.

Cohen, D., Ra’anan. Z., 1983. The production of the freshwater prawn *Macrobrachium rosenbergii* in Israel. III. Density effect of all-male tilapia hybrids on prawn yield characters in polyculture. Aquaculture 35, 57-71.

Corbin, J.S., Fujimoto, M.M., Iwai, T.U., Jr., 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P. (Ed), CRC Handbook of Mariculture. CRC Press, Boca Raton, FL, pp. 391-423.

Coyle, S.D., Najeeullah, T., Tidwell, J.H., 1996. Evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawns *Macrobrachium rosenbergii*. J. Appl. Aqua. 6, 57-66.

Dewan, S., Wahab, M.A., Beveridge, M.C.M., Rahman, M.H., Sarkar, B.K., 1991. Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carp fry and fingerlings grown in extensively managed, rain-fed ponds in Bangladesh. Aquacul. Fish. Manage. 22, 277-294.

García-Pérez, A., Alston, D.E., Cortés-Maldonado, R., 2000. Growth, survival, yield and size distribution of freshwater prawn *Macrobrachium rosenbergii* and tilapia *Oreochromis niloticus* in polyculture and polyculture systems in Puerto Rico. J. World Aquac. Soc. 31(1), 446-451.

Hatcher, B.G., 1983. Grazing in coral substrate ecosystems. In: Barnes, D.J. (Ed.), Perspective of coral substrates. Brian Clouston Publ., Manuka, pp.164-179.

Hay, M.E., 1991. Fish-Seaweed interactions on coral substrates: effects of herbivorous fishes and adaptations of their prey. In: Sale, P.F. (Ed.), The ecology of fishes on coral substrates. Academic Press, London, pp. 96-119.

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M., 2000. The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture 186, 45–60.

Jacoby, J.M., 1987. Alteration in periphyton characteristics due to grazing in a cascade foothill stream. Freshwat. Biol. 18, 495-508.

Karplus, I., Hulata, G., Peretz, G., 1990. Effect of age at metamorphosis on population structure and production of *Macrobrachium rosenbergii* in polyculture ponds. Bamidgeh 42(4), 122-127.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquaculture 235, 303-314.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Rooij, J.M., Beveridge, M.C.M., Baird, V., Verdegem, M.C.J., van Dam, A.A., 2001. Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. Aquac. Res. 32, 189–197.

Makarevich, T.A., Zhukova, T.V., Ostapenya, A.P., 1992. Chemical composition and energy value of periphyton in a mesotrophic lake. Hydrobiol. 28(1), 30-34.

Martino, C., Wilson, J.L., 1986. Behavioral interactions of tilapia, crawfish, and freshwater prawns in aquaculture. Aquaculture Magazine 12(4), 36-37.

Montgomery, W.L., Gerkling, S.D., 1980. Marine microalgae as foods for fishes: an evaluation of potential food quality. Enviorn. Biol. Fishes 5, 143-153.

New, M.B., 2005. Freshwater prawn farming: global status, recent research and a glance at the future. Aquac. Res. 36, 210-230.

Perschbacher, P.W., Lorio, W.J., 1993. Filtration rates of catfish pond phytoplankton by Nile tilapia *Oreochromis niloticus.* J. World Aqua. Soc. 24(3), 434-437.

Rakocy, J.E., McGinty, A.S., 1989. Pond Culture of Tilapia. SRAC Publication No. 280, pp. 1-4.

Ramesh, M.R., Shankar, K.M., Mohan, C.V., Varghese, T.J., 1999. Composition of three plants substrates for enhancing carp growth through bacterial biofilm. Aquac. Eng. 19, 119-131.

Rouse, D.B., Kahn. B.M., 1998. Production of Australian red claw *Cherax quadricarinatus* in polyculture with Nile tilapia *Oreochromis niloticus*. J. World Aqua. Soc. 29(3), 340-344.

Swamikannu, X., Hoagland, K.D., 1989. Effect of snail grazing on the diversity and structure of a periphyton community in a eutrophic pond. Can. J. Fish. Aquat. Sci. 46, 1698-1704.

Tidwell J.H., Cycle, S., Van Arnum, A., Weibel, C., 2000. Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. J. World Aqua. Soc. 31, 452-458.

Tidwell, J.H., Bratvold, D., 2005. Utility of added substrates in shrimp culture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 247-268.

Tidwell, J.H., Coyle, S.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, S.J., D'Abramo, L.R., Fuller, M.J., 1997. Relative prawn production and benthic macroinvertebrate densities in unfed, organically fertilized, and fed pond systems. Aquaculture 149, 227-242.

Tidwell, J.H., Webstar, C.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, Jr., S.J., D'Abramo, L.R., Daniels, W.H., Fuller, M.J., Montañez, J.L., 1995. Effects of complete and supplemental diets and organic pond fertilization on production of *Macrobrachium rosenbergii* and associated benthic microinvertibrate populations. Aquaculture 138, 169-180.

Turker, H., Eversole, A.G., Burne, D.E., 2003. Effect of temperature and phytoplankton concentration on Nile tilapia (*Oreochromis niloticus*) filtration rate. Aquac. Res. 34, 453-459.

Uddin, M.S., Farzana, A., Fatema, M.K., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2006. Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia-prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development. Aquaculture (in press).

Uddin, S., Azim, M.E., Wahab, A., Verdegem, M.C.J., 2006. The potential of mixed culture of genetically improved farmed tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. Aquac. Res. 37, 241-247.

Valenti, W.C., 1993. Freshwater prawn culture in Brazil. World Aquaculture 24, 30-34.

Valenti, W.C., New, M.B., 2000. Grow-out systems-Monoculture. In: New, M.B., Valenti, W.C. (Eds.), Freshwater prawn farming. The farming of *Macrobrachium rosenbergii,* Blackwell Science, Osney Mead, Oxford, England, pp. 157-176.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1-31.

Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S., 1999. The potential of periphyton based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton). Aquac. Res. 30, 409-419.

Willis, S.A., Berrigan, M.E., 1977. Effects of stocking size and density on growth and survival of *Macrobrachium rosenbergii* (de Man) in ponds. Proceedings of the World Mariculture Society 8, 251-264.

Wohlforth, G.W., Hulata, G., Karplus, I., Halevy, A., 1985. Polyculture of freshwater prawn *Macrobrachium rosenbergii* in intensively matured ponds, and the effect of stocking rate of prawns and fish on their productions characteristics. Aquaculture 46, 143-156.

Zimmermann, S., New. M.B., 2000. Grow-out systems polyculture and integrated culture. In: New, M.B., Valenti, W.C. (Eds.), Freshwater prawn farming. The farming of

Macrobrachium rosenbergii, Blackwell Science, Osney Mead, Oxford, England, pp.187-202.

Table 1

Comparison of means (± S.D., $N=3$) of yield parameters of tilapia and prawn in 20,000 ha^{-1} (TP-20), 30,000 ha^{-1} (TP-30) and 40,000 ha^{-1} (TP-40) treatments. If main effects are significant, then means followed by the different superscript letter in each row indicate significant difference at 0.05 level based on Tukey-HSD test

Species/yields parameters	TP-20	TP-30	TP-40
O. niloticus			
Individual stocking weight (g)	1.92±0.10	1.84±0.30	1.92±0.06
Individual harvesting weight (g)	122.8±39.7^{a}	115.9±31.8^{a}	94.6±27.1^{b}
Survival (%)	82.9±2.2^{ab}	86.1±1.2^{a}	78.2±4.1^{b}
Individual weight gain (g)	120.82±7.9^{a}	114.06±5.6^{a}	92.68±4.6^{b}
SGR (% day^{-1})	3.96±0.05	3.95±0.16	3.71±0.06
FCR	1.06±0.16	1.01±0.09	1.14±0.20
Net yields (kg ha^{-1})	1505±81^{b}	2209±79^{a}	2162±87^{a}
M. rosenbergii			
Individual stocking weight (g)	1.96±0.33	2.26±0.20	2.04±0.19
Individual harvesting weight (g)	50.5±16.5	47.7±15.8	41.3±16.3
Survival (%)	57.0±4.0^{a}	50.6±4.5^{a}	39.1±4.3^{b}
Individual weight gain (g)	48.5±8.4	45.4±5.3	39.3±1.2
SGR (% day^{-1})	3.09±0.31	2.90±0.17	2.87±0.09
Net yields (kg ha^{-1})	136±30	163±28	141±19
Combined net yields (kg ha^{-1}105 day^{-1})	1641±66^{b}	2372±90^{a}	2303±169^{a}

Table 2

A comparison of economics within three stocking densities of tilapia and prawn polyculture with periphyton substrates. Calculation was done based on 1 ha pond and 120 days experimental period.

Items	Amount used	Price rate	TP-20	TP-30	TP-40
Fixed/common costs					
Land rental cost	1 ha	15000 ha^{-1} y^{-1}	4930	4930	4930
Labor (Pond cleaning, substrate installation, feed application and fish harvesting)	100 man-day	75 $manday^{-1}$	7500	7500	7500
Rotenone	12 kg	240 kg^{-1}	2880	2880	2880
Lime	250 kg	6 kg^{-1}	1500	1500	1500
Cowdung	3000 kg	0.50 kg^{-1}	1500	1500	1500
Urea	350 kg	6 kg^{-1}	2100	2100	2100
TSP	350 kg	14 kg^{-1}	5250	5250	5250
Bamboo (Substrate can be used for 6 times)	4660 culms	25 $culms^{-1}$	19417	19417	19417
Subtotal			45077	45077	45077
Variable costs					
Fish feed		14 kg^{-1}	22433	31262	32858

Tilapia fry	0.50 fry^{-1}	7500	11250	15000
Prawn juvenile	3 juvenile^{-1}	15000	22500	30000
Subtotal		44933	65012	77858
Total		90010	110089	122935
Interests on inputs	10%	2959	3619	4042
(for 4 months)	annually			
Total inputs		92969	113708	126977
Financial returns				
Fish sale	60 kg^{-1}	92040	134960	133120
Prawn sale	350 kg^{-1}	47600	57050	49700
Total returns		139640	192010	182820
Net margin		46671	78302	55843
Net profit margin (%)		50	69	44

Currencies are given in Bangladesh Taka, BDT (US$=67BDT)

Figure Captions

Figure. 1. Abundance of algae (A) and zooplankton (B) per litre pond water in three treatments throughout the experimental period. Values are means (± S.D.) of three ponds (*N=3*) per sampling date in each treatment.

Figure. 2. Abundance of algae (A) and zooplankton (B) in periphyton mats per unit surface area of bamboo substrate throughout the experimental period. Values are means (± S.D.) of three ponds (*N=3*) per sampling date in each treatment.

Figure 3. Average chlorophyll *a* concentrations of water column in different treatments during the experimental period. Values are means (±S.D.) of three replicated ponds per sampling date (*N=3*) in each treatment.

Figure. 4. Periphyton ash free dry matter (AFDM) mg cm^{-2}(A), chlorophyll *a* (B), pheophytin *a* µg cm^{-2} (C) and autotrophic index (D) per unit surface area of bamboo substrates over the experimental period. Values are means (± S.D.) of three ponds (*N=3*) per sampling dates in each treatment.

Figure. 5. Relationship between fish stocking density and net fish production.

Figure 1

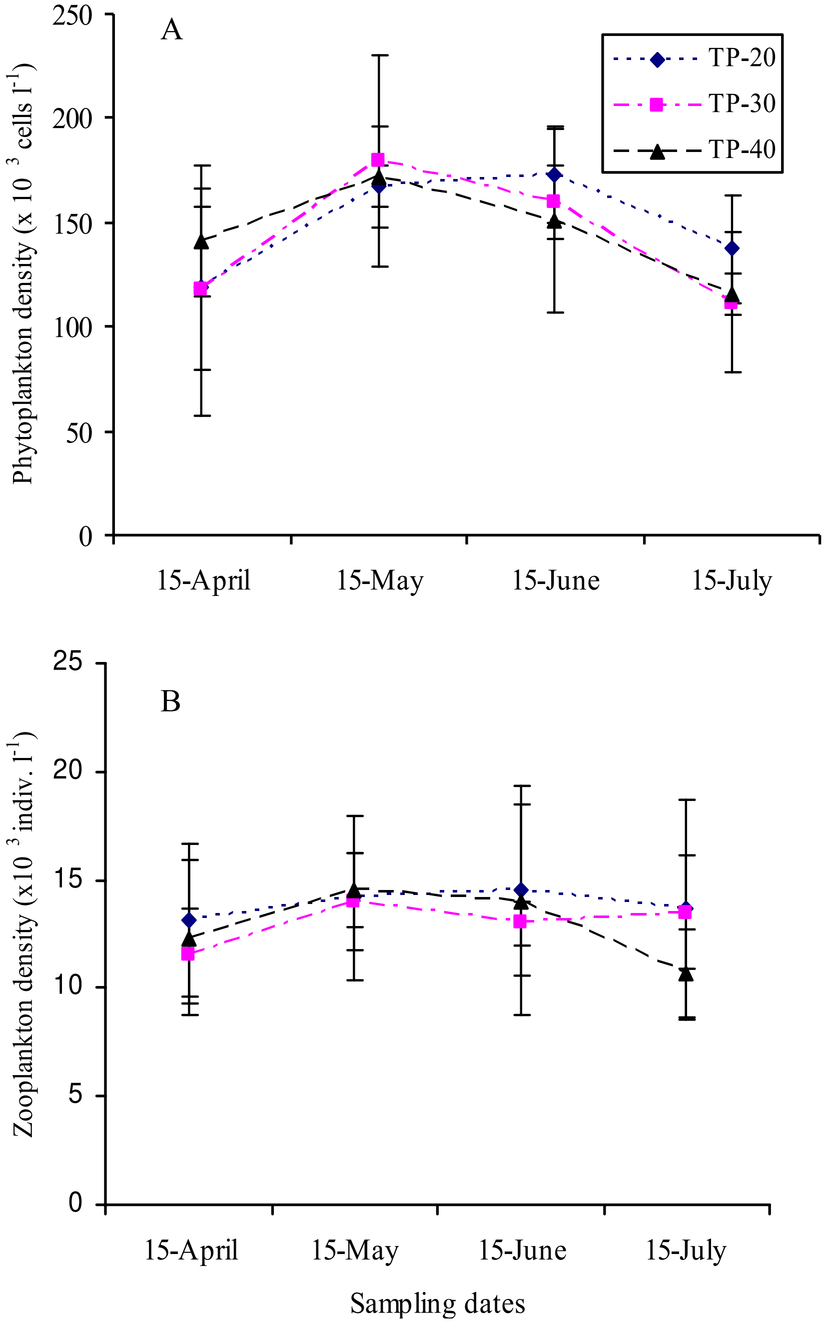

A
Phytoplankton density (x 10 3 cells l-1)
0
50
100
150
200
250
TP-20
TP-30
TP-40
15-April
15-May
15-June
15-July
B
Zooplankton density (x10 3 indiv. l-1)
0
5
10
15
20
25
15-April
15-May
15-June
15-July
Sampling dates

Figure 2

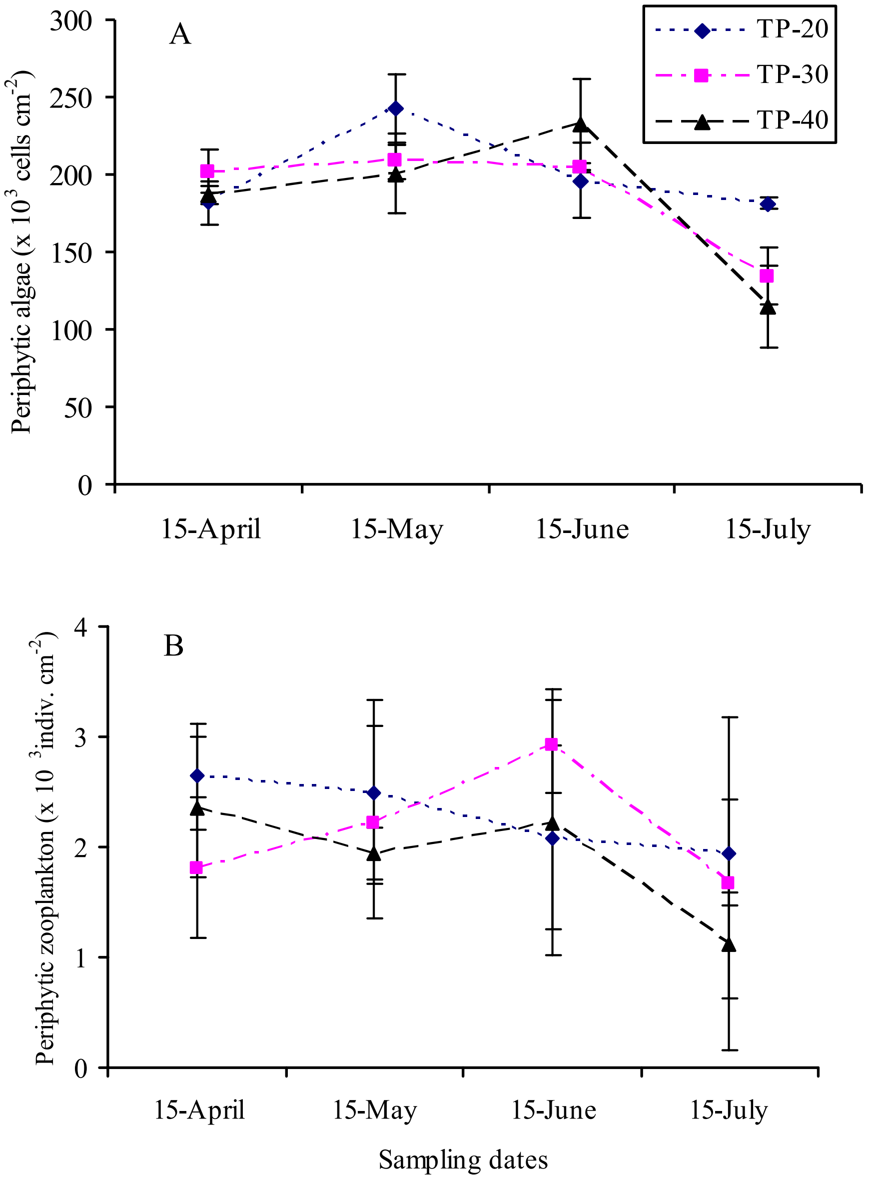

A
Periphytic algae (x 10³ cells cm⁻²)
300
250
200
150
100
50
0
TP-20
TP-30
TP-40
15-April
15-May
15-June
15-July
B
Periphytic zooplankton (x 10³ indiv. cm⁻²)
4
3
2
1
0
15-April
15-May
15-June
15-July
Sampling dates

Figure 3

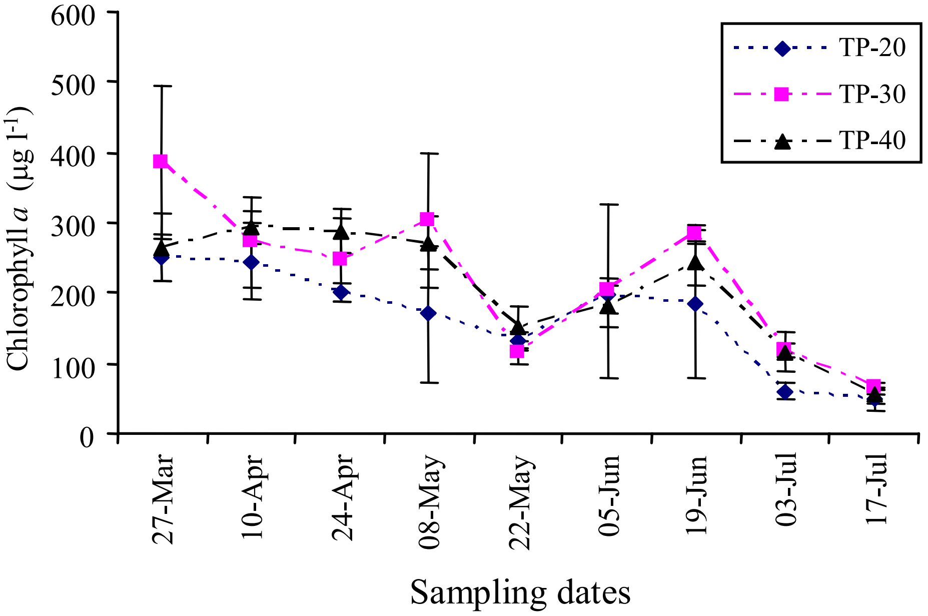
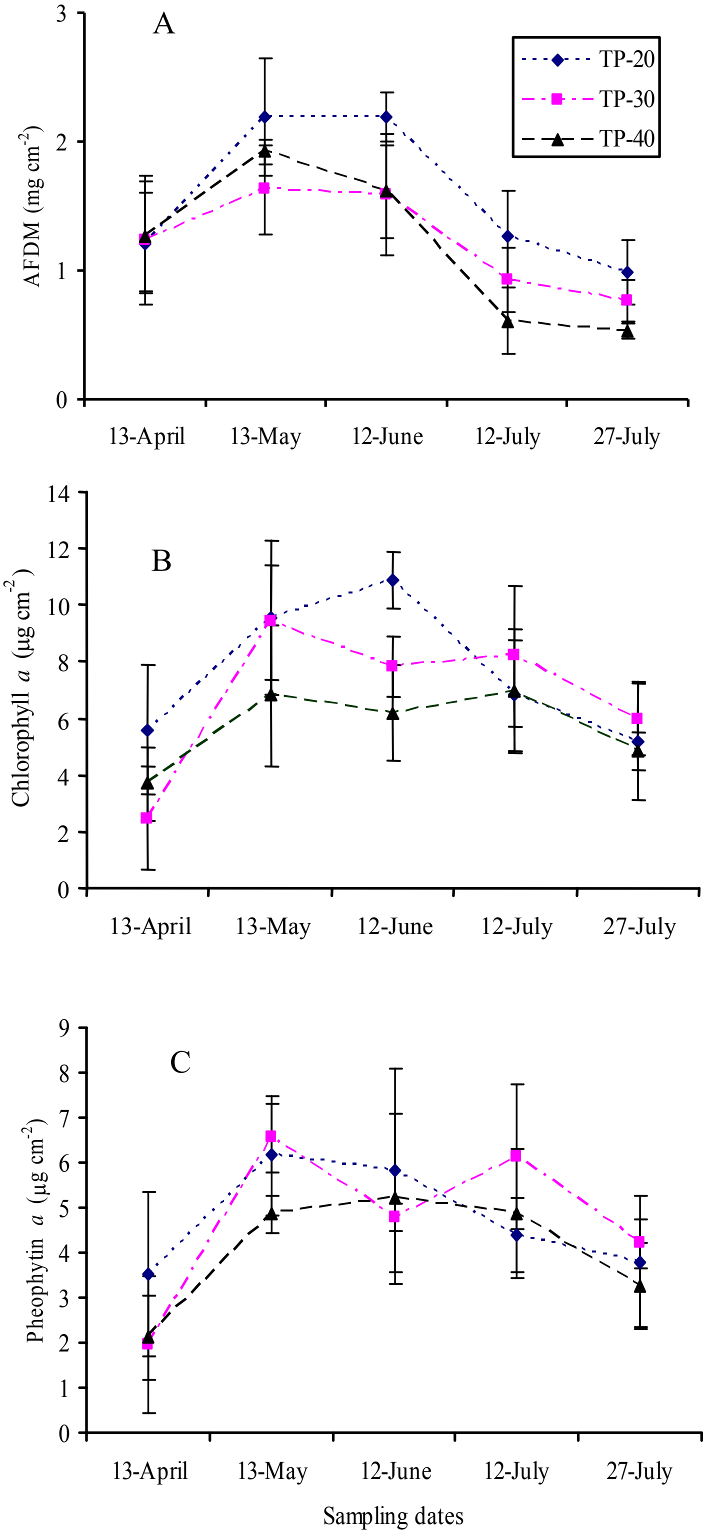

Chlorophyll *a* (µg l-1)
600
500
400
300
200
100
0
27-Mar
10-Apr
24-Apr
08-May
22-May
05-Jun
19-Jun
03-Jul
17-Jul
Sampling dates
TP-20
TP-30
TP-40

Figure 4


A
AFDM (mg cm^{-2})
0
1
2
3
TP-20
TP-30
TP-40
13-April
13-May
12-June
12-July
27-July
B
Chlorophyll *a* (µg cm^{-2})
0
2
4
6
8
10
12
14
13-April
13-May
12-June
12-July
27-July
C
Pheophytin *a* (µg cm^{-2})
0
1
2
3
4
5
6
7
8
9
13-April
13-May
12-June
12-July
27-July
Sampling dates

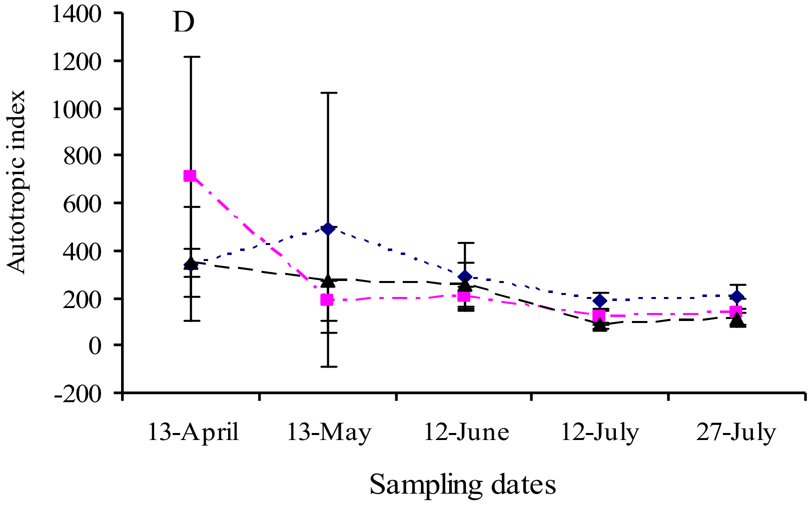
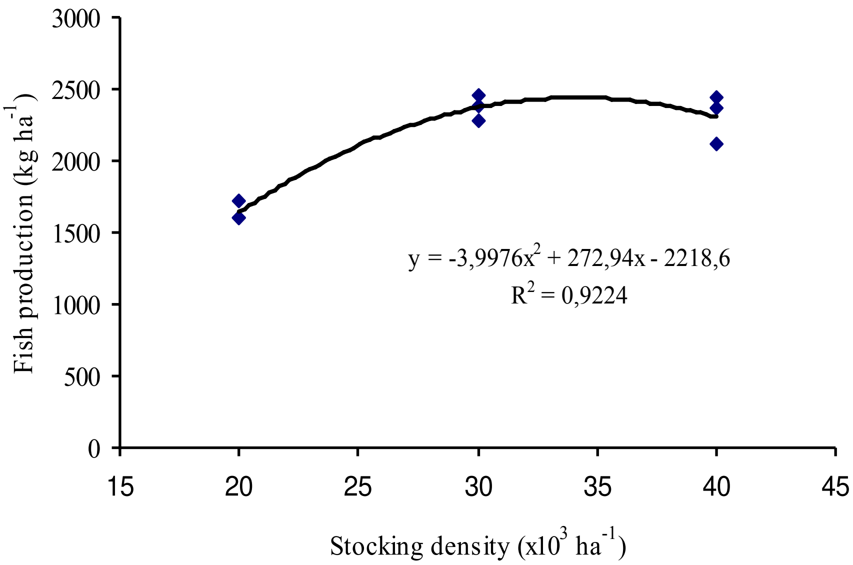

D
Autotropic index
1400
1200
1000
800
600
400
200
0
-200
13-April
13-May
12-June
12-July
27-July
Sampling dates

Figure 5


y = -3,9976x2 + 272,94x - 2218,6
R2 = 0,9224
Fish production (kg ha-1)
Stocking density (x103 ha-1)
0
500
1000
1500
2000
2500
3000
15
20
25
30
35
40
45

Chapter 5

Comparison of substrates and supplemental feeding on growth and production of tilapia (*Oreochromis niloticus*) and fresh water prawn (*Macrobrachium rosenbergii*) polyculture: a bioeconomic approach

Chapter 5

Comparison of substrates and supplemental feeding on growth and production of tilapia *(Oreochromis niloticus)* and fresh water prawn (*Macrobrachium rosenbergii*) polyculture: a bioeconomic aspect

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Abstract

The present on-farm research investigated the effects of substrates and supplemental feeding on growth and production of tilapia *(Oreochromis niloticus)* and fresh water prawn (*Macrobrachium rosenbergii*) in polyculture system. Four treatments were tried in triplicate: substrate plus feed (herein called treatment T- SF), no substrate plus feed (T-S_0F), substrate plus no feed (T-SF_0) and no feed and substrate (control). All ponds were stocked with tilapia and freshwater prawn juveniles at a stocking density of 30,000 ha^{-1} with the ratio of 75 and 25% of tilapia and freshwater prawn, respectively. In the substrate based system the ponds were provided with bamboo poles that results 60% of additional surface area for periphyton growth and in feed driven system the fish were fed with commercial 25% protein diet at 2-3% tilapia body weight day^{-1}.

