The Potential of Periphyton-based Aquaculture Production Systems

Doctoral Dissertation

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Mohd. Ekram-Ul-Azim

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Stellingen (Propositions)

1. *Labeo rohita* is a predominantly periphyton grazer, realizing excellent growth and production in periphyton-based aquaculture systems.
   (this Thesis)

2. By providing a substrate area similar to the pond water surface area, both autotrophic and fish production can be doubled.
   (this Thesis)

3. The expanded aquaculture industry relying heavily on fish meal poses a threat, not only to capture fisheries, but also to itself.
   (Naylor et al., 2000 in Nature)

4. More attention should be given to research of polyculture and integrated cultures, especially targeting fish species that feed low in the food chain.
   (Professor E.A. Huisman in his farewell message, January, 2001)

5. The practice of aquaculture should be pursued as an integral component of development, contributing towards sustainable livelihoods for poor sectors of the community, promoting human development and enhancing social well-being.
   (Bangkok declaration, Aquaculture development beyond 2000)

6. Mechanization and automation save money, time and resources. However, if applied in a dogmatic way, they reduce creativity and enhance the wealth of few at the expense of many.

7. In a world where freshwater will become increasingly scarce, increasing fish production concurrently with sustainability is a priority as well as a challenge for scientists.

8. Machee Bhatee Bangali (Fish and rice make the People of Bangladesh).
   (Bangladeshi proverb)

Stellingen belonging to the thesis
"The potential of periphyton-based aquaculture production systems"
M.E. Azim
Wageningen, 10 December, 2001

Dedicated to
my wife and daughter

Abstract

Azim, M.E., 2001. The potential of periphyton-based aquaculture production systems. Ph.D. Thesis, Fish Culture and Fisheries Group, P.O. Box 338, 6700 AH Wageningen, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, The Netherlands.

The overall objective of this study was to determine the technical and economical performance of periphyton-based aquaculture systems in Bangladesh. It thus addressed one of the key constraints for the poor to benefit from fish culture - limited access to resources such as fertilizers and feeds - while trying to maximize the conversion of these resources into fish. The suitability of four locally available substrates for periphyton growth was evaluated and the optimal fertilization regime determined. The production and growth of four indigenous species, rohu *Labeo rohita* (Hamilton), catla *Catla catla* (Hamilton), kalbaush *L. calbasu* (Hamilton) and gonia *L. gonius* (Linnaeus), was evaluated under single species and polyculture conditions. The species were selected on the basis of their feeding behaviour, ease of culture and high market demand. The periphyton production rate was around 2.5 g of ash free dry matter m^{-2} d^{-1}. Considering the total periphyton substrate area in the pond, this rate can support an estimated fish production of 5,000 kg ha^{-1} y^{-1}. Periphyton production effectively doubled the autotrophic C production, while no trade-off in production between phytoplankton and periphyton communities was observed. The nutritional quality of periphyton was adequate to support the dietary needs of the experimental fish. Nitrogen retention in fish in substrate-based systems was about 1.6 times higher than in control systems without substrate. As periphyton substrate, bamboo (*Bambusa* sp.) proved better than hizol branch (*Barringtonia* sp.), bamboo side shoot or jute stick (*Corchorus* sp.) in terms of nutritional quality and periphyton productivity. Jute stick, however, provided better economical returns.

In single species culture, fish yields of rohu and kalbaush increased on average 80% compared to control systems without substrate, whereas with gonia, no significant production increase was observed. In periphyton-based polyculture systems, 70-180% greater fish production compared to controls was obtained. A three species periphyton-based polyculture technology was developed. Using the 75 m^2 experimental freshwater ponds, provided with a substrate surface area approximately equal to the pond water surface area and stocked at the rate of 6,000 rohu, 4,000 catla and 1,500 kalbaush (total 11,500 juveniles) per hectare, a fish production of 2,306 kg was achieved within a 90-days culture period.

A pilot scale trial in an extended number of household ponds, distributed over different agro-ecological zones of the country, has been proposed before dissemination of this technology for wider use as a means of poverty alleviation and nutritional security in Bangladesh and elsewhere in the region.

Key words: Periphyton; Biofilm; Artificial substrates; Pond productivity; Tropical aquaculture; Monoculture; Polyculture; Fertilization; Proximate composition; Stocking ratio; Stable isotope ratio; Nutrient efficiency; Production economics; Indian major carps; *Catla catla*; *Labeo rohita*; *Labeo gonius*; *Labeo calbasu*.

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Chapter 1

General Introduction

Status of inland aquaculture production in Bangladesh

Since the 1970s, global aquaculture production has increased 10 percent annually and is now the fastest growing food production sector in many countries. Today, Asia's share is about 92 percent of total aquaculture in the world. About 60 percent of the total production is produced in freshwater. Finfish accounts for approximately 98 percent of the freshwater aquaculture production. The development of inland aquaculture is seen as an important factor contributing to food security in Asia, particularly in the land-locked countries (FAO, 2000). The top ten inland aquaculture producing countries and their freshwater aquaculture production are shown in Table 1. The production is dominated by Asian countries particularly China, India, Indonesia and Bangladesh, which together account for 90 percent of the world aquaculture production of freshwater species. Although Bangladesh ranks fourth in respect to total freshwater aquaculture production, the country has the highest production per unit surface area in the world (Table 1).

Fish and fisheries have been an essential part of the life and culture of the people of Bangladesh from time immemorial. Bengali people were popularly referred to as *Macche-Bhate Bangali* (fish and rice make Bengali). The country is blessed with vast inland water bodies comprising 283,792 ha of floodplains, 1,031,563 ha of rivers and estuaries, 114,161 ha of beels (natural shallow depressions connected to open waters), a 68,800 ha man-made reservoir (Lake Kaptai), 215,000 ha of inland pond and ditches, 5,488 ha of oxbow lakes and 141,353 ha of shrimp farms. The total fish production in 1999-2000 was 1.66 million metric tonnes (FRSS, 2001). Inland fisheries contributed 1.32 million metric tonnes of which 49 percent came from aquaculture especially from pond culture (85 percent).

The fisheries sub-sector in Bangladesh plays an important role in alleviating protein deficiency, generating employment, reducing poverty and earning foreign exchange. This sub-sector contributes 5 percent to GDP representing 16.7 percent of the agriculture sector. Fish and fishery products are the third export commodity of Bangladesh contributing 6.28 percent of the total export value. About 1.2 million people are engaged full-time, and 12 million people part-time in this sector. Fish is the major animal protein source contributing 63 percent of total intake. Annual per capita fish consumption is about 12 kg against the minimum requirement of about 18 kg (FRSS, 2001). Nowadays, fish prices are beyond the reach of poor people. Supplies lag severely behind demand of the fast growing population, in spite of the recent growth in inland fish production. Prospects to obtain more animal protein from livestock are not bright, because there is an increased competition to grow food grains for human population instead of producing food grains and leaving lands fallow to provide food for livestock. Therefore, fish production in Bangladesh has to double to ensure the minimum supply of protein to the human population.

Table 1

The top-10 freshwater aquaculture production countries in 1996 (Source: FAO, 1998)

Country	Production (in x1000 metric tonnes)	Percent of world production	Fish production per square kilometer (kg)
China	10,895	76.7	1,139
India	1,300	9.1	405
Indonesia	378	2.7	193
Bangladesh	355	2.5	2,465
USA	281	2.0	29
Thailand	208	1.5	405
Philippines	90	0.6	300
Taiwan	79	0.6	2,194
Chile	54	0.4	71
Egypt	53	0.4	53
Other countries	520	3.7	-
Total	14,213	100.0	

The annual growth in inland fisheries production through capture fisheries in Bangladesh increased 5 percent annually over the last ten years but is gradually reducing (Figure 1). Possible causes are (1) habitat destruction due to construction of flood control, drainage, irrigation embankments and barrages over the major rivers in the upstream areas, (2) over-fishing, (3) water abstraction for irrigation and (4) reclamation of land for human settlement. These developments have also caused siltation, soil erosion, and destruction of rich mangrove forests. These problems have further been aggravated by the increased pollution from agricultural inputs, industrial wastes, sewage runoff as well as municipal wastes thrown out to the major rivers along the major cities of the country (NCS, 1996).

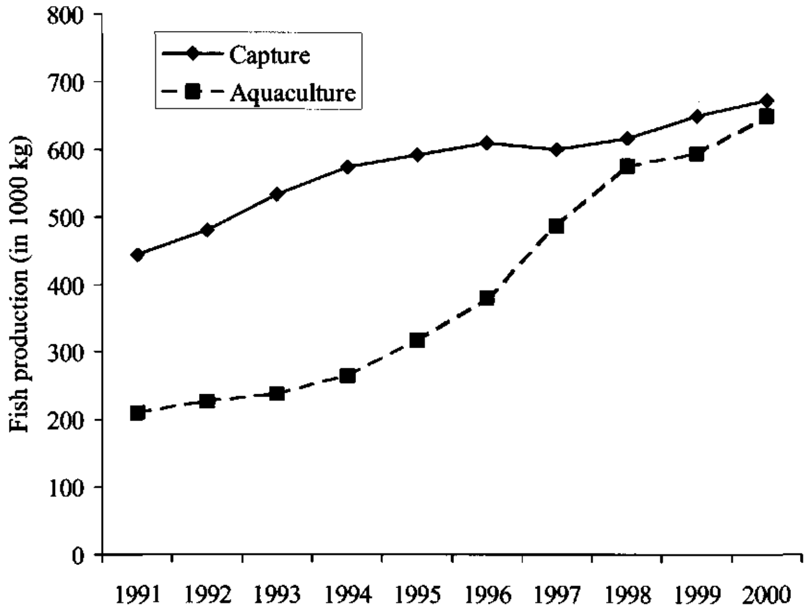

Figure 1. Trends of inland capture and aquaculture production in Bangladesh (Source: FRSS, 2001)

Contrarily, since 1991, the annual growth rate in aquaculture production is about 14 percent (Figure 1). The growth in production was due to (1) expansion of pond culture area by re-using derelict ponds and by excavating new ones and (2) increased productivity, mainly on the basis of technological innovations proposed by research institutes and extension agencies (Gupta et al., 1999; Alam and Thomson, 2001).

Most of the fish farmers in Bangladesh traditionally apply cow manure, household wastes and compost to their fishponds on an irregular basis. Inorganic fertilizers are used in some commercial farms and projects to enhance natural production. Farmers also apply supplementary feeds to their ponds on an irregular basis. The common supplementary feeds in Bangladesh are rice bran, wheat bran, oil cake and some other agricultural wastes. However, there is a severe competition for these agricultural wastes, mainly for raising livestock and for fuel (Azim et al., 1998). Nowadays, alternative sources like aquatic vascular plants and soft plant leaves, especially fresh duckweed (*Lemna* sp.) and banana leaves, are being used in Bangladesh (Azim and Wahab, 1998).

Traditionally, several combinations of 6-7 species of carp are raised in polyculture systems in Bangladesh. This culture practice often lacks a sound scientific basis. Farmers are often disappointed because of the complex nature of the technology, and find it difficult to obtain fingerlings of all species at time of stocking (Wahab et al., 1995). In addition, some of the exotic species stocked together with indigenous species exerted antagonistic behaviour, or competed for the same dietary resources (Dewan et al., 1991; Wahab et al., 1994). There is a need to develop simpler polyculture production systems, using less species, and preferably indigenous ones.

Rural aquaculture in Bangladesh is increasingly recognised as a way to improve the livelihoods of poor people (Lewis, 1997; Gupta et al., 1999; Hussain, 1999). Aquaculture is not only seen as an important contributor to development, but also as an important tool for increasing food security (FAO, 2000). Rural aquaculture, including enhanced fisheries and culture-based fisheries, has made significant contributions to the alleviation of poverty. This was done directly by small-scale farming households raising aquatic organisms for domestic consumption and/or income generation. Indirectly this was done by providing employment and by supplying low-cost fish in local markets. In Bangladesh, where most fish farmers are relatively poor, there is vast potential for the poorer section of the society to become new aquaculturists. There are wide opportunities for the poor to integrate aquaculture into their existing farming systems and thus, there is still room for growth in aquaculture production, the majority to be realized through efforts by poor people (Lewis, 1997; FAO, 2000; Alam and Thomson, 2001).

Periphyton-based aquaculture

The term 'periphyton' in classical limnology refers to the microfloral community living attached to the surfaces of submerged objects in water (Wetzel, 1983). This definition does not include fungal, bacterial, protozoan and other attached animal components, which are included in the German word 'Aufwuchs'. Depending on the substrate types, periphyton communities are again subdivided as 'Epilithon' grown on rock, 'Epipelon' on mud or silt, 'Epipsammon' on sand and 'Epiphyton' on submerged macrophyte substrates. In microbiology, periphyton is often referred to as 'Biofilms' (Nielsen et al., 1997; Shankar and

Mohan, 2001). In aquaculture, the term periphyton has been used in a broader sense. Throughout this thesis, periphyton is defined as the entire complex of sessile aquatic biota attached to the substratum including associated detritus and microorganisms. Thus, the periphyton community comprises bacteria, fungi, protozoa, phytoplankton, zooplankton, benthic organisms and a range of other invertebrates and their larvae. Any material providing surface area, including coral reef, branches of different trees, higher aquatic plants, bamboo, PVC pipes, etc., can be used for periphyton production.

The idea of periphyton-based aquaculture was originally derived from traditional fishing methods, such as the 'acadjas' of Côte 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 bamboo are established in lakes, lagoons or rivers, and the fish are attracted by the provision of shelter from predators, a suitable breeding habitat and the availability of natural food. These unique tools used in capture fisheries have recently been considered as models for novel periphyton-based aquaculture systems in managed ponds, mostly in the resource-poor countries.

State of knowledge

Brush parks in open water bodies

Welcome (1972) first reported on the 'acadjas' systems in the coastal lagoons in several West-African countries and particularly in Benin. Productions in the range of 4-20 tonnes ha^{-1} y^{-1} have been reported from acadja systems. However, the acadja practices caused serious social and environmental conflicts (Pliya, 1980): conflicts between the acadja owners and lagoon fishermen since the acadja attracts most of the fishes from the wild stock; conflicts between acadja and navigation since the acadja occupies considerable space; environmental degradation due to deforestation from intensive needs for wood.