About 29 genera of algae and 9 genera of zooplankton were identified from pond water and it shows significant ($P < 0.05$) differences for Chlorophyceae, Bacillariophyceae and Cyanophyceae group in treatment with substrate and feeding (T-SF) than control. There were no significant ($P > 0.05$) difference for periphyton biomass in terms of ash free dry matter (AFDM), chlorophyll *a* and pheophytin *a* in substrate based system. The feed conversion ratio (FCR) of tilapia was significantly higher in treatment with substrate and feeding (T-SF) than in treatment with feeding (T-S_0F. The combined net yields varied significantly ($P < 0.05$) among four treatments and production were 59, 48 and 47% higher in ponds with substrate with feed (T-SF), feeding alone (T-S_0F) and substrate alone (T-SF_0), respectively, compared to the control. There were no ($P > 0.05$) significant difference between only substrate and feed driven treatments. Net profit margin was highest in treatment T-SF_0 (53%) followed by T-S_0F (52%), T-SF (50%), and T-control (10%) treatments. Although more experiments are needed to optimize periphyton density and the culture period, but the results show that periphyton can replace or complement supplemental feeding in tilapia and freshwater prawn polyculture.

Keywords: Periphyton, tilapia, freshwater prawn, polyculture, substrates, profit margin

Introduction

Global farmed fish and shellfish production has more than doubled in the past 15 years and such growth relieves pressure on wild fisheries. Farming carnivorous species requires more inputs of wild fish for feed, while about 80% of carp and 65% of tilapia worldwide are farmed without the use of modern compound feeds–feeds formulated from multiple ingredients (Naylor et al., 2000). Tilapia is one of the first fish species that is cultured world-wide (Popma and Masser, 1999). During last few decades, Nile tilapia, both as a subsistence and commercial crop, became a dominant component of fisheries sector in many Asian countries with an average annual growth rate of 13% (Dey and Gupta, 2000). Now a day there is a tendency towards tilapia culture and intensification of aquaculture using artificial feeds in Bangladesh. Fresh water prawn is a most popular species for their taste, growth and high international market value. The popularity of prawn has encouraged the development of prawn culture globally and for this reason freshwater prawn culture has been gaining importance day by day. Considering these farmers in Bangladesh are highly interested to culture freshwater prawns because potential profits are higher than for other types of culture.

Many trials have demonstrated that fish production from the ponds provided with substrate for periphyton is higher than that of substrate free ponds (Hem and Avit, 1994; Wahab et al., 1999; Azim et al., 2001; Keshavanath et al., 2004; Uddin et al., 2006a). These studies showed that some species such as rohu (*Labeo rohita*), mahseer (*Tor khudree*) and tilapia (*Oreochromis niloticus*) are very suitable for periphyton-based aquaculture. Although tilapia is known to be a periphyton grazer (Dempster et al., 1993; Keshavanth et al., 2004; Uddin et al., 2006a), and in recent years, substrate-based systems were tested in freshwater prawn (*Macrobrachium rosenbergii*) culture (Tidwell et. al., 1998; 2000) and reported positive and encouraging result. However, the role of supplementary feeds in semi-intensive polyculture systems is not straightforward as there are complex interactions among natural food organisms, supplementary feeding practices, environmental parameters and different fish species cultured (Azim, 2001). From environmental and economic point of view to examine the options to make aquaculture systems more resource efficient are worth looking. In recent years, a series of experiment has been carried out to develop a substrate-based tilapia-prawn polyculture system in Bangladesh. Trials have demonstrated that tilapia and prawn production from substrate-based system is higher than that of substrate free systems and addition of freshwater prawn to tilapia ponds resulted in higher production of both species in periphyton-based ponds (Uddin et al., 2006a,b).

To this end, using a preoptimized periphyton-based polyculture package with tilapia and freshwater prawn, the present study was carried out to (i) compare traditional, improved traditional, substrate-based and substrate plus feed systems; (ii) compare the effects of periphyton substrates and supplemental feeding on their growth, survival and production; (iii) see if periphyton substrates could minimize the need for supplemental feed; and (iv) compare cost-benefits of different production systems.

Materials and methods

Study area and experimental design

The experiment was carried out in 12 earthen farmer's ponds of Guatala village in Mymensingh district, 25 km from Bangladesh Agricultural University (BAU), Mymensingh, for a period of 140 days during August-December, 2004. The ponds were rectangular in shape with area ranging from 115 to 270 m^2 and water depth ranging from 1.0 to 1.5 m. Ponds were different in shape, size and bottom conformation and dependent on rainfall for water supply; and well exposed to sunlight. The experiment was carried out in 2-factorial design (with and without periphyton substrates and supplemental feed) resulting in four treatments in triplicate: substrate plus feed (herein called treatment T- SF), substrate plus no feed (T-SF_0), feed plus no substrate (T-S_0F), and no feed and no substrate (control). Ponds were stocked with a preoptimized tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) juveniles at a stocking density of 30,000 ha^{-1} and with the ratio of 75 and 25% of tilapia and freshwater prawn, respectively (Uddin et al., 2006).

Pond preparation and fish stocking

All pond embankments were renovated and aquatic vegetation was cleaned off manually from the ponds and predators and fishes were eradicated using rotenone. Since the experiment was started in the rainy season, ponds were already filled up by precipitation. Pond draining or drying was not possible because of the multipurpose use of rural ponds. In substrate treatments ponds, bamboo poles (with an average periphery of 0.27m each) were driven vertically into the pond bottom, the upper portion extending above the water surface, excluding a 1-m-wide perimeter water surface from the pond dike. Each pond was provided with the number of bamboo poles (5-6 poles m^{-2}) that resulted in an additional 60% effective submerged substrate surface area of the total pond water surface.

The day of substrate installation is named Day 1 of the experiment. On Day 2, powdered lime ($CaCO_3$) was broadcasted over the water surface at a rate of 250 kg ha^{-1}. Ponds were fertilized with semi-decomposed cow manure, urea and triple super phosphate (TSP) at 3,000, 100 and 100 kg ha^{-1}, respectively on Day 6. Juveniles of tilapia (*Oreochromis niloticus*, 3.03 g) and freshwater prawn (*Macrobrachium rosenbergii*, 5.92 g) were stocked on Day 16 according to the design, when periphyton was shown to grow on the substrates. The tilapia juveniles were collected from Bangladesh Fisheries Research Institute, Mymensingh and prawn juveniles were collected from Zhalak Hatchery, Gouripur, Mymensingh. Stocking was done in the morning, care being taken for temperature acclimatization to the pond conditions.

Measurement of plankton biomass and composition

Plankton biomasses in terms of chlorophyll *a* concentration of pond water were determined fortnightly. A known amount of water sample were filtered through micro-fibre glass filter paper (Whatman GF/C) using a vacuum pressure air pump. The filter paper was kept in a test tube containing 10 ml 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 hours. Later, Chlorophyll *a* was determined using a spectrophotometer (Milton Roy Spectronic, Model 1001 plus) at 664 and 750 nm wavelength following Boyd (1979).

Determination of Periphyton biomass

The periphyton biomass growing on bamboo substrates, viz. dry matter (DM) and pigment concentration (chlorophyll *a* and pheophytin *a*) were determined monthly following standard methods (APHA, 1992), starting from Day 30. In substrate treatments, from each pond, three poles were randomly selected and two 2x2 cm^2 of periphyton samples were collected at 30, 60 and 90 cm depths bellow from the water surface. The periphyton samples were collected with a sharp blade from the surface area of the substrate, care being taken not to remove any of the substrate itself, and the material transferred to pre-weighed and □avourab pieces of aluminium foil. After sampling, the poles were replaced in their original positions, marked and excluded from subsequent sampling. One sample was used to determine DM and ash contents, and another sample was used to determine chlorophyll *a* and pheophytin *a*. Periphyton biomass (DM in mg cm^{-2}, ash-free dry matter [AFDM] in mg cm^{-2}, and pigment concentration chlorophyll *a* and pheophytin *a* both in µg cm^{-2}) content were determined as described in Azim et al. (2001).

Post stocking management

All ponds were fertilized fortnightly with urea and TSP at the rate of 50 kg ha^{-1} each until harvesting. In feed treatments, commercial pelleted fish feed (25% crude protein) procured from the local market was applied to the ponds at a rate of 3% of total tilapia biomass for the first three months and 2% of the tilapia biomass for the rest of the experimental period. Tilapias were sampled at monthly intervals using cast net. Weight of approximately 10% of total number of tilapias were measured to estimate the tilapia biomass and to adjust the feeding rate under the feed treatments. The fish were cultured for a period of 120 days. At the end of the experiment, bamboo poles were removed from ponds under substrate treatments, water was pumped out of the ponds and all fish and prawn were collected, pond-wise number of individuals were counted, and bulk weights of tilapia and freshwater prawn were taken using a balance (Denver-xp-3000; precision=0.1 g). Weight gain per fish was calculated by deducting the average initial weight from the average final weight. Specific Growth Rate (SGR; % body weight gain day^{-1}) was estimated as:

SGR = [Ln(final weight) - Ln(initial weight) x 100]/ cultured period (days).

Net yield= Total biomass at harvest – total biomass at stock

Economical analysis

A simple algebraic economical analysis was performed to estimate the net return and profit margins 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 = Interests on inputs. The wholesale price per kg of tilapia and prawn were 60 and 350 taka, respectively. The prices of inputs and fish and prawn correspond to the Mymensingh wholesale market price in 2004 and are expressed in Bangladeshi taka (BDT).

Statistical analysis

Yield parameters and cost-benefit ratio were compared by two-way ANOVA with addition of substrate (with and without) and feeding (with and without) as main factors. A split-plot ANOVA design (repeated measurements) was used for periphyton and phytoplankton biomass using treatment as a main factor and time as a sub factor (Gomez & Gomez, 1984). Again, a one-way ANOVA was used to compare the fish production and plankton population within the treatments. The arcsine transformation was used for comparing percentage data before statistical analysis but percent values are reported. The effects were tested at 5% level of significance using statistical package, SAS version 9.1 (SAS Institute, Cary, NC 27513, USA).

Results

Plankton biomass and composition

The plankton communities in pond water consisted of four groups of algae and two groups of zooplankton in all the trials. Twenty nine genera of algae belonging to Bacillariophyceae (7), Chlorophyceae (14), Cyanophyceae (5) and Euglenophyceae (3) were found in all treatments during the last day of the experiment. The total number of Bacillariophyceae, Chlorophyceae and Cyanophyceae were significantly higher in substrate with feed treatments than in control and there were no significant different within only substrate and feed driven systems (Table 1).

Phytoplankton biomass in terms of chlorophyll *a* concentrations was found to be higher in treatment T-SF (201 μgl^{-1}) than in other treatments (112-132 $\mu g\ l^{-1}$) and were significant differences (P = 0.0001) among sampling dates. Phytoplankton chlorophyll *a* content was relatively higher in the last part of the experiment for all the treatments. It showed that chlorophyll *a* concentrations were static in control pond with a lower value but some fluctuations occurred with other treatments (Figure 1). Treatment-time interaction was apparent (P = 0.002).

Periphyton biomass

Periphyton biomass in terms of DM and AFDM contents per surface area of substrate in feed with substrate (T-FS) ponds were higher than in only substrate (T-SF$_0$) ponds (P = 0.0001 for DM and P = 0.007 for AFDM). There were no time effect on DM (P = 0.75) and AFDM (P = 0.95) among sampling dates indicating that the quality of periphyton did not changed with time. Periphyton DM density increased and had reached 2.62 mg cm^{-2} in feeding (T-SF) treatment and was static up to next sampling date and then decreased steadily till last date. But in treatment without feeding (T-SF$_0$) periphyton DM decreased steadily reaching at 2.65 mg cm^{-2} in the second sampling dates (Figure 2A). For the first month of the experiment, periphyton AFDM density increased and had reached 1.81 mg cm^{-2} in feeding (T-SF) treatment and 1.89 mg cm^{-2} in treatment without feeding, and there after periphyton AFDM decreased steadily up to the last date of the experiment (Figure 2A) in both treatments. On the other hand ash percent of periphyton dry matter increased up to the second month of the experiment and then decreased steadily. The ash percent of periphyton dry matter ranged from 19-39% (Figure 2B).

There were differences in periphyton chlorophyll *a* among sampling dates (P = 0.009) with higher values during on day 30 and ranging from 6.53 in T-SF$_0$ to 8.74 µg cm^{-2} in T-SF, and there after density decreased steadily to around 4.00 µg cm^{-2} in both treatments on the last date of the experiment (Figure 3A). In case of pheophytin *a* concentration, it shows a static level for only treatment without feeding (T-SF$_0$), but in treatment with feeding (T-SF), it showed more fluctuations (Figure 3B).

Fish yield parameters

Outcomes of two-way ANOVA, considering substrate and feeding as factors, on yield parameters are given in Table 2. Effects of substrate and feed were significant on survival, final weight gain and net yield for both the cultured species. The interaction between substrate and feed significantly affect on tilapia final weight (P = 0.0274) and net yield (P =0.0342), but it was not significant for prawn. Survival, individual harvested weight and net yields of tilapia and freshwater prawn were significantly higher in ponds with substrate and/or feed than in control. The net yield of both tilapia and freshwater prawn were significantly higher in with substrate and feeding treatment (T-SF) among the treatments, and there was no significant difference within feeding (T-S$_0$F) and substrate (T-SF$_0$) treatments but were significantly higher than in control. The combined net yields varied significantly among four treatments, with 59% higher production in ponds with substrate and feed (T-SF), 48% higher in ponds with feeding alone (T-S$_0$F) and 47% higher in substrate alone (T-SF$_0$) compared to the control (one-way ANOVA and Tukey test; Table 2).

Cost-benefit analysis

The cost-benefit analysis of different treatments is given in Table 4. Net profit margin was found to be higher (*P* = 0.0001) in all treatments than in control (two-way ANONA). No difference (*P* = 0.15) was found for net profit margin between both feed and/or substrate driven systems. The substrates, supplemental feed and the fresh water prawn juveniles were main expensive inputs. When compared among the treatments for percent profit margin, substrate based system was better than either treatments with feed and substrate.

Discussion

The enumerated plankton population in fish ponds indicates that the number was highest in with substrate and feed based ponds and lowest in the control ponds (Table 1). In substrate with feed treatments, total number of Bacillariophyceae, Chlorophyceae and Cyanophyceae were significantly higher than in control. The phytoplankton biomass in terms of chlorophyll *a* was also found lower in the control ponds (Figure 1). This representing that the fishes were more reliant on plankton in the control ponds since no feed and/or substrate were provided in these ponds. Perschbacher and Lorio (1993) reported that tilapia promoted a very effective biological control over phytoplankton. The genera of plankton population identified from pond water were representative of that found in Bangladesh fishponds (Dewan et al., 1991; Wahab et al., 1999).

In the only periphyton based system, the steady decrease of periphyton biomass (DM) after one month showed the effects of grazing pressure by the fish, but in case of ash percent it shows the same trends after second month. This suggests that the organic part of the periphyton mats was grazed selectively by the fish keeping left the inorganic part in there early growing stage, and in the following dates the ash percent decreased steadily indicates that the fishes grazed on substrate unselectively. Fish size has a strong effect on the preference for periphyton (Dempster et al., 1995; Azim et al., 2003) and grazing rates per unit body weight are higher in the bigger one (Keshavanath et al., 2004). The ash content increased when the periphyton communities grow older (Makarevich et al., 1992). In some experiments with other organisms such as snails and insect larvae (Jacoby 1987; Swamikannu and Hoagland, 1989), grazing resulted in lowering periphyton biomass. Fish grazing on attached algal mat in coral reefs or on low-quality detritus are known the best part to maximize their dietary protein and energy ratio (Montgomery and Gerkling, 1980; Bowen et al., 1995). It is also 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).

Addition of substrates enhanced survival and production of both tilapia and freshwater prawn. Substrate had effect on survival, final weight and net yield of tilapia. Fish grazing on attached algae on coral reefs or on detritus known to select the best part to maximize their dietary protein and energy ratio (Montgomary and Gerking, 1980; Bowen et al., 1995). Substrate contributed to the individual harvesting weight and net yield of tilapia with 31 and 83%, respectively higher weight compared to the control (Table 2). Keshavanath et al. (2004) reported that the gross production of red tilapia with additional 100% surface area was 112% higher than substrate free system. This indicates that the amount of increased substrate area installed in the ponds will increase production (Azim et al., 2004b). Substrate also resulted into higher survival, final weight and net yield of prawn. Addition of substrate in ponds increased prawn production by 14% and average size by 13% (Cohen et al., 1983), and addition of substrate was more effective in intensively stocked, aerated system (Ra'anan et al., 1984).

Substrate also resulted in highest survival, harvesting weight and net yield when combined with feeding for both the cultured species compared to only feeding and substrate systems. The combined net yields varied significantly among four treatments, with 59% higher production in ponds with substrate and feed, 48% higher in ponds with feeding alone and 47% higher in substrate alone compared to the control. For finfish, the reported increase in production due to substrates ranged from 30-115% in carp monoculture (Wahab et al., 1999; Keshavanath and Gangadhar, 2005), 30-210% in carp polyculture (Azim and Wahab, 2005) and 40-127% in tilapia-prawn polyculture (Uddin et al., 2006b), depending on several factors.

In the present experiment, FCR of tilapia was significantly higher in periphyton with feed driven ponds than only feed driven system. This indicates that the periphyton compensated for the absence of supplemental feeding with the feed and feeding level applied. The similar observation was made by Keshavanath et al. (2004) during tilapia culture in periphyton based systems. This is mainly because of natural food in the form of periphyton colonized on bamboo substrates 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). In this experiment the amount of periphyton ingested by tilapia can be estimated using the contribution of substrate to additional net tilapia yield in substrate and feed driven system than feed driven system (346 kg ha^{-1} 120 d^{-1}; from Table 2) and using a reported periphyton FCR value of 1.34 on ash free dry matter basis (Azim et al., 2003). To produce 346 kg ha^{-1} fish, about 467 kg AFDM periphyton per ha pond (6,000 m^{2} ha^{-1} additional surface area) was needed which is equivalent to about 0.64 g AFDM m^{-2} d^{-1} (or 0.84 g dry matter, 30% ash). It is interesting that the ingested AFDM m^{-2} d^{-1} by tilapia is 1.66 g for only substrate-based system compared to substrate with feed system, and is almost double. This indicated that tilapia used periphyton more efficiently in only substrate-based system than in feeding with substrate-based system.

Net yields of both tilapia and freshwater prawn increased due to provision of substrate and/or feed in ponds over control despite the fact that the observation was not possible to confirm whether freshwater prawn grazed on periphyton since they mainly inhabit the deeper region either on the substrates or simply crawling over on the bottom, but tilapias were regularly observed grazing on the substrates for periphyton in the substrate based ponds. There are evidences that prawns in their natural habitats prefer to forage on animals like trichopterans, chironomids, oligochates, nematodes, gastropods and zooplankton (Corbin et al., 1983; Coyle et al., 1996; Tidwell et al., 1997). There is also evidence that substrate based systems enhanced the production of benthos in the culture systems (Azim, 2001). However, there were also substrate and feeding effect on growth and production of freshwater prawn and it indicated that when substrate and feed were provided, tilapia mainly depended on periphyton or supplemental feed for food leaving natural autotrophic food for freshwater prawn.

Efforts have been made to analyze and compare the economics of the traditional non-fed, semi-intensive feed-driven, substrate-based and substrate with feed-based fish production systems in the present study (Table-4). To make the analysis realistic this experiment was performed in the ponds of progressive fish farmers who are engaged with fish farming for last 5-7 years. The size of the cultured fish and prawn did not attain the optimum market size during 120 days culture period. As the experiment was conducted in rainfed ponds and aquaculture was not possible for year round by the farmers, which resulted lower prize of their products and benefit as well. Apparently, the net profit margin (%) is still higher in substrate-based system. Although all input costs are considered in this analysis, in reality, most of the small scale fish farmers in Asia use their own resources fully or partly like lands, labours, substrates, manures, etc. derived from their farming systems and household wastes. Therefore, the input costs in reality would be very low and net benefit would be higher. The periphyton-based aquaculture in well-managed practice might be a profitable business (Azim et al., 2004a). However, it can be concluded that the substrate-based system replaces or complement supplemental feeding in tilapia-prawn polyculture.

A major issue that needs attention is the use of bamboo (*Bambusa* sp.) in the periphyton-based production systems. The demand of bamboo as substrate may have negative environmental and social impacts on other users (Bunting et al., 2001), but the farmers are commonly used bamboo, bamboo side shoots and tree branches in their ponds to reduce poaching, improve fish health and increased production. Bamboo is used as house building material, raw material in cottage industry, household fuel energy, raw material in paper and hardboard industry and as brush shelter in fisheries. However, there would not be any constraint for substrates since the fish production and profit margins are considerably higher in periphyton-based system. Considering all these the production of this self-sustaining plant can be increased easily on fallow land and other possible substrates used in “khata” fisheries in Bangladesh like branches of the Shaora tree (*Balanostreblus illicifolius*), Gab tree (*Diospyros peregrine*) Babla tree (*Acacia nilotica*) etc. can be used as the alternative use of bamboo (Wahab and Kibria, 1994). But these substrates should be tested for any adverse effects on water quality and for their ability to sustain a periphyton assemblage.

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References

APHA, 1992. Standard Methods for the Examination of the Water and Wastewater. American Public Health Association, Washington, DC.

Azim, M.E., 2001. The potential of periphyton-based aquaculture production systems. Ph.D. Thesis. Fish Culture and Fisheries Group, Wageningen University, The Netherlands, 219 pp.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia, *Oreochromis niloticus*. L. Aquac. Res. 34, 85-92.

Azim, M.E., Wahab, M.A., 2005. Periphyton-based pond polyculture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 207-222.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Milstein, A., Verdegem, M.C.J., 2001. Optimization of fertilization rates for maximizing periphyton growth on artificial substrates and implications for periphyton-based aquaculture. Aquac. Res. 32, 749-760.

Azim, M.E., Rahaman, M.M., Wahab, M.A., Asaeda, T., Little, D.C., Verdegem, M.C.J., 2004a. Periphyton-based pond polyculture system: a bioeconomic comparison of on-farm and on-station trials. Aquaculture 242, 381–396.

Azim, M.E., Wahab, M.A., Biswas, P.K., Asaeda, T., Fujino, T., Verdegem, M.C.J., 2004b. The effect of periphyton substrate density on production in freshwater polyculture ponds. Aquaculture 232, 441–453.

Bowen, S.H., Lutz, E.V., Ahlgren, M.O., 1995. Dietary protein and energy as determinants of food quality: tropic strategies compared. Ecology 76, 899-907.

Boyd, C.E., 1979. Water Quality in Warmwater Fish Ponds. Auburn University, Craftmaster Printers, Opelika, Alabama, 359 pp.

Bunting, S.W., Chowhan, G., Wahab, M.A., Beveridge, M.C.M., 2001. the potential of periphyton-based pond aquaculture in the livelihoods of small-scale rural farming households in Bangladesh. In: Keshavanath, P., Wahab, M.A. (Eds.), Periphyton-based aquaculture and its potential in rural development. Summary of an EC-INCO-DC funded workshop. Asian Fisheries Society, Indian Branch, Mangalore. pp. 39-40.

Cohen, D., Ra'anan, Z., Rappaport, U., Arieli, Y., 1983. The production of freshwater prawn *Macrobrachium rosenbergii* in Israel: improved conditions for intensive monoculture. Bamidgeh, 35, 31-37.

Corbin, J.S., Fujimoto, M.M., Iwai, T.U., Jr., 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P. (Ed), CRC Handbook of Mariculture. CRC Press, Boca Raton, FL, pp. 391-423.

Coyle, S.D., Najeeullah, T., Tidwell, J.H., 1996. Evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawns *Macrobrachium rosenbergii*. J. Appl. Aqua. 6, 57-66.

Dempster, P.W., Beveridge, M.C.M., Baird, D.J., 1993. Herbivory in the tilapia *Oreochromis niloticus*: A comparison of feeding rates of phytoplankton and periphyton. J. Fish Biol. 43, 385-392.

Dempster, P.W., Baird, D.J., Beveridge, M.C.M., 1995. Can fish survive by filter feeding on microparticles? Energy balance in tilapia grazing on algal suspensions. J. Fish Biol. 47, 7-17.

Dewan, S., Wahab, M.A., Beveridge, M.C.M., Rahman, M.H., Sarkar, B.K., 1991. Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carp fry and fingerlings grown in extensively managed, rain-fed ponds in Bangladesh. Aquacul. Fish. Manage. 22, 277-294.

Dey, M.M., Gupta, M.V, 2000. Socioeconomics of disseminating genetically improved Nile tilapia in Asia: an introduction. Aquaculture Economics and Management 4(1/2), 5-11.

Gomez, K.A., Gomez A.A., 1984. Statistical procedures for agricultural research, 2nd edition. John Wiley and Sons, New York, 680 pp.

Hatcher, B.G., 1983. Grazing in coral substrate ecosystems. In: Barnes, D.J. (Ed.), Perspective of coral substrates. Brian Clouston Publ., Manuka, pp.164-179.

Hay, M.E., 1991. Fish-Seaweed interactions on coral substrates: effects of herbivorous fishes and adaptations of their prey. In: Sale, P.F. (Ed.), The ecology of fishes on coral substrates. Academic Press, London, pp. 96-119.

Hem, S., Avit, J.L.B., 1994. First results on “acadjas □avoura” as an extensive aquaculture system (West Africa). Bull. Mar. Sci. 55 , 1040-1051.

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M., 2000. The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture 186, 45-60.

Jacoby, J.M., 1987. Alteration in periphyton characteristics due to grazing in a cascade foothill stream. Freshwat. Biol. 18, 495-508.

Keshavanath, P., Gangadhar, B., 2005. Research on periphyton-based aquaculture in India. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 223-236.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquaculture 235, 303-314.

Makarevich, T.A., Zhukova, T.V., Ostapenya, A.P., 1992. Chemical composition and energy value of periphyton in a mesotrophic lake. Hydrobiol. 28(1), 30-34.

Milstein, A., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2003. The effects of periphyton, fish and fertilizer dose on biological processes affecting water quality in earthen fish ponds. Env. Biol. Fish. 68, 247-260.

Montgomery, W.L., Gerkling, S.D., 1980. Marine microalgae as foods for fishes: an evaluation of potential food quality. Enviorn. Biol. Fishes 5, 143-153.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of Aquaculture on world fish supplies. Nature 405, 1017-1024.

Perschbacher, P.W., Lorio, W.J., 1993. Filtration rates of catfish pond phytoplankton by Nile tilapia *Oreochromis niloticus.* J. World Aqua. Soc. 24(3), 434-437.

Pompa, T., Masser, M., 1999. Tilapia life history and biology. Southern Regional Aquaculture Centre, SAARC Publication No. 283 p.

Ra'anan, Z., Cohen, D., Rappaport, U., Zohar, G., 1984. The production of the freshwater prawn *Macrobrachium rosenbergii* in Israel. The effect of added substrates on yields in a monoculture situation. Bamidge 36, 35-40.

Swamikannu, X., Hoagland, K.D., 1989. Effect of snail grazing on the diversity and structure of a periphyton community in a eutrophic pond. Can. J. Fish. Aquat. Sci. 46, 1698-1704.

Tidwell J.H., Cycle, S., Van Arnum, A., Weibel, C., 2000. Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. J. World Aqua. Soc. 31. 452-458.

Tidwell, J.H., Coyle, S.D., Schulmeister, G., 1998. Effects of added substrate on the production and population characteristics of freshwater prawns *Macrobrachium rosenbergii* in ponds. J. World Aqua. Soc. 29(1), 17-21.

Tidwell, J.H., Coyle, S.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, S.J., D'Abramo, L.R., Fuller, M.J., 1997. Relative prawn production and benthic macroinvertebrate densities in unfed, organically fertilized, and fed pond systems. Aquaculture 149, 227-242.

Tidwell, J.H., Coyle, S.D., VanArnum, A., Weibel, C., 2002. Effects of substrate amount and orientation on production and population structure of freshwater prawns *Macrobrachium rosenbergii* in ponds. J. World Aqua. Soc. 33 (1), 63-69.

Uddin, M.S., Farzana, A., Fatema, M.K., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2006a. Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia-prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development. Aquaculture (in press).

Uddin, S., Azim, M.E., Wahab, A., Verdegem, M.C.J., 2006b. The potential of mixed culture of genetically improved farmed tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. Aquac. Res. 37, 241-247.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1-31.

Wahab, M.A., Kibria, M.G., 1994. Khata and Kua fisheries - Unusual fishing methods in Bangladesh. Aquaculture News 18, 24.

Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S., 1999. The potential of periphyton based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton). Aquac. Res. 30, 409-419.

Table 1

Abundance of plankton (cells or colonies l^{-1}) in pond water during last day of the experiment. Mean values followed by different superscript letter in each row indicate where main effect were significant ($P > 0.05$) based on Tukey test.

Group/Genus	Treatments			
	SF	S$_0$F	SF$_0$	Control
Bacillariophyceae				
Actinella	167±167	0	333±167	0
Cyclotella	833±167	667±441	500±289	333±167
Fragillaria	1167±928	1333±441	833±333	333±167
Melosira	1000±764	667±167	833±441	167±167
Navicula	3167±601^{a}	1833±601^{ab}	3000±577^{a}	500±289^{b}
Surirella	500±0^{ab}	833±167^{a}	167±167^{b}	0
Tabellaria	3167±601	1500±1041	1833±333	333±333
Total	10000±2255^{a}	6833±1922^{ab}	7500±1756^{ab}	1667±333^{b}
Chlorophyceae				
Actinastrum	833±167	833±167	833±167	500±289
Ankistrodesmus	500±289	500±289	500±0	333±167
Botryococcus	1000±577	1333±726	1333±667	833±167
Chlorella	45667±4372^{a}	28333±3723^{b}	35167±2804^{ab}	26500±1323^{b}
Closterium	500±0	333±167	167±167	333±167
Gonatozygon	333±167	1167±1167	500±289	833±441
Oocystis	3167±1302	1500±500	3500±1607	2000±764
Palmella	8167±1302^{a}	2333±882^{b}	3500±1323^{ab}	2667±882^{b}
Pediastrum	7833±1641	5000±1732	4000±1323	3000±1443
Scenedesmus	2333±1202	3167±1093	5167±1167	2167±1202
Spirogyra	4833±2848	1500±289	1000±289	1333±601
Tetraedron	2333±1590	1500±1041	667±167	1333±167
Ulothrix	2167±726	2167±1014	3167±726	1167±601
Volvox	1333±333	500±289	833±167	500±289
Total	81000±9005^{a}	50167±6797^{b}	60333±6126^{ab}	43500±500^{b}
Cyanophyceae				
Anabaena	6833±1093	4667±1364	9500±2082	5167±1364
Aphanocapsa	500±289	500±289	500±289	167±167
Gomphosphaeria	3333±333	3500±1041	4000±1155	1000±289
Microcystis	39667±3321	25000±2500	32000±3403	24667±4055
Oscillatiria	1000±764	500±289	833±601	500±500
Total	51333±2088^{a}	34167±1667^{bc}	46833±2682^{ab}	31500±4163^{c}
Euglenophyceae				
Euglena	1167±441	1667±928	1667±441	1000±500
Phacus	1167±333	1000±289	1167±441	1167±167
Trachelomonas	500±289	833±441	500±289	667±167
Total	2833±1014	3500±289	3333±726	2833±833

Rotifera				
Brachionus	1833±882	1167±333	3667±2186	2167±167
Filinia	1000±500	167±167	167±167	0
Keratella	500±289	500±289	333±167	333±167
Lecane	2000±1041	667±167	500±289	500±0
Total	5333±1014	2500±500	4667±2242	3000±289
Crustacea				
Cyclops	1000±289	1500±289	1333±167	1333±441
Daphnia	333±167	333±167	167±167	500±289
Moina	333±167	0	167±167	0
Nauplius	2333±1014	1333±601	1500±289	1167±441
Sida	167±167	833±601	1167±333	167±167
Total	4167±882	4000±577	4333±167	3167±333

Table 2

Outcomes of two-way ANOVA comparing yield parameters between substrate (with or without substrate) and feeding level (with or without feed).

Species	Effect	*df*	Survival			Final weight			Net yield		
			MS	*F*	P	MS	*F*	P	MS	*F*	P
Tilapia	Substrate(S)	1	281	23.42	**0.0013**	1457	27.29	**0.0008**	801501	59.65	**0.0001**
	Feed (F)	1	417	34.72	**0.0004**	1618	30.30	**0.0006**	1059171	78.83	**0.0001**
	S×F	1	15	1.27	0.2924	387	7.25	**0.0274**	87332	6.50	**0.0342**
	Error	11	12			53			13436		
Prawn	Substrate(S)	1	922	29.45	**0.0006**	437	22.26	**0.0015**	15365	40.99	**0.0002**
	Feed (F)	1	200	6.29	**0.0354**	320	16.32	**0.0037**	7440	19.85	**0.0021**
	S×F	1	17	0.54	0.4847	0.27	0.01	0.9095	195	0.52	0.4911
	Error	11	31			20			375		

Significant effects are printed bold at 0.05 significance level.

Table 3

Comparison of means (±S.D.) of yield parameters of tilapia and freshwater prawn among treatments.

Species	Feeding	Substrate	Survival (%)	SGR (% day^{-1})	FCR	Final weight (g)	Net yield (kg ha^{-1})
Tilapia	Yes	Yes	76±2	2.50±0.05	1.23±0.02	120±6	1940±95
	Yes	No	68±3	2.47±0.05	1.39±0.06	109±7	1594±171
	No	Yes	66±4	2.40±0.10	-	108±5	1517±109
	No	No	54±4	2.08±0.13	-	74±10	829±62
Prawn	Yes	Yes	58±7	2.10±0.05	-	38±5	153±28
	Yes	No	43±2	1.80±0.16	-	26±5	73±12
	No	Yes	52±8	1.82±0.26	-	28±6	95±23
	No	No	32±4	1.40±0.20	-	16±2	32±8

Table 4

A comparison of economics within substrate with/and feed based tilapia and freshwater prawn polyculture systems. Calculation was done based on 1 ha pond and 120 days culture period.

Items	Amount used	Price rate	T- SF	T-S_0F	T-SF_0	Control
Inputs						
Land rental cost	1 ha	15,000 ha^{-1} y^{-1}	5,753	5,753	5,753	5,753
Tilapia fry	22,500 pieces	0.50	11,250	11,250	11,250	11,250
Prawn juvenile	7,500 pieces	3.00	22,500	22,500	22,500	22,500
Lime	250 kg	6	1,500	1,500	1,500	1,500
Cow dung	3,000 kg	0.5	1,500	1,500	1,500	1,500
Urea	450 kg	6	2,700	2,700	2,700	2,700
TSP	450 kg	15	6,700	6,700	6,700	6,700
Bamboo	4389 culms 5 times use	22	24,138	-	24,138	-
Fish feed		14	32,999	29,443	-	-
Labour for pond cleaning, stocking and harvesting	30 man-day	75	2,250	2,250	2,250	2,250
Labour for substrate installation and removal	90 man-day	75	6,750	-	6,750	-

Labour for feed application	30 man-day	75	2,250	2,250	-	-
Total			120,340	85,846	85,091	54,203
Interest on inputs		10%	4,616	3,295	3,264	2,079
Grand total			124,956	89,141	88,355	56,282
Tilapia sale		60	122,473	100,837	96,454	48,152
Prawn sale		350	58,201	29,012	37,527	13,546
Total sale proceed			180,674	129,849	133,981	61,698
Net margin			59,809	44,686	46,142	5,762
Net profit margin %			50	52	53	10

Currencies are given in Bangladesh Taka, BDT (US$=67BDT)

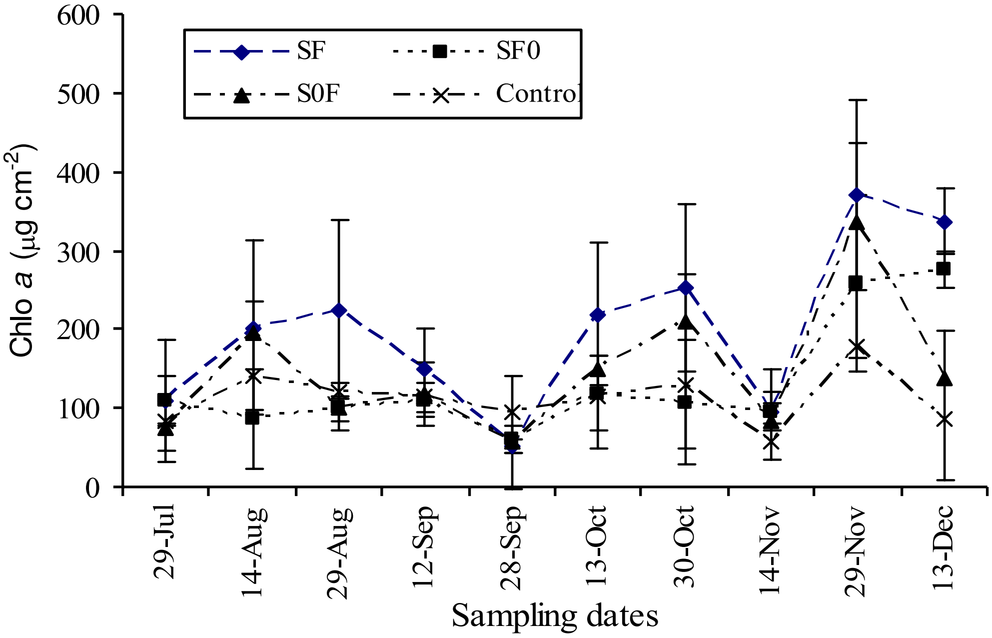
Figure Captions

Figure 1. Average chlorophyll *a* concentrations of water column in different treatments during the experimental period. Values are means (±S.D.) of three replicated ponds per sampling date (*N*=*3*) in each treatment.

Figure 2. Periphyton ash free dry matter (AFDM) mg cm^{-2} (A) and ash % (B) per unit surface area of bamboo substrates over the experimental period. Values are means (± S.D.) of three ponds (*N*=*3*) per sampling dates in each treatment.

Figure 3. Periphyton chlorophyll *a* (A) and pheophytin *a* µg cm^{-2} (B) per unit surface area of bamboo substrates over the experimental period. Values are means (± S.D.) of three ponds (*N*=*3*) per sampling dates in each treatment.

Figure 1.



600
500
400
300
200
100
0
Chlo a (μg cm-2)
SF
SF0
S0F
Control
29-Jul
14-Aug
29-Aug
12-Sep
28-Sep
13-Oct
30-Oct
14-Nov
29-Nov
13-Dec
Sampling dates

Figure 2

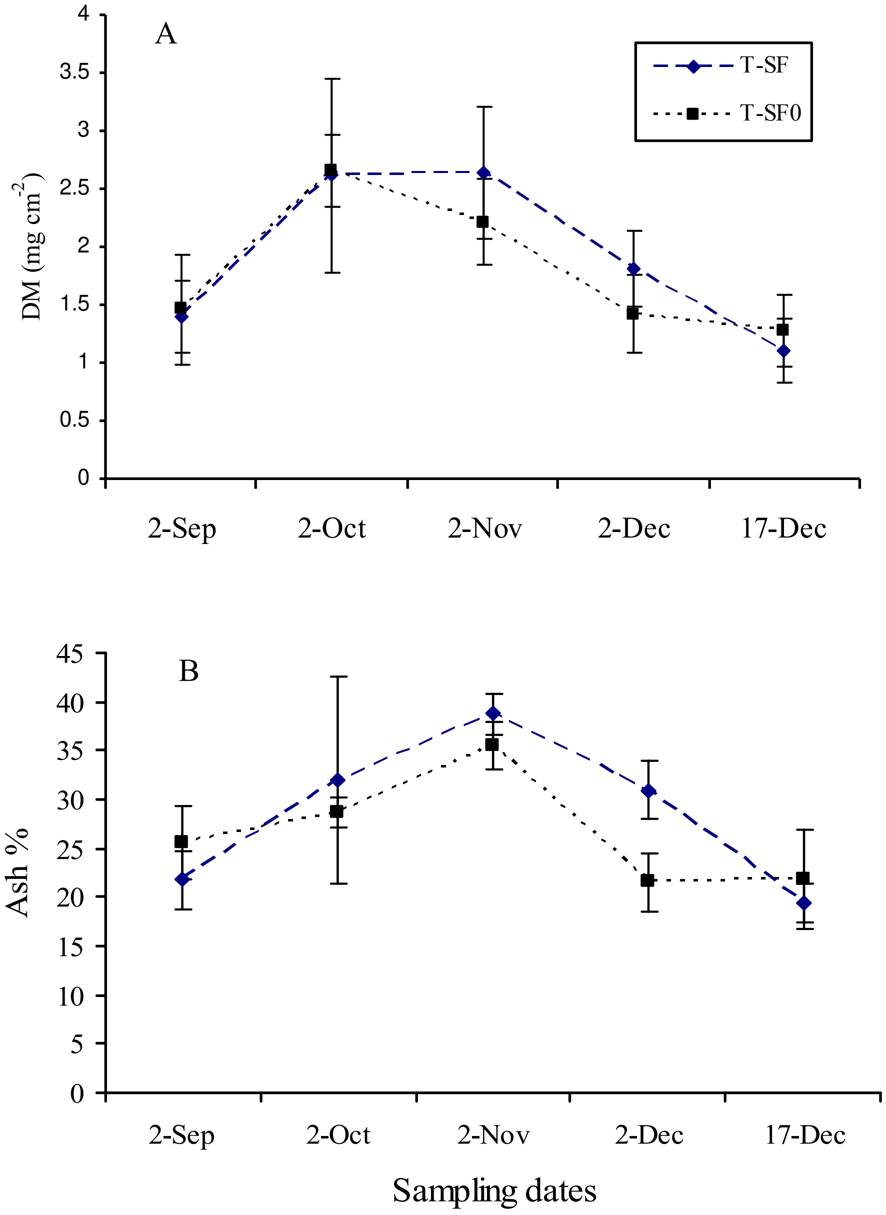
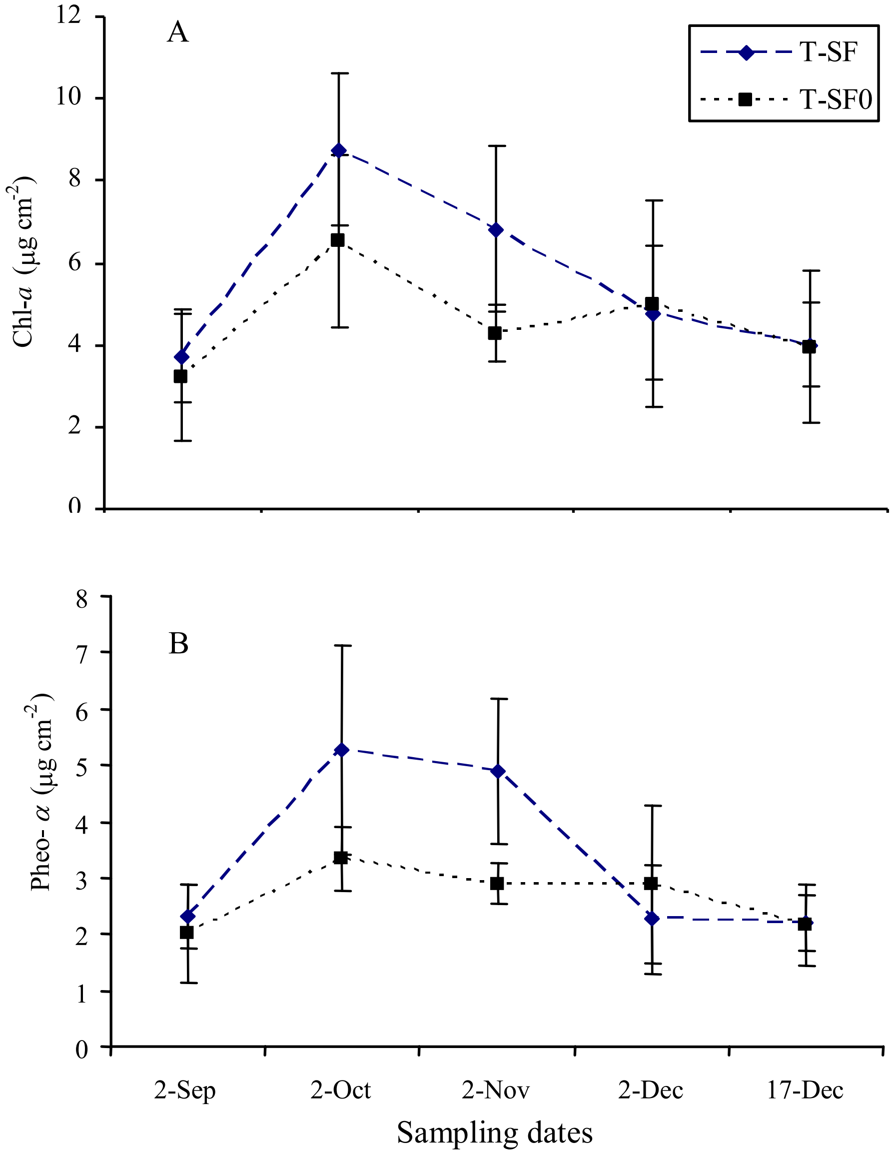

A
T-SF
T-SF0
DM (mg cm^{-2})
0
0.5
1
1.5
2
2.5
3
3.5
4
2-Sep
2-Oct
2-Nov
2-Dec
17-Dec
B
Ash %
0
5
10
15
20
25
30
35
40
45
2-Sep
2-Oct
2-Nov
2-Dec
17-Dec
Sampling dates

Figure 3.


A
12
10
8
6
4
2
0
Chl-a (μg cm^{-2})
T-SF
T-SF0
B
8
7
6
5
4
3
2
1
0
Pheo-a (μg cm^{-2})
2-Sep
2-Oct
2-Nov
2-Dec
17-Dec
Sampling dates

Chapter 6

The effects of stocking density, periphyton substrate and supplemental feed on biological processes affecting water quality in earthen tilapia-prawn polyculture ponds

Chapter 6

The effects of stocking density, periphyton substrate and supplemental feed on biological processes affecting water quality in earthen tilapia-prawn polyculture ponds

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Abstract

The technical and economic potentials of tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems in South Asia are under investigation in an extensive research programme. This article is a further analysis of data from four experiments carried out in that framework, to explore periphyton, fish, prawn and feed effects on water quality. Factor analysis and ANCOVA models applied to a data matrix of water quality parameters in ponds with and without artificial substrates (bamboo poles), monoculture and polyculture of tilapia and freshwater prawn, and with or without feed, allowed to identify the underlying ecological processes governing the tilapia-prawn system, and construct conceptual graphic models of the periphyton-environment relationships observed. The main sources of water quality variability (factors) were autotrophic (photosynthesis and nutrient uptake) and heterotrophic (respiration and decomposition) activities that affect water quality in opposite directions. The second variability source was related to the cycling of decomposition on the bottom - nutrient liberation into the water column - algae biomass synthesis and sedimentation on to the bottom. The analysis of the relationships between both factors and the growth rates of tilapia and prawn in the different systems studied allowed a better understanding of the functioning of tilapia-prawn ponds. The use of substrates for periphyton growth is a low cost culture method that resulted in a more favorable environment for the cultured organisms (organic loading avoided) and at the same time provided an extra source of food for them. The synergistic relationships between tilapia and prawn through their effects on pond ecology indicate that their joint culture is technically feasible. The good growth rates and environmental conditions obtained at relatively high stocking densities of both target organisms is a good indication of the economic viability of this technology.

Key words: tilapia, freshwater prawn, water quality, periphyton-based aquaculture, feed, multivariate statistics,

Introduction

Bangladesh is one of the top 10 aquaculture-producing countries, contributing 657,000 tons by quantity and 1,159 US$ by value (FAO, 2002). Aquaculture in Bangladesh is increasingly recognized as a way to improve the livelihoods of poor people (Gupta et al., 1999; Azim et al., 2003a). There is a large scope for further growth in aquaculture production in this country, the majority to be realized through the efforts by poor people (Lewis, 1997; Alam and Thompson, 2001). The traditional pond inputs in Bangladesh are cow/chicken manures, urea, TSP, rice bran, wheat bran, oil cake and some other agricultural and home wastes. As a resource-constrained country, there is a severe competition for these agricultural wastes. Important uses are raising (aquatic or terrestrial) livestock and fuel energy. Alternative means of increasing fish production are essential if aquaculture by resource poor farmers is to grow further. Periphyton-based aquaculture systems offer the possibility of increasing both primary productivity and food availability for fish (Legendre et al., 1989; Hem and Avit, 1994; Guiral et al., 1995; Wahab et al., 1999; Huchette et al., 2000; Azim et al., 2001, 2005). Periphyton is a preferable natural food of herbivorous and omnivorous fish species, especially of Indian major carps (Azim et al., 2002; Keshavanath et al., 2002) and tilapias (Legendre et al., 1989; Hem and Avit 1994; Azim et al., 2003b).

Tilapias are currently having important impacts on poor people in developing countries, both as cultured species in household-management systems and through access to fish produced in informal and formal fisheries. Nile tilapia (*Oreochromis niloticus*) is one of the first fish species that was cultured in the world (Popma and Masser, 1999). During the last few decades, Nile tilapia, both as a subsistence and commercial crop, became a dominant component of the aquaculture sector in many Asian countries, including China, Indonesia, Bangladesh, Malaysia, the Philippines, Thailand, Vietnam, Myanmar and Sri Lanka. Tilapias are regarded as an omnivorous species and capable of feeding on benthic and attached algal and detrital aggregates (Bowen, 1982; Dempster et al., 1993; Azim et al., 2003c). Tilapia relies on periphyton and microbial flocs to collect sufficient nutrients to fulfil its basic requirements, as the collection of isolated cells of phytoplankton by filter feeding requires a too high energy expenditure to be sustainable in the long term (Dempster et al., 1993).

Fresh water prawn (*Macrobrachium rosenbergii*) is a popular species for their taste, growth rate and high international market value. The popularity of prawn has encouraged the development of its culture globally and for this reason freshwater prawn culture is gaining importance day by day (New, 2002). Shrimp exports from Bangladesh are increasing steadily due to a rising demand and favourable prices in the international markets. Two single species viz., brackish water tiger shrimp (*Penaeus monodon*) and the freshwater giant prawn (*Macrobrachium rosenbergii*) make up almost 100% of shrimp exports, with prawn contributing 25-30% to the total export by quantity and value (DoF, 2004). However, although tiger shrimp contributes the majority of the exports, further expansion of culture area and increased culture intensity are not possible due to viral disease outbreaks and environmental degradations in the coastal region (Johnson and Bueno, 2000). The social and environmental conflicts associated with marine shrimp farming are making freshwater prawn farming more attractive (New, 2000). At present only 5-7% water areas are used for freshwater prawn culture and it can be cultured in the vast freshwaters throughout the country (DoF, 2004).

Considering their traditional experience with polyculture, Bangladeshi farmers are showing growing interest in culturing tilapia and freshwater prawn together. In doing so, farmers can sell their high value prawns besides a part of their tilapia harvest in the market, keeping the other part for home consumption. They are desperately looking for a low cost tilapia and prawn culture technologies, which are economically viable and technically feasible. Therefore, developing a viable technology of tilapia and freshwater prawn polyculture in periphyton based systems for rural aquaculture may be a desirable option for poverty alleviation and nutritional security in Bangladesh as well as other countries in the region as a whole.

This paper combines data obtained in four different experiments carried out at the Bangladesh Agricultural University to evaluate the technical and economic potential of polyculture of Nile tilapia and freshwater giant prawn in periphyton-based systems. Each experiment had different objectives, which were either to (1) compare tilapia monoculture and polyculture of tilapia with freshwater prawn with and without periphyton substrates (Uddin et al., 2006a in press), (2) optimize the stocking ratios of tilapia and freshwater prawn in periphyton-based systems (Uddin et al., 2006b), (3) explore the effect of combined stocking densities on production of tilapia and prawn in periphyton-based systems (in preparation), and (4) compare the effects of substrates and supplemental feeding on growth and production of tilapia and fresh water prawn in periphyton based systems (in preparation).

In the present study the water quality data gathered in those experiments were merged into a single dataset and reanalyzed using multivariate statistics, to gain deeper insights into the functioning of the ecosystem, and relationships between the large numbers of variables (Prein and Milstein, 1988). The objective was to explore the effects of tilapia and prawn density, presence of substrates for periphyton growth and supplemental feed on the water quality of earthen tilapia-prawn polyculture ponds.