The "katha" fishery in Bangladesh (also called "jhag", "katta" or "jhata"; Wahab and Kibria, 1994) is similar to the acadja fishery. Kathas are constructed from the branches of local trees such as hizol (*Barringtonia* sp.), wax jambu (*Eugenia* sp.) or babla (*Acacia* sp.). These branches are piled up between a number of bamboo poles fixed in the bottom to keep the structure in place and to delimit the size of the katha. Kathas are usually built in secondary rivers or canals or in beel areas. Water hyacinth (*Eichhornia crassipes*) may be used to cover the katha. The whole structure can be 6-10 m long, 2-6 m wide and approximately 1-2 m deep. Kathas are usually operated for 5-7 months each year in dry season and are fished 3-4 times during each production period. For fishing, the katha is encircled with a net and all branches are removed. Harvests range from 100 to 1,000 kg, depending on the size of the katha and productivity of the water bodies.

Ahmed and Hambrey (1999) surveyed the Katha fisheries in a manmade resrvoir Lake Kaptai (area 68,800 ha) in Bangladesh. It is estimated that about 1,000 brush shelters (area 0.02-0.12 ha each) were in operation around the reservoir and fished twice a year. A total of 483 tonnes of fish was harvested annually, accounting for about 8 percent of the total catch from the reservoir. The difference between this fishing technique used in this reservoir and that used elsewhere in Bangladesh was that it used locally available feeds and scents to attract fish. However, it was concluded that this type of fishing posed a serious threat to the natural stocks as mostly small fish are harvested.

In Cambodia, kathas are named 'samarahs' (Shankar et al., 1998) providing fish yields of 1-4 tonnes ha^{-1} per fishing season. Generally, samarahs are rectangular in shape and more than 70 m^2 in area. Tree branches are used as substrate, coupled with floating aquatic weeds like water hyacinth which cover the entire surface area. The floating weeds are kept in place with ropes. Fish attracted to the samarahs, are harvested 60 days after tree branches are laid in the water, by encircling the area with a net. Some fishermen even provide feed like rice bran to attract and fatten the fish in the area. However, fish productions per area of samarahs were not reported in the literature.

Hem and Avit (1994) compared three 625 m^2 enclosures in Ivory Coast: one without substrate, one with a 100 m^2 acadja of a floating macrophyte, *Echinochloa pyramidalis*, and one with a 100 m^2 traditional acadja made of the usual tree branches. Fish recruited to these systems naturally through the 14-mm mesh surrounding nets. After 12 months, a total fish biomass of 11.7, 18.2 and 80.5 kg, respectively, was harvested from the three enclosures. *Sarotherodon melanotheron* contributed 80 percent of the total biomass in the enclosure with tree branches. Subsequent trials with different sizes of acadja (200-2,500 m^2) yielded on average 1.8 tonnes ha^{-1} y^{-1}. Because of the high requirements for wood, additional trials were done with bamboo poles (10 sticks m^{-2}, approximately 6 cm diameter) leading to average yields of 8.3 tonnes ha^{-1} y^{-1}. Intensive culture of two brackish water tilapia, *Tilapia guineensis* and *S. melanotheron* fed with pelleted diet (30 percent protein) was also tried and it was reported that these two species were not suitable for intensive culture but gave promising results in extensive acadja systems (Legendre et al., 1989).

Fish production trials in closed water bodies

Although substrate-based aquaculture was initiated in Africa, no pond-based trial was reported in the region. Some periphyton-based pond trials were carried out in Asia in the nineties. Shrestha and Knud-Hansen (1994) conducted two experiments in Thailand where plastic sheets and bamboo poles (7.7 m^2 extra surface area per tank) were used vertically as substrates in concrete tanks (2.5 x 2 x 1.1 m). Sex-reversed all-male Nile tilapia (*Oreochromis niloticus*) were stocked at a density of 3 fish per m^2 and grown over a 56 days period. Although there was evidence that the fish utilized periphyton from the substrates, there was no difference in net fish yield between tanks with and without plastic sheets as substrate. In another experiment, bamboo substrates resulted in greater net fish yield (3.43 g m^{-2} d^{-1}) than plastic sheets (2.51 g m^{-2} d^{-1}), but the contribution of bamboo substrate was not quantified due to absence of a treatment without bamboo substrate.

The pioneering work on the Indian subcontinent was carried out at the North-west Fisheries Extension Project (NFEP), Parbatipur aquaculture complex in Bangladesh by Faruk-ul-Islam (1996), who investigated the effect of 40 vertical split bamboo panels (locally called chatai, each side 0.56 m^2) on tilapia production in 80 m^2 trial ponds. Tilapia production in a four months culture period in ponds with and without bamboo chatai, were 640 and 600 kg ha^{-1} respectively, which was not statistically significant. The bamboo chatais were severely infested with molluscs (snails), which competed with fish for food. In conclusion, these two initial works conducted in Asia were not encouraging because of modest increases in fish production with additional substrate or due to poor economic performance.

The second fish production trial conducted at the NFEP complex was more successful (NFEP, 1997). The trial was carried out in eight fertilized earthen ponds of 103-140 m^2 for a period of 168 days. Four ponds were provided with bamboo trimmings inserted vertically into the pond bottom (17 poles m^{-2}, diameter not mentioned) and four were without substrates as control. Juveniles of rohu, *Labeo rohita* (individual weight 11.5 g) were stocked at a density of 1 fish per m^2 in each pond. Net production in substrate and control ponds were 570 and 180 kg ha^{-1}, respectively; a three-fold increase in production in the substrate treatment. However, overall production of this trial was low because the trial commenced in August and continued until January. During winter season, low temperature (16°C) and short photoperiods may have limited fish production.

Shankar et al. (1998) from India reported that the growth of *Oreochromis mossambicus* and *Cyprinus carpio* increased 48 and 50 percent, respectively, in fertilized 1 m^2 cement tanks provided with sugarcane bagasse as substrate compared to ponds without substrate during a 91-days culture period. Based on this result, a further investigation was carried out in three 25 m^2 cement cisterns (Ramesh et al., 1999). Sugarcane bagasse was suspended in cisterns fertilized with cow dung and urea, and only fertilized cisterns were used as control. At a stocking density of 10,000 fish ha^{-1}, growth of *C. carpio* and *L. rohita* was higher by 47 and 48 percent respectively, compared to the control. The combined fish production was 1,235 kg ha^{-1} in substrate ponds during a 133 days culture period.

In a recent trial, Keshavanath et al. (2001) tested three substrate types with masheer, *Tor khudree* in 25 m^3 concrete tanks in India and reported net yields of 400 and 450 kg ha^{-1} in tanks with PVC and bamboo substrates, respectively for a period of 90 days. In the bagasse treatment, 100 percent fish mortality had occurred. However, no conclusion about the effect of periphyton substrate on fish production could be made, because there was no control without substrate in this experiment.

The substrate-based systems were also tested in freshwater prawn culture at Kentucky University, USA (Tidwell et al., 1998). In three randomly selected ponds (0.04 ha each), artificial substrate was added to increase available surface area with approximately 20 percent. Three control ponds received no substrate. Added substrate consisted of PVC frames with horizontal plastic mesh and vertical suspended seines. Juvenile prawns were stocked into all ponds at a density of 59,280 ha^{-1} and fed with a commercial diet (32 percent protein) twice daily. At harvest, average individual weight and total yield were significantly higher in ponds with added substrate (37 g and 1,268 kg ha^{-1}, respectively) than in control ponds (30 g and 1,060 kg ha^{-1}, respectively). In another study, the same substrates were added to increase available substrate area with 80 percent which produced a significant increase in total production by 18 percent. Feed conversion ratios were decreased due to added substrates (Tidwell et al., 1999). However, the purpose of using substrates was mainly to provide shelter rather than growing periphyton for food in these experiments.

Periphyton biomass and composition

Most research on periphyton development and ecology was conducted in natural water bodies concentrating on system productivity, food web interactions, trophic status, taxonomic composition, bioassay and periphyton sampling methods (Aloi, 1990). The most reported periphyton productivity was on coral reef environment which ranged from 1 to 3 g C m^{-2} d^{-1} (Carpenter, 1986; Polunin, 1988; Klumpp and Polunin, 1989; Van Rooij et al., 1998). However, scientist from Ivory Coast and France were the first to explore the productivity of acadja system. Guiral et al. (1993) estimated a total autotrophic production of 9.9 g C m^{-2} d^{-1}, which was 4.5 times higher than that of the lagoon water and reported that 80 percent of the acadja productivity was due to periphyton.

Konan-Brou and Guiral (1994) reported that algal concentrations through the proliferation of periphytic species on the submerged branches led to a five-fold increase in terms of dry matter (DM) and an eight-fold increase in terms of chlorophyll *a* within the acadja compared with the lagoon waters. The maximum biomass was found near the compensation depth (highest organic matter around 40 cm and total pigment around 70 cm below the surface). Two explanations for this were offered: the cells near the water surface deteriorate because of tidal action and periodical drying, and photo-inhibition may occur near the water surface. About 17 genera of algae belong to Bacillariophyceae (10 genera), Chlorophyceae (3), Cyanobacteria (2), Pheophyceae (1) and Rhodophyceae (1) were identified. The most dominating genera were *Rhizoclonium*, *Coleocheate*, *Lyngbya*, *Scytonema*, *Audouinella*, *Pleurocladia*, *Cymatopleura* and *Nitzschia*.

Shrestha and Knud-Hansen (1994) determined 0.93-1.71 and 0.78-0.80 mg cm^{-2} periphyton DM colonized on plastic baffles in tanks without and with tilapia, respectively. In bamboo periphyton, DM was 0.62 mg cm^{-2} under the same grazing pressure by fish.

Keshavanath et al. (2001) reported 0.54-1.86 mg cm^{-2} periphyton DM in treatments without fish, whereas it was 0.24-0.88 mg cm^{-2} in treatments with fish depending on the substrate types. Total pigment, highly depending on the substrate types, ranged from 2.71 to 12.7 μg cm^{-2} in treatment without fish and 0.63-25.69 μg cm^{-2} in treatment with fish. Ash contents were 37-54 percent in ungrazed and 38-58 percent in grazed conditions. It has been concluded that bamboo seems a superior substrate than PVC or sugarcane bagasse.

Many factors can influence periphyton production in aquaculture ponds, but no complete overview is yet available. However, there are considerable literature data on factors influencing periphyton production in open waters, the most relevant ones have been cited here. Differences in nutrient limitations for algal periphyton biomass were determined using additions of N and P supplied by nutrient-diffusing artificial substrates (Fairchild et al., 1985). Sealed clay flowerpots were filled with nine nutrient treatments and submerged at 0.5 m depth in Douglas Lake, Michigan and diffused N and P to their outer surfaces in proportion to internal concentrations. After 51 days, the pots were scraped and analyzed for attached algae. Total algal biomass in terms of chlorophyll *a* ranged from 0.17 $\mu g\ cm^{-2}$ for pots without added nutrients to 15.7 $\mu g\ cm^{-2}$ for pots treated with both N ($NaNO_3$ at 0.5 mol l^{-1}) and P (K_2HPO_4 at 0.05 mol l^{-1}). Chlorophyll *a* on pots containing only P (0.05, 0.5 mol l^{-1}) increased 6- to 10-fold over control, whereas pots containing only N (0.05, 0.5 mol l^{-1}) increased 1.5- to 2-fold.

Bothwell (1988) conducted year-round phosphate enrichment experiments in experimental troughs to determine the relationship between external concentrations of orthophosphate and the growth rates of lotic periphytic diatom communities. Maximum growth rate occurred at a phosphate concentrations of approximately 0.3-0.6 $\mu g\ l^{-1}$ and varied seasonally with temperature.

The mechanisms of microbial mats development on grass clippings have been looked into by Bender et al. (1989). They suggested that bacteria, probably from the sediments, did the first colonization of the substrates. In tanks without sediments, colonization and periphyton development took longer than in tanks with sediments. Rice et al. (2000) performed a biofilm experiment in a parallel plate flow cell reactor with a glass substratum. Bacterial cells made the transition from a planktonic state into a sessile state. Under the same nutrient conditions, when planktonic and secondary biofilm cells both colonized the substrate, the periphyton community developed faster than when only primary biofilm cells developed on the substrate.

Vymazal and Richardson (1995) investigated the effects of season and substrate types on periphyton biomass. Maximum biomasses on three macrophytes, *Eleocharis vivipara*, *E. cellulosa* and *Nymphaea odorata* were 118, 16 and 6 mg DM cm^{-2}, respectively and occurred in summer and early autumn; winter and spring periphyton biomass was very low.

The effects of seasonal changes on acadjas were also investigated by Arfi et al. (1997). In the rainy season, river discharge and cloud cover increase, leading to a reduced salinity in the lagoon and a drop in photosynthesis. These environmental changes lead to a decrease in phytoplankton settling on the substrates and an overall reduction in periphyton production and biomass. The amount of dry matter colonizing the substrates was 3.5-4.0 mg cm^{-2} in dry season compared to 0.5 mg cm^{-2} in wet season.

Very little information on nutritional quality of periphyton in aquaculture ponds is available. Montgomery and Gerking (1980) reported proximate composition of 16 periphytic algae grown on granite boulders suspended at 1 m depth in the lower Gulf of California. Protein, lipid carbohydrate and ash contents were 8-10, 2-5, 52-60 and 25-38 percent on dry matter basis, respectively, depending on the group of algae. Energy content ranged from 10 to 14 kJ g^{-1}. Polunin (1988) estimated an average protein content of 15 percent from periphyton collected from coral reef.

Lane (1991) determined the proximate composition of periphyton grown on glass slides submerged in Perdido Bay, Florida and reported 60-66 percent ash, 5.3-8.6 percent protein, 10.4-46.1 percent lipid and 3.0-4.3 percent carbohydrate on ash free dry matter basis depending on the seasons. Napolitano et al. (1996) reported spatial variations in taxonomic compositions as well as lipid contents of periphyton (2.8-8.7 percent lipid on ash free dry matter basis).

In a recent study, Ledger and Hildrew (1998) determined biochemical composition of periphyton grown on small and large stonnes and bedrock collected from an acid lake. Protein, lipid and carbohydrate ranged 2.18-3.2, 0.04-0.29 and 29-33 percent, respectively, depending on the substrates and season. Periphyton biomass also showed a consistent temporal pattern with peak in spring and early summer and decreased subsequently.

As the periphyton community grows older, its ash content increases and the relative content of carbon and nitrogen, and the energy and trophic value decreases (Makarevich et al., 1993; Huchette et al., 2000). However, Huchette et al. (2000) reported approximately 30-70 percent ash contents under ungrazed and 20-30 percent under grazed conditions in periphyton dry matter collected from aquaculture cages. In the same experiment, approximate ash free dry matter contents of 0.7-0.9 mg cm^{-2} on ungrazed and 0.5-0.9 mg cm^{-2} on grazed substrates were reported.

Periphyton feeding by fish

Some laboratory-based data on periphyton feeding are reported as well. In a digestibility study with Nile tilapia (*Oreochromis niloticus*) and silver carp (*Hypophthalmichthys molitrix*) in plastic tanks (58 cm x 15 cm x 8 cm), Ekpo and Bender (1989) showed that periphytic mats grown on silaged grass had a dry matter digestibility of 62 and 60 percent for Nile tilapia and silver carp, respectively. Protein digestibility was 81 and 75 percent, respectively.

A quantitative comparison of the grazing of young Nile tilapia feeding on the planktonic *Microcystis aeruginosa* and periphytic *Oscillatoria* was made in Stirling, UK (Dempster et al., 1993). Ingestion rates of fish in the periphyton treatment were approximately 10 times higher than those observed in fish offered phytoplankton alone. Highest biomass ingestion rates were observed in the combined periphyton plus phytoplankton treatment, corresponding to 25 times higher values than those obtained in the phytoplankton treatment. The reason given was that the phytoplankton reduced disturbance by causing turbidity and that, as a result, fish spent more time feeding on periphyton when phytoplankton was present. This indicated a greater potential for substrate-based pond culture when both plankton and periphyton are available as fish food.

Quantitative data on algal ingestion by filter-feeding were incorporated into a bio-energetic model, demonstrating that under most conditions tilapias are unable to meet their maintenance requirements and hence lose weight (Dempster et al., 1995). It was concluded that the apparently high volumes of algae ingested by tilapias must be achieved by particulate feeding on either aggregations of algae in the water column or scums of cyanobacteria or periphytic mats.

Role of periphyton in the aquatic food web

There are two basic food sources for all organisms in extensive and semi-intensive ponds: primary productivity from algae, and added organic matter as feed. In the first case, algae produce organic matter by using solar energy and carbon dioxide through photosynthesis that can be utilized indirectly through secondary trophic levels (zooplankton, benthos, invertebrates, etc.) and/or directly through grazing by fish. In the latter case, organic matter applied as supplemental feed enhances fish production. In both cases, heterotrophic micro-organisms (bacteria, protozoa, fungi) are essential components of the food web because these organisms decompose organic matter and release nutrients, which can again be utilized by algae or consumed by the fish (Colman and Edwards, 1987; Moriarty, 1997). However, a common assumption, particularly in aquaculture, has been that the phytoplankton community is the most important in terms of energy fixation and fuelling of the food web. Research has shown, however, that macrophytes and periphyton are a significant, and often the dominant, contributor to primary production (Moss, 1998).

To date, strategies adopted for reducing inputs and increasing efficiencies of aquaculture production have focused on optimization of exploitation of the phytoplankton-based food web. However, the other option to optimize the exploitation of the periphyton-based food web remains unexplored. There is little information on periphyton-based food webs, especially in managed ecosystems such as ponds. Figure 2 offers a preliminary theoretical framework for the mechanism of periphyton-based food webs.

(1) **Periphyton biomass and productivity:** Periphyton biomass as well as productivity might be influenced by both ecological (temperature, sunlight etc.) and operational processes (fish stocking density, fertilization, substrate density and type etc.). Grazing of periphyton by fish or invertebrate grazers can reduce biomass but at the same time increase productivity of periphyton (Hatcher, 1983; Hay, 1991).

(2) **Periphyton loop:** When substrates are installed in aquatic systems, the flux of nutrients through the periphyton "loop" is expected to increase besides an increased flux of nutrients through phytoplankton. In consequence, the overall nutrient efficiency of the system increases. Sometimes a trade-off between phytoplankton and periphyton productivity occurs, decreasing the relative importance of the phytoplankton loop.

(3) **Periphyton detritus:** Similar to dead aquatic organisms (plankton, invertebrates) and fish faeces, dead periphyton may contribute to the amount of detritus present in the pond. The greatest advantage of periphyton compared to phytoplankton is that after dying, dead cells remain in place, providing a rich source of organic nutrients for the heterotrophs (bacteria and others) associated with the periphyton layer. Processing of this organic matter yields inorganic nutrients that can be utilized by the living algae again (Wetzel, 1983). On the other hand, if the grazing pressure is insufficient in relation to periphyton production, periphyton mats may be dislodged from the substrate thereby contributing to the turbidity in the water column.

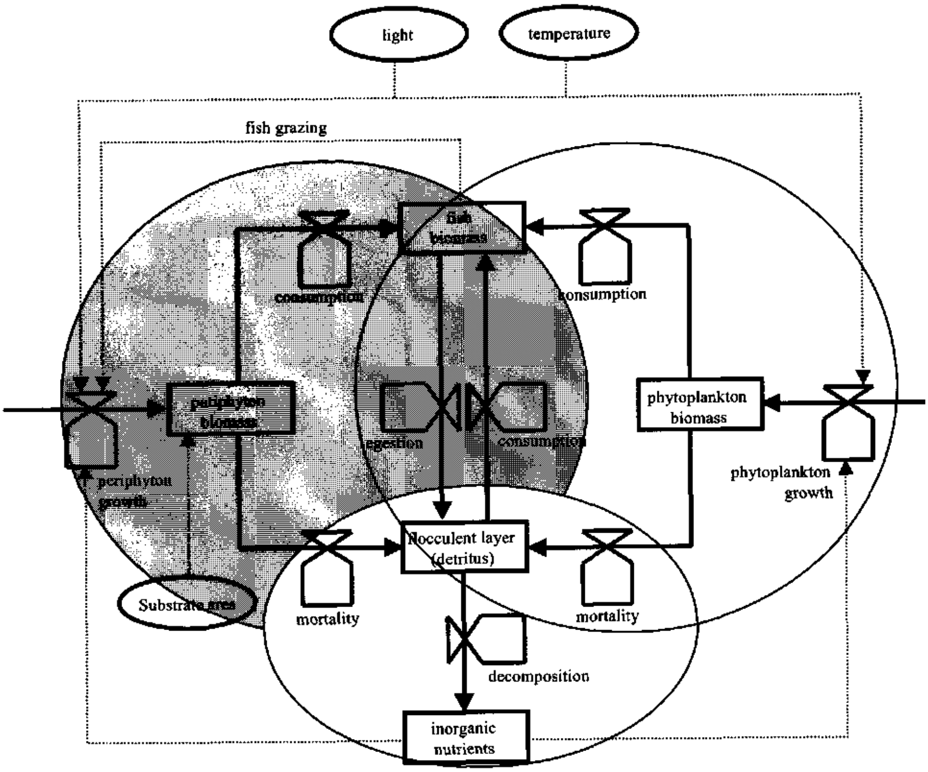

Figure 2. Preliminary conceptual model of a periphyton-based aquaculture system. Only major rate and state variables are shown. The shaded area indicates the "periphyton loop", the interest of this research, but the effects on the whole system will be monitored.

Rationale of approach

Asian pond production systems are becoming increasingly reliant on external resources (feed, fertilizers) to supplement or to stimulate autochthonous food production in ponds. As a result, resources that were previously used elsewhere are now claimed for fish production. In addition, the increased costs for production exclude the poorer sectors of the community from participation (O'Riordan, 1992; Beveridge and Phillips, 1993; NACA, 1995). For aquaculture development to be sustainable, two key issues must be addressed: the sustainable use of resources and the development of the World's poor (Brundtland, 1987; NACA, 2000).

Bangladesh is a least developed country, where more than 75 percent of households spend 90 percent of income on basic needs (BBS, 1995). Many can not afford to provide even rudimentary supplementary feeds for their fish ponds (O'Riordan, 1992). Developing sustainable technologies to increase fish production is essential if aquaculture by resource-poor farmers is to grow further. Periphyton-based aquaculture is such a low cost technology. Periphyton-based systems offer the exciting possibility of increasing primary production per unit area and of increasing the availability of food to fish per unit resource use. The hypothesis is that part of the nutrients lost in traditionally managed ponds are converted into periphyton (as an additional food) and subsequently into fish and that because of increasing fish grazing efficiencies a smaller fraction of the primary production is processed through the microbial loop. As a result, farmers should be able to maintain, or even enhance, fish production levels while applying less nutrients. Although research results showed that periphyton-based systems potentially can enhance pond production, a more systematic and comprehensive approach should be developed before this technique can be used by resource poor farmers in resource constrained countries, such as Bangladesh. Therefore, this study has been formulated to provide an advanced knowledge over the current state of art and to fill up the gaps and shortfalls on periphyton-based aquaculture systems.

Objectives, hypotheses and outline of the thesis

Considering the potential of rural aquaculture in Bangladesh, as well as the fact that there is a scarcity of aquaculture inputs in this country, efforts are being made to explore the usage of various unused locally available substrates for aquaculture practices. If locally available and cheap substrates could be provided to fishponds it may reduce the need for costly feeds and fertilizers and increase production and profitability. However, the technique of increasing periphyton on such materials has not been evaluated. It has not yet been screened for different fish species and fish densities and species combinations, which might be appropriate for use in periphyton based production systems. To overcome the complexity of a 6-7 species polyculture system with indigenous and exotic species, in the present study, efforts have been made to develop a periphyton-based polyculture technique with three indigenous carp species of complementary feeding habits. The effects of increasing periphyton production on the production of the whole system have yet to be determined.

This Ph.D. thesis starts with a general introduction (this chapter) and concludes with a general discussion (Chapter 9). The remaining chapters are organised around the objectives of the research. The principal objectives of this Ph.D. project are to determine the effect of periphyton substrate on fish production and to understand the food web linked to periphyton with the goal to improve nutrient utilisation in aquaculture systems. As a result, the viability of low-input aquaculture for resource-poor farmers is enhanced.

The specific objectives are as follows:

I. To evaluate the locally available substrates in Bangladesh and optimize fertilization for maximizing periphyton production on substrates.

This objective sets out to quantify periphyton production on locally available substrates, and in different fertilization regimes in absence of fish to collect the baseline information (described in Part I of the thesis). Three substrates were evaluated: bamboo, bamboo side shoot (kanchi) and hizol tree branches, which have been described in Chapter 2. Another cheaper substrate, jute stick along with bamboo and kanchi has been evaluated in presence of fish and described in Chapter 8 (under Part IV). It was hypothesized that a considerable amount of high quality periphyton will be produced on artificial substrates. Another experiment under this objective was carried out to optimize fertilization for enhancing periphyton production on substrates and described in Chapter 3. In this experiment, the hypothesis was that there were linear relationships between periphyton biomass and fertilizer doses. Effects of substrates on water quality parameters and fish production potential based on the periphyton productivity have also been assessed.

II. To select suitable indigenous fish species for periphyton-based aquaculture.

After preliminary screening based on laboratory trials, three indigenous species, rohu, *Labeo rohita* (Hamilton), gonia, *Labeo gonia* (Linnaeus) and kalbaush, *Labeo calbasu* (Hamilton) have been tested in monoculture in earthen ponds with and without substrates to confirm the effects of periphyton on the growth and production of individual fish species. This objective is described in Part II of the thesis. It was hypothesized that the increased fish production could be better achieved in the substrate-based systems than in the control system. In Chapter 4, periphyton-based culture potential of rohu and gonia and in Chapter 5, that of kalbaush has been reported. Effects of fish grazing on periphyton communities have also been discussed.

III. To evaluate a three species polyculture production system by exploring different stocking densities, species ratios and combinations.

Objective III explores the possibility of polyculture with indigenous carp species with different niches in periphyton-based aquaculture ponds. Two experiments were carried out under this objective and described in Part III. In the first experiment, a periphyton consuming fish species (*L. rohita*, based on the findings of the objective II), and a planktivorous, surface feeding fish species (*Catla catla*) were selected and their optimum stocking ratio was determined. It was hypothesized that total fish production could be increased when these two fish species with complementary feeding habits are cultured together in ponds. Chapter 6 describes the optimization of the stocking ratio of these two native species in polyculture system.

In the second experiment, the addition of a bottom dwelling fish species, kalbaush (*L. calbasu*) in the previously optimized system (based on the findings of Chapter 6) was evaluated and optimized. The hypothesis was that the bottom dwelling as well as periphyton feeding kalbaush will have synergistic interactions in the polyculture system and hence would further increase fish production. The results are described in Chapter 7.

IV. To compare fish production, nitrogen conversion efficiency and economics among fertilized, fertilized plus fed (feed-driven), and three substrate-based production systems.

The final experiment compared the effect of the addition of three locally available substrates to traditional non-fed ponds on production using the previously optimized three species polyculture system and results are described in Chapter 8 (under Part IV). The effect of adding substrate was also compared to the effect of supplementary feeding. In this experiment, it was hypothesized that the enhancement of periphyton production in the system will increase the proportion of nutrients assimilated into algal and fish biomass thereby increasing profitability than non-periphyton systems. Cost-benefit analysis of the traditional and periphyton-based systems has been presented.