Materials and Methods

The experiments were carried out in earthen ponds at the Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, and in farmer ponds in the nearby villages Montola, Goneshampur and Goatala. Weekly or fortnightly water quality data collected in those experiments were merged in a single dataset. Treatments were re-named according to their tilapia-prawn-substrate-feed combination in the ponds (Table 1). Thirteen combinations were represented in the global dataset, which no longer reflect the experimental design of the original experiments.

The earthen fishponds at BAU are 75 m^2 with a mean water depth of 1.2 m, and in the nearby villages range from 125 to 275 m^2 with a mean water depth of 1.25 m. The ponds of the on-station experiments are rain-fed, fully exposed to prevailing sunlight, pond embankments have regular shape and are covered with grass. Water levels were maintained by supplying deep tube-well water whenever needed. The ponds of the on-farm experiments are partially shaded by trees, fully depend upon rainfall, and have pond embankments with irregular shape. In both locations, before starting, the ponds were repaired, manually cleaned of aquatic vegetation and unwanted fishes were removed using rotenone and netting. Powered lime ($CaCO_3$) was broadcasted over the water surface at a rate of 250 kg ha^{-1}. Substrates for periphyton growth, 5-6 bamboo (*Bambusa* sp.) poles per meter, each 2 m long with a mean diameter of 5.5 cm, were posted vertically into the bottom mud in substrate treatment ponds, excluding a one meter wide perimeter close to the dike. This resulted in an additional surface for periphyton development equaling about 60% of the pond surface area. All ponds were fertilized with the standard traditional dose of fertilizer used in Bangladesh, consisting of semi-decomposed cattle manure, urea and triple super phosphate (TSP) at the rates of 3000, 100 and 100 kg ha^{-1}, respectively (Azim et al., 2001). Subsequently, all ponds were fertilized fortnightly with urea and TSP at the rate of 50 kg ha^{-1}, until harvesting.

Fry of the GIFT (Genetically Improved Farmed Tilapia) strain of Nile tilapia and freshwater giant prawn post larvae (PL) juveniles were stocked at the densities indicated in Table 1. Stocking was carried out 2 weeks after substrate installation, when the periphyton biomass had developed on the bamboo poles. In 2003, tilapia was stocked 15 days after prawn stocking, while in 2004 both were stocked the same day. A low protein (25% crude protein) pelleted fish feed procured from the market was applied daily at the rates indicated in Table 1.

Starting one week after substrate installation, water quality sampling was carried out with the vertical haul of a tube sampler covering 4-5 feet depth, as it was representative of all layers of the water column, between 09:00 and 10:00 h, weekly in the on-station experiments and fortnightly in the on-farm experiments. In situ measurements included pH, temperature, dissolved oxygen (with electrodes) and water transparency (with Secchi disc). Water samples were collected and filtered through Whatman GF/C filters for nutrient analyses. Total ammonium nitrogen (TAN), nitrate (NO_3-N) and orthophosphate (PO_4-P) were determined using HACH kits. The algae remaining on the Whatman filters were used to determine planktonic chlorophyll-$\underline{a}$ concentrations following Boyd (1979).

Data were analyzed using uni- and multivariate statistics as exploratory methods. As such, no a priori hypothesis exists. Ecological processes that account for the main variability of the measured variables were identified through factor analysis (Kim and Mueller, 1978; Milstein, 1993). The purpose of factor analysis is to explain the relationships among a set of variables in terms of a limited number of new variables, which are assumed to be responsible for the co-variation among the observed variables. From the several available techniques to extract factors, principal components (Seal, 1964; Jeffery, 1978) calculated from the correlation matrix among variables were used here. The first factor extracted from that matrix is the linear combination of the original variables, which accounts for as much of the variation contained in the samples as possible. The second factor is the second such function that accounts for most of the remaining variability, and so on. The factors are independent of one another, have no units and are standardized variables (normal distribution, mean=0, variance=1). The coefficients of the linear functions defining the factors are used to interpret their meaning, using the sign and relative size of the coefficients as an indication of the weight to be placed upon each variable. The effects of the treatment applied (combination of tilapia density, prawn density, substrates for periphyton growth and feed, as described in Table 1) and sampling month on each original variable and the factors extracted were tested with the General Linear Model (GLM) used as analysis of covariance (ANCOVA), with pond area as covariate. Differences between levels of the combined variable ‘treatment’ were tested with Scheffe’s multi-comparison test of means. For interpretation purposes, to tell apart the effects of tilapia density, prawn density, substrate and feed on water quality, the multi-comparison test results performed on the combined variable ‘treatment’ were compared in subgroups of treatments as indicated in Table 2, which also includes the definition of ‘low’ and ‘high’ tilapia and prawn densities. For each variable, treatments that presented at least one common letter in the Scheffe’s mean multicomparison test were considered not significantly different at the 0.05 level. The analyses were run using the procedures FACTOR and GLM of the SAS statistical package.

Results

The results of the ANCOVAs of tilapia and prawn harvesting weight and growth rate are shown in Table 3. The models were significant, each variable explaining over 80% of the variance. The covariate was significant only for prawn variables, accounting for 20% of their explained variance and indicating higher prawn harvesting weight and growth in smaller ponds. Comparing the mean multicomparisons by treatment given in Table 3 as indicated in Table 2, it can be seen that no model showed significant differences due to periphyton or feed. Prawn density had a significant effect only in periphyton ponds that received feed and had high tilapia density. Under these conditions, tilapia performance was better when prawn were absent or at high density (2 prawn m^{-2}), and prawn growth rate was higher at low (0.75 prawn m^{-2}) than at high prawn density, with intermediate growth rate values not significantly different from either at 1 prawn m^{-2}. Tilapia density affected prawn performance only at high prawn density, with better prawn growth at the highest tilapia density (3 tilapia m^{-2}) than when tilapia was absent, and intermediate values not significantly different from either at intermediate tilapia density. Tilapia performance (both, harvesting weight and growth) was lower when its density was the highest than when it was 0.5-2 tilapia m^{-2} in high prawn density ponds or 1 tilapia m^{-2} in low prawn density ponds.

The results of the ANCOVAs of each water quality variable are shown in Table 4. The models were all significant, accounting for 78% of temperature variability (r^2= 0.78) and 30-50% of the variability of the other parameters. The rather low r^2's indicate that there is/are other important source(s) of variability among the data than those tested. The covariate accounted for 14% of the variability explained by the Secchi model, explaining water transparency was higher in larger ponds. Pond area accounted for 2-5% of dissolved oxygen, pH and nitrate explained variability, with lower oxygen and pH and higher nitrate in larger ponds. The other variables were not significantly affected by pond area. Month accounted for 60% of the explained variability of temperature and maximally for 25% of the other variables, with patterns as shown in the mean multicomparisons by month section of Table 4. Except for temperature, treatment as main effect and in combination with month (treatment*month cross effect) accounted for 64-93% of the explained variability. Comparing the mean multicomparisons by treatment section of Table 4 as indicated in Table 2, several significant effects are revealed. Periphyton had a significant effect only when feed was supplied and prawn density was low. Under these conditions the presence of substrates for periphyton growth resulted in decreased Secchi, phosphate and nitrate, and increased chlorophyll *a* and pH in the water column. Feed supply increased chlorophyll *a* when periphyton was present, and increased Secchi and nitrate when periphyton was absent. Under low prawn density the presence of 1 tilapia m^{-2} resulted in higher Secchi and lower oxygen, pH and ammonium than with 3 tilapia/m^{2}. Under high prawn density Secchi was higher when tilapia was absent or up to 1 m^{-2}, oxygen and pH were lower at 1 tilapia m^{-2} than at higher densities, and ammonium was higher when tilapia was absent. In the absence of periphyton, Secchi was higher when there were 0.75 prawn m^{-2} than without prawns, while at 2 prawn m^{-2} it had intermediate values not significantly different from either. Oxygen and pH were higher and nutrients were lower at 0.75 prawn m^{-2} than without or with 2 prawn m^{-2}. In the presence of periphyton and at high tilapia density, Secchi was higher when prawns were present than when they were absent, and phosphate was higher in the 0.75 prawn m^{-2} density. In the presence of periphyton and low tilapia density Secchi was higher at 1 prawn m^{-2} than at 0.5 prawn m^{-2}, and ammonium was higher at 2 prawn m^{-2} than at lower prawn densities.

The results of the factor analysis and of the ANCOVAs of the factors and their main effects mean multi-comparison analyses are presented in Table 5, and Figure 1 shows the treatment*month cross effects. Combined, two factors accounted for 51% of the total variability in water quality. The ANCOVA models were significant and accounted for 64% and 49% of the factors' variability.

The first factor (FACTOR1) accounted for 32% of the overall data variability. It is a bipolar factor, with two groups of variables, each group holding variables that are positively correlated within the group and negatively correlated with the other group. High negative values of FACTOR1 indicate water with high dissolved oxygen, pH and to a lower extent planktonic chlorophyll-a (high negative coefficients) combined with low nutrient levels (high positive coefficients) (Table 5). This factor reflects the opposite effects of autotrophic and heterotrophic activity on water quality. The variables with the highest coefficients (> 0.5) are affected by both processes, while variables correlated only with photosynthesis present mid-value coefficients (0.4 to 0.5). Respiration decreases dissolved oxygen concentration and pH and decomposition releases nutrients into the water. Photosynthesis has the opposite effect on those variables, and also increases algal biomass (chlorophyll-a) that reduces water transparency (Secchi depth). The ANCOVA model applied accounted for 64% of the factor's variability, most of which related to treatment either as main effect (38%) or as cross effect with month (46%). The covariable pond area accounted for only 6% of the factor variability, with a positive correlation. Since low FACTOR1 values indicate higher autotrophic and lower heterotrophic activity, in smaller ponds algal activity dominated. Month as main effect accounted for 10% of FACTOR1 variability, autotrophic activity decreasing and heterotrophic activity increasing from April to November (Table 5, Mean multicomparisons by month section). Comparing the mean multicomparisons by treatment section of Table 4 as indicated in Table 2, several significant effects are revealed. Substrates for periphyton growth increased autotrophic and decreased heterotrophic activity (decreased FACTOR1) when feed was supplied and prawn density was low, but not under the remaining combinations of conditions tested. The addition of feed increased autotrophic activity in periphyton based ponds, and increased heterotrophic activity in ponds without periphyton. Under low prawn density autotrophic activity was higher and heterotrophic activity was lower when 1.5-3 tilapia m^{-2} were present than when only 1 tilapia m^{-2} were stocked. At high prawn density autotrophic activity was higher and heterotrophic activity was lower when 3 tilapia m^{-2} were present than when 0-1 tilapia m^{-2} were stocked, ponds with 2 tilapia m^{-2} not being significantly different from either. In ponds without periphyton and high tilapia density, autotrophic activity was lower and heterotrophic activity was higher at low prawn density than either when prawns were not stocked or were in high density. In ponds without periphyton the autotrophic and heterotrophic activity balance was not affected by prawn density when tilapia density was high. But when tilapia density was low, autotrophic activity was higher and heterotrophic activity was lower in 0.5 prawn/m^2 ponds than in 1 prawn m^{-2} ponds, those with higher prawn densities being intermediate and not significantly different from either. The treatment*month cross effect is shown in Figure 1a. The figure shows that the treatments carried out only at BAU (stocked in April and June) the FACTOR1 values were relatively constant dominated by autotrophic activity throughout the culture period, while those carried out in the villages (stocked in July) mostly were dominated by heterotrophic activity with either rather constant or increasing positive FACTOR1 values until September. Thus, this cross effect includes autotrophic-heterotrophic activity differences related to the experimental location (either BAU or villages). If the ANCOVA is run with experimental location as main effect instead of month*treatment cross effect, the coefficient of determination (r^2) falls to 0.55 and the significance level of pond area is reduced to P=0.03.

This is related to the confounding between the new variable and the covariable, since ponds at BAU are smaller than in the villages and the experiments at BAU included spring months and in the villages not.

The second factor (FACTOR2) accounted for a further 19% of the overall data variability. It shows positive correlation between ammonia, phosphate and chlorophyll-a, and negative correlation with Secchi (Table 5). This factor reflects the role of TAN and phosphate in phytoplankton biomass synthesis in the water column and their relation with decomposition processes occurring on the pond bottom. The more TAN and phosphate are available in the water column, the higher the phytoplankton biomass synthesis (chlorophyll-*a*) that increases water turbidity (lower Secchi). The higher the water turbidity, the more organic material will settle on the pond bottom and be decomposed by bacteria that in turn release nutrients, including ammonium and phosphates into the water column. The ANCOVA model applied accounted for 49% of the factor's variability, most of which related to treatment either as main effect (32%) or as cross effect with month (43%). The covariable pond area accounted for only 2% of the factor variability, with a negative correlation. Thus, higher phytoplankton biomass synthesis in the water column and decomposition on the pond bottom occurred in smaller ponds. Month as main effect accounted for 23% of FACTOR2 variability, with higher values in May and by the end of the culture period. Comparing the treatments as indicated in Table 2 it can be seen that FACTOR2 was significantly affected by tilapia and prawn densities but not by periphyton or feed. While under high prawn density tilapia density had no effect on FACTOR2, under low prawn density higher phytoplankton biomass and decomposition occurred in ponds with 2.25 than in ponds with 1-2 tilapia m^{-2}, ponds with 3 tilapia m^{-2} not being significantly different from either. In ponds without periphyton and with high tilapia density, FACTOR2 was higher with 0.75 than with 2 prawn m^{-2}, and intermediate values occurred in ponds without prawns. In periphyton ponds there was no prawn density effect when tilapia was absent or at low density. But when tilapia density was high, higher phytoplankton biomass and decomposition occurred in ponds with 0.75 prawn m^{-2} than when prawns were absent, and intermediate values occurred at higher prawn densities. Over the treatment and the month main effects also occurred treatment*month cross effect (Figure 1b), with different time patterns in the different treatments. Phytoplankton biomass synthesis and decomposition on the bottom increased in some treatments throughout the culture period, while in others it increased the first few months to peak in June (early stocked treatments) or September (summer stocked), and then decreased.

Discussion

In aquatic systems the values measured in water samples reflect the result of a wide range of biological and chemical processes that occur simultaneously in different compartments of the system and affect water quality. In traditional earthen fish ponds (without substrates) processes of decomposition and synthesis occurring in the pond bottom and in the water column, affect water quality parameters in opposite directions. In ponds with substrates both types of processes also occur in the periphyton mats, resulting in synergistic and competitive relationships among them (Azim et al., 2003). In the present analysis, FACTOR1 mainly shows the balance/dominance of autotrophic and heterotrophic processes that mainly affect the oxygen balance in the pond. Secondarily FACTOR1 shows synergistic relationships between those processes that are more clearly shown by FACTOR2: decomposition supplies nutrients for biomass synthesis that supplies particles for decomposition. Synergistic and competitive relationships were identified by Milstein et al. (2003) in a similar study with data from Indian carp polyculture in periphyton based ponds receiving different levels of fertilizers. The fish were catla (*Catla catla*) and rohu (*Labeo rohita*) that feed in the water column and periphyton like our tilapia, and kalbaush (*Labeo calbasu*) that is a bottom feeder like prawn. In that study the same ecological processes herein identified were recognized in their FACTOR1 and FACTOR2. Besides, and since in their system different fertilization levels were applied, the third factor showed ammonium supply as the main limiting factor for nitrification, related to competition between autotrophic organisms and nitrifying bacteria.

The relationships between the different elements in each treatment as indicated in Tables 3 and 5 allowed constructing conceptual graphic models for each treatment. In Figures 2 to 4 the number of fish and prawn reflect their density, the size is relative to the corresponding growth rate, and the thickness of the arrows indicate the intensity of the effect represented (FACTOR1, FACTOR2, food). To avoid confusion, in the graphs and in the following text the concepts of autotrophic and heterotrophic activities and not the value of FACTOR1 is considered. Thus increases of both, autotrophic and heterotrophic activities are represented by upward arrows and their decreases by downward arrows.

Figure 2a shows the relationships among the ecosystem elements under high tilapia and low prawn densities when no substrates and feed are provided. FACTOR1 shows heterotrophic activity including the effects of respiration and decomposition, and autotrophic activity including the effects of photosynthesis, nutrient uptake and algal biomass that affect turbidity. FACTOR2 shows particle sedimentation, decomposition on the bottom that liberates TAN and phosphate into the water column that are used for particle synthesis. Prawn activity on the pond bottom reintroduces sedimented particles and nutrients in the water column. The main food sources are phytoplankton for tilapia and detritus for prawn. Contribution of tilapia and prawn faeces to detritus and their oxygen uptake for respiration are not explicitly indicated. Addition of substrates (Figure 2b *vs.* 2a) should have contributed to increase autotrophic activity through photosynthesis and nutrient uptake of its attached algae and FACTOR2 through biomass dislodgment and its sedimentation on the pond bottom. On the other hand, respiration of the heterotrophic periphyton community and reduction of periphyton biomass through grazing by the cultured organisms act in the opposite direction, and as a result both factors did not change. The feed added in ponds without substrates (Figure 2c *vs.* 2a) was consumed by tilapia and prawns, so that no increase in organic matter accumulation on the pond bottom occurred and FACTOR2 did not change. Together with this, biological processing of the organic loading involved in feed addition led to respiration increase in the ponds and to nutrients release into the water column, which added to the nutrients reintroduced due to prawn activity on the bottom. The nutrient levels then were over the phytoplankton requirements, the overall balance moving towards dominance of heterotrophic activity effects on water quality. Still in ponds without substrates, in the absence of prawns nutrient reintroduction in the water column by prawn activity did not occur, so that feed addition had a fertilizer effect on phytoplankton and did not produce that organic loading effect. This resulted in increased autotrophic and reduced heterotrophic activities and no significant change in FACTOR2 (Figure 3a *vs* 2c). With increased prawn density (Figure 3c *vs* 2c) prawn activity on the pond bottom increased and with it consumption of feed and detrital organic matter increase. This also resulted in the elimination of the organic loading effect of feed addition, and reduced accumulation of organic matter on the bottom and hence its decomposition and nutrient liberation into the water column. As a result heterotrophic activity and FACTOR2 decreased. In ponds with substrates, organic matter and nutrients originated from feed are partly trapped by periphyton (van Dam et al., 2002), and had a fertilization effect on phytoplankton and the autotrophic periphyton (Figure 2d *vs* 2b). Together with this, the heterotrophic periphyton contributed to the organic loading process (Figure 2d *vs* 2c). The result of these processes was a decrease in heterotrophic activity, an increase of autotrophic activity and no changes in FACTOR2. In the absence of prawns in periphyton-based ponds prawn activity did not resuspend particles from the bottom and dislodged them from substrates, so that less organic material was available for decomposition and FACTOR2 decreased (Figure 3b *vs* 2d). In these ponds, increasing prawn density increased intra-specific competition that led to reduced prawn growth rate (Figure 3d *vs* 2d). The increased activity on the pond bottom and periphyton substrates by the larger amount of prawns might have increased nutrients and particles flow that increased food resources for tilapia, which showed higher harvesting weight and growth rate. However, tilapia and prawn grazing and respiration counteracted the autotrophic activity and decomposition effects on water quality, so that the factors did not show significant differences in relation to the low prawn density ponds.

The effects of tilapia density could be studied only in periphyton ponds that received feed. When prawn density was low (up to 1 prawn m^{-2}), a 50% increase in tilapia density from 1 m^{-2} to 1.5 m^{-2} led to an increase of autotrophic activity (Figure 4c *vs* 4b), probably due to the grazing activity of tilapia that might have kept the phytoplankton and periphyton populations in a fast growing stage avoiding their aging. This stimulating tilapia effect has already been reported in phytoplankton (e.g.: Milstein and Svirsky, 1996) and periphyton (e.g.: Huchette and Beveridge, 2003). The increased autotrophic activity was sufficient to cover the tilapia and prawn nutritional needs (similar growth rates) in spite of the increased tilapia density. The increased grazing activity of the larger tilapia population led to an efficient utilization of organic matter in the ponds, which did not allow deposition on the bottom and accumulation of TAN and phosphate in the water (FACTOR2 did not change). Further tilapia density increases did not affect autotrophic activity (FACTOR1 in Figure 4b *vs* 3b, 2d and 4d). However, when tilapia density increased over 2 m^{-2} the corresponding increased feed inputs, tilapia excretion and periphyton dislodgements produced by tilapia grazing and swimming near the substrates might have increased particle availability for decomposition on the bottom and liberation of decomposition products into the water column (FACTOR2 in Figure 4c, 4b and 3b *vs* 2d and 4d). Intra-specific competition also increased, resulting in lower tilapia growth rate at the density of 3 tilapia m^{-2} (Figure 4d).

At high prawn density (1-2 prawn m^{-2}) the increase in tilapia density showed some differences. When no tilapia was present (Figure 4a) the effects of autotrophic activity on water quality dominated. The addition of tilapia up to a density of 1 fish m^{-2} did not significantly change the factors and the prawn growth rate (Figure 4c *vs* 4a). When tilapia density increased over 2 fish m^{-2} (Figure 3d and 4d *vs* 4a and 4c) autotrophic activity increased as did under low prawn density when tilapia density was over 1.5 fish m^{-2}, but the increase of FACTOR2 did not occur. Under high tilapia and prawn densities the increased particle availability on the bottom due to the corresponding increased feed inputs, tilapia and prawn excretion and periphyton dislodgements produced by tilapia and prawn grazing and swimming near the substrates was consumed by the larger number of prawns. Hence less particles accumulated on the bottom and less decomposition products were liberated into the water column, so that FACTOR2 did not change. The feed supplied and the ecological processes described allowed good prawn and tilapia growth rates. However, only at the highest density (3 tilapia m^{-2}) tilapia produced a significant positive effect on prawn growth rate (Figure 4d *vs* 4a), while intraspecific competition significantly reduced its own growth rate (Figure 4d *vs* 4c and 3d).

The analysis of the interaction of management procedures (stocking densities, feeding, placing substrates for periphyton development) with the ecological processes developing in the pond bottom and the water column as identified by the multivariate analysis applied to combined data of several experiments, allowed a better understanding of the functioning of tilapia-prawn ponds. The use of substrates for periphyton growth is a low cost culture method that resulted in a more favorable environment for the cultured organisms (organic loading avoided) and at the same time provided an extra source of food. The synergistic relationships between tilapia and prawn through their effects on pond ecology indicate that their joint culture is technically feasible. The good growth rates and environmental conditions obtained at relatively high stocking densities of both organisms is a good indication of the economic viability of this technology. Therefore, the technology of tilapia and freshwater prawn polyculture in periphyton based systems is recommended for poverty alleviation and nutritional security in rural Bangladesh as well as other countries in the South and Southeast region as a whole.

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References

Alam, F.M., Thomson, K.J., 2001. Current constraints and future possibilities for Bangladesh fisheries. Food Policy 26, 297-313.

Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M., 2005. Periphyton: ecology, exploitation and management. CABI Publishing, Cambridge, USA. pp. 319.

Azim, M.E., Milstein, A., Wahab, M.A., Verdegam, M.C.J., 2003. Periphyton–water quality relationships in fertilized fishponds with artificial substrates. Aquaculture 228,169-187.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Milstein, A. Verdegem, M.C.J., 2001. Optimization of fertilization rates for maximizing periphyton growth on artificial substrates and implications for periphyton-based aquaculture. Aquac. Res. 32, 749-760.

Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2003a. Status of aquaculture and fisheries in Bangladesh. World aquaculture 34(4), 37-40, 67.

Azim, M.E., Verdegem, M.C.J., Singh, M., van Dam, A.A., Beveridge, M.C.M., 2003b. The effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. Aquac. Res. 34, 685-695.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003c. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia, *Oreochromis niloticus* L. Aquac. Res. 34, 85-92.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A. Beveridge, M.C.M., 2002. Evaluation of polyculture of Indian major carps in periphyton-based ponds. Aquaculture 213, 131-149.

Bowen, S.H., 1982. Feeding, digestion and growth-qualitative considerations. In: Pullin, R.S.V., Lowe-McConnell. (Eds.), The Biology and Culture of Tilapias. ICLARM Conf. Proc., vol. 7. pp. 141-156.

Boyd, C.E., 1979. Water Quality in Warmwater Fish Ponds. Auburn University, Craftmaster Printers, Opelika, Alabama, 359 pp.

Dempster, P.W., Beveridge, M.C.M., Baird, D.J., 1993. Herbivory in the tilapia *Oreochromis niloticus*: a comparison of feeding rates on phytoplankton and periphyton. J. Fish Biol. 43, 385–392.

DoF, 2004. Fishery Statistical Year Book of Bangladesh, 2002-2003. Fisheries Resource Survey System. Department of Fisheries. Dhaka, Bangladesh. 18 pp.

FAO, 2002. The State of World Fisheries and Aquaculture. The United Nations Food and Agricultural Organization, Rome, Italy. www.fao.org.

Guiral, D., Gourbault, N., Helleouet, M.N., 1995. Sediment nature and meiobenthos of an artificial reef (Acadja) used for extensive culture. Oceanol. Acta. 18, 543-555.

Gupta, M.V., Mazid, M.A., Islam, M.S., Rahman, M., Hussain, M.G., 1999. Integration of aquaculture into the farming system of the floodprone ecosystem of Bangladesh: an evaluation of adoption and impact. ICLARM Tech. Rep. 56. Manila.

Hem, S., Avit, J.L.B., 1994. First results on “acadja-enclos” as an extensive aquaculture system (West Africa). Bull. Mar. Sci. 55, 1038–1049.

Huchette, S.M.H., Beveridge, M.C.M., 2003. Technical and economical evaluation of periphyton-based cage culture of tilapia (*Oreochromis niloticus*) in tropical freshwater cages. Aquaculture 218, 219–234

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M., 2000. The impact of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture 186, 45-60.

Jeffers, J.N.R., 1978. An introduction to systems analysis: with ecological applications. Edward Arnold, London, 198 pp.

Johnson, S.K., Bueno, S.L.S., 2000. Health management. In: New, M.B., Valenti, W.C. (Eds.), Freshwater prawn culture: The farming of *Macrobrachium rosenbergii*. Blackwell Science Ltd. UK, pp. 239-258.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2002. The effect of periphyton and supplemental feeding on the production of the indigenous carps, *Tor khudree* and *Labeo fimbriatus*. Aquaculture 213, 207-218.

Kim, J.O., Mueller, C.W., 1978. Factor analysis. Statistical methods and practical issues. Quantitative applications in the Social Sciences 14. Sage University.

Legendre, M., Hem, S., Cisse, A., 1989. Suitability of brackish water tilapia species from the Ivory Coast for lagoon aquaculture. II.- growth and rearing methods. Aquat. Living Resour. 2, 81-89.

Lewis, D., 1997. Rethinking aquaculture for resource-poor farmers: perspective from Bangladesh. Food Policy 22, 533-546.

Milstein, A., Svirsky, F., 1996. Effect of fish species combinations on water chemistry and plankton composition in earthen fish ponds. Aquac. Res. 27: 79-90

Milstein, A., 1993. Factor and □avourabl correlation analyses: basic concepts, data requirements and recommended procedures. In: Prein, M., Hulata, G., Pauly, D. (Eds.), Multivariate methods in aquaculture research: case studies of tilapias in experimental and commercial systems. ICLARM Stud. Rev. 20. pp. 24-31.

Milstein, A., Azim, M.E., Wahab, M.A., Verdegem., 2003. The effects of periphyton, fish and fertilizer dose on biological processes affecting water quality in earthen fish ponds. Env. Biol. Fish. 68, 247-260.