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PART I

Evaluation of substrates and optimization of fertilization rate for periphyton production: implications in aquaculture

Chapter 2

The effect of artificial substrates on freshwater pond productivity and water quality and the implications for periphyton-based aquaculture

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Abstract

As a first step in assessing the viability of periphyton-based fish production in South Asian pond aquaculture systems, the effect of artificial substrates on development of periphyton and on water quality was evaluated. Earthen ponds (10 m × 7.5 m) were provided with an artificial substrate constructed from poles of either bamboo, kanchi or hizol tree branches (1.0 m^2 artificial substrate m^{-2} pond water surface). Higher periphyton biomass, in terms of dry matter (4.89 mg cm^{-2}) and chlorophyll *a* (11.51 μg cm^{-2}) developed on hizol and bamboo, respectively. Periphyton ash content was higher on hizol (41%) than on the other two substrate types (29%). Protein content of the periphyton growing on bamboo (38% of ash free dry matter) was 50% higher than that on the other two substrate types. Maximum periphyton productivity of 1.01, 1.38 and 1.03 g C m^{-2} d^{-1} were obtained for bamboo, hizol and kanchi substrates, respectively. Taxonomic composition of periphyton showed a rapid development of a relatively stable community with little differences between the substrate types. In total, 56 genera of algal periphyton and 35 genera of phytoplankton were identified. Based on a periphyton productivity estimate of 2.17-2.83 g AFDM m^{-2} d^{-1}, periphyton alone can sustain an estimated fish production of 5,000 kg ha^{-1} y^{-1} through the addition of a substrate area equivalent to 100% of the pond water surface area.

Introduction

Asia accounts for about 90% of the world's aquaculture production, the bulk of which is from ponds and rice fields (FAO, 2000). Pond production systems in Southern Asian countries are becoming increasingly reliant on external resources (feed, fertilizers) to supplement or stimulate autochthonous food production for pond fish. In most feed-driven pond production systems, only about 15-30% of nutrient inputs is converted into harvestable products, the remainder being lost to the sediments, effluent water and the atmosphere (Acosta-Nassar et al., 1994; Gross et al., 2000). Intensive pond production systems are also reliant on the environment at large to disperse and assimilate wastes (Beveridge and Phillips, 1993). Adoption of periphyton-based aquaculture through the addition of artificial substrates into existing pond systems, could improve the conversion of nutrients into harvestable products.

Periphyton is defined here as the entire complex of sessile biota attached to the substratum, plus associated detritus and microorganisms. The idea is originally derived from traditional fishing method, such as the 'acadjas' of Côte d'Ivoire (Welcomme, 1972), the 'samarahs' of Cambodia (Shankar et al., 1998) and the 'katha' fisheries of Bangladesh (Wahab and Kibria, 1994), where tree branches are placed in shallow open waters to attract fish and enhance productivity. Preliminary data reported by Hem and Avit (1994) suggested that fish yields in an 'acadja-enclos' could be up to 8 tonnes ha^{-1} y^{-1}, 8 times higher than in control areas without artificial substrate. Increased food availability and better protection from predators may explain the high yields. The results from experiments in aquaculture ponds, where stocking and predation are more controlled, vary from no effect (Shrestha and Knud-Hansen, 1994; Faruk-ul-Islam, 1996; Azim et al., 2001a) to a 40-80% increase in fish yield in ponds with artificial substrates compared to control ponds (Ramesh et al., 1999; Wahab et al., 1999; Azim et al., 2001a). However, yields were highly variable within and between substrate types, and the design of the trials allowed no conclusion about the causal factors responsible for this difference. The periphyton productivity and proximate composition were not quantified or qualified in any of these experiments.

As a first step in assessing the viability of periphyton-based fish production in South Asian pond aquaculture systems, this experiment was designed to 1) estimate the quantity and quality of periphyton grown on artificial substrates of three locally available plant materials in the absence of fish and 2) determine the effects of substrates for periphyton on water quality. The potential of substrate-based aquaculture in this region has also been discussed.

Materials and methods

Pond facilities and design

The field trial was carried out in twelve earthen ponds (10 m × 7.5 m, mean water depth 1.2 m) at the Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, over a 6 weeks period between May and July 1998. Three substrate types plus one control were evaluated in triplicate using a complete randomized design.

Substrate selection and pond preparation

Three different substrates were used: bamboo (*Bambusa* sp.) poles, kanchi (bamboo side shoot) and hizol (*Barringtonia* sp.) branches, which were collected from adjacent villages. Of the several types of locally available bamboos, *Bambusa* sp. was chosen as it is less useful for house building purposes. Kanchi was selected because of its wide availability and low price. Moreover, farmers can collect this substrate from the homestead garden, virtually without any cost. Hizol is a floodplain tree from which fishermen cut branches to construct brush-parks that attract fish in open inland waters in Bangladesh (Wahab and Kibria, 1994).

Ponds were drained and renovated and all aquatic weeds and other organisms were removed. Quicklime (CaO) was applied to the pond bottom at the rate of 250 kg ha^{-1}. Maintaining a substrate free perimeter, an effective area of 8 × 5 m^2 was planted with vertical poles/branches of 2 m length one week after liming. Bamboo poles (mean diameter 5.47 cm) were driven vertically into the pond bottom, the upper portion extending above the water surface, at a density of 9 poles m^{-2}, yielding a total submerged substrate area of 74.2 m^2 per pond, approximating that of the pond water surface area (75 m^2). Similar substrate areas were obtained for the other two substrates by planting 34 kanchi poles (mean diameter 1.47 cm) and 13 hizol branches (mean diameter 3.84 cm) per m^2. Three ponds received no substrate and served as controls.

Water supply and fertilization

After the substrates were installed, the ponds were filled with ground water from a nearby deep tube well. The water depth in each pond was monitored daily (fluctuating from 1.15-1.35 m) and maintained by adding deep tube well water to replace losses at weekly intervals. A traditional schedule of fortnightly fertilization for aquaculture ponds with semi-decomposed cattle manure, urea and triple super phosphate (TSP) at the rates of 3,000, 100 and 100 kg ha^{-1}, respectively, was started immediately after pond filling and maintained throughout the experimental period.

Determination of periphyton biomass

Starting one week after substrate installation, the periphyton biomass growing on the substrates, *viz.* dry matter (DM), pigment concentrations (chlorophyll *a* and pheophytin *a*), ash free dry matter (AFDM), ash percentage and autotrophic index (AI) were determined weekly following standard methods (APHA, 1992). From each pond, three poles were selected by random number tables and two 2 x 2 cm^2 samples of periphyton were taken at each of four depths (0, 30, 60 and 90 cm below the water surface) per pole. The areas were carefully scraped with a scalpel blade to remove all periphyton without (visually) affecting the substrate. After sampling, the poles were replaced in their original positions, marked and excluded from subsequent sampling. One sample of the two was used to determine total dry matter and ash content. The material was collected on pre-weighed and labeled pieces of aluminium foil, dried at 105°C until constant weight (24 h in a Memmert stove, Model UM/BM 100-800), and kept in a dessiccator until weighed (BDH, Model 100A; precision 0.1 mg). Because the individual dry matter samples were too small to allow reliable determination of ash content, 2 x 2 cm^2 samples from all depths, poles and replicate ponds were pooled per sampling day. They were then transferred to a muffle furnace and ashed at 450°C for 6 h and weighed. DM, AFDM and ash content were determined by weight differences. Ash content was not determined at the final sampling date; instead, samples were dried and stored at -20°C for later energy content and proximate analysis at the laboratory of Fish Culture and Fisheries Group of Wageningen University and Research Centre (WUR), Netherlands.

The another sample was used to determine chlorophyll *a* and pheophytin *a* concentrations following standard methods (APHA, 1992). Upon removal, the material 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 sec with a tissue grinder, refrigerated for 4 h and centrifuged for 10 min at 2,000-3,000 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*, pheophytin *a* and autotrophic index were calculated using the following equations APHA (1992):

$$\text{Chlorophyll } a\ (\mu g\ cm^{-2}) = [26.7(664_b - 665_a)V_1]/(V_2L)$$

$$\text{Pheophytin } a\ (\mu g\ cm^{-2}) = [26.7\{1.7(665_a) - 664_b\}V_1]/(V_2L)$$

where, V_1 = volume of extract, ml; V_2 = volume of sample, cm^{-2}; L = light path length of cuvette, cm; 664_b, 665_a = optical density of 90% acetone extract before and after acidification, respectively.

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

Study of taxonomic composition of periphyton and plankton

In addition to the two samples taken from four depths, an extra 2 x 2 cm^2 periphyton sample was collected from each sampled pole at 25 cm depth for determination of the periphyton community composition. Samples were collected on a weekly basis starting after one week of substrate installation. Pooled samples from three poles from each pond were re-suspended in 50 ml distilled water and preserved in 5% buffered formalin in sealed plastic vials. After vigorous shaking, a 1 ml subsample was transferred to a Sedgewick-Rafter cell (S-R cell) divided in 1,000 squares, upon which the number of colonies (algae) or individuals (invertebrates) were counted in 10 randomly selected squares under a binocular microscope (Swift, M-4000; magnification 40×). Taxa were identified to genus level using keys from Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976) and Bellinger (1992). Periphyton numbers were estimated using the following formula:

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

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

Plankton samples were collected weekly by passing 5 l of water from water column at five locations of each pond with a plankton net (mesh size 45µm). The concentrated samples were preserved in small plastic bottles with 5% buffered formalin. Plankton numbers were estimated using an S-R cell. One ml concentrated sample was placed to the counting chamber of the S-R cell and was left to stand for 15 min to allow plankton to settle. Then the plankton on 10 randomly selected fields of the chamber were counted under a binocular microscope (Swift, M-4000). Plankton density was calculated using the following formula:

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

where N = the number of plankton cells or units per litre of original water; P = the number of plankton counted in ten fields; C = the volume of final concentrate of the sample (ml); L = the volume (litres) of the pond water sample. Identification of plankton to genus level was carried out using the keys mentioned above for periphyton.

Analysis of proximate composition and energy content of periphyton

Because of the low biomass of the samples, proximate composition and energy content were determined stoichiometrically from C:H:N ratios, following the method of Gnaiger and Bitterlich (1984). With this method, sample as small as 1 mg DM can be used to determine the proximate composition of the AFDM. Each sample was used in triplicate for CHN analysis. The CHN content of the dry matter samples was corrected for ash fractions according to equation (1) in Gnaiger and Bitterlich (1984):

$$W_i = [{}_{tot}W_i - ({}_{ash}W_i \times W_{ash})] / (1 - W_{ash});$$

where, i represents nitrogen, carbon or hydrogen; W_i is the organic fraction of i in ash-free biomass, ${}_{tot}W_i$ is the total mass of i in the total dry biomass, ${}_{ash}W_i$ is the inorganic fraction of i in the ash, and W_{ash} is the mass fraction of ash in the dry weight.

Protein content of the AFDM was calculated using the nitrogen to protein conversion factor of 5.78 proposed by Gnaiger and Bitterlich (1984) who found this to be a more appropriate value for bacteria, algae and aquatic invertebrates than that of 6.25 that is usually applied. Subsequently, lipid, carbohydrate, residual water and caloric content were calculated from the mass fractions of organic C, H, and N in the AFDM (Gnaiger and Bitterlich, 1984).

Water quality monitoring

Temperature and dissolved oxygen content, pH and water transparency (Secchi disc depth) were measured daily. Total alkalinity, total ammonia (NH_4^+-N), nitrate (NO_3-N), phosphate (PO_4-P) and chlorophyll *a* of water were measured weekly. Determination of water quality parameters started on the first day of the experiment and was carried out between 0900 and 1000 h on each sampling day. Temperature and DO of both surface and bottom water were measured with a DO meter (YSI, model 58) and pH with a pH electrode (Jenway, model 3020). Total alkalinity was determined titrimetrically following Stirling (1985). Chlorophyll *a* was determined spectrophotometrically after filtering samples through Whatman GF/C filters and subsequent acetone extraction of the filtrate following Boyd (1979). Water samples were filtered before the nutrients were analyzed using a Hach kit (DR 2000).

Statistical analyses

Daily and weekly water quality parameters were compared by split-plot ANOVA (repeated measurements) with treatments (substrate types and control) as the main factor and time as the sub-factor (Gomez and Gomez, 1984) using the SAS 6.12 program (SAS Institute Inc., Cary, NC 27513, USA):

$$Y_{ijk} = \mu + S_i + e_{ij} + T_k + (SxT)_{ik} + e_{ijk}$$

where, Y_{ijk} = observed value; μ = overall mean; S_i = effect of treatments (i = 4); e_{ij} = error 1 (j = 3 replicates); T_k = effect of sampling date (k = 42 for daily and k = 6 for weekly water quality); $(SxT)_{ik}$ = interaction of substrate type and sampling date; e_{ijk} = error 2.

Periphyton DM and pigment parameters (means of three poles per pond) were analyzed in a split-split-plot ANOVA with substrate type as the main factor, depth as the first sub-factor and sampling date as the second sub-factor:

$$Y_{ijkl} = \mu + S_i + e_{ij} + D_k + e_{ijk} + T_l + (SxT)_{il} + (DxT)_{kl} + (SxDxT)_{ikl} + e_{ijkl}$$

where, Y_{ijkl} = observed value; μ = overall mean; S_i = effect of substrate type (i = 3); e_{ij} = error 1 (j = 3 replicates); D_k = effect of depth (k = 4); e_{ijk} = error 2; T_l = effect of sampling date (l = 6); $(SxT)_{il}$ = interaction of substrate type and sampling date; $(DxT)_{kl}$ = interaction of depth and sampling date; $(SxDxW)_{ikl}$ = interaction of substrate type, depth and date; e_{ijkl} = error 3.

Again, DM and chlorophyll *a* were analyzed within each substrate type separately in a split-plot design with depth as main factor and sampling date as sub-factor. If a main effect was significant, the ANOVA was followed by a Tukey-HSD test at 0.05 level. Ash percentage, AFDM and autotrophic index were not considered for ANOVA because of small sample numbers. Periphyton and plankton taxonomic data were analyzed by Systat 5.0, using the non-parametric Kruskal-Wallis test. The assumption of normal distributions and homogeneity of the variances were checked before analyses. In case of significant deviations from normality or heterogeneous variances, means were compared using the non-parametric Kruskal-Wallis test. When the latter confirmed the results from the ANOVA, only the ANOVA results were presented.

Results

Periphyton biomass

There were no significant differences ($P > 0.05$) between substrate types but depths and sampling dates had significant effects ($P < 0.01$) on periphyton dry matter (DM) and pigment concentrations. Mean (±S.E.) DM was highest on hizol (4.89±0.26 mg cm^{-2}) and lower on bamboo and kanchi (3.10±0.20 mg cm^{-2}) (Table 1). The development of periphyton DM was more or less similar on all three substrates during the first three weeks, increasing from 1.67±0.41-2.29±0.58 mg cm^{-2} on Day 7 to 3.56±0.82-4.93±0.53 mg cm^{-2} on Day 21. During the second half of the experiment, however, mean DM was highest on hizol (4.92±0.61-9.04±1.44 mg cm^{-2}), intermediate on bamboo (3.33±1.02-4.27±1.31 g cm^{-2}) and lowest on kanchi (2.07±0.41-2.87±0.51 g cm^{-2}) (Figure 1A). Although there was no substrate-depth interaction, the substrate-time interaction was apparent for periphyton DM indicating that the pattern of DM development throughout the experimental period varied with substrate types

Table 1
Mean values (and S.E.) of periphyton biomass. Figures are means of samples from four depths, three poles, three ponds and six sampling dates ($N = 216$) for dry matter, chlorophyll *a* and pheophytin *a* and only six sampling dates ($N = 6$) for ash free dry matter, Ash content and autotrophic index for each combination of substrate.

Parameters	**Substrate types**		
	Bamboo	**Hizol**	**Kanchi**
Dry matter (mg cm^{-2})	3.05 (0.20)	4.89 (0.26)	3.12 (0.20)
Chlorophyll *a* (μg cm^{-2})	11.51 (0.56)	8.30 (0.45)	8.83 (0.62)
Pheophytin *a* (μg cm^{-2})	2.17 (0.32)	2.31 (0.24)	0.91 (0.25)
Ash content (%)	29 (3)	41 (5)	29 (1)
Ash free dry matter (mg cm^{-2})	2.17 (0.15)	2.87 (0.23)	2.22 (0.33)
Autotrophic index	189 (62)	346 (62)	251 (62)

(Figure 1A). There were significant variations ($P < 0.01$) in DM contents among different depths of the hizol and kanchi substrates (Figure 2A). The differences occurred between 30 and 90 cm depth for both the substrates (Tukey test). The bamboo substrate, however, showed more or less similar periphyton DM values at different depths ($P > 0.05$).

Mean (±S.E.) chlorophyll *a* concentrations on bamboo, hizol and kanchi were 11.51±0.56, 8.30±0.45 and 8.83±0.62 μg cm^{-2}, respectively (Table 1). Chlorophyll *a* concentrations increased steadily during the first four weeks for bamboo and hizol and during the first three weeks for kanchi; thereafter they levelled off (Figure 1B). Mean chlorophyll *a* during the second half of the experiment was higher on bamboo than on kanchi (interaction of time and substrate; Figure 1B).The significant substrate-time and substrate-depth interactions ($P < 0.05$) for chlorophyll *a* indicated that the concentrations of the pigment at different sampling dates and substrate depths followed different patterns depending on substrate types. It was significantly higher ($P < 0.01$) at 0 and 30 cm depths in comparison with 60 and 90 cm in bamboo substrate (Figure 2B; Tukey test). On kanchi substrate, differences were confined to 0 and 90 cm depth. There were no significant differences ($P > 0.05$) among different depths for hizol substrate.

Pheophytin *a* concentrations on bamboo, hizol and kanchi were 2.17±0.32, 2.31±0.24 and 0.91±0.25 μg cm^{-2}, respectively, and increased steadily during the first three weeks for

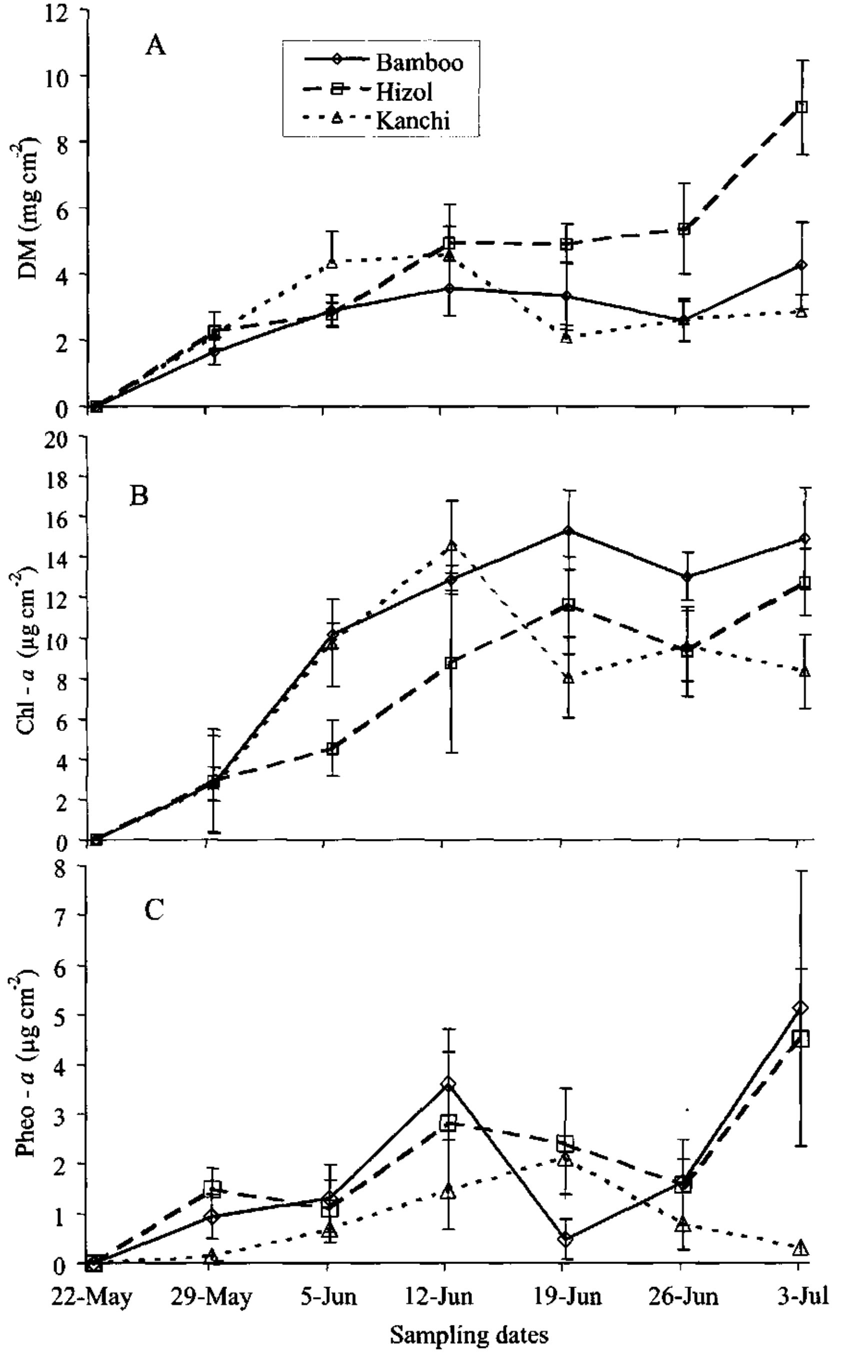

Figure 1. Amounts of periphyton biomass per unit surface area during the experimental period (A) Dry matter (B) Chlorophyll *a* and (C) Pheophytin *a*. Values are means (±S.E.) of four depths, three poles and three ponds per substrate types ($N = 36$).

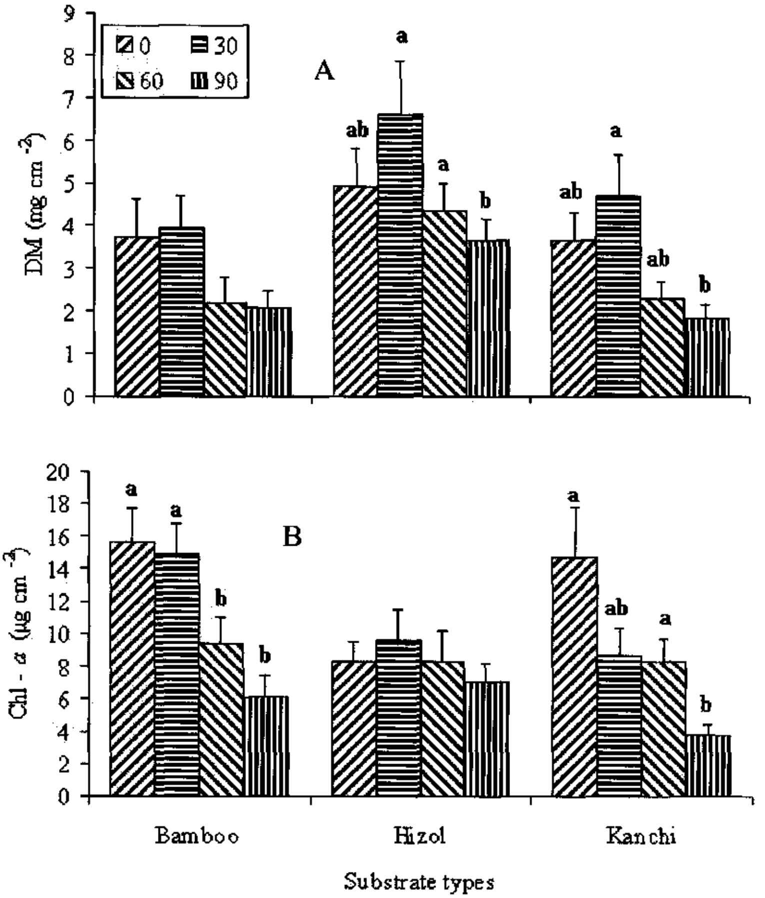

Figure 2. Amounts of periphyton biomass along the different depth of the substrate from water surface (A) Dry matter (B) Chlorophyll *a*. Values are means (and S.E.) of three poles, three ponds and six sampling dates per substrate type ($N = 54$). If main effects are significant, then bars followed by different letters among different depths of the same substrate are significantly different ($P > 0.05$) based on Tukey test.

bamboo and hizol and during the first four weeks for kanchi; then they decreased (Table 1; Figure 1C). However, they increased sharply again on bamboo and hizol during the last week. There were substrate-time interactions ($P < 0.05$) in pheophytin *a* concentrations and they did not vary significantly ($P > 0.05$) among different depth of the substrate.

Ash percentage of periphyton on hizol (41±5%) was higher than on either bamboo or kanchi (29±1-3%) (Table 1) during the entire experimental period. The peak value was found during Week 4 on bamboo and hizol substrates, whereas on kanchi substrate it did not change markedly during the different sampling weeks. The AFDM values showed a similar trend to those of DM. The AI was lower on bamboo (189±62) than either on kanchi (251±62) or on hizol (346±62) (Table 1) and decreased with time. However, no statistical analysis was performed for ash, AFDM and AI because samples from replicated ponds were pooled during the laboratory analysis.

Energy content and proximate composition of periphyton

The results of the elemental C:H:N analysis are summarized in Table 2. The caloric value of all periphyton samples ranged between 19 and 20 kJ per gram AFDM. Periphyton protein levels on bamboo (38% of AFDM) were higher than those on hizol (25%) and kanchi (26%). Lipid content was estimated at 7 and 9% of AFDM for the periphyton derived from bamboo and hizol, respectively. For kanchi a value as low as 0.5% lipid content was obtained. Carbohydrate content was estimated at 46% of AFDM for bamboo, 64% for hizol and 73% for kanchi.

Table 2
Proximate composition and energy content of periphyton samples, as estimated stoichiometrically from elemental C:H:N ratios. N, C and H are expressed as weight fractions in the ash free dry matter (AFDM) fraction of the periphyton samples. The values are obtained using the equations of Gnaiger and Bitterlich (1984) and actual residual water fraction of the periphyton samples.

Substrate types	Residual water (%)	N (%AFDM)	C (%AFDM)	H (%AFDM)	Energy (kJ g^{-1} AFDM)	Protein (%AFDM)	Lipid (%AFDM)	Carbo-hydrate (%AFDM)
Bamboo	8.0	6.6	46.5	7.2	19.6	38.3	7.3	46.4
Hizol	1.9	4.3	48.7	6.9	20.4	24.8	9.2	64.0
Kanchi	0.5	4.6	46.6	6.5	18.8	26.4	0.5	72.6

Taxonomic composition of periphyton and plankton

There was no significant difference (non-parametric ANOVA, $P > 0.05$) in numbers of different group of algal periphyton among substrates as well as among sampling dates, except for Rotifera and a few species of phytoplankton. On average, a total of 60 periphyton genera was identified on bamboo, 57 on hizol and 55 on kanchi (Table 3). Chlorophyceae were most abundant (168-223 $\times 10^3$ cells/colonies cm^{-2}) and most speciose (29 genera, six rarely occurred) on all substrates, followed by Bacillariophyceae (97-156 $\times 10^3$ cells/colonies cm^{-2}; 13 genera, three rarely occurred), Cyanophyceae (102-146 $\times 10^3$ cells/colonies cm^{-2}; 10 genera) and Euglenophyceae (21-29 $\times 10^3$ cells/colonies cm^{-2}; four genera). Eight genera of zooplankton belonging to Crustacea (two genera, rarely occurred) and Rotifera (six genera, five rarely occurred) were also identified.

Among Chlorophyceae, *Closterium*, *Cosmarium*, *Pediastrum* and *Scenedesmus* were significantly higher ($P < 0.05$) in numbers in hizol ponds. Abundance of Rotifera was significantly higher ($P < 0.05$) in bamboo substrate. *Fragillaria*, *Gomphonema*, *Navicula* and *Nitzschia* were the most dominant genera of Bacillariophyceae. Whereas *Chlorella*, *Gonatozygon* and *Scenedesmus* were the most dominant genera of Chlorophyceae, *Chroococcus*, *Lyngbya* and *Microcystis* were the most commonly represented ones of Cyanophyceae, whereas *Difflugia* was the most abundant genus of Euglenophyceae.