New, M.B., 2000. History and global status of freshwater prawn farming. In: New, M.B., Valenti, W.C. (Eds.), Freshwater Prawn culture: the farming of *Macrobrachium rosenbergii*. Blackwell, Malden, Massachusetts, pp.1-11.

New, M.B., 2002. Farming freshwater prawns: a manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). FAO Fisheries Technical Paper no.428, FAO, Rome, Italy, 212pp.

Pompa, T., Masser 1999. Tilapia life history and biology. Southern Regional Aquaculture Centre, SAARC Publication No. 283.

Prein, M., Milstein, A., 1988. Techniques for handling large pond and farm datasets. Aquabyte 1, 4-5.

Seal, H.L., 1964. Multivariate statistical analysis for Biologists. Methuen, London.

Uddin, M.S., Farzana, A., Fatema, M.K., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2006a. Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia-prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development. Aquaculture (in press).

Uddin, M.S., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2006b. The potential of mixed culture of genetically improved farmed tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. Aquac. Res. 37, 241-247.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1–31.

Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S.. 1999. The potential of periphyton-based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton). Aquac. Res. 30, 409–419.

Table 1. Samples used and treatment names in this analysis.

Treatment name[1]	number of ponds	period	days of culture	tilapia density fish/m^2	prawn density prawn/m^2	substrates for periphyton	feed	place
_t2p0F	3	Jun-Nov 2003	150	2	0	no	yes[2]	BAU
_t2p2F	3	Jun-Nov 2003	150	2	2	no	yes[2]	BAU
_t2.25p0.75F	3	Jul-Nov 2004	140	2.25	0.75	no	yes[5]	village
_t2.25p0.75_	3	Jul-Nov 2004	140	2.25	0.75	no	no	village
Pt0p2F	3	Jul-Nov 2003	140	0	2	yes	yes[3]	village
Pt0.5p1.5F	3	Jul-Nov 2003	140	0.5	1.5	yes	yes[3]	village
Pt1p1F	3	Jul-Nov 2003	140	1	1	yes	yes[3]	village
Pt1.5p0.5F	3	Jul-Nov 2003	140	1.5	0.5	yes	yes[3]	village
	3	Apr-Jul 2004	120				yes[4]	BAU
Pt2p0F	3	Jun-Nov 2003	150	2	0	yes	yes[2]	BAU
	3	Jul-Nov 2003	140				yes[3]	village
Pt2p2F	3	Jun-Nov 2003	150	2	2	yes	yes[2]	BAU
Pt2.25p0.75F	3	Apr-Jul 2004	120	2.25	0.75	yes	yes[4]	BAU
	3	Jul-Nov 2004	140				yes[5]	village
Pt2.25p0.75_	3	Jul-Nov 2004	140	2.25	0.75	yes	no	village
Pt3p1F	3	Apr-Jul 2004	120	3	1	yes	yes[4]	BAU

[1] Treatment name: first character indicates periphyton presence (P) or absence (_); numbers following letters t and p are respectively the densities /m^2 of tilapia and prawn; last character indicates feed application (F) or not (_).

[2] 3% of tilapia biomass

[3] First 60 days: 5% of tilapia and prawn biomass, then 3%.

[4] First 30 days: 5% of tilapia biomass, then 2%.

[5] First 90 days: 3% of tilapia biomass, then 2%.

Table 2. Comparisons between treatments.

To test the effect of	Under conditions			Treatments to be compared
	1	2	3	
periphyton	feed	high tilapia density (2 m^{-2})	no prawn	Pt2p0F ; _t2p0F
		high tilapia density (2.25 m^{-2})	low prawn density (0.75 m^{-2})	Pt2.25p0.75F ; _t2.25p0.75F
		high tilapia density (2 m^{-2})	high prawn density (2 m^{-2})	Pt2p0F ; _t2p2F
	no feed	high tilapia density (2.25 m^{-2})	low prawn density (0.75 m^{-2})	Pt2.25p0.75_ ; _t2.25p0.75_
feed	high tilapia density (2.25 m^{-2})	low prawn density (0.75 m^{-2})	periphyton	Pt2.25p0.75F ; Pt2.25p0.75_
			no periphyton	_t2.25p0.75F ; _t2.25p0.75_
tilapia density	periphyton	feed	low prawn density (0-1 m^{-2})	Pt1p1F ; Pt1.5p0.5F ; Pt2p0F ; Pt2.25p0.75F ; Pt3p1F
			high prawn density (1-2 m^{-2})	Pt0p2F ; Pt0.5p1.5F ; Pt1p1F ; Pt2p2F ; Pt3p1F
prawn density	feed	no periphyton	high tilapia density (2-2.25 m^{-2})	_t2p0F ; _t2.25p0.75F ; _t2p2F
		periphyton	high tilapia density (2-3)	Pt2p0F ; Pt2.25p0.75F ; Pt3p1F ; Pt2p2F
			low tilapia density (0-1.5 m^{-2})	Pt1.5p0.5F ; Pt1p1F ; Pt0.5p1.5F ; Pt0p2F

Table 3. Results of ANCOVA and Scheffe mean multicomparisons of fish and prawn parameters. r^2 = coefficient of determination. Significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant. %SS = percentage of total sums of squares. Cov = covariable sign. Mean multicomparisons: same letters in each column indicate no significant differences at the 0.05 level. a>b>….

	Tilapia harvesting weight	**Tilapia growth rate**	**Prawn harvesting weight**	**Prawn growth rate**
	(g)	(g/day)	(g)	(g/day)
ANCOVA MODELS				
Significance	***	***	***	***
r^2	0.82	0.82	0.82	0.83
Variance Source	Sign.	Sign.	Sign. %SS Cov	Sign. %SS Cov
pond area	ns	ns	*** 20 -	*** 20 -
treatment	***	***	*** 80 .	*** 80 .
Mean multicomparisons by treatment (n)				
_t2p0F (3)	162 abcd_	1.2 abcd_		
_t2p2F (3)	152 abcd_	1.2 abcd_	16 __c	0.11 __c
_t2.25p0.75F (3)	109 _bcde	0.8 __cde	26 abc	0.19 abc
_t2.25p0.75_ (3)	75 ____e	0.6 ____e	16 __c	0.11 __c
Pt0p2F (3)			16 __c	0.12 __c
Pt0.5p1.5F (3)	180 ab___	1.4 ab___	22 abc	0.15 abc
Pt1p1F (3)	173 abc__	1.3 abc__	22 abc	0.16 abc
Pt1.5p0.5F (6)	144 abcd_	1.2 abcd_	36 abc	0.28 abc
Pt2p0F (6)	172 abc__	1.3 abc__		
Pt2p2F (3)	199 a____	1.5 a____	20 _bc	0.14 _bc
Pt2.25p0.75F (6)	118 _bcde	0.9 _bcde	43 a__	0.34 a__
Pt2.25p0.75_ (3)	108 __cde	0.8 __cde	28 abc	0.21 abc
Pt3p1F (3)	95 ___de	0.7 ___de	41 ab_	0.33 ab_

Table 4. Results of ANCOVA and Scheffe mean multicomparisons of each water quality variable. r^2 = coefficient of determination. Sign= Significance levels: +=0.1, * = 0.05, ** = 0.01, *** = 0.001, ns = not significant. %SS = percentage of total sums of squares. Cov = covariable sign. Mean multicomparisons: same letters in each column indicate no significant differences at the 0.05 level. a>b>…. (n)= number of observations.

ANCOVA MODELS		Temp. (°C)	Secchi (cm)	Chl-a (µg/l)	DO (mg/l)	pH	PO4_P (mg/l)	NH3_N (mg/l)	NO3_N (mg/l)
Model significance		***	***	***	***	***	***	***	***
r^2		0.78	0.48	0.32	0.39	0.42	0.53	0.48	0.46
Var.Source		Sign %SS Cov	Sign %SS Cov	Sign %SS Cov	Sign %SS Cov	Sign %SS Cov	Sign %SS Cov	Sign %SS Cov	Sign %SS Cov
pond area		ns 0 -	*** 14 +	ns 0 -	*** 3 -	** 2 -	ns 0 +	ns 0 -	*** 5 +
treatment		*** 4	*** 29	*** 25	*** 43	*** 21	*** 54	*** 19	*** 35
month		*** 60	*** 22	*** 15	*** 12	*** 23	*** 7	*** 22	*** 25
treatment*month		*** 36	*** 35	*** 60	*** 42	*** 54	*** 39	*** 59	*** 35
Mean multicomparisons by treatment (n)									
_t2p0F	(72)	29.3 ab___	28 __cde	173 abc	6.3 a___	7.8 a____	0.44 ____e	0.29 __cd	0.041 __c
_t2p2F	(72)	29.3 ab___	31 _bcde	167 abc	6.1 ab__	7.7 a____	0.40 ____e	0.27 __cd	0.027 __c
_t2.25p0.75F	(30)	26.6 ___de	39 ab___	130 _bc	3.9 __cd	6.6 ___de	1.63 a____	0.67 ab__	0.298 a__
_t2.25p0.75_	(30)	27.1 __cde	28 __cde	112 __c	3.5 ___d	6.5 ____e	1.32 ab___	0.40 abcd	0.077 _bc
Pt0p2F	(30)	28.4 abc__	39 ab___	152 abc	6.1 ab__	6.9 _bcde	0.61 ___de	0.70 a___	0.075 _bc
Pt0.5p1.5F	(30)	27.9 _bcde	36 abc__	173 abc	5.6 ab__	7.3 abcd_	0.67 __cde	0.32 __cd	0.080 _bc
Pt1p1F	(30)	28.1 _bcd_	43 a____	161 abc	4.6 _bcd	6.8 __cde	0.75 __cde	0.51 abc_	0.074 _bc
Pt1.5p0.5F	(84)	29.2 ab___	31 _bcde	171 abc	5.8 ab__	7.3 abcd_	0.52 ___de	0.23 __cd	0.051 __c
Pt2p0F	(102)	29.2 ab___	34 _bcd_	183 abc	5.8 ab__	7.4 abc__	0.43 ____e	0.26 __cd	0.054 __c
Pt2p2F	(72)	29.2 ab___	27 ___de	205 ab_	6.3 a___	7.5 ab___	0.36 ____e	0.33 __cd	0.027 __c
Pt2.25p0.75F	(84)	28.5 abc__	26 ___de	223 a__	5.3 abc_	7.4 abc__	0.99 _bcd_	0.39 _bcd	0.072 _bc
Pt2.25p0.75_	(30)	26.5 ____e	26 ___de	131 _bc	3.9 __cd	6.8 __cde	1.14 _bc__	0.42 abcd	0.147_b_
Pt3p1F	(54)	29.8 a____	23 ____e	216 ab_	6.4 a___	7.6 ab___	0.46 ____e	0.16 ___d	0.041 __c
Mean multicomparisons by month (n)									
Apr	(45)	27.4 ____e_	27 _bc	152 _b	6.5 ab_	7.5 ab__	0.46 _bc	0.19 _b	0.016 __c
May	(45)	30.3 ab____	24 __c	237 a_	6.7 a__	7.5 ab__	0.50 _bc	0.17 _b	0.031 __c
Jun	(84)	31.1 a_____	33 a__	181 _b	5.8 abc	7.3 abcd	0.37 __c	0.23 _b	0.024 __c
Jul	(138)	29.0 __cd__	33 a__	157 _b	5.4 __c	7.6 a___	0.58 abc	0.29 _b	0.051 _bc
Aug	(102)	28.8 __cd__	33 a__	184 ab	5.2 __c	7.2 _bcd	0.75 ab_	0.27 _b	0.046 _bc
Sep	(114)	29.4 _bc___	29 ab_	192 ab	5.7 _bc	7.4 abc_	0.76 ab_	0.51 a_	0.097 ab_
Oct	(102)	28.4 ___d__	31 ab_	169 _b	5.3 __c	7.1 __cd	0.72 ab_	0.31 _b	0.125 a__
Nov	(90)	25.1 _____f	27 _bc	180 _b	5.3 __c	7.0 ___d	0.82 a__	0.52 a_	0.087 ab_

Table 5. Results of factor analysis, ANCOVA and Scheffe mean multicomparisons of water quality data. Factor coefficients in bold were used for interpretation. r^2 = coefficient of determination. Sign= Significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant. %SS = percentage of total sums of squares. Cov = covariable sign. Mean multicomparisons: same letters in each column indicate no significant differences at the 0.05 level. a>b>…. n= 720 observations.

Factors:		**FACTOR1**			**FACTOR2**		
Secchi		**0.42**			**-0.63**		
DO		**-0.70**			0.13		
pH		**-0.71**			0.10		
NH_4_N		**0.42**			**0.52**		
NO_3_N		**0.56**			0.28		
PO_4_P		**0.62**			**0.51**		
Chlo_a		**-0.48**			**0.56**		
Variance Explained (%)		32			19		
Interpretation		autotrophic *vs.* heterotrophic activity			phytoplankton biomass synthesis in the water column and decomposition processes on the pond bottom		
ANOVA MODELS							
Model significance		***			***		
r^2		0.64			0.49		
Variance Source		Sign	%SS	Cov	Sign	%SS	Cov
pondarea		***	6	+	***	2	-
treatment		***	38	.	***	32	.
month		***	10	.	***	23	.
treatment*month		***	46	.	***	43	.
Mean multicomparisons by treatment (n)							
_t2p0F	(72)	____ef			abc		
_t2p2F	(72)	____ef			_bc		
_t2.25p0.75F	(30)	a_____			a__		
_t2.25p0.75_	(30)	_b____			abc		
Pt0p2F	(30)	_bcd__			abc		
Pt0.5p1.5F	(30)	__cde_			_bc		
Pt1p1F	(30)	_bc___			__c		
Pt1.5p0.5F	(84)	___def			_bc		
Pt2p0F	(102)	___def			_bc		
Pt2p2F	(72)	____ef			abc		
Pt2.25p0.75F	(84)	___def			a__		
Pt2.25p0.75_	(30)	_b____			ab_		
Pt3p1F	(54)	_____f			abc		
Mean multicomparisons by month (n)							
Apr	(45)	__cd			__c		
May	(45)	___d			ab_		
Jun	(84)	_bcd			__c		
Jul	(138)	abc_			__c		
Aug	(102)	ab__			_bc		
Sep	(114)	ab__			a__		
Oct	(102)	a___			abc		
Nov	(90)	a___			a__		

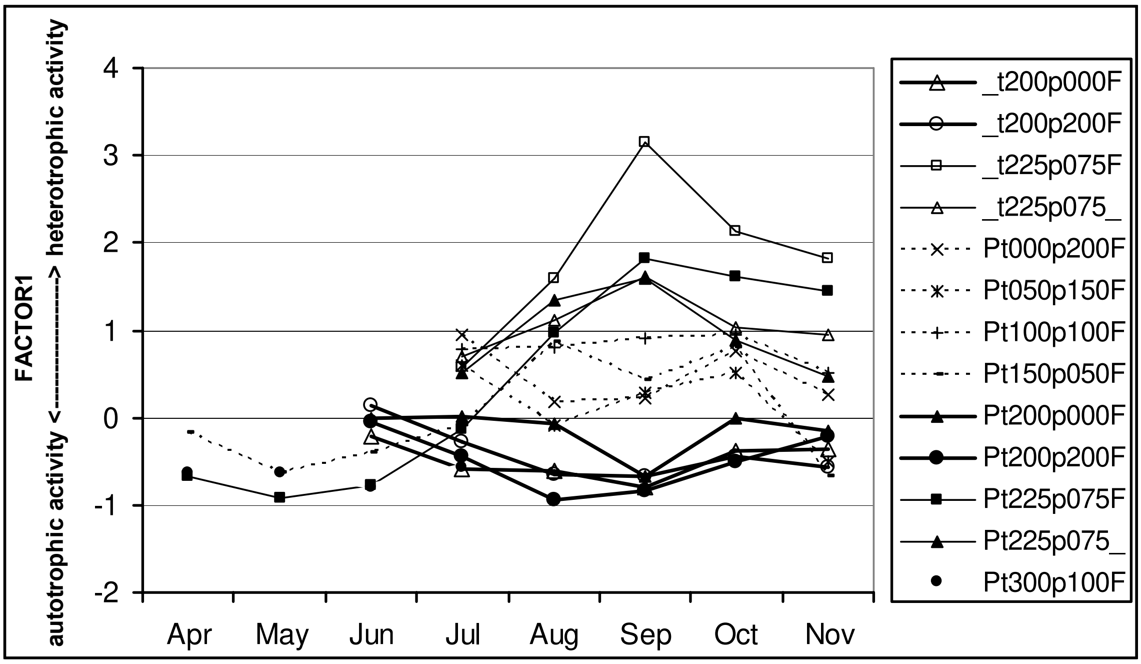
FIGURE LEGENDS

Figure 1. FACTOR1 and FACTOR2 treatment*month cross effects.

Figure 2. Conceptual representation of the fish, prawn, water quality significant interactions observed under high tilapia and low prawn densities. Left graphs without substrates, right graphs with substrates. Upper graphs without feed addition, lower graphs with feed addition. Size of organisms reflects changes in growth rate and width of arrows represent importance of effects.

Figure 3. Conceptual representation of the fish, prawn, water quality significant interactions observed under high tilapia density and feed addition. Left graphs without substrates, right graphs with substrates. Upper graphs without prawns, lower graphs with high prawn density. Size of organisms reflects changes in growth rate and width of arrows represent importance of effects.

Figure 4. Conceptual representation of the fish, prawn, water quality significant interactions observed in ponds with substrates and feed addition, under different tilapia densities. Size of organisms reflects changes in growth rate and width of arrows represent importance of effects.



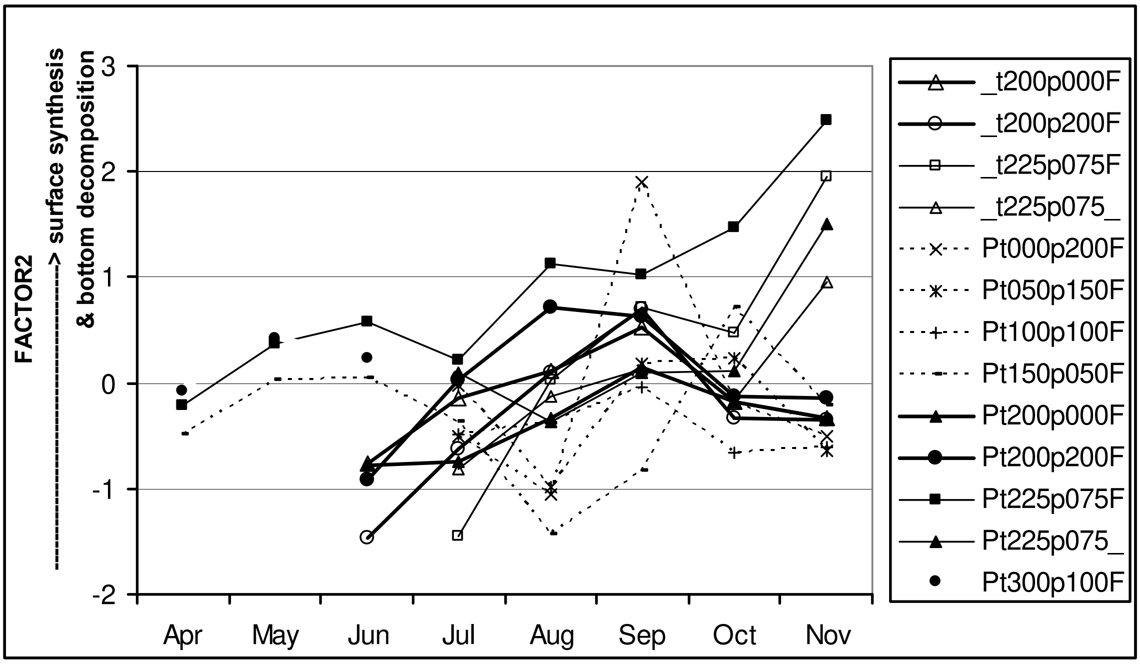
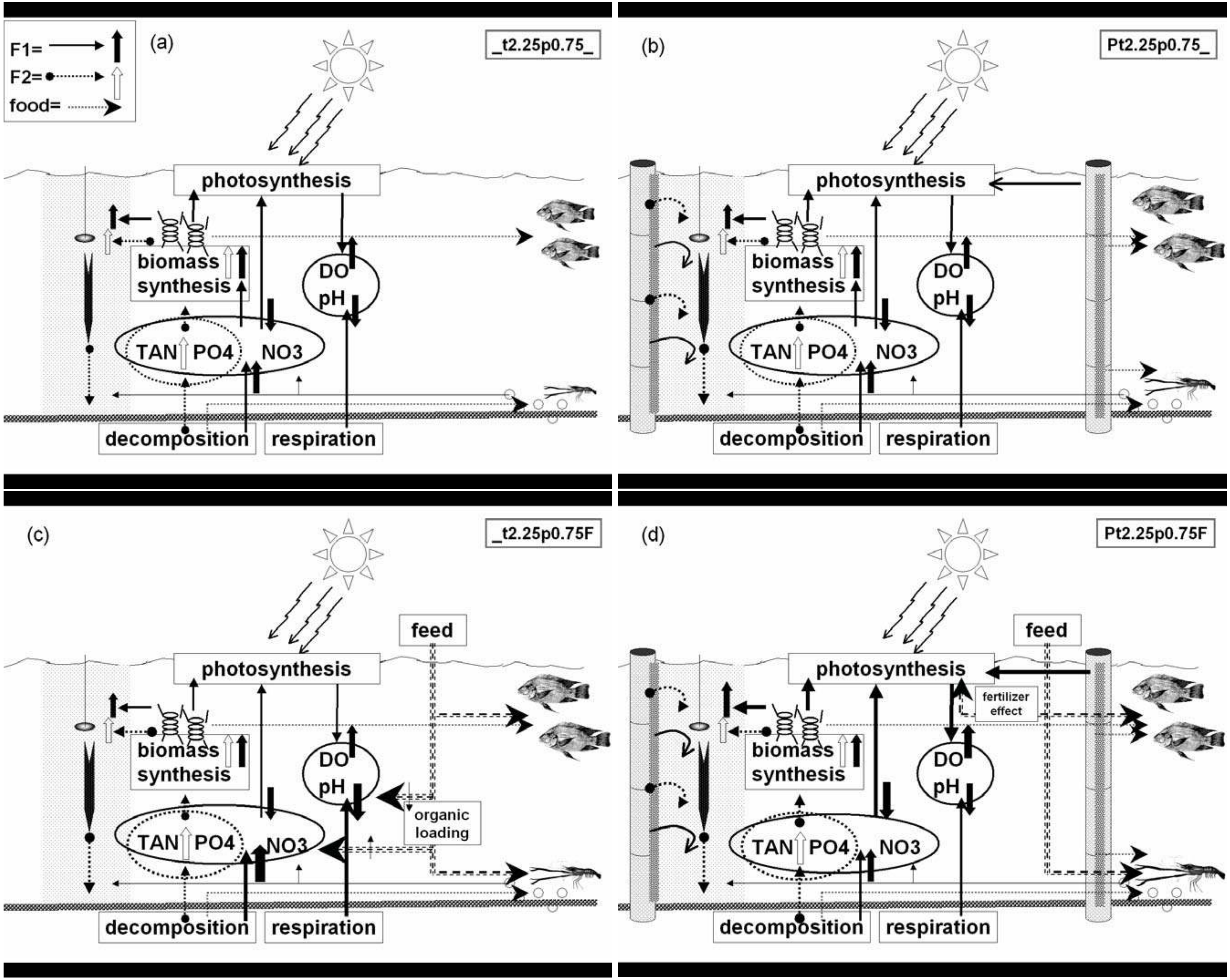