Plankton comprised 35 genera of phytoplankton belonging to Bacillariophyceae (five genera), Chlorophyceae (19 genera, two rarely occurred), Cyanophyceae (seven genera) and Euglenophyceae (four genera, one rarely occurred), and 12 genera of zooplankton belonging to Crustacea (five genera, one rarely occurred) and Rotifera (seven genera, one rarely occurred) (Table 4). There were no significant differences (non-parametric ANOVA, $P > 0.05$) in numbers of the different group of plankton.

Cyanophyceae were the most dominant group in bamboo and kanchi ponds (72-73 x 10^3 units l^{-1}) whereas the Chlorophyceae were the most dominant group in hizol and control ponds (63-66 x 10^3 units l^{-1}) .

Actinella and *Navicula* were the most dominant genera of Bacillariophyceae. *Chlorella* was the most dominant genus of Chlorophyceae. *Chroococcus* and *Microcystis* were the most dominant genera of Cyanophyceae while *Euglena* was the dominant genus of the Euglenophyceae. There were significantly higher numbers of *Ceratium* in hizol ponds.

Table 3

Abundance of periphyton (cells or colonies cm^{-2}) during the experimental period. Numbers are means of three ponds and six sampling dates. Numbers in small italics are standard errors (N = 18). On the right, the Kruskal-Wallis statistic (K-W, df = 2) and probability (P) are shown for a non-parametric ANOVA between substrate types.

Group/Genus	**Bamboo**		**Hizol**		**Kanchi**		***K-W***	***P***
PHYTOPLANKTON								
Bacillariphyceae								
Achnanthes	3611	*1604*	5833	*1654*	2778	*799*	2.828	0.243
Actinella	1389	*1389*	0		0		2.000	0.368
Cocconeis	3333	*1811*	5069	*1406*	1528	*704*	4.940	0.085
Cyclotella	3819	*967*	6250	*1325*	4583	*663*	2.229	0.328
Cymbella	3403	*1297*	1875	*744*	2708	*1184*	0.612	0.737
Fragillaria	19375	*5149*	25417	*4395*	17500	*3890*	2.717	0.257
Gomphonema	22847	*6927*	15972	*4467*	9306	*1655*	1.335	0.513
Melosira	7083	*3105*	1111	*677*	6250	*2064*	5.227	0.073
Microphora	0		1528	*1528*	0		2.000	0.368
Navicula	56319	*16003*	63889	*11845*	32778	*5535*	5.524	0.063
Nitzschia	16875	*6351*	17222	*3753*	12292	*4212*	1.811	0.404
Penium	903	*903*	0		0		2.000	0.368
Synedra	11736	*4496*	12708	*3086*	7500	*2214*	2.719	0.257
Total	150694	*40028*	156875	*21784*	97222	*16646*	5.142	0.076
Chlorophyceae								
Actinastrum	2431	*1606*	3264	*1263*	1250	*808*	2.964	0.227
Ankistrodesmus	9792	*3726*	9722	*3031*	7639	*3877*	1.278	0.528
Ceratium	556	*556*	2083	*839*	1528	*530*	4.969	0.083
Chaetophora	5764	*2196*	3264	*1883*	1597	*1151*	4.129	0.127
Characium	2292	*1210*	6042	*3104*	7986	*2081*	5.237	0.073
Chlorella	44375	*5424*	55347	*4823*	44583	*4749*	2.940	0.230
Cladophora	556	*556*	208	*208*	0		1.020	0.601
Closterium	7222	*3220*	17083	*4042*	4028	*1823*	10.950	**0.004**
Coelustrum	2708	*971*	4931	*1778*	1806	*641*	0.706	0.702
Coleochaete	4375	*1989*	4514	*2123*	3542	*1364*	0.178	0.915
Cosmarium	486	*337*	2639	*669*	0		17.353	**0.000**
Crucigenia	347	*347*	2083	*1217*	278	*278*	3.360	0.186
Draparnaldia	7083	*2700*	2778	*1459*	4792	*2276*	0.974	0.614
Gloeocapsa	347	*347*	694	*476*	347	*347*	0.530	0.767
Gonatozygon	18472	*2859*	23958	*3563*	21319	*3161*	1.883	0.390
Microspora	625	*625*	0		556	*431*	2.002	0.367
Microthamnion	1597	*1229*	694	*694*	2708	*1239*	3.528	0.171
Mougeotia	9167	*1802*	10000	*2039*	9792	*2147*	0.059	0.971
Oedogonium	417	*417*	417	*417*	972	*549*	1.540	0.463

Group/Genus	Bamboo		Hizol		Kanchi		*K-W*	*P*
Oocystis	2153	*1111*	1250	*881*	833	*606*	0.899	0.638
Pediastrum	1736	*744*	5556	*1533*	1667	*802*	6.888	**0.032**
Scenedesmus	29514	*8817*	38681	*7519*	11111	*2612*	10.576	**0.005**
Stigeoclonium	17569	*4949*	7014	*2096*	17222	*6806*	1.995	0.364
Tetraedon	1944	*784*	1458	*771*	1042	*443*	0.651	0.722
Tetraspora	13403	*3659*	13611	*3103*	12778	*3224*	0.057	0.972
Triplocerus	1042	*1042*	0		556	*556*	1.020	0.601
Ulothrix	8472	*1603*	5208	*1791*	7917	*1874*	3.065	0.216
Volvox	486	*418*	0		208	*208*	2.076	0.354
Zygnema	347	*347*	694	*487*	0		2.076	0.354
Total	195278	*18385*	223194	*21263*	168056	*12707*	4.508	0.105
Cyanophyceae								
Anabaena	3889	*1389*	4722	*1699*	1667	*763*	2.021	0.364
Aphanigominon	208	*208*	556	*556*	69	*69*	0.003	0.999
Aphanocapsa	5833	*1730*	10139	*3108*	2431	*1145*	4.183	0.123
Chroococcus	18264	*3355*	28056	*3332*	21250	*3284*	2.972	0.226
Gloetrichia	2708	*1283*	1319	*1319*	694	*565*	3.567	0.168
Gomphosphaeria	1042	*537*	3819	*1466*	2708	*1140*	2.528	0.282
Lyngbya	15486	*4623*	32083	*7597*	30278	*9109*	2.416	0.299
Microcystis	32778	*6531*	41944	*7959*	51389	*8998*	2.865	0.239
Oscillatoria	18403	*2339*	17847	*3718*	12361	*2440*	3.386	0.184
Rivularia	3472	*1772*	5764	*2336*	4167	*1796*	0.382	0.826
Total	102083	*11787*	146250	*15113*	127014	*15299*	4.849	0.089
Euglenophyceae								
Difflugia	12708	*2432*	12778	*2431*	10486	*1499*	0.146	0.930
Euglena	833	*335*	1806	*702*	972	*447*	0.516	0.773
Phacus	3750	*736*	5347	*1133*	4514	*679*	1.562	0.458
Trachelomonas	7361	*1474*	8403	*1610*	5278	*1082*	1.803	0.406
Total	24653	*3535*	29236	*3638*	21250	*2158*	2.128	0.345
ZOOPLANKTON								
Crustacea								
Monostyla	972	*344*	833	*335*	903	*438*	0.248	0.883
Nauplius	0		69	*69*	0		2.000	0.368
Total	972	*344*	903	*332*	903	*438*	0.291	0.865
Rotifera								
Ascomorpha	5556	*804*	3056	*537*	3472	*843*	7.180	**0.028**
Asplanchna	139	*139*	278	*162*	69	*69*	1.682	0.431
Brachionus	278	*191*	0		0		4.077	0.130
Filinia	0		0		139	*139*	2.000	0.368
Keratella	0		347	*243*	208	*152*	2.121	0.346
Trichocerca	486	*305*	139	*95*	0		3.124	0.210
Total	6458	*1002*	3819	*696*	3889	*1035*	6.581	**0.037**

Table 4

Abundance of plankton (cells or colonies l^{-1}) during the experimental period. Numbers are means of three ponds and six sampling dates. Numbers in small italics are standard errors (N=18). On the right, the Kruskal-Wallis statistic (K-W, df=3) and probability (P) are shown for a non-parametric ANOVA between treatments.

Group/Genus	Bamboo		Hizol		Kanchi		Control		*K-W*	*P*
PHYTOPLANKTON										
Bacillariophyceae										
Actinella	1694	*497*	4472	*1212*	1528	*463*	2583	*633*	5.456	0.141
Cyclotella	750	*438*	1028	*494*	1250	*382*	972	*419*	2.819	0.420
Fragillaria	778	*229*	1444	*431*	1167	*556*	583	*300*	4.892	0.180
Navicula	1583	*364*	2750	*770*	2389	*777*	3306	*901*	1.815	0.612
Surirella	278	*199*	1306	*434*	972	*378*	889	*691*	6.430	0.092
Total	5083	*1047*	11000	*2271*	7306	*1769*	8333	*1886*	4.220	0.239
Chlorophyceae										
Actinastrum	889	*471*	2389	*954*	1917	*725*	1833	*690*	3.219	0.359
Ankistrodesmus	1917	*499*	1472	*363*	2500	*645*	2361	*716*	1.241	0.743
Botryococcus	833	*573*	694	*382*	1444	*1122*	556	*392*	0.630	0.890
Ceratium	972	*423*	17417	*7876*	6833	*3705*	10389	*6888*	9.445	**0.024**
Chlorella	15028	*4667*	26861	*8143*	29111	*9205*	27444	*6504*	4.140	0.247
Closterium	250	*136*	333	*157*	417	*292*	1556	*857*	2.040	0.564
Cosmarium	556	*404*	500	*283*	694	*280*	1083	*495*	1.713	0.634
Crucigenia	306	*233*	722	*413*	167	*167*	833	*551*	1.515	0.679
Gonatozygon	2944	*878*	3556	*753*	3944	*1016*	4750	*1162*	1.567	0.667
Oocystis	1222	*791*	472	*353*	1444	*581*	2694	*1581*	4.498	0.212
Pediastrum	1306	*373*	1556	*454*	778	*272*	1889	*780*	1.521	0.677
Scenedesmus	1444	*480*	889	*301*	833	*437*	3306	*941*	5.914	0.116
Selenastrum	306	*172*	1278	*787*	139	*97*	56	*56*	2.672	0.445
Spirogyra	0		56	*56*	639	*557*	56	*38*	2.339	0.505
Synedra	0		0		167	*167*	0		3.000	0.392
Treubaria	1222	*678*	167	*90*	500	*352*	972	*555*	1.190	0.755
Tetraedron	2083	*661*	2194	*562*	1583	*540*	2028	*555*	1.234	0.745
Volvox	167	*90*	306	*147*	861	*586*	417	*237*	0.373	0.946
Zygnema	1389	*567*	2250	*1048*	2722	*1077*	3944	*2525*	0.774	0.856
Total	32833	*5617*	63111	*13069*	56694	*11159*	66167	*9999*	6.311	0.097
Cyanophyceae										
Anabaena	3917	*1539*	15000	*9532*	5139	*2078*	2833	*762*	0.519	0.915
Aphanocapsa	14444	*3684*	8556	*3257*	5806	*1870*	6806	*1806*	4.485	0.214
Chroococcus	12222	*9343*	2472	*762*	36111	*22445*	8417	*2880*	3.807	0.283
Gomphosphaeria	14111	*3405*	9389	*1780*	13222	*3188*	18083	*5629*	0.305	0.959
Merismopedia	806	*343*	250	*141*	250	*173*	1056	*408*	4.593	0.204
Microcystis	24944	*13470*	21278	*13549*	8833	*2356*	7167	*1421*	1.553	0.670

Group/Genus	**Bamboo**		**Hizol**		**Kanchi**		**Control**		***K-W***	***P***
Oscillatoria	2722	*2383*	2389	*1933*	2722	*2088*	4111	*3882*	0.824	0.844
Total	73167	*15062*	59333	*23834*	72083	*22899*	48472	*6231*	2.364	0.500
Euglenophyceae										
Euglena	32139	*14412*	25500	*10945*	7306	*1079*	16389	*7324*	3.958	0.266
Gymnodinium	0		2722	*1861*	0		2500	*2124*	5.667	0.129
Phacus	694	*280*	806	*379*	278	*173*	917	*401*	2.219	0.528
Trachalomonas	2806	*851*	6500	*2058*	3111	*794*	9417	*3031*	3.112	0.375
Total	35639	*14504*	35528	*11316*	10694	*1434*	29222	*10222*	3.500	0.321
ZOOPLANKTON										
Crustacea										
Cyclops	2000	*604*	3667	*874*	3944	*1280*	2222	*625*	3.536	0.316
Daphnia	56	*56*	56	*56*	56	*38*	194	*108*	1.835	0.607
Diaphanosoma	167	*167*	389	*335*	833	*644*	194	*141*	1.239	0.744
Diaptomus	0		861	*517*	0		333	*333*	6.317	0.097
Nauplius	2083	*465*	4083	*799*	3028	*774*	3111	*726*	2.652	0.448
Total	4306	*1031*	9056	*2209*	7861	*2147*	6056	*1267*	3.302	0.347
Rotifera										
Asplanchna	1667	*823*	2028	*549*	1556	*746*	2194	*586*	2.253	0.522
Brachionus	2917	*1532*	2056	*538*	1778	*681*	3000	*725*	3.944	0.268
Filinia	500	*274*	611	*241*	1222	*432*	1333	*506*	3.043	0.385
Keratella	1083	*339*	1194	*366*	1694	*479*	2194	*563*	2.259	0.520
Lecane	250	*141*	250	*147*	0		56	*56*	5.471	0.140
Polyarthra	167	*114*	194	*167*	444	*315*	1111	*808*	0.457	0.928
Trichocerca	222	*158*	750	*316*	361	*201*	694	*341*	2.516	0.472
Total	6806	*1684*	7083	*833*	7056	*1375*	10583	*1664*	4.351	0.226

Water quality parameters

Means (and ranges) of daily monitored water quality data by substrate and control ponds are given in Table 5. Substrate type had no significant effect ($P > 0.05$) on daily water quality parameters other than bottom DO. There were significant effects of sampling date ($P < 0.05$) on all daily monitored water quality parameters. Surface and bottom temperatures varied between 28-33.7°C and 27.6-32.4°C, respectively. Although mean Secchi depth was higher in the control ponds (46 cm) than in the substrate ponds (36-43 cm), differences were not statistically significant. The presence of substrates significantly affected mean bottom DO values (control = 3.0 mg l^{-1}; substrate 2.2-2.5 mg l^{-1}; Tukey test). The pH fluctuated between

7.5 and 9 during the first half of the experiment, dropping to between 7 and 7.5 during the second half. During the final week of the trial, pH increased to around 9 in all treatments.