Figure 1. FACTOR1 and FACTOR2 treatment*month cross effects.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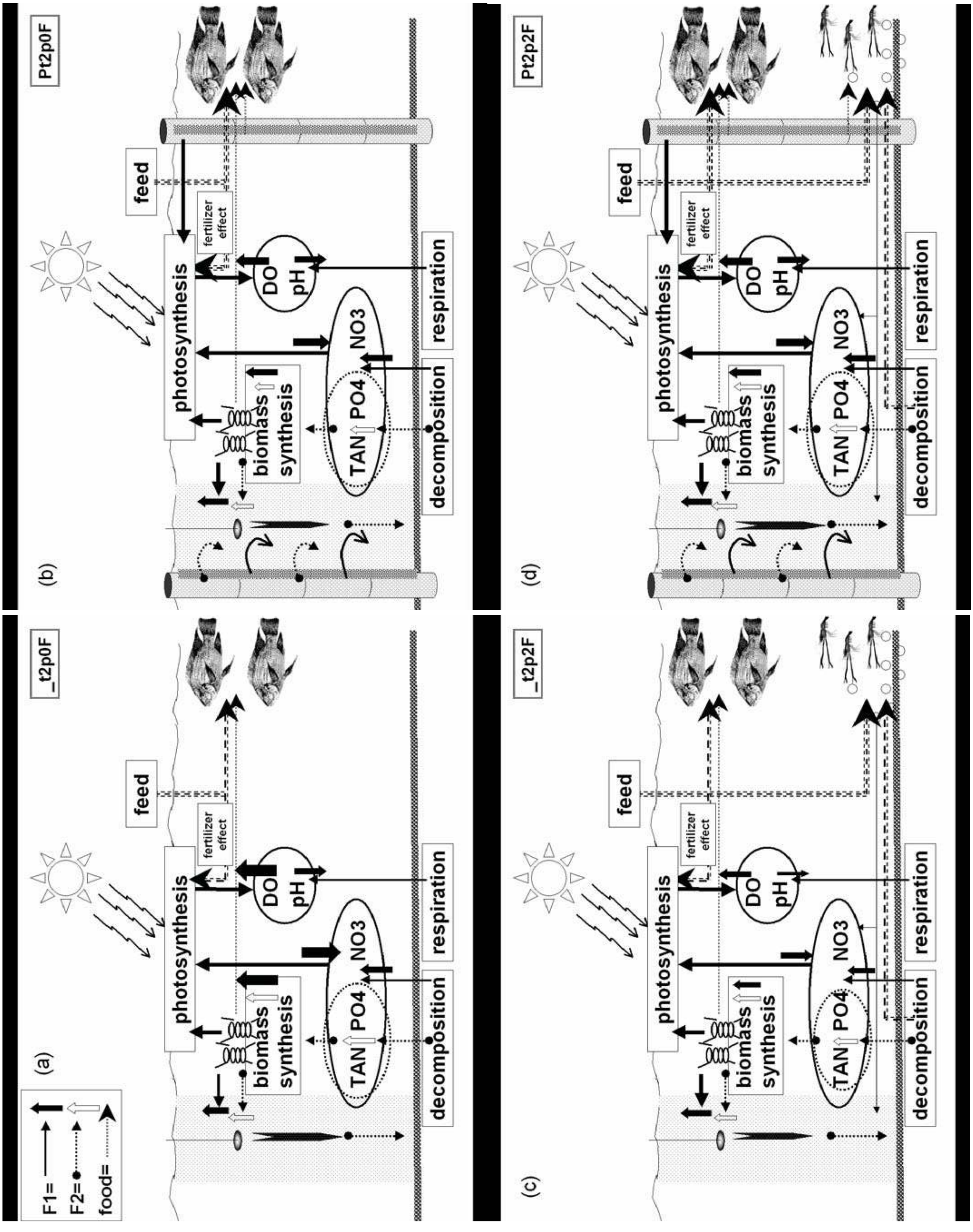
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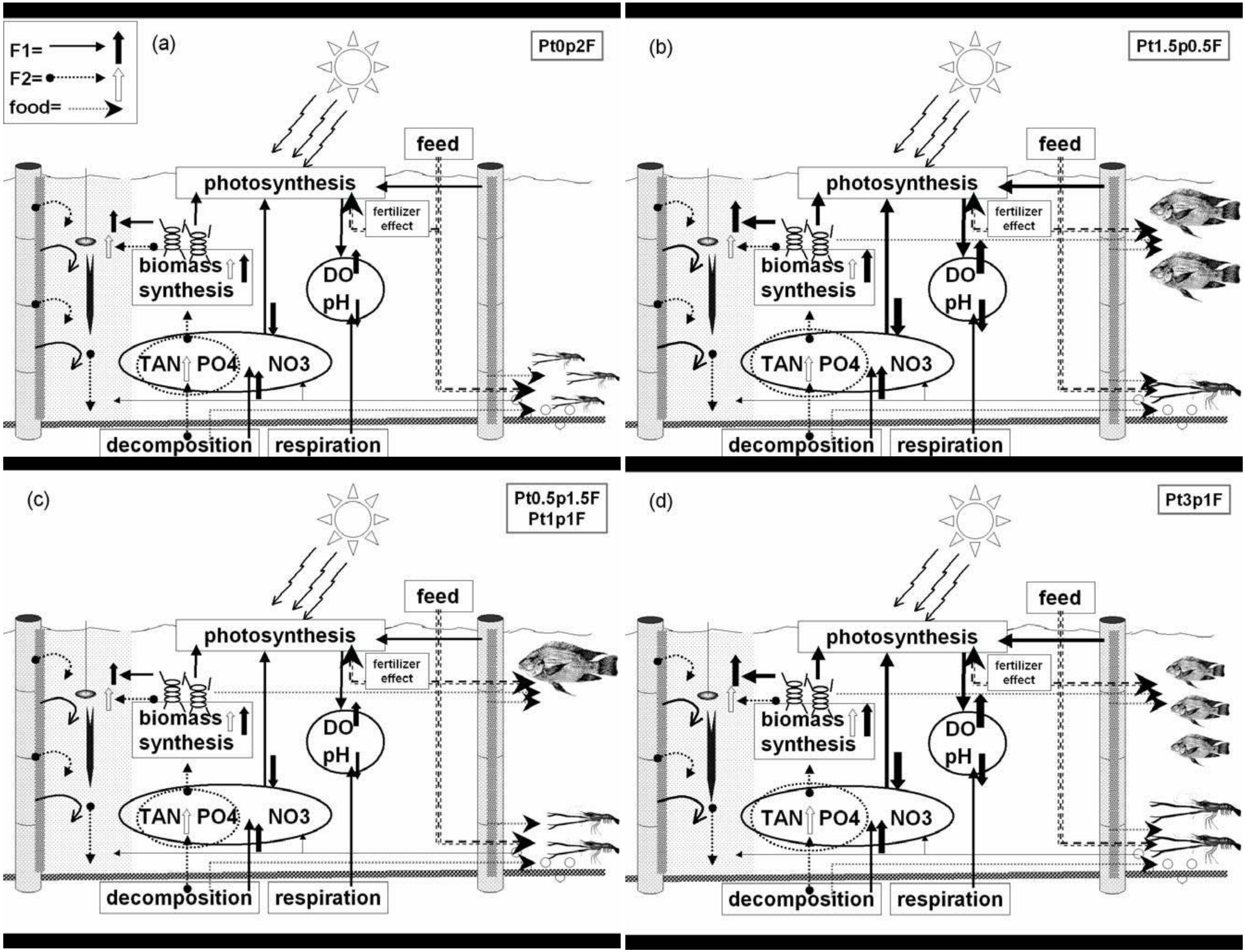
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Chapter 7

General Discussion

Introduction

Aquaculture production in ponds consumes nutrient applied as inorganic or organic fertilizers, and/or feed. The majority of the fish farmers in Asia are poor and unable to buy expensive inputs like formulated feeds. In stead, cheap organic wastes produced on-farm or within the household can be used, but the conversion efficiencies are in general low, reaching 20% at best (Davenport et al., 2003). The rest is discharged or accumulates in the sediment, and only a minor fraction is subsequently recycled within the farming system. More research on how to improve the environmental performance of low-cost aquaculture systems, while simultaneously increasing income and food security is needed (NACA, 2000). This thesis research project to develop an environmental and economic sustainable tilapia-freshwater prawn culture system contributes to this need.

The aim of this research was to improve production, input efficiency and benefits from Nile tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) polyculture in ponds with or without substrate addition for periphyton development. This chapter summarizes the major research results, outlines strength and weakness of the followed approach and discusses the applicability of research finding. On the basis of this analysis suggestions for further research are given.

The choice of bamboo poles as substrate

Bamboo (*Bambusa* sp.) poles were used as substrate for periphyton development because its effect on production is well documented and the material is broadly available in south Asia (Keshavanath et al., 2001; Azim et al., 2002). In periphyton based production systems, bamboo proved to be a better substrate than PVC. The better performance of bamboo may be due to a better surface structure favorable to attachment or to the leaching of nutrients from the bamboo itself. Jones et al. (2002) considers bamboo a good carbon source, and hence it could stimulate colonization and productivity of periphytic communities. The lower periphyton growth on synthetic materials like PVC could also be due to leaching of chemicals that adversely affect the periphyton community (Keshavanath et al., 2001). But there is strong competition in using bamboo with other household activities like house building materials, raw materials for cottage industry, household fuel energy and raw materials for paper and hardboard industry (Azim, 2001). It is also laborious and expensive to remove the bamboo poles before netting the ponds at harvest and partial harvesting of fish is impractical in bamboo-installed ponds. The dependency on expensive bamboo could be reduced if cheaper and easily available alternative substrates were found. Identifying alternatives for bamboo in substrate-based pond production systems is a priority for further research. There are some low-cost materials like Gab tree (*Diospyros peregrine*), Babla tree (*Acecia nilotica*), Shaora tree *(Balanostreblus illicifolius*) and Epil epil (*Leucaena lencocephala*) that could be used to reduce cost and increase profitability. Nevertheless, in our experiments, bamboo was chosen because large poles can be easily standardized in ponds. The amount of periphyton biomass (dry matter) growing on bamboo substrate ranged from 0.43 to 5.24 mg cm^{-2} which was in the same density range as reported by other authors and was uniform between experiments (Chapter 2-5). The periphyton biomass grown on substrate can be influenced by water depth (Konan-Brou and Guiral, 1994; Light and Beardall, 1998; Keshavanath et al., 2001), nutrient availability (Elwood et al., 1981; Fairchild et al., 1985; Vermaat and Hootsmans, 1994), grazing pressure (Hatcher and Larkum, 1983; Hansson et al., 1987; Hay, 1991; Vermaat and Hootsmans, 1994; Huchette et al., 2000), and environmental factors such as light (Meulemans and Ross, 1985) and temperature (Meulemans and Ross, 1985; Sommer et al., 1986; Bothwell, 1988; Vermaat and Hootsmans, 1994).

Synergism between tilapia and prawn in polyculture system

A successful polyculture system consists of simultaneously rearing two or more species with different feeding habits and space utilization with the goal to maximize production (Zimmermann and New, 2000). Different stocking densities and ratios of tilapia and freshwater prawn were tested. The highest production and net return were obtained with a 3 tilapia:1 prawn ratio and a combined stocking density of 20,000 individuals per ha (Chapter 3). The advantage of stocking freshwater prawns at such a low density is that costs for the farmer remain small while the profit margin increase considerably (New, 2000).

Both Nile tilapia and freshwater prawn prefer similar temperature ranges, so far are not plagued with major disease problems, and reach market size in Bangladesh within 5 months of culture (Rouse and Kahn, 1998). It is known that freshwater prawns feed on benthic organisms (Tidwell et al., 1995), detritus and feces (Zimmermann and New, 2000). Therefore, freshwater prawns benefit directly from tilapia feed wastes and feces, and indirectly from sediment enrichment, stimulating the development of benthic organisms. No extra feed or changes in pond management are needed when stocking low densities of freshwater prawn (Chapter 2) in tilapia ponds. In tilapia-prawn polyculture, total production increased without supplying more feed. Hence, the nutrient input efficiency improved (dos Santos and Valenti, 2002). Wohlfarth et al. (1985) suggested that in tilapia and prawn polyculture, the observed individual growth of each species are largely independent and additive. However, at the very low prawn densities used in our experiments, tilapia production increased due to the addition of freshwater prawns. This effect was further enhanced with the addition of bamboo substrate for periphyton development. There was synergism with a low density of prawns, but this effect diminished at higher densities. Why this happens is not well understood yet and an interesting topic for further research.

Periphyton as alternative to artificial feed

Many trials have demonstrated that production from ponds provided with substrate for periphyton development is higher than from substrate free ponds (Hem and Avit, 1994; Wahab et al., 1999; Azim et al., 2001; Keshavanath et al., 2004). These studies showed that some species such as rohu (*Labeo rohita*), mahseer (*Tor khudree*) and tilapia (*Oreochromis niloticus*) are very suitable for periphyton-based aquaculture. The nutritive value of periphyton can be regarded as appropriate for dietary needs of Indian major carps (Azim, 2001) and tilapia (Azim et al., 2003a). The optimum dietary protein content for Nile tilapia is reported to be 30-40% (Siddique et. al., 1988). The protein content of periphyton in our experiments felt within this range with 24-28% of dry matter (Chapter 5); similar to protein levels reported by Azim et al. (2003a).

The feed conversion ratio (FCR) was 13% lower in fed periphyton-based ponds compared to substrate free fed ponds. To produce an additional 345 kg ha^{-1} of tilapia 464 kg ash free dry matter (AFDM) of periphyton was eaten considering a FCR of 1.34 for periphyton (Azim et al., 2003b). Following the same reasoning, nearly twice as much, 922 kg periphyton AFDM was eaten by tilapia in non-fed periphyton-based ponds (Chapter 5). This indicates that tilapia used periphyton more efficiently in the absence of supplemental feed. Similar productions were obtained in non-fed periphyton-based ponds and substrate-free fed ponds, suggesting that that periphyton can nearly replace completely supplemental feeding in tilapia-freshwater prawn polyculture. Keshavanath et al. (2004) reached similar conclusions with a monoculture of tilapia comparing the effect of substrate addition on production in fed and non-fed ponds. The effect of supplemental feeding in semi-intensive polyculture systems on production is not straightforward as there are complex interactions among natural food organisms, feeding practices, water quality parameters and cultured fish species. This partially explains the wide range of production increases due to periphyton development found in literature. In monoculture with the column feeder rohu (*Labeo rohita*) or the bottom feeder kalbaush (*Labeo calbasu*) production increased 80% while in polyculture with the addition of the surface feeder catla (*Catla catla*) the production increased nearly 300% due to substrate-addition (Azim, 2001).

Selective grazing by tilapia on periphyton

The periphyton biomass (dry matter) decreased over time due to tilapia grazing. The periphyton productivity was (Chapter 2) about 1.23 g AFDM m^{-2} d^{-1} (or 1.76 g dry matter, 30% ash). The ash content of periphyton ranged between 16 and 55% (Chapter 2-5). The highest periphyton dry matter concentration was 4.5 mg cm^{-2} with 55% ash in prawn monoculture under on-farm conditions (Chapter 3). The periphyton biomass in terms of dry matter and chlorophyll *a* concentration increased in the first 3-4 weeks of each experiment but decreased steadily subsequently (Chapter 2-5). Initially, when there was abundant periphyton available, tilapia grazed selectively on the organic rich parts of the periphyton mats avoiding sections rich in inorganic matter. On the following dates, with increasing tilapia density, the ash percentage in the periphyton decreased steadily indicating that grazing became less selective. This is well-known strategy of herbivores to meet their nutritional requirement using low quality feeds (Bowen et. al., 1995). Huchette et al. (2000) reported approximately 30-70% ash content under ungrazed and 20-30% under grazed conditions (dry matter basis) in periphyton collected from cages. In our experiment too, the periphyton was grazed intensively. Tilapia is known to be a periphyton grazer (Dempster et al., 1993; Keshavanth et al., 2004) and grazing itself keeps the periphyton productive (Hatcher, 1983; Hay, 1991; Huchette et al., 2000).

Effects of periphyton on survival and production

Submerged substrates create habitat for nitrifying and denitrifying bacteria, and those bacteria can improve water quality by converting toxic nitrogenous wastes into less toxic forms through nitrification or remove it through denitrification. A link was found between reduced levels of total ammonia nitrogen and increased production in periphyton-based ponds (Otoshi et al., 2006).

Although the underlying mechanisms need further study, substrate addition positively influenced survival and production of prawn and tilapia. For prawn, substrate addition in unfed ponds resulted in a 75% higher survival. In contrast, feed addition to substrate free ponds resulted in a 35% higher survival. Similarly, net prawn production was 127% higher after substrate addition in unfed ponds and 110% higher after feeding in substrate free ponds. For tilapia, substrate addition in unfed ponds resulted in a 10% higher survival, while feed addition to substrate free ponds resulted in a 12% higher survival. The production of tilapia increased 45% after substrate addition to unfed ponds, while administrating feed to substrate-free ponds resulted in a 22% higher tilapia production (Chapter 2 and 5).

Conflicting effects of substrate addition on prawn and shrimp production have been reported in literature. Sandifer et al. (1987) reported that artificial substrate, in the form of fiberglass screens, enhanced shrimp survival but did not increase growth rates of nursery-sized *L. vannamei*. Samocha et al. (1993) reported that the addition of vertical netting did not improve survival, growth, and feed conversion ratio (FCR) of *L. vannamei* reared under intensive culture conditions. Bratvold and Browdy (2001) reported that shrimp weight, survival and production were enhanced in the presence of AquaMats^{TM} during the grow-out phase. More recently, it was found that in nursery ponds, production and growth rate were significantly higher and FCRs significantly lower for *L. vannamei* and *P. monodon*, in presence of AquaMats^{TM} compared to AquaMats^{TM}-free ponds (Moss and Moss, 2004; Arnold et al., 2006). The feeding efficiency in prawn ponds improved proportionately with the amount of substrate surface area installed (Tidwell et al., 2000). It is likely that an increase in natural biota provided by the added substrate enhanced growth of freshwater prawn, but further investigation to determine which components of the natural biota are contributing to prawn growth will be important to optimize this technology further.

Plankton and periphyton ingested by the fishes

About 60 genera of plankton were identified in the water column, compared to 48 genera in periphyton collected from on-station experiments (Chapter 2, 4) and 38 genera in periphyton from on-farm trials. There was a partial overlap between the genera identified in the water column and in the periphyton (Chapter 5). Some oligochaete, polychaete and other benthic organisms were occasionally identified in the periphyton. Grazing on periphyton in aquaculture ponds is important, compared to lakes and rivers where death followed by heterotrophic decomposition is the principal route of nutrient recycling from periphytic communities (Wetzel, 2005). Most nutrients are recycled within the periphyton mat itself, the exchange with the surrounding water being a minor fraction of the total nutrient flux (Verdegem et. al., 2005). Tilapias are reported feeding on benthic and attached (periphyton) algal and detrital aggregates (Bowen, 1982; Dempster et al., 1993; Azim et al., 2003a) and 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). There is also evidence that substrate based systems enhanced the production of benthos in the culture systems (Azim, 2001; Tidwell and Bratvold, 2005). The similar periphyton biomass observed in presence of absence of freshwater prawn (Chapter 3) suggests that prawns either did not eat periphyton or selectively picked up animal biomass from the mixed (autotrophic and heterotrophic) periphyton biofilms.

It was not possible to distinguish between periphytic and planktonic portions in the gut contents; nevertheless, an attempt was made to compare the feeding niches of tilapia and freshwater prawn. Tilapia’s electivity indices were negative for all zooplankton and positive for all phytoplankton groups except Bacillariophyceae (Figure 1), indicating that tilapia preferred phytoplankton above zooplankton (Chapter 2). The characteristic diet of adult tilapia is plant material and/or detritus of plant origin, like phytoplankton, benthic algae, macrophytes and periphyton. In literature *Oreochromis* species are often described as microphagous (Caulton, 1977; Lowe-McConnell, 1982; Beveridge and Baird, 1999), but also algae and algae-derived detritus from sedimented phytoplankton, benthic algae, periphytic algae, or cyanobacterial surface scum can be important (Beveridge and Baird, 1999). There is a large overlap in electivity indices between tilapia and prawn. A striking difference is the preference of tilapia for Cyanophyceae compared to the avoidance of Cyanophyceae by prawn. It remains an interesting question if food preferences of both species are fixed. Most tilapia have a very flexible diet and are able to digest blue-green algae (Moriarty and Moriarty, 1973; Turker et al., 2003). It is uncertain if prawns exhibit a similar dietary flexibility. However, the wide range of organisms eaten by freshwater prawn does not exclude a high degree of dietary flexibility. Freshwater prawn food items reported include benthic macro-invertebrates like oligochaetes, chironomids, gastropods, lamellibranchs, insects, nematodes and microalgae (Schroeder, 1983; Tidwell et. al., 1995).

The most reliable method to determine food preferences in fish is to use a combination of food item availability and stomach sampling. With crustaceans, such as prawns, this method might be less reliable, due to incidental ingestion of nutritionally unimportant items, small stomach size, small prey size, and mastication of food items before ingestion (Brown et al., 1992). The unidentified portion of the gut content of prawn was about 35 percent compared to 20% for tilapia.

Prawn size at stocking

The survival rate of freshwater prawn in the on-farm trials (Chapter 3 and 5) was higher when juveniles were stocked rather than PLs. After stocking in grow-out ponds, juvenile freshwater prawns are more resistant to predation (New and Singholka, 1985), cannibalism, and fluctuating environmental conditions (Ling, 1969, Fujimura and Okamoto, 1972) than PLs. Prawn survival is important, as the cost of PLs or juveniles is high for farmers. Stocking juveniles is more expensive but is largely compensated by the better survival and production. Cohen et al. (1981) even suggested that polyculture with carnivorous fish might be possible when stocking large sized freshwater prawn juveniles.

Economic returns

The international market typically requires 450-700 g tilapia. Under favorable culture condition this size is obtained within 5-6 months. In semi-tropical regions freshwater prawns are generally cultured in single batch whereas in the tropics an all-year continuous harvest culture system is practiced. Due to constraints with availability of experimental facilities and seasonal limitations, experiments for this thesis lasted 102-150 days. This growth period was too short for tilapias and prawns to reach an optimal market size. In consequence, prices obtained for tilapia and prawn were suboptimal, reducing benefits (Chapter 5). Nevertheless, a comparison between treatments was possible, and in general a higher net profit margin was obtained in the substrate-based system (Figure 2). Interestingly, although the contribution of prawn to the total production in quantity, as compared to tilapia, was only 4-7%, it contributed 22-32% to the total sales value. Since the market price of prawn is many times higher than that of tilapia, prawn contributed significantly to sales income, even with the low quantities produced.

Water quality and stocking density in periphyton-based tilapia-prawn polyculture systems

The interaction between bottom surface area and decomposition showed positive correlation between total ammonia nitrogen, phosphate and chlorophyll-a, and negative correlation with Secchi (Chapter 6). This reflects the role of total ammonia nitrogen and phosphate in phytoplankton biomass synthesis in the water column and their relation with decomposition processes occurring on the pond bottom. The more ammonia-N and phosphate were available in the water column, the higher the phytoplankton biomass synthesis (chlorophyll-a), causing higher water turbidity and lowering Secchi depth visibility. However, due to added substrate no adverse effects on water quality parameters were observed. Substrate addition for periphyton development had only a significant effect on water quality when feed was supplied and prawn density was low. In periphyton-based ponds periphyton improved water quality by trapping suspended solids and through nitrification. Substrate addition lowered Secchi depth visibility, and phosphate and nitrate concentrations, and increased chlorophyll *a* concentration and pH in the water column (Chapter 6). The close linkage between autotrophic and heterotrophic processes in periphyton mats speeds up nutrient cycling and positively influences water quality (Azim et al., 2003b, Milstein et al., 2003).

Considering yield and profits, the best tilapia-freshwater prawn farming system is with a 3 tilapia:1 prawn stocking ratio at a combined stocking density of 30,000 ha^{-1} (Chapter 3 and 4). The good water quality observed indicates the technology is also sustainable. Potential pond productivity is insufficiently used in monoculture of freshwater prawn due to antagonistic interactions, hence the need for polyculture (New, 1990). Prawn polyculture has a potentially higher net return than prawn monoculture (Rouse and Stickney, 1982). The benefits from fish-freshwater prawn polyculture are further enhanced by the introduction of substrates for periphyton development. At the stocking densities applied in this study, periphyton offers a good alternative to supplemental feeding. The future challenge is to identify cheap substrates, and to lower labor requirements in periphyton based production systems.

Conclusions and further perspectives

The technical and economic potentials of tilapia (*Oreochromis niloticus*) and prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems in South Asia was investigated. This study demonstrated that pond fish production significantly increased with the introduction of substrates and reduced the need for supplemental feeding. Commercial viability may ultimately reside in the judicious and economical use of feed, which can represent 40-60% in operational cost. Tacon and De Silva (1997) emphasized the need to reduce feed costs in aquaculture operations. Stocking prawns in polyculture with finfish has the potentials of increasing total yields as well as farm income, particularly as prawns have a higher commercial value locally than the commonly cultured fish species. Some ecological advantages of periphyton-based tilapia-prawn polyculture, such as improved water quality (thus potentially reduce water exchanges), further increase the sustainability of this form of aquaculture.

There is a growing consumer’s perception, especially in the developed world, that organically produced foods are safer and healthier for both human beings and the environment. One of the main difficulties of organic aquaculture is that fish feeds must be organic in origin. This strongly limits the use of the main sources of protein used in conventional aquaculture feeds and increases the cost of feeding. On the other hand, organic standards encourage the use of food sources of biological origin not suitable for human consumption. Periphytic communities are such a food source fitting the criteria for ecological and organic aquaculture. This opens opportunities to produce and promote organic products in export and domestic markets while providing opportunities to small-scale farmers to significantly benefit from such a development.

The major strength of this research was that it investigated the combined effects of stocking densities of fish and crustaceans, addition of substrate for periphyton development and artificial feed, considering water quality, nutrient accumulation, natural food accumulation and feeding indices. Another strength was the mix of on-station and on-farm (adaptive) action research involving considerable numbers of farmers to participate in the research and to become owner of the research results. Economical analysis were performed both under on-station and on-farm condition and it was assessed that the technology is applicable under a broad range of circumstances, from the commercial level to resource-poor and marginal farmers. Modeling of periphyton-based systems also will be useful to determine the optimum combinations of substrates, fish stocking densities and relative contribution of feed and periphyton to the fish production in tilapia-prawn polyculture systems. A major drawback of the present study was the inability to analysis the gut content of tilapia and prawn in combination with stable isotope analysis. When experimental animals consume food from two or more different sources with different natural stable isotope ratios, the fraction that each food contributed to the diet can be calculated from the resulting stable ratios in the fish (Fry et al., 1983; Schroeder, 1983; Anderson et al., 1987). Therefore, studies with stable isotopes can help in elucidating food webs in ponds with periphyton-based systems and are recommended for further study.

References

Anderson, P.K., Parker, P.L., Lawrence, A., 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond grow out system. J. World Aquac. Soc. 18, 148-155.

Arnold, S.J., Sellars, M.J., Crocos, J.P., Coman, G.J., 2006. Intensive production of Juvenile tiger shrimp *Penaeus monodon*: An evaluation of stocking density and artificial substrates. Aquaculture 261, 890-896.

Azim, M.E., 2001. The potentials of periphyton-based Aquaculture production systems. Ph.D. Thesis. 219 pp.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., 2002. Evaluation of polyculture with Indian major carps in periphyton-based pond. Aquaculture 213, 131–149.

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., Beveridge, M.C.M., 2003a. Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia, *Oreochromis niloticus* L. Aquac. Res. 34, 85-92.

Azim, M.E., Verdegem, M.C.J., Singh, M., van Dam, A., Beveridge, M.C.M., 2003b. The effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. Aquac. Res. 34, 685-695.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2001. The potential of periphyton-based culture of two Indian major carps, rohu *Labeo rohita* (Hamilton) and gonia (*Labeo gonius* Linnaeus). Aquac. Res. 32, 209–216.

Beveridge, M.C.M., Baird, D.J., 1999. Diet, feeding and digestive physiology. In: Beveridge, M.C.M., McAndrew, B.J. (Eds.), Tilapias: Biology and Exploitation. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 59-87.

Bothwell, M.L., 1988. Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. Can. J. Fish. Aquat. Sci. 45, 261-270.

Bowen, S.H., 1982. Feeding digestion and growth qualitative considerations. In: Pullin, R.S.V., Lowe-McConnel, R.H. (Eds.), The Biology and Culture of Tilapias. ICLARM Conf. Proc. 7, Manila. pp. 141-156.

Bowen, S.H., Lutz, E.V., Ahlgren, M.O., 1995. Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76, 899-907.

Bratvold, D., Browdy, C.L., 2001. Effect of sand sediment and vertical surfaces (AquaMats^{TM}) on production, water quality and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture 195, 81–94.

Brown, P.B., Wetzel, J.E., Spacie, A., Konopka, A., 1992. Evaluation of nutritionally occurring organisms as food for juvenile crayfish, *Procambarus clarkii*. J. World Aquac. Soc. 23, 211-216.

Caulton, M.S., 1977. A quantitative assessment of the daily ingestion of Panicum repens L. by *Tilapia rendalli* Boulenger (Cichlidae) in Lake Karibia. Trans. Rhod. Sci. Assoc. 58(6), 38-42.

Cohen, D., Ra'anan, Z., Brody, T., 1981. Population profile development and morphotypic differentiation in the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). J. World Aquac. Soc. 12(2), 231-243.

Corbin, J.S., Fujimoto, M.M., Iwai, T.U.Jr., 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. In: McVey, J.P. (Ed.), CRC Handbook of Mariculture. CRC Press, Boca Raton, FL, pp. 391-423.

Coyle, S.D., Najeeullah, T., Tidwell, J.H., 1996. Evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawns *Macrobrachium rosenbergii*. J. Appl. Aquat. 6, 57-66.

Davenport, J., Black, K., Burnell, G., Cross, T., Culloty, S., Ekaratne, S., Furness, B., Mulcahy, M,. Thetmeyer, H., 2003. Aquaculture, the Ecological Issue. Blackwell Publishing, Oxford, UK.

Dempster, P.W., Beveridge, M.C.M., Baird, D.J., 1993. Herbivory in the tilapia *Oreochromis niloticus*: a comparison of feeding rates on phytoplankton and periphyton. J. Fish Biol. 43, 385–392.

dos Santos, M.J.M., Valenti, W.C., 2002. Production of Nile tilapia *Oreochromis niloticus* and freshwater prawn *Macrobrachium rosenbergii* stocked at different densities in polyculture system in Brazil. J. World Aqua. Soc. 33, 369-376.

Elwood, J.W., Newbold, J.D., Tribble, A.F., Stark, R.W., 1981. The limiting role of phosphorus in a woodland ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62, 146-158.