Table 5
Mean values of daily water quality parameters. Values are means of three ponds and 44 sampling dates ($N = 132$). The range of observed values is given in parentheses.

Parameters	**Substrate types**			
	Bamboo	**Hizol**	**Kanchi**	**Control**
Surface temperature	30.4	30.5	30.4	30.7
(°C)	(28.1-33.7)	(28.1-33.7)	(28.0-33.5)	(28.2-33.7)
Bottom temperature	29.8	29.9	29.89	30.1
(°C)	(27.6-31.9)	(27.6-32.0)	(27.7-31.9)	(27.6-32.4)
Secchi depth (cm)	43	38	36	46
	(16-120)	(19-88)	(10-111)	(19-95)
Surface DO (mg l^{-1})	5.8	5.8	5.3	5.9
	(0.8-14.7)	(0.4-14.2)	(0.4-13.5)	(0.4-14.8)
Bottom DO (mg l^{-1})	2.4	2.5	2.2	3.0
	(0.2-10.5)	(0.1-7.2)	(0.1-7.2)	(0.3-9.1)
pH range	6.5-9.8	6.7-9.3	6.5-9.4	6.7-9.9

Substrate type did not affect ($P > 0.05$), but there was an effect of sampling date ($P < 0.05$) on all weekly monitored water quality parameters (Table 6). Alkalinity decreased slightly over the experimental period from around 140 to 110 mg l^{-1}, except in the kanchi treatment where it rose to 140 in the last two weeks. Nitrate fluctuated between 1 and 4 mg l^{-1} with higher values during the last two weeks of the experiment for all substrates as well as in the control ponds. Total ammonia values were around 0.2 mg l^{-1} during the first four weeks and then rose to between 0.6 and 1.2 depending on the substrate type. Phosphate fluctuated in all substrate treatments with the highest concentration in the kanchi treatment in Week 4 (1.6 mg l^{-1}). Pond water chlorophyll *a* values showed a cyclic pattern in all three substrates with values between 100 and 400 µg l^{-1}, except for the control ponds where 600 µg l^{-1} was the highest concentration in Week 3; thereafter it decreased below 100 μg l^{-1} till the last day of the experiment.

Table 6

Mean values of weekly water quality parameters. Figures are means of three ponds and seven sampling dates ($N = 21$). The range of observed values is given in parentheses.

Parameters	**Substrate types**			
	Bamboo	**Hizol**	**Kanchi**	**Control**
Total alkalinity	126	120	132	121
(mg l^{-1})	(90-184)	(84-162)	(95-166)	(91-156)
Nitrate nitrogen	2.34	2.30	2.78	2.27
(mg l^{-1})	(1.0-3.8)	(0.7-3.8)	(1.0-5.7)	(0.7-4.1)
Total ammonia	0.43	0.28	0.46	0.31
(mg l^{-1})	(0-1.48)	(0-2.13)	(0-1.38)	(0-0.95)
Phosphate	0.60	0.44	0.81	0.43
phosphorous (mg l^{-1})	(0.07-1.74)	(0-2.39)	(0.03-2.7)	(0.05-1.13)
Chlorophyll *a*	139	165	153	107
(μg l^{-1})	(1-589)	(7-646)	(1-518)	(4-468)

Discussion

Periphyton productivity

Periphyton biomass as measured by DM and pigment concentrations, differed significantly between depth with higher values in the upper 0-60 cm depth. These results are in agreement with the findings of Konan-Brou and Guiral (1994) and Keshavanath et al. (2001) who reported maximum periphytic biomass levels coinciding with photosynthetic compensation depths.

Ponds with phytoplankton blooms can produce 2-4 g C m^{-2} d^{-1} (Delincé, 1992). An estimate of phytoplankton productivity in the experimental ponds can be made from the increase in chlorophyll *a* concentration of pond water during Week 2 of the experiment. On average, the increase in chlorophyll *a* was 268 μg l^{-1} during that week, equivalent to an estimated production of 1.17–1.53 g C m^{-2} d^{-1} (assuming 47% C in phytoplankton DM, 1 mg Chl-*a* per 65-85 mg DM; Reynolds, 1984; Dempster et al., 1993; APHA, 1992). Whereas, from the biomass increase of the periphyton during the first week of the experiment, when clean substrates were first colonized, periphyton productivity was 2.17-2.83 AFDM m^{-2} d^{-1} depending on substrate types. Highest periphyton productivity in terms of carbon was calculated for hizol with 1.38 g C m^{-2} d^{-1} followed by kanchi (1.03 g C m^{-2} d^{-1}) and bamboo (1.01 g C m^{-2} d^{-1}) (C content from Table 2). Based on maximum periphyton productivity values as observed in the present trial, pond productivity is approximately doubled as a result of the periphyton-bearing substrate.

Despite a peak in Week 3, mean chlorophyll *a* concentration of water in the control ponds was not higher than in the ponds with substrates (Table 6). Therefore, periphyton production was additional to phytoplankton production. Regular fertilization of all ponds was conducted throughout the trial, resulting in persistent high dissolved N and P concentrations and avoidance of nutrient limitation conditions.

After Week 3, periphyton biomass more or less stabilized (except for the hizol treatment), probably due to algal competition for substrate, nutrients and light, self-shading and decreased productivity of older periphyton. A sharp increase in biomass in the hizol treatment was observed during the last week (Figure 1A), possibly because of the inadvertent inclusion of hizol bark in the periphyton samples. Huchette et al. (2000) reported that the periphyton communities grazed by tilapia were younger, healthier and more productive. Although fish were absent in this experiment, grazing by zooplankton, molluscs and other invertebrates did occur. We identified several zooplankton genera both attached on the substrates and in pond water. Macrobenthic organisms, especially chironomid larvae, were observed on the substrates, but became detached from the poles during sampling. However, taxonomic analysis of the sessile component showed rapid development of a relatively stable community with little differences between substrate types (Table 3).

Periphyton nutritional quality

The ash content of the periphyton varied over time as well as between substrates. The higher ash content on hizol substrates might be caused by the surface of hizol being much rougher than that of the other two substrate types, thereby trapping more sediment particles. However, the ash content of periphyton samples from bamboo and kanchi was less than 30%, which can be considered reasonable for herbivorous fish (Yakupitiyage, 1993). Protein content of the periphyton from bamboo was much higher (38% AFDM) than from hizol and kanchi (Table 2). Still, 25-26% protein and an energy level of 19-20 kJ g^{-1} AFDM in the periphyton from hizol and kanchi compares well with some other vegetative materials used in aquaculture (Hepher, 1988; Yakupitiyage, 1993; Dempster et al., 1995). Dempster et al. (1995) reported 28-55% protein and 5-18% lipid in some algal species. Hepher (1988, cited from other literatures) reported 18-31% protein, 4-10% lipid and 27-48% ash contents on dry matter basis for planktonic algae in ponds. Makrevich et al. (1993) and Huchette et al. (2000) reported that periphyton ash contents were higher without grazing by fish and increased with time. The low estimated lipid value for kanchi (0.5%) may be an artifact of the small sample size, resulting in highly variable residual water values, as observed in the deviations associated with mean residual water fractions of dried samples (Table 2). Periphyton can be a good fish feed provided that the fish species used can harvest it. However, although periphyton production on hizol was comparatively high, bamboo is superior in terms of higher protein and pigment contents and lower ash content. Hem and Avit (1994) and Keshavanath et al. (2001) also reported bamboo as a superior substrate.

The periphyton AI values of bamboo, hizol and kanchi were 189, 346 and 251, respectively, which indicated that higher amount of algae colonized on bamboo substrate than on hizol and kanchi (APHA, 1992). The AI values also decreased with time in this experiment indicating that AFDM of non-algal origin dominated in periphyton DM at initial stage. Huchette et al. (2000) reported AI values ranged from 150 to 300 in ungrazed and 300 in grazed conditions. Bender et al. (1989) explained the mechanisms of biomass development of microbial mats on the substrates. They suggested that there is an interaction between the periphyton and the detrital matter on the bottom of a tank that is necessary for the periphyton to develop. According to them, the first colonization of the substrates is done by bacteria, probably from the sediments. Assuming that 1 mg chlorophyll *a* can be derived from 65-85 mg algal AFDM (Dempster et al., 1993; Reynolds, 1984; APHA, 1992), algae comprised 34-45%, 19-25% and 26-34% of the periphytic biomass of bamboo, hizol and kanchi, respectively. The bulk of the periphyton organic matter is thus not of an algal nature.

Effects of artificial substrates on water quality

Although DO concentrations were suitable for fish culture throughout the experimental period, exceptionally low DO values were recorded on a few occasions. Bottom DO concentrations were significantly higher in the control ponds than in the other treatments, differences being approximately 0.5-1 mg l^{-1} (Table 5). This may be an indication of reduced water mixing due to the presence of the substrates, but the difference seems not important. In fact, there was little difference among treatments in all the other water quality parameters. The higher Secchi disc visibility in control ponds could be related to the absence of shading due to substrate itself and dislodgment of periphyton from the substrates. Konan-Brou and Guiral (1994) reported a reduction in the euphotic layer in acadjas in Côte d'Ivoire through shading effects caused by bamboo.

Plankton abundance was similar in all substrate and control ponds but chlorophyll *a* of water was lower in control ponds during the last three weeks of the experiment, despite the fact that same rates of fertilization were applied to all substrate and control ponds. This may be an important advantage of periphyton-based systems since there was no trade-off between periphyton and phytoplankton production. Higher inorganic nutrients were recorded from ponds provided with substrates than control ponds although they were not statistically different.

The potential of periphyton-based aquaculture systems

Filter-feeding on small planktonic algae may not fully meet the energy requirements of most herbivorous carp and tilapia species (Dempster et al., 1995). Besides phytoplankton, these herbivorous fish generally require larger-sized food sources such as benthic algae, algal-based detritus or higher aquatic plants that can be harvested more efficiently (Dempster et al., 1993; Yakupitiyage, 1993). Benthic algal mats rarely grow on bottoms in highly eutrophic ponds due to light limitation. They need some hard substrate in euphotic layer of the ponds to grow which is absent in traditional fish ponds. In the present study, a more diverse algal (56 genera) community on substrates was found than in pond water (35 genera), some 30 algal genera being exclusive to the periphyton communities. In addition, other periphytic compositions such as heterotrophic microorganisms, zooplankton, benthic macroinvertebrates and organic matters can also be consumed by many fish species (Prejs, 1984; Horn, 1989).

According to Miller and Falace (2000), there are two mechanisms for increasing fish production in artificial reefs-based systems : 1) the additional shelter provided by the substrate allows more of the resources to flow into fish biomass, and 2) the new primary production and attached benthic secondary production fostered by the artificial substrate support a new food web, part of which will end up in fish biomass. The highest values for periphytic algae are probably those reported for benthic algal turfs on coral substrates, the most productive natural ecosystems in tropical waters, which range from 1-3 g C m^{-2} d^{-1} (e.g. Wanders, 1976; Polovina, 1984; Carpenter, 1985; Polunin, 1988; Van Rooij et al., 1998). In the present study, the combined production of phytoplankton and periphyton in tropical aquaculture ponds could achieve comparable production figures (1.17–1.53 g C m^{-2} d^{-1} from phytoplankton and 1.01-1.38 g C m^{-2} d^{-1} from periphyton). However, pond productivity could be further increased if plankton and periphyton are optimally grazed by fish (Hatcher, 1983; Hay, 1991; Huchette et al., 2000) and ponds are optimally fertilized.

In a grazing trial in laboratory with Nile tilapia (*Oreochromis niloticus*), protein conversion ratio (protein consumed/increment of fish biomass) and food utilization rate (food consumed/total food offered) of periphyton DM were 0.48 and 0.68, respectively (Azim et al., unpublished data). Based on a productivity estimate of 0.59-0.83 protein m^{-2} d^{-1} (calculated from 2.17-2.83 g AFDM m^{-2} d^{-1} and % protein from Table 2) from periphyton, a fish production of 1.23-1.73 g fresh weight m^{-2} d^{-1} can be achieved, equivalent to 4,500-6,300 kg ha^{-1} y^{-1} from periphyton alone. Although this is a rather bold extrapolation for complex pond ecosystem, this figure is indeed comparable to the results from other studies. A maximum production of 8,000 kg ha^{-1} y^{-1} of tilapia (*Sarotherodon melanotheron*) was achieved in acadja-enclos in the Ebrie Lagoon, Ivory Coast (Hem and Avit, 1994). Ramesh et al. (1999) reported a maximum production of 3,390 kg ha^{-1} y^{-1} in a polyculture with rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*) of which sugarcane baggase (used as substrate) contributed about 1,343 kg ha^{-1} y^{-1}. In a monoculture trial, Azim et al. (2001a) recorded a total production of rohu of 5,800 kg ha^{-1} y^{-1}, of which 2,520 kg ha^{-1} y^{-1} was contributed with periphyton grown on bamboo substrates. In another polyculture trial with rohu and catla (*Catla catla*), Azim et al. (2001b) reported a net yield of 6,700 kg ha^{-1} y^{-1} in periphyton-based system compared to a net yield of 2,340 kg ha^{-1} y^{-1} in the control system without substrate. According to them, an increased production of 4,360 kg ha^{-1} y^{-1} in substrate-based system was achieved not only because of added substrate but also from synergistic interactions of the two fish species. However, production is likely to be influenced by a range of factors, such as age, size, species and food and feeding habit of fish, availability of other food sources in ponds, environmental parameters, etc.

Conclusions

Bamboo is recommended as substrate for periphyton growth, in view of its production of high quality periphyton, its availability in the tropics, ease of use and durability. Periphyton substrates do not have any adverse effect on water quality parameters. By supplying a substrate area equal to the pond surface, the periphyton alone could support a fish production of around 5,000 kg ha^{-1} y^{-1}. More research is needed to determine optimum substrate density and fertilization strategies and to select the fish species combinations that achieve the highest production. Other factors, such as the economic viability of the potential substrate materials will be important in determining how this technology can be applied under field conditions in resource poor countries.

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Chapter 3

Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-based aquaculture

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Abstract

The effects of four rates of application of fertilizer, with cow manure (3,000 kg ha^{-1}), urea (100 kg ha^{-1}) and TSP (100 kg ha^{-1}) (treatment F), treatment F x 0.5 (treatment 0.5F), treatment F x 1.5 (treatment 1.5F) and treatment F x 2 (treatment 2F), on periphyton, plankton and water quality in tropical freshwater ponds were studied. The highest periphyton biomass in terms of dry matter (3.27 mg cm^{-2} substrate), ash free dry matter (2.06 mg cm^{-2} substrate) and chlorophyll *a* (7.49 μg cm^{-2} substrate) developed in treatment 1.5F. Ash content of periphyton was lower in treatment 1.5F (38% of dry matter) than in other treatments (57-66% of dry matter). Total ammonia and chlorophyll *a* of water increased with fertilization rate. Treatment 1.5F (cow manure, urea and TSP at rates of 4,500, 150 and 150 kg ha^{-1}, respectively) appears to be the optimum, yielding high quantity and quality periphyton. By supplying a substrate area for periphyton equivalent to the pond water surface, it was estimated that this level of fertilization could support a fish production of around 5,000 kg ha^{-1} y^{-1}, without recourse to supplementary feed.

Introduction

In Bangladesh and elsewhere in the region fertilization is widely used to increase fish yields from ponds. Fertilization stimulates both the autotrophic and heterotrophic production chains, which in turn enhances fish production (Wohlfarth and Schroeder, 1979; FAO, 1983; Wohlfarth et al., 1985; Sharma and Olah, 1986; Li, 1987; Zhu et al., 1990; Maclean et al., 1994; Milstein et al., 1995; Garg and Bhatnagar, 1996, 2000). However, Hickling (1962) and Hepher (1988) have demonstrated that fish production in fertilized ponds does not increase in direct proportion to increased fertilizer additions and that above a certain level, increasing fertilizer rates does not further increase fish yields.

Periphyton-based aquaculture systems offer the possibility of increasing both primary production 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., 2001a), an important consideration in resource-constrained countries. In recent years, a range of substrate-based aquaculture systems has been developed with the aim of increasing the production of periphyton per unit fertilizer input (NFEP, 1997; Ramesh et al., 1999; Wahab et al., 1999; Azim et al., 2001b; Keshavanath et al., 2001), thereby increasing economic viability.

However, a dose-response relationship for fertilizer in periphyton-based systems with respect to phytoplankton and periphyton (as an additional food source) availability has not been developed.

The present experiment was carried out to optimize the fertilizer dose for the production of periphyton grown on bamboo substrate in earthen ponds by comparing periphyton biomass, plankton biomass and water quality parameters. The potential of periphyton-based aquaculture systems is also considered. Periphyton is defined here as the entire complex of sessile biota attached to the substratum, plus associated detritus and microorganisms.

Materials and methods

Pond facilities and design

The trial was carried out in twelve earthen ponds (10 m × 7.5 m, mean water depth 1.2 m) at the Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, over a 6-weeks period during July-September 1998. The experiment had a completely randomized design, with four treatments and three replicates of each. The traditionally practiced fortnightly fertilizer dose (partially decomposed cow manure, urea and triple super phosphate (TSP) at the rates of 3,000, 100 and 100 kg ha^{-1}, respectively) in aquaculture ponds in Bangladesh was used as standard. This standard is equivalent to 66 kg N ha^{-1} and 60 kg P ha^{-1}, and is herein called treatment F. In the other treatments fertilization rates were reduced by half (treatment 0.5F), increased in 50% (treatment 1.5F) and doubled (treatment 2F). Bamboo (*Bambusa* sp.) was used as substrate for periphyton growth in view of its production of high quality periphyton, its availability, ease of use and durability (Hem and Avit 1994; Ramesh et al. 1999; Keshavanath et al. 2001; Azim et al., 2001c).

Substrate installation and pond preparation

Prior to the trial, ponds were renovated, aquatic vegetation removed and all small fish and other larger aquatic organisms eradicated. Maintaining a substrate free perimeter, an effective pond area of 8×5 m^2 was planted with bamboo poles (mean length = 2.0 m; mean diameter = 5.5 cm) driven vertically into the pond bottom, the upper portion extending above the water surface, at a density of 9 poles m^{-2}. The bamboo substrates added an effective surface area of about 75 m^2 per pond, approximating the pond water surface area. Ponds were subsequently filled with water and treated with fertilizers according to the experimental design on Day 1, followed by applications fortnightly.

Determination of periphyton biomass

The periphyton biomass growing on the substrates, *viz.* dry matter (DM) and pigment concentrations (chlorophyll *a* and pheophytin *a*), were determined weekly following standard methods (APHA, 1992), beginning on Day 7. From each pond, three poles were selected by random number tables and two 2 x 2 cm^2 samples of periphyton were taken at each of four depths (0, 30, 60 and 90 cm below the water surface) per pole. The areas were carefully scraped with a scalpel blade to remove all periphyton and no (visually detectable) substrate. After sampling, the poles were replaced in their original positions, marked and excluded from subsequent sampling.

One sample of the two was used to determine total DM and ash content. The material was collected on pre-weighed and labeled pieces of aluminium foil, dried at 105°C until constant weight (24 h in a Memmert stove, Model UM/BM 100-800), and kept in a dessiccator until weighed (BDH, Model 100A; precision 0.1 mg). Because the individual dry matter samples were too small to allow reliable determination of ash content, 2 x 2 cm^2 samples from all dates, poles and replicate ponds per treatment and depth were pooled. They were then transferred to a muffle furnace and ashed at 450°C for 6 h and weighed. The DM, ash free dry matter (AFDM) and ash content were determined by weight differences (APHA, 1992).

The remaining 2 x 2 cm^2 sample was used to determine chlorophyll *a* and pheophytin *a* content following standard methods (APHA, 1992). Upon removal, the material 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 s with a tissue grinder, refrigerated for 4 h and centrifuged for 10 min at 2,000-3,000 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 and autotrophic index (AFDM/Chl-*a*) were calculated using the equations given in APHA (1992).

Determination of plankton biomass

Water samples for chlorophyll *a* analyses were collected together with nutrient analyses on a weekly basis. A known amount of water was filtered through Whatman GF/C filters. The filtered water samples were used for nutrient analyses and filter papers were used to determine chlorophyll *a* concentrations. The filter papers with plankton were put into a plastic tube, 5 ml 90% acetone was added and the filter paper was ground with a tissue grinder. After the filter was crushed, a further 5 ml of 90% acetone was added, stirred and the tubes transferred to a refrigerator for 24 h. The tubes were centrifuged for ten minutes at 3,000 rpm, the supernatant was decanted into1 cm glass cuvettes and absorption measured at 665 and 750. Chlorophyll *a* was determined following Boyd (1979):

$$\text{Chlorophyll } a \ (\mu g \ l^{-1}) = 11.9(E_{665}-E_{750})V/L\text{x}1000/S$$

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).

On the last sampling day, the taxonomic composition of plankton was studied. Plankton samples were collected by passing 5 l of water taken from five locations of each pond through a plankton net (mesh size 45µ). The concentrated samples were then carefully transferred to a measuring cylinder and made up to a standard volume of 100 ml with distilled water. Samples were preserved in small plastic bottles with 5% buffered formalin. Plankton numbers were estimated using Sedgewick Rafter counting cell (S-R cell). One ml concentrated sample was placed on to the counting chamber of the S-R cell and was left to stand for 15 min to allow plankton to settle. The phytoplankton on 10 randomly selected fields of the chamber were counted under a binocular microscope (Swift, M-4000). Plankton density was calculated using the following formula:

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

where N = the number of plankton cells or units per litre of original water; P = the number of plankton counted in ten fields; C = the volume of final concentrate of the sample (ml); L = the volume (litres) of the pond water sample. Identification of plankton to genus level was carried out using the keys from Ward and Whipple (1959), Prescott (1962) and Bellinger (1992).

Water quality monitoring

Determination of water quality parameters started on Day 1 of the experiment and was carried out between 0900 and 1000 h. Temperature and dissolved oxygen content, pH and water transparency (Secchi disc depth) were measured daily. Total alkalinity, total ammonia (NH_4^+-N), nitrate (NO_3-N) and phosphate (PO_4-P) were measured weekly. Temperature and DO of both surface and bottom water were measured with a DO meter (YSI, model 58) and pH with a pH electrode (Jenway, model 3020). Total alkalinity was determined titrimetrically following Stirling (1985). Water samples were filtered before the nutrients were analyzed using a Hach kit (DR 2000) and following standard methods.

Statistical analyses

Daily and weekly water quality parameters were compared by split-plot ANOVA with fertilization level as the main factor and time as the sub-factor (Gomez and Gomez, 1984) using the SAS 6.12 program (SAS Institute Inc., Cary, NC 27513, USA):

$$Y_{ijk} = \mu + S_i + e_{ij} + T_k + (SxT)_{ik} + e_{ijk}$$

where Y_{ijk} = observed value; μ = overall mean; S_i = effect of fertilization level (i = 4); e_{ij} = error 1 (j = 3 replicates); T_k = effect of sampling date (k = 45 for daily and k = 7 for weekly water quality); $(SxT)_{ik}$ = interaction of fertilization level and sampling date; e_{ijk} = error 2.

Periphyton DM and pigment parameters (means of three poles per pond) were analysed in a split-split-plot ANOVA with fertilization level as the main factor, depth as the first sub-factor and sampling date as the second sub-factor:

$$Y_{ijkl} = \mu + S_i + e_{ij} + D_k + e_{ijk} + T_l + (SxT)_{il} + (DxT)_{kl} + e_{ijkl}$$

where, Y_{ijkl} = observed value; μ = overall mean; S_i = effect of fertilization level (i = 3); e_{ij} = error 1 (j = 3 replicates); D_k = effect of depth (k = 4); e_{ijk} = error 2; T_l = effect of sampling date (l = 6); $(SxT)_{il}$ = interaction of substrate type and sampling date; $(DxT)_{kl}$ = interaction of depth and sampling date; e_{ijkl} = error 3.

The interaction of fertilization level, depth and date was added to error 3 because their interaction was found to be non-significant ($P > 0.05$). If a main effect was significant, the ANOVA was followed by a Tukey test. Ash percentage, AFDM, AI and plankton abundance were compared among treatment and depth by one-way ANOVA because of small sample numbers.

Results

Periphyton biomass

Periphyton biomass values associated with different fertilizer doses, both overall and according to depth, are presented in Table 1. There were significant differences ($P < 0.05$) between fertilizer doses, between depths of substrates and between sampling dates for periphyton DM, chlorophyll *a*, AFDM, ash percentage and AI values. There was no interaction ($P > 0.05$) between fertilization level and substrate depth, indicating that the fertilizer level affected periphyton in a similar way at all bamboo depths. By contrast, the significant interactions ($P < 0.05$) for periphyton DM and chlorophyll *a* between fertilization levels and sampling dates indicated that the pattern of periphyton development differed with fertilization level (Figure 1). A similar trend was found for substrate depth and sampling date

Table 1

Mean values of periphyton biomass. Figures by fertilization level are means from four depths, three ponds and six sampling dates (N = 72) for dry matter (DM), chlorophyll *a* and pheophytin *a* and only four depths (N = 4) for ash free dry matter (AFDM), ash content and autotrophic index (AI) for each combination of fertilizer doses. If main effects are significant, means followed by different superscripts in the same row for fertilization level and same column for depth indicate differences at 0.05 level of significance based on Tukey test.

Parameter	Substrate Depth (cm)	Fertilization level 0.5F	F	1.5F	2F	Mean (depth)
	0	1.09	1.80	2.97	1.67	1.88^{b}
Dry matter	30	1.38	3.37	4.96	1.86	2.89^{a}
(mg cm^{-2})	60	1.04	3.03	2.94	2.11	2.28^{ab}
	90	1.02	1.91	2.20	1.26	1.60^{b}
Mean (fertilization level)		1.13^{c}	2.53^{ab}	3.27^{a}	1.72^{bc}	
	0	67	65	41	58	58
Ash content	30	58	67	34	65	56
(%)	60	48	64	34	54	50
	90	55	67	41	59	56
Mean (fertilization level)		57^{a}	66^{a}	38^{b}	59^{a}	
	0	0.36	0.62	1.74	0.69	0.85
Ash free dry	30	0.58	1.11	3.26	0.65	1.40
matter (mg cm^{-2})	60	0.54	1.09	1.94	0.97	1.14
	90	0.46	0.64	1.30	0.51	0.73
Mean (fertilization level)		0.49^{b}	0.87^{b}	2.06^{a}	0.71^{b}	
	0	4.62	5.39	6.33	4.53	5.22^{bc}
Chlorophyll *a*	30	7.96	7.44	7.29	6.78	7.37^{ab}
(µg cm^{-2})	60	8.19	9.05	11.89	4.19	8.33^{a}
	90	3.84	5.76	4.46	2.40	4.12^{c}
Mean (fertilization level)		6.15^{ab}	6.91^{a}	7.49^{a}	4.48^{b}	
	0	0.10	0.14	0.44	0.64	0.33
Pheophytin *a*	30	0.16	0.42	0.95	0.95	0.62
(µg cm^{-2})	60	0.10	0.37	0.34	0.33	0.29
	90	0.24	0.59	0.09	0.28	0.30
Mean (fertilization level)		0.15	0.38	0.45	0.55	
	0	78	115	275	152	155
AI	30	73	149	447	96	191
(AFDM/Chl-*a*)	60	66	120	163	231	145
	90	120	111	291	212	184
Mean (fertilization level)		84^{b}	124^{b}	294^{a}	173^{ab