Fairchild, G.W., Lowe, R.L., Richardson, W.B., 1985. Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. Ecology 66, 465-472.

Fry, B., 1983. Fish and shrimp migrations in the Gulf of Mexico analysed using stable C, N and S isotope ratios. Fish Bull. 81, 789-801.

Fujimura, T., Okamoto, H., 1972. Notes on progress in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. In: Pillay, T.V.R. (Ed.), Coastal Aquaculture in the Indo-Pacific Region. Fishing News Books, Blackwell Science, Oxford, pp. 313-327.

Hansson, L.A., Johansson, L., Persson, L., 1987. Effects of fish grazing on nutrient release and succession of primary producers. Limnol. Oceanogr. 32, 723-729.

Hatcher, B.G., Larkum, A.W.D., 1983. An experimental analysisof factors controlling the standing crop of the epilithic algal community on a coral reef. J. Exp. Mar. Biol. Ecol. 69, 61-84.

Hay, M.E., 1991. Fish-seaweed interactions on coral substrates: effects of herbivorous fishes and adaptations of their prey. In: Sale, P.F. (Ed.), The ecology of fishes on coral substrates. Academic Press, London, pp. 96-119.

Hem, S., Avit, J.L.B., 1994. First results on "acadja-enclos" as an extensive aquaculture system (West Africa). Bull. Mar. Sci. 55, 1038-1049.

Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J., Ireland, M., 2000. The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. Aquaculture 186, 45-60.

Ivlev, V.S., 1961. Experimental ecology of the feeding of fish. Yale University Press, New Haven, Connecticut, USA, 302 pp.

Jones, J.I., Young, J.O., Eaton, J.W., Moss, B., 2002. The influence of nutrient loading, dissolve inorganic carbon and higher tropic levels on the interaction between submerged plants and periphyton. Journal of Ecology 90, 12-24.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Dam, A.A., Beveridge, M.C.M., Verdegem, M.C.J., 2004. Effect of bamboo substrate and supplemental feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* × *Oreochromis niloticus*). Aquaculture 235, 303-314.

Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Rooij, J.M., Beveridge, M.C.M., Baird, V., Verdegem, M.C.J., van Dam, A.A., 2001. Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. Aquac. Res. 32, 189-197.

Konan-Brou, A.A., Guiral, D., 1994. Available algal biomass in tropical artificial habitats. Aquaculture 119, 175-190.

Light, B.R., Beardall, J., 1998. Distribution and spatial variation of benthic microalgal biomass in a temperature, shallow-water marine system. Aquat. Bot. 61, 39-54.

Ling, S.W., 1969. Methods of rearing and culturing *Macrobrachium rosenbergii* (De Man). FAO Fisheries Report 57(3), 606-619.

Lowe-McConnell, R.H., 1982. Tilapias in fish communities. In: Pullin, R.S.V., Lowe-McConnell, R.H. (Eds.), The Biology and Culture of Tilapias. ICLARM Conf. Proc. 7, Manila. pp. 83-114.

Meulemans, J.T., Roas, P.J., 1985. Structure and architecture of periphytic community on dead reed stems in lake Maarsseveen. Archive für Hydrobiologie 102, 487-502.

Milstein, A., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., 2003. The effects of periphyton, fish and fertilizer dose on biological processes affecting water quality in earthen fish ponds. Env. Biol. Fish. 68, 247-260.

Moriarty, C.M. Moriarty, D.J.W., 1973. Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis nigripinnis* in Lake George, Uganda. J. Zool. (London), 171, 209-255.

Moss, K.R.K., Moss, S.M., 2004. Effects of artificial substrate and stocking density on the nursery production of Pacific white shrimp *Litopenaeus vannamei*. J. World Aquac. Soc. 35, 536-542.

NACA, 2000. Aquaculture development beyond 2000: The Bangkok declaration and strategy. Conference on aquaculture development in the third millennium. Bangkok.

New, M.B., 1990. Freshwater prawn culture: a review. Aquaculture 88, 99-143.

New, M.B., 2000. history and global status of freshwater prawn farming. In: New, M.B., Valenti, W.C. (Eds.), Freshwater Prawn culture: the farming of *Macrobrachium rosenbergii*. Blackwell, Malden, Massachusetts, pp.1-11.

New, M.B., Singhola, S., 1985. Freshwater prawn farming. A manual for the culture of *Macrobrachium rosenbergii*. FAO Fisheries Technical Paper 225 (Rev.1). FAO, Rome.

Otoshi, C.A., Montgomery, A.D., Matsuda, E.M., Moss, S.M., 2006. Effects of artificial substrate and water source in growth of juvenile Pacific white shrimp, *Litopenaeus vannamei*. J. World Aquac. Soc. 37(2), 210-213.

Rouse, B.D., Stickney, R.R. 1982. Evaluation of the production potential of *Macrobrachium rosenbergii* in monoculture and polyculture with *Tilapia aurea*. J. World Mari, Soc. 13, 73-85.

Rouse, D.B., Kahn. B.M., 1998. Production of Australian red claw *Cherax quadricarinatus* in polyculture with Nile tilapia *Oreochromis niloticus*. J. World Aquac. Soc. 29(3), 340-344.

Samocha, T.M., Lawrence, A.L., Biedenbach, J.M., 1993. The effect of vertical netting and water circulation pattern on growth and survival of *Penaeus vannamei*. J. Appl. Aquacult. 2, 55-64.

Sandifer, P.A., Hopkins, J.S., Stokes, A.D., 1987. Intensive culture potentials of *Penaeus vannamei*. J. World Aquac. Soc. 18, 94-100.

Schroeder, G.L., 1983. Sources of fish and prawn growth in polyculture ponds as indicated by δC analysis. Aquaculture 35, 29-42.

Siddique, A.Q., Hawladar. M.S., Adam, A.A., 1988. Effects of diatory protein levels on growth, feed conversion and protein utilization in fry and young Nile Tilapia, *Oreochromis niloticus*. Aquaculture 70, 63-73.

Sommer, U., Gliwicz, Z.M., Lampert, W., Duncan, A., 1986. The PEG-model of seasonal succession of planktonic events in freshwaters. Archiv für Hydrobiologie 106, 433-471.

Tacon, A.G.J., De Silva, S.S., 1997. Feed preparation and feed management strategies within semi-intensive fish farming systems in tropics. Aquaculture 151, 379-404.

Tidwell J.H., Cycle, S., Van Arnum, A., Weibel, C., 2000. Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. J. World Aquac. Soc. 31, 452-458.

Tidwell, J.H., Bratvold, D., 2005. Utility of added substrates in shrimp culture. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 247-268.

Tidwell, J.H., Coyle, S.D., Webster, C.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, S.J., D'Abramo, L.R. Dainels, H.W., Fuller, M.J., 1997. Relative prawn production and benthic macroinvertebrate densities in unfed, organically fertilized, and fed pond systems. Aquaculture 149, 227-242.

Tidwell, J.H., Webstar, C.D., Sedlacek, J.D., Weston, P.A., Knight, W.L., Hill, Jr., S.J., D'Abramo, L.R., Daniels, W.H., Fuller, M.J., Montañez, J.L., 1995. Effects of complete and supplemental diets and organic pond fertilization on production of *Macrobrachium rosenbergii* and associated benthic microinvertibrate populations. Aquaculture 138, 169-180.

Turker, H., Eversole, A.G., Brune, D.E., 2003. Filtration of green algae and cyanobacteria by Nile tilapia, *Oreochromis niloticus*, Lower Case in the Partitioned Aquaculture System. Aquaculture 215, 93-101.

Verdegem, M.C.J., Eding, E.H., Sereti. V., Munubi, R.N., Satacruz-Reyes, R.A., van Dam, A.A., 2005. Similarities between microbial and periphytic biofilms in Aquaculture systems. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 191-205.

Vermaat, J.E., Hootsmans, M.J.M., 1994. Periphyton dynamics in a temperature-light gradient. In: Van Vierssen, W., Hootsmans, M.J.M., Vermaat, J.E. (Eds.), Lake Veluwe, a macrophyte-dominated system under eutrophication stress, Geobotany 21. Kluwer Academic Publishers, The Netherlands, pp. 193-212.

Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S., 1999. The potential of periphyton based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton). Aquac. Res. 30, 409-419.

Wetzel, R.G., 2005. Periphyton in the aquatic ecosystem and food webs. In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, Exploitation and Management. CABI Publishing, Wallingford, UK, pp. 51-69.

Wohlforth, G.W., Hulata, G., Karplus, I., Halevy, A., 1985. Polyculture of freshwater prawn *Macrobrachium rosenbergii* in intensively matured ponds, and the effect of stocking rate of prawns and fish on their productions characteristics. Aquaculture 46, 143-156.

Zimmermann, S., New. M.B., 2000. Grow-out systems polyculture and integrated culture. In: New, M.B., Valenti, W.C. (Eds.), Freshwater prawn farming. The farming of *Macrobrachium rosenbergii,* Blackwell Science, Osney Mead, Oxford, England, pp.187-202.

Figure captions

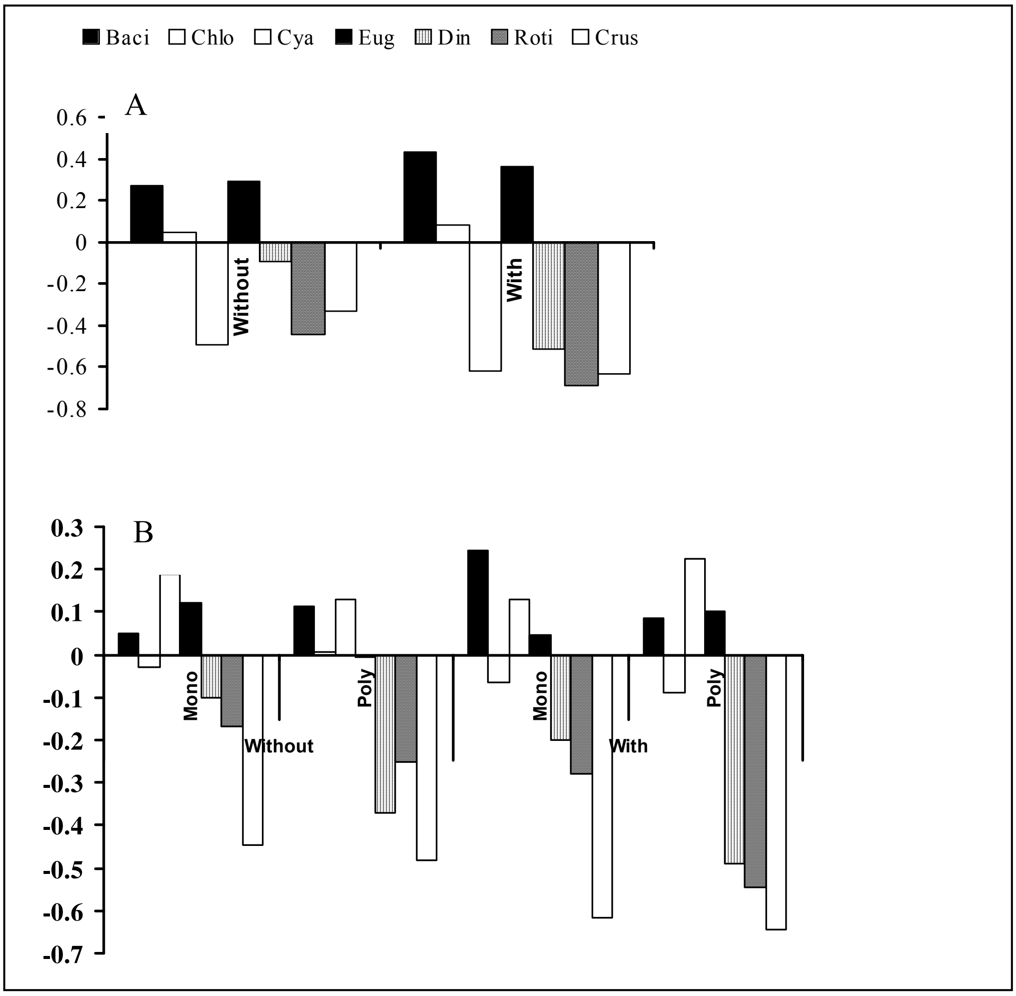
Figure 1.

Electivity indices of prawn (A) and tilapia (B) in different groups of plankton in different treatments. Mono amd Poly indicate monoculture of tilapia and polyculture of tilapia with prawn, and Without and With indicate the presence or absence of bamboo as substrate, respectively. Baci, Chlo, Cya, Eug, Din, Toti and Crus indicate Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, Dinophyceae, Rotifera and Crustaceae, respectively. Values on Y axis indicate the electivity indices (EI), calculated as, EI = (Pg-Pw)/(Pg+Pw)(Ivlev, 1961), where Pg is the relative content of any food ingredient in the gut expressed as % of the total ration, and Pw is the relative proportion of the similar item in the pond water expressed as %. Positive values of EI indicate the selection of a particular food item while a negative value indicates avoidance.

Figure 2.

Total expenditure and income from different cultured systems and their relationship with net profit margin (%). FS, FS_0, F_0S and Control indicate treatment with both feed and substrate, with feed plus no substrate, no feed plus substrate and without both feed and substrate, respectively.

Figure 1


Baci
Chlo
Cya
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Crus
A
0.6
0.4
0.2
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-0.4
-0.6
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Without
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0.3
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Mono
Poly
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Mono
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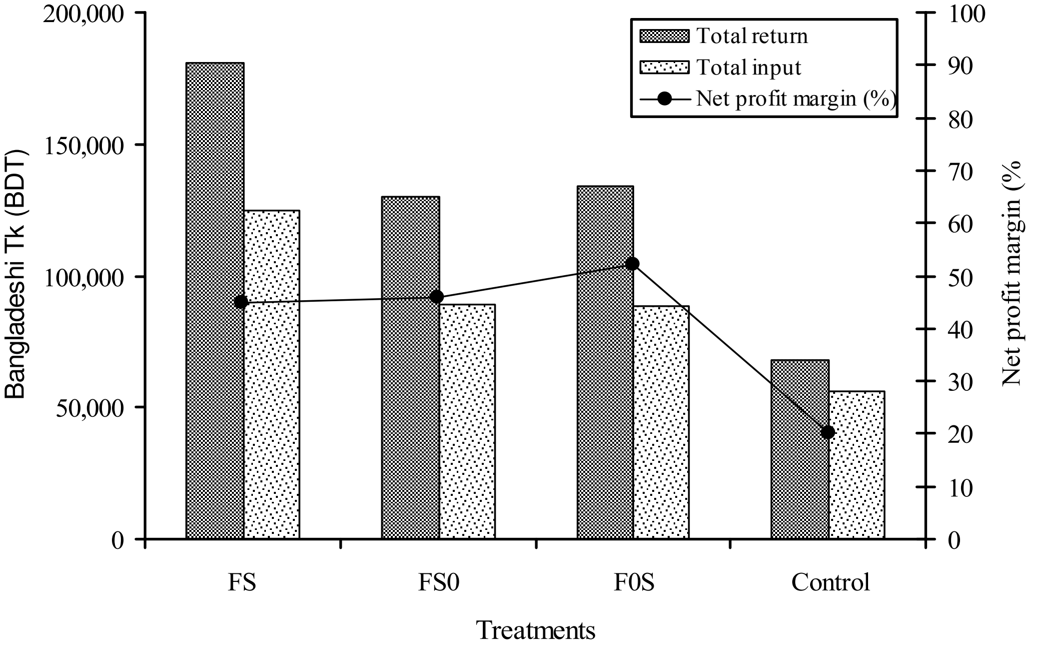
Figure 2



Bangladeshi Tk (BDT)
200,000
150,000
100,000
50,000
0
Net profit margin (%
100
90
80
70
60
50
40
30
20
10
0
Total return
Total input
Net profit margin (%)
FS
FS0
F0S
Control
Treatments

Summary

In recent years, the concept of periphyton-based aquaculture has been tested and applied in aquaculture. Positive effects of substrate addition for periphyton development included increasing food supply and providing shelter for culture animals. The aim of this project was to develop a low-cost culture technology for resource-poor farmers in South Asia. The technology is an extension of traditional brush-park fisheries in which bacteria, protozoa, fungi, phytoplankton, zooplankton, benthic organisms and a range of other invertebrates colonized the substrates installed in rivers and lakes, attracting many fishes. These microbial communities are an excellent food for fishes or crustaceans. The effects of the addition of substrates to ponds to stimulate periphyton development have been studied intensively, but for tilapia-prawn polyculture the methodology still had to be optimized. This thesis focused on the effects of substrate and artificial feed addition on the overall nutrient dynamics, growth, production and economics of tilapia-prawn polyculture. A stepwise approach was followed.

The first step was to quantify the effects of substrate and prawn addition to tilapia ponds on total productivity and to quantify the contribution of substrates to tilapia and prawn production. In the second step, the best tilapia:prawn stocking ratio for periphyton ponds was determined. The third step was to optimize the stocking density in periphyton ponds while using the previously determined best stocking ratio. In the fourth step, the optimized tilapia-prawn polyculture package was compared to a traditional (fertilizer, no feed, no substrate), a fed traditional and a periphyton-based fed production system in terms of total fish production and economic benefits. The final step combined all data from the previous experiments into a multivariate analysis looking at the relationships between production and pond ecology related parameters.

In the first study, a considerable overlap was found between the feeding niches of tilapias and prawns (Chapter 2). Substrate addition improved the food conversion ratio in tilapia ponds by 32%, while stocking of prawns resulted in an additional 12% improvement. On average, substrate addition resulted in a 40% higher net yield of tilapia in monoculture and 56% in tilapia-prawn polyculture. The individual weight gain of tilapia increased by 30% due to addition of substrates in both mono- and polyculture ponds. Substrate addition resulted in

9% higher survival and 45% higher tilapia production while prawn survival increased 75% and production 127%. The highest total yield (2,445 kg ha^{-1} tilapia and 141 kg ha^{-1} prawn) over a 145 days culture period was recorded in periphyton-based tilapia-prawn polyculture ponds. The positive effects on survival and production of tilapia of additional prawn stocking indicated mixed culture of these two species is promising and options for further optimization should be explored.

In Chapter 3, the tilapia:prawn stocking ratio in the periphyton-based production system was optimized. The survival of tilapia was higher in polyculture whereas that of prawn was higher in monoculture. The periphyton biomass decreased with increased stocking density of tilapia, indicating the preferential feeding of tilapia on periphyton. The highest production (1,623 kg tilapia and 30 kg prawn ha^{-1}) was recorded in the combination of 75% tilapia and 25% prawn at a total density of 20,000 ha^{-1}. The cost benefit analysis revealed that the monoculture of tilapia and addition of prawn to the tilapia ponds at any ratio were profitable.

Chapter 4 explored the optimum stocking density of tilapia and prawn in periphyton-based polyculture ponds. Total stocking densities of 20,000, 30,000 and 40,000 individual ha^{-1} were tested. Periphyton biomass on the substrates increased during the first 2 months of culture and then decreased for all stocking densities. Survivals of tilapia and freshwater prawn were higher at low and medium (83-86% for tilapia and 51-57% for prawn) stocking densities than at high (78% for tilapia and 39% for prawn) stocking density. The combined net yield of tilapia and freshwater prawn were higher at medium (2,372 kg ha^{-1}) and high (2,303 kg ha^{-1}) than at low (1,641 kg ha^{-1}) stocking density. The net profit margin was highest (69%) at medium (30,000 ha^{-1}) and lowest (44%) at high (40,000 ha^{-1}) stocking density.

Chapter 5, compared tilapia-prawn production in non-fed periphyton-based ponds with traditional (fertilizer, no feed, no substrate), fed traditional and periphyton-based fed ponds. The food conversion ratio (FCR) of tilapia was 13% lower in periphyton-based fed ponds than in fed traditional ponds. Both substrate and feed addition influenced tilapia harvesting weight but not prawn harvesting weight. Survival, final weight gain and net yield of both tilapia and prawn were highest in periphyton-based fed ponds and lowest in traditional ones. The absence of significant differences in survival, harvesting weight and net yield of both tilapia and prawn between periphyton-based and feed driven ponds indicate that periphyton is a good alternative to supplemental feeding. The combined net yield was 59% higher in periphyton-based fed ponds, 48% in fed traditional and 47% in non-fed periphyton-based ponds compared to traditional ponds. The net profit margin was similar between fed traditional and non-fed periphyton-based ponds. Interestingly, although the contribution of prawn to the total production in quantity, as compared to tilapia, was very low (4-7%), it contributed 22-32% to the total sales value. Therefore, even at low density, freshwater prawns contributed an important fraction of farming revenue in tilapia-prawn periphyton-based ponds.

In Chapter 6, all water quality, periphyton and fish production data from the previous four experiments were merged into a single dataset and re-analysed using multivariate statistics, to gain deeper insights into the functioning of the ecosystem. The objective was to explore the effects of tilapia and prawn density, presence of substrates for periphyton growth and supplemental feed on the water quality in tilapia-prawn polyculture ponds. The main sources of water quality variability were due to photosynthesis, nutrient uptake, respiration and decomposition. Substrates for periphyton development resulted in a more favourable environment for the cultured organisms and provided an extra source of food for culture animals. The positive effects on the overall pond ecology supported the conclusions from the previous studies that tilapia:prawn polyculture in periphyton-based ponds is a reliable production system that improves farming benefits considerably.

In the final discussion (Chapter 7), the applicability of this new technology was reviewed, giving attention to developmental aspects and contemplating ideas for future research.

Samenvatting

Recent werd het concept van het gebruik van periphyton in de aquacultuur veelvuldig getest en toegepast. Positieve effecten van substraattoevoeging voor de ontwikkeling van periphyton zijn een toename van het voedselaanbod en het verstrekken van schuilplaatsen voor de gekweekte dieren. De doelstelling van dit project was voor arme boeren in zuidoost Azië een betaalbare technologie te ontwikkelen die gebaseerd is op een traditionele visserijtechniek bestaande uit het creëren van 'vegetatieparken' in open water. Op deze vegetatie ontwikkelen gemeenschappen van bacteriën, protozoën, schimmels, fytoplankton, zooplankton, benthos en andere kleine ongewervelde diertjes die een rijke voedselbron zijn en vissen of garnalen. Substraattoevoeging in visvijvers imiteert deze 'vegetatieparken'. Deze techniek werd uitvoerig onderzocht en wordt veelvuldig toegepast, maar werd nooit geoptimaliseerd voor tilapia-zoetwatergarnaal[1] polycultuur. Daarom concentreerde deze studie zich op de effecten van substraattoevoeging en bijvoederen op de nutriënthuishouding, groei, productie en economische haalbaarheid van tilapia-garnaal polycultuur systemen. De studie werd in verschillende stappen uitgevoerd.

De eerste stap kwantificeerde de effecten van substraat- en garnaaltoevoeging in tilapia vijvers op de totale productiviteit. Verder werd ook gekeken wat het effect was van substraattoevoeging op de productie van enerzijds tilapia en anderzijds garnaal. De volgende stap was het bepalen van de optimale tilapia-garnaal bezettingsratio. In stap drie werd de totale bezettingsdichtheid geoptimaliseerd, gebruik makende van de in stap 2 bepaalde bezettingsratio. In stap 4 werden de totale productie en de economische haalbaarheid vergeleken in vijvers bezet met de optimale tilapia-garnaal bezettingsratio en -dichtheid, met of zonder substraattoevoeging en met of zonder bijvoedering. In stap 5 werden alle data van de voorgaande experimenten gecombineerd in een multivariate analyse waarbij het verband tussen productiegerichte en ecologische parameters werd onderzocht.

In de eerste studie werd er een aanzienlijke overlap tussen de voedingniches van tilapia en garnaal gevonden (Hoofdstuk 2). Substraattoevoeging verbeterde de voederconversie in tilapia vijvers met 32%. Het uitzetten van garnalen in tilapia vijvers verbeterde de voederconversie met 12%. Gemiddeld veroorzaakte substraattoevoeging een

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[1] zoetwatergarnaal wordt verder in de samenvatting verwezen naar als 'garnaal'

40% toename van de netto tilapiaproductie in monocultuur en 56% toename in tilapia-garnaal systemen. De individuele gewichtstoename van tilapia werd 30% verhoogd dank zij substraattoevoeging, zowel in mono- als in polycultuur. Substraattoevoeging resulteerde ook in een 9% betere overleving en een 45% hogere tilapiaoogst, terwijl bij garnaal de overleving 75% beter en de oogst 127% hoger lag. De hoogste totale opbrengst (2445 kg ha^{-1} tilapia en 141 kg ha^{-1} garnaal) na een 145-dagen productiecyclus werd gehaald in tilapia-garnaal periphyton vijvers. De positieve effecten van het uitzetten van garnaal op tilapia-overleving en -productie waren veelbelovend en er werd besloten te proberen tilapia-garnaal polycultuur verder te optimaliseren.

In Hoofdstuk 3 werd de tilapia-garnaal bezettingsratio in periphyton vijvers geoptimaliseerd. De overleving van tilapia was hoger in polycultuur. Daarentegen was de overleving van garnaal beter in monocultuur. De op het substraat aanwezige periphyton biomassa nam af bij toenemende bezettingsdichtheid van tilapia, wat er op wees dat tilapias bij voorkeur grazen op periphyton. De hoogste productie (1623 kg tilapia en 30 kg garnaal) werd verkregen bij een combinatie van 75% tilapia en 25% garnaal en een gecombineerde bezettingsdichtheid van 20,000 dieren ha^{-1}. Monocultuur van tilapia en alle geteste tilapia-garnaal bezettingsratios waren winstgevend.

In Hoofdstuk 4 werd onderzocht wat de optimale gecombineerde bezettingsdichtheid is bij een bezettingsratio of 3 tilapia : 1 garnaal. Bezettingsdichtheden van 20,000, 30,000 en 40,000 dieren ha^{-1} werden getest. De beschikbare periphytonbiomassa nam toe tijdens de eerste 2 maanden van de teelt en nam daarna af bij alle bezettingsdichtheden. De overleving van tilapia en garnaal waren hoger bij bezettingsdichtheden van 20,000 en 30,000 dieren ha^{-1} (83-86% voor tilapia en 51-57% voor garnaal) dan bij 40,000 dieren ha^{-1} (78% voor tilapia en 39% voor garnaal). Bij een gecombineerde bezettingsdichtheid van 20,000 dieren ha^{-1} was de totale productie lager (1,641 kg ha^{-1}) dan bij 30,000 (2,372 kg ha^{-1}) en 40,000 (2,303 kg ha^{-1}) dieren ha^{-1}. Het netto winst percentage was het hoogst bij een bezettingsdichtheid van 30,000 dieren ha^{-1} (69%) en het laagst bij 40,000 dieren ha^{-1} (44%).

In Hoofdstuk 5 werd een vergelijking gemaakt tussen vijversystemen bezet met de optimale tilapia-garnaal bezettingsratio en -dichtheid bepaald in Hoofdstukken 3 en 4. Alle vijvers werden bemest. In de helft van de vijvers werd substraat voor periphytonontwikkeling aangebracht (= periphyton vijvers), de andere vijvers waren substraatvrij. Van deze beide vijvertypes werd de helft bijgevoerd, de andere helft niet. De voederconversie was 13% lager in periphyton vijvers met bijvoedering dan in substraatvrije vijvers met bijvoedering. Substraattoevoeging en bijvoedering beïnvloedden het oogstgewicht van tilapia maar niet van garnaal. Overleving, individueel eindgewicht en netto productie waren het hoogst in gevoederde tilapia-garnaal periphyton vijvers, en het laagst in substraatvrije, niet-gevoerde vijvers. Het feit dat er geen verschil in effect van substraattoevoeging en van bijvoedering was op overleving, individueel eindgewicht en netto productie van zowel tilapia als garnaal duidt erop dat substraattoevoeging een goed alternatief is voor bijvoedering. De totale productie was 59% hoger in gevoederde periphyton vijvers, 48% in gevoederde substraatvrije vijvers en 47% in niet gevoederde periphyton vijvers dan in substraatvrije niet gevoederde vijvers. De netto winstmarge was vergelijkbaar tussen gevoederde substraatvrije en niet gevoederde periphyton vijvers. Ook als droeg garnaal slechts 4-7% bij aan de totale productie, garnaal vertegenwoordigde wel 22-32% van de totale verkoopswaarde van de oogst. Dus zelf bij de gebruikte lage bezettingsdichtheid droeg garnaal significant bij aan de inkomsten uit tilapia-garnaal polycultuur.

In Hoofdstuk 6 werden alle waterkwaliteits-, periphyton- en visproductiedata van de voorgaande 4 experimenten samengebracht in 1 databestand en geanalyseerd met multivariate technieken, met als doel ons inzicht in het functioneren van het vijverecosysteem te verdiepen. Daarbij werd vooral gekeken hoe factoren als tilapia- en garnaal-bezettingsdichtheid, aanwezigheid van substraat voor periphytonontwikkeling en bijvoedering de waterkwaliteit beïnvloeding in tilapia-garnaal vijvers. Variatie in waterkwaliteit werd vooral veroorzaakt door fotosynthese, nutriëntopname, respiratie en afbraakprocessen. Substraattoevoeging verbeterde de leefomgeving voor tilapia en garnaal en verschafte een extra voedselbron. De gevonden positieve effecten op de vijverecologie bevestigen het gevonden beeld van de vorige experimenten dat tilapia-garnaal polycultuur een betrouwbaar productiesysteem is dat het inkomen uit aquacultuur significant verbetert.

In de algemene discussie (Hoofdstuk 7) wordt, met aandacht voor ontwikkelingsrelevante aspecten, ingegaan op de toepasbaarheid van deze nieuwe technologie en worden mogelijkheden voor vervolgonderzoek verkend.

সারাংশ

সাম্প্রতিক কালে পেরিফাইটন ভিত্তিক মাছচাষ কার্যক্রমের গবেষণা করা হয়েছে এবং প্রাপ্ত ফলাফল মাছচাষের ক্ষেত্রেও প্রয়োগ করা হয়েছে। সাবস্ট্রেট স্থাপনের মাধ্যমে পেরিফাইটন উৎপাদনের ইতিবাচক দিকগুলো হল চাষযোগ্য প্রাণীর জন্য অধিকতর খাদ্য সরবরাহ ও আশ্রয়স্থলের সংস্থান করা। এই প্রকল্পের মুল উদ্দ্যেশ্য ছিল দক্ষিণ এশিয়ার দরিদ্র মৎস্য চাষীদের জন্য স্বল্প ব্যয় ভিত্তিক একটি লাগসই মাছচাষ কৌশল উদ্ভাবন করা। এটি হলো প্রথাগত কাঠা-কুয়া ভিত্তিক মৎস্য ব্যবস্থাপনার একটি পরিবর্তিত রূপ, যাতে নদী, হ্রদ বা অন্য কোন জলাশয়ের পানিতে বাঁশ, গাছের ডাল প্রভৃতি সাবস্ট্রেট হিসাবে স্থাপন করা হয় এবং স্থাপনকৃত সাবস্ট্রেটের উপরিভাগে ব্যাকটেরিয়া, এককোষী প্রাণী, ছত্রাক, উদ্ভিদপ্লাঙ্কটন, প্রাণীপ্লাঙ্কটন, তলদেশীয় জীব এবং অনেক অমেরুদন্ডী প্রাণী জন্মায় যা বিভিন্ন প্রজাতির মৎস্যকুলকে আকৃষ্ট করে। সাবস্ট্রেটের গায়ে জন্ম নেয়া এই অণুজীবসমুহ মাছ এবং চিংড়ি জাতীয় প্রাণীর জন্য উৎকৃষ্ট খাদ্য। পুকুরে সাবস্ট্রেট স্থাপনের মাধ্যমে পেরিফাইটন জন্মানো ও বর্ধন প্রক্রিয়া নিবিড়ভাবে পর্যবেক্ষণ করা হলেও তেলপিয়া-চিংড়ির মিশ্রচাষে এই ধরণের গবেষণা অদ্যাবধি করা হয়নি। এই থিসিসে পুকুরের সার্বিক পুষ্টির গতিবিদ্যা, মাছের দৈহিক বৃদ্ধি, মোট মাছ উৎপাদন এবং আয়-ব্যয় এর উপর সাবস্ট্রেট ও সম্পূরক খাদ্যের প্রভাব নিরুপণের বিষয়ে আলোকপাত করা হয়েছে। নিম্নে ধারাবাহিক বর্ণনা দেয়া হল।

গবেষণার প্রথম ধাপে তেলপিয়া চাষের পুকুরে মোট উৎপাদনের উপর সাবস্ট্রেট ও চিংড়ির প্রভাব এবং তেলাপিয়া ও চিংড়ি উৎপাদনে সাবস্ট্রেটের প্রভাব নিরুপণ করা হয়। দ্বিতীয় ধাপে, পেরিফাইটন ভিত্তিক মাছ চাষ পদ্ধতিতে তেলাপিয়া ও চিংড়ির মজুদ হারের সর্বোৎকৃষ্ট অনুপাত নিরুপণ করা হয়। তৃতীয় ধাপে পূর্ব নিরুপিত সর্বোৎকৃষ্ট মজুদ অনুপাতকে ঠিক রেখে হেক্টর প্রতি সর্বাধিক গ্রহণযোগ্য মজুদ ঘনত্ব নিরুপণ করা হয়। চতুর্থ ধাপে পূর্বে প্রাপ্ত সর্বোৎকৃষ্ট মজুদ অনুপাত ও ঘনত্বকে নির্ভর করে প্রচলিত পদ্ধতি (সারসহ, সম্পূরক খাদ্যবিহীন, সাবস্ট্রেট ব্যতীত), সম্পূরক খাদ্যসহ প্রচলিত পদ্ধতি এবং সম্পূরক খাদ্যসহ ও সম্পূরক খাদ্য ব্যতীত পেরিফাইটন ভিত্তিক তেলাপিয়া-চিংড়ি চাষের একটি গবেষণা করা হয় এবং এই পদ্ধতিগুলির মধ্যে মোট মাছের উৎপাদন ও আয়-ব্যয়ের তুলনামুলক পর্যালোচনাও করা হয়। শেষ ধাপে পূর্ববতী সকল গবেষণার ডাটাসমুহ একটি ডাটাসেটে সন্নিবেশিত করা হয় এবং বহুমাত্রিক বিশ্লেষণের মাধ্যমে মাছের উৎপাদন ও পুকুরের বাস্তুসংস্থানিক বিষয়াদির মধ্যে আন্ত:সম্পর্ক নিরুপণ করা হয়।

প্রথম গবেষণায়, তেলাপিয়া ও চিংড়ির প্রাকৃতিক খাদ্যে উল্লেখযোগ্য পরিমাণে সামঞ্জস্যপূর্ণতা দেখা যায় (অধ্যায়-২)। তেলাপিয়া চাষের পুকুরে সাবস্ট্রেট স্থাপন করায় খাদ্য রুপান্তর অনুপাত ৩২% হ্রাস পায়, অপরদিকে একই পুকুরে চিংড়ি মজুদের ফলে উক্ত অনুপাত অতিরিক্ত ১২% হ্রাস পায়। সাবস্ট্রেট স্থাপনের ফলে তেলাপিয়ার একক চাষের

ক্ষেত্রে প্রকৃত মোট উৎপাদন গড়ে ৪০%, তেলাপিয়া-চিংড়ির মিশ্র চাষে প্রকৃত মোট উৎপাদন ৫৬% এবং উভয় প্রকার (একক ও মিশ্র) চাষের ক্ষেত্রে তেলাপিয়ার দৈহিক বর্ধনের হার ৩০% বৃদ্ধি পায়। সাবস্ট্রেট স্থাপনের ফলে তেলাপিয়ার বাঁচার হার ৯% ও উৎপাদন ৪৫% এবং চিংড়ির বাঁচার হার ৭৫% ও উৎপাদন ১২৭% বৃদ্ধি পায়। পেরিফাইটন ভিত্তিক তেলাপিয়া-চিংড়ির মিশ্র চাষে ১৪৫ দিনে সর্বোচ্চ মোট উৎপাদন প্রতি হেক্টরে ২,৪৪৫ কেজি তেলাপিয়া এবং ১৪১ কেজি চিংড়ি পাওয়া গেছে। তেলাপিয়ার সাথে চিংড়ি যোগ করার ফলে তেলাপিয়ার বাঁচার হার ও উৎপাদনে ভাল প্রভাব - এই দুই প্রজাতির মিশ্র চাষের একটি উজ্জ্বল সম্ভাবনা লক্ষ্য করায় এ ধরণের মিশ্রচাষে প্রজাতিদ্বয়ের সর্বোৎকৃষ্ট মজুদ অনুপাত নির্ধারণ করার প্রয়োজনীয়তা দেখা দেয়।

তৃতীয় অধ্যায়ে, পেরিফাইটন ভিত্তিক চাষে তেলাপিয়া-চিংড়ি মজুদের সর্বোৎকৃষ্ট অনুপাত নির্ধারণ করা হয়। মিশ্রচাষে তেলাপিয়া এবং একক চাষে চিংড়ির বাঁচার হার অপেক্ষাকৃত বেশি ছিল। তেলাপিয়ার মজুদ হার বৃদ্ধির সাথে সাথে পেরিফাইটনের জীবভর কমতে থাকায় প্রতীয়মান হয় যে, পেরিফাইটন তেলাপিয়ার উৎকৃষ্ট খাদ্য। হেক্টর প্রতি মোট ২০,০০০ পোনার মধ্যে ৭৫% তেলাপিয়া এবং ২৫% চিংড়ি মজুদের ফলে ১২৫ দিনে সর্বোচ্চ ১,৬২৩ কেজি তেলাপিয়া এবং ৩০ কেজি চিংড়ি উৎপাদিত হয়েছে। আয়-ব্যয় বিশ্লেষণ করে দেখা যায় যে, তেলাপিয়ার একক চাষ এবং তেলাপিয়ার সাথে যে কোন অনুপাতে চিংড়ির মিশ্র চাষ উভয়ই লাভজনক।

চতুর্থ অধ্যায়ে, পেরিফাইটন ভিত্তিক তেলাপিয়া-চিংড়ি মিশ্র চাষের উভয় প্রজাতির সর্বাধিক গ্রহণযোগ্য মজুদ ঘনত্বকে নিরুপণ করা হয়েছে। প্রতি হেক্টরে উভয় প্রজাতির সর্বমোট ২০,০০০, ৩০,০০০ এবং ৪০,০০০ পোনা মজুদ করে গবেষণা পরিচালনা করা হয়। সাবস্ট্রেটের গায়ে উৎপাদিত পেরিফাইটন জীবভর প্রথম দুই মাসে বৃদ্ধি পেলেও পরবর্তী কালে সকল মজুদ ঘনত্বের ক্ষেত্রেই তা কমতে থাকে। তেলাপিয়া এবং চিংড়ির বাঁচার হার নিম্ন এবং মধ্যম ঘনত্বে (তেলাপিয়ার ক্ষেত্রে ৮৩-৮৬% এবং চিংড়ির ক্ষেত্রে ৫১-৫৭%) বেশী ঘনত্বের (তেলাপিয়ার ক্ষেত্রে ৭৮% এবং চিংড়ির ক্ষেত্রে ৩৯%) তুলনায় অধিক ছিল। উভয় প্রজাতির হেক্টর প্রতি ১০৫ দিনে সম্মিলিত প্রকৃত উৎপাদন মধ্যম মজুদ ঘনত্বে (২,৩৭২ কেজি) ও উচ্চ মজুদ ঘনত্বে (২,৩০৩ কেজি) নিম্ন মজুদ ঘনত্বের (১,৬৪১ কেজি) তুলনায় বেশি ছিল। প্রকৃত মুনাফা মধ্যম মজুদ ঘনত্বে (প্রতি হেক্টরে ৩০,০০০ পোনা) সর্বোচ্চ (৬৯%) এবং উচ্চ মজুদ ঘনত্বে (প্রতি হেক্টরে ৪০,০০০ পোনা) সর্বনিম্ন (৪৪%) ছিল।

পঞ্চম অধ্যায়ে, সম্পূরক খাদ্যবিহীন পেরিফাইটন ভিত্তিক তেলাপিয়া-চিংড়ি মিশ্র চাষের উৎপাদনের সাথে প্রচলিত পদ্ধতি (সার সহ, সম্পূরক খাদ্যবিহীন, সাবস্ট্রেট ব্যতীত), সম্পূরক খাদ্যসহ প্রচলিত পদ্ধতি এবং সম্পূরক খাদ্যসহ পেরিফাইটন ভিত্তিক পদ্ধতিতে তেলাপিয়া-চিংড়ি মিশ্র চাষের উৎপাদনের তুলনা করা হয়েছে। সম্পূরক খাদ্যসহ প্রচলিত পদ্ধতিতে চাষের তুলনায় সম্পুরক খাদ্যসহ পেরিফাইটন ভিত্তিক চাষের ক্ষেত্রে খাদ্য রুপান্তর অনুপাত ১৩% কম পরিলক্ষিত হয়। সাবস্ট্রেট স্থাপন এবং সম্পূরক খাদ্য প্রয়োগ তেলাপিয়ার আহরণকালীন ওজনকে প্রভাবিত করলেও চিংড়ির

আহরণকালীন ওজনকে প্রভাবিত করেনি। তেলাপিয়া এবং চিংড়ি উভয়ের বাঁচার হার, চূড়ান্ত ওজন বৃদ্ধি এবং প্রকৃত উৎপাদন খাদ্যসহ পেরিফাইটন ভিত্তিক চাষ পদ্ধতিতে সর্বোচ্চ এবং পেরিফাইটন ব্যতীত প্রচলিত পদ্ধতিতে সর্বনিম্ন পাওয়া গেছে। পেরিফাইটন ভিত্তিক চাষ এবং সম্পূরক খাদ্যসহ চাষ উভয়ের ক্ষেত্রে তেলাপিয়া ও চিংড়ির বাঁচার হার, আহরণকালীন ওজন এবং প্রকৃত উৎপাদনের মধ্যে তাৎপর্যপূর্ণ পার্থক্য না থাকায় প্রতীয়মান হয় যে, পেরিফাইটন সম্পূরক খাদ্যের একটি উত্তম বিকল্প। তেলাপিয়া ও চিংড়ির সম্মিলিত প্রকৃত উৎপাদন, প্রচলিত পদ্ধতির তুলনায় খাদ্যসহ পেরিফাইটন ভিত্তিক পদ্ধতি, খাদ্যসহ প্রচলিত পদ্ধতি এবং খাদ্য প্রয়োগ বিহীন পেরিফাইটন ভিত্তিক পদ্ধতিতে যথাক্রমে ৫৯%, ৪৮% এবং ৪৭% বেশী ছিল। খাদ্যসহ প্রচলিত পদ্ধতি এবং খাদ্য প্রয়োগ বিহীন পেরিফাইটন ভিত্তিক পদ্ধতি উভয়ের বেলায় প্রকৃত মুনাফা প্রায় একই রকম ছিল। মজার বিষয় হলো, মোট উৎপাদনে চিংড়ির অবদান (৪-৭%) তেলাপিয়ার তুলনায় বেশ কম হলেও বিক্রয় মূল্যের দিক থেকে চিংড়ির অবদান (২২-৩২%) উল্লেখ করার মত ছিল। সুতরাং পেরিফাইটন ভিত্তিক তেলাপিয়া-চিংড়ি চাষে কম ঘনত্বের চিংড়িও খামারের আয়ে গুরুত্বপূর্ণ অবদান রাখতে পারে।

ষষ্ঠ অধ্যায়ে, পুর্বোক্ত চারটি গবেষণায় প্রাপ্ত পানির গুণাগুণ, পেরিফাইটন ও মৎস্য উৎপাদন সংক্রান্ত উপাত্তসমূহ একত্রীভূত করে একটি একক ডাটাসেট তৈরী করা হয় এবং বাস্তুসাংস্থানিক অন্তর্নিহিত তাৎপর্য অনুধাবনের জন্য উক্ত ডাটাসেট পুনরায় বহুমাত্রিক পরিসংখ্যানগত বিশ্লেষণ করা হয়। মূল উদ্দেশ্যে ছিল তেলাপিয়া ও চিংড়ির মজুদ ঘনত্ব, পেরিফাইটনের জন্য সাবস্ট্রেটের উপস্থিতি এবং সম্পুরক খাদ্য প্রয়োগ তেলাপিয়া-চিংড়ির মিশ্র চাষের পুকুরের পানির গুণাগুণের উপর যে প্রভাব বিস্তার করে তা নিরুপণ করা। পানির গুণাগুণের মূল পার্থক্যসমুহ মূলতঃ সালোকসংশ্লেষন, পুষ্টি গ্রহণ, শ্বসন এবং পচনের উপর নির্ভরশীল। পানিতে সাবস্ট্রেট স্থাপনের ফলে উৎপাদিত পেরিফাইটন চাষের আওতাধীন মাছের জন্য অতিরিক্ত খাদ্য এবং অধিকতর উপযোগী পরিবেশ সৃষ্টি করে। পুর্বোক্ত গবেষণা সমূহে সমর্থিত হয় যে, পুকুরের সার্বিক বাস্তুসংস্থানের উপর সাবস্ট্রেটের ভাল প্রভাব পেরিফাইটন ভিত্তিক তেলাপিয়া-চিংড়ি মিশ্র চাষের একটি গ্রহণযোগ্য উৎপাদন পদ্ধতি যা খামারের আয়কে উল্লেখযোগ্য পরিমাণে বৃদ্ধি করে।

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Md. Sharif Uddin

Md. Sharif Uddin

Curriculum Vitae

Md. Sharif Uddin was born on 16 February, 1964 in the village Jaduboyra of the district Kushtia, Bangladesh. He is the eldest son of Mr. Md. Mahtab Uddin and Mst. Rizia Khatun. He completed his primary education at the Jaduboyra Primary School, secondary education (SSC) at the Jaduboyra High School and higher secondary education (HSC) at the Kumarkhali College. He obtained his Bachelor degree of Science in Fisheries (Honours) in 1986 from Bangladesh Agricultural University, Mymensingh and Master of Science (M.Sc.) in Aquaculture and Management in 1987 from the same University. He obtained first class in all the degrees.

He started his career as Farm Manager in the Department of Fisheries (DoF) in 1990, and as Bangladesh Civil Service (BCS) Officer for BCS (Fisheries) cadre service he joined as Upazilla (Subdistrict) Fisheries Officer in 1991. He had an opportunity to get a Diploma on General Aquaculture from KIFTC (Kanagawa International Fisheries Training Center), Japan. Md. Sharif Uddin enrolled as PhD student in December 2001 in the Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh with the financial support of the DFID-B through FTEP-II, Department of Fisheries (DoF), Bangladesh. Finally, he was awarded with the Sandwich Scholarship under the INREF-Pond and the EU-funded PondLive project of the Aquaculture and Fisheries Group, Wageningen University and Research Centre (WUR), the Netherlands to obtain PhD degree.

He attended the conference of the 7th Asian Fisheries Forum held at Penang, Malaysia and presented a piece of his PhD research. He was awarded with the best student abstract award by the Asian Fisheries Society and World Aquaculture Society, Asia-Pacific Chapter. He also attended two international and one national conference and presented papers in relation to his PhD project. Mr. Sharif is an active member of different renowned societies such as Krishibid Institution of Bangladesh, Bangladesh Fisheries Society, Bangladesh Fisheries Research Forum, Asian Fisheries Society, World Aquaculture Society etc.

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List of Publications

Thesis related articles:

Uddin, M.S., Azim, M.E., Wahab, A., Verdegem, M.C.J., 2006. The potential of mixed culture of genetically improved farmed tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. Aquac. Res. 37, 241-247.

Uddin, M.S., Farzana, A., Fatema, M.K., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia-prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development. Aquaculture (in press).

Uddin, M.S., Rahman, S.M.S., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., Verreth, J.A.J. The effects of stocking density on production and economics of Nile tilapia *(Oreochromis niloticus)* and freshwater prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems. Ready to submit.

Uddin, M.S., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., Verreth, J.A.J. Comparison of substrates and supplemental feeding on growth and production of tilapia *(Oreochromis niloticus)* and freshwater prawn (*Macrobrachium rosenbergii*) polyculture: a bioeconomic aspect. Ready to submit.

Uddin, M.S., Milsten, A., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., Verreth, J.A.J. The effects of stocking density, periphyton substrate and supplemental feed on biological processes affecting water quality in earthen tilapia-prawn polyculture ponds. Ready to submit.

Other publications:

Azad, M.S., **Uddin, M.S.,** Kadir, M.A., Hossain, M.A.R., 2004. Rearing of post larvae of freshwater prawn, *Macrobrachium rosenbergii* in hapa system in pond environment. Progress. Agric. 15(1), 213-218.

Fatema, M.K., **Uddin, M.S.,** Rahman, S.M.S., Wahab, M.A., 2005. Community composition of plankton and periphyton on tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) monoculture and polyculture in periphyton based systems. Progress. Agric. 16(2), 183-191.

Fatema, M.K., **Uddin, M.S.,** Wahab, M.A., Haq, M.S., Ahmed. Z.F., 2003. Effects of substrate used and fish species combination on water quality parameters and composition of plankton and periphyton. Bangladesh J. Fish. 26 (1-2), 73-82.

Hossain, M.F., Fatema, M.K., Haq, M.S. Haque, M.M., **Uddin, M.S.,** 2005. Inclusion of Small Indigenous Species (SIS) *Puntius ticto* in carp polyculture system. Bangladesh J. Fish. 29 (1-2), 69-78.

Kunda, M., Dewan, S., Uddin, M.J., Karim, M., Kabir, S., **Uddin, M.S.,** Length-weight relationship, condition factor and relative condition factor of *Macrobrachium rosenbergii* in rice fields. (Accepted in Asian Fisheries Science).

Kunda, M., Dewan, S., Uddin, M.J., Yousuf, A.M., **Uddin, M.S.,** 2004. Length-weight relationship, Condition factor and Relative condition factor of *Barbodes gonionotus*, *Amblypharyngodon mola* and *Cyprinus carpio* in rice fields. Progress.Agric. 15(1), 159-165.

Mridha, M.A.R., Lipi, S.Y., Narijo, N.T. **Uddin, M.S.,** Kabir, M.S., Karim, M., 2005. Determination of Bio-chemical composition of *Cirrhinus reba* (Hamilton-1822) from Jessore, Bangladesh. J. Sc. & Tech. Univ. Peshwar 29 (1), 1-5.

Mridha, M.A.R., Narijo, N.T., **Uddin, M.S.,** Kabir, M.S., Chowdhury, M.B.R., Kadir, M.A., 2005. Investigation of bacterial flora in Catla (*Catla catla*) associated with EUS (epizootic ulcerative syndrome) in a commercial fish farm. Sindh Univ. Res. Jour. (Sci. Ser.) 37(1), 1-8.

Mridha, M.A.R., Narijo, N.T., **Uddin, M.S.,** Kabir, M.S., Karim, M., Chowdhury, M.B.R., 2005. Resistance of *Aeromonas* spp. in the fish, *Catla catla*, against some antibacterial agents. Pakistan J. Zool. 37(2), 158-161.

Mridha, M.A.R., **Uddin, M.S.,** Chowdhury, M.B.R., Kabir, M.S., Nareijo, N.T., 2004. Investigation of the Resistance of *Pseudomonas* sp. in the carp *Catla catla* to some Antibacterial Agents. Sindh Univ. Res.Jour. (Sci. Ser.), 36(1), 19-24.

Nahiduzzaman, M., Saklaen, G., **Uddin, M.S.,** Mridha, M.A.R., Azad, M.S., 2006. Performance assessment of sustainable aquaculture practices in small-scale farming system of Bogra and Joypurhat district in Bangladesh. Bangladesh J. Prog. Sci. & Tech. 4(2), 187-190.

Rahman, M.M., Nahiduzzaman, M., Khan, M.R.I., Azad, M.S., **Uddin, M.S.,** 2007. Effects of stocking size on growth, survival and yield of carps in polyculture systems. Bangladesh J. Prog. Sci. & Tech. 5(1), 177-180.

Uddin, M.S., Azim, M.E.,. Wahab, M.A, Haq , M.S., 2004. Optimization of stocking ratios of the GIFT strain of Nile Tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) in periphyton-based system. Proc. ISTA 6, Manila, 600-609.

Uddin, M.S., Fatema, M.K., Rahman, S.M.S., Ahmed, Z.F., Wahab, M.A., 2005. Feeding relation and dietary overlap of tilapia (*Oreochromis niloticus*) and prawn (*Macrobrachium rosenbergii*) and its effect on pond fish production in a periphyton based system. Progress. Agric. 16(2), 169-181.

Uddin, M.S., Islam, M.A., Mridha, M.A.R., Zaman, M.N., 2001. Effects of stocking densities on the growth of catfish *Clarias batrachus* (Linnaceus). Bangladesh J. Fish. Vol. 24 (1-2), 101-108.

Uddin, M.S., Verdegem, M.C.J., Azim, M.E., Wahab, M.A., 2007. Periphyton-based tilapia-prawn polyculture. New dimension in aquaculture. May support organic status. Global Aquaculture Advocate 10(1), 50-53.

Presentations:

Uddin, M.S., Farzana, A., Wahab, M.A., Fatema, M.K., Azim, M.E., 2004. A bioeconomic evaluation of tilapia monoculture and polyculture with freshwater prawn with and without periphyton substrates. 7th Asian Fisheries Forum. 30 Nov.-4 Dec. 2004, Penang, Malaysia, AFF (pp. 82).

Uddin M.S., Fatema, M.K., Wahab, M.A., Azim, M.E., 2005. Effects of periphyton substrates and addition of freshwater prawn (*Macrobrachium rosenbergii*) on pond ecology and tilapia (*Oreochromis niloticus*) production. World Aquaculture Society. World Aquaculture, May 09-13, 2005, Bali, Indonesia, WAS (pp. 662).

Wahab, M.A., Rakibullah, A.J.M., **Uddin, M.S.,** Masud, O.A., 2005. Evaluation of production performance of GIFT strain of Tilapia (*Oreochromis niloticus*) in polyculture with common carp (*Cyprinus carpio*) and with two major carps (*Catla catla* & *Labeo rohita*). World Aquaculture Society. World Aquaculture, May 09-13, 2005, Bali, Indonesia, WAS (pp. 685).

Azim, M.E., **Uddin, M.S.,** Rahman, S.M.S., Wahab, M.A., Verdegem, M.C.J., 2006. Optimum stocking density of Nile tilapia (*Oreochromis niloticus*) and Freshwater prawn (*Macrobrachium rosenbergii*) in periphyton-based polyculture system. 9-13 May, 2006, Florence, Italy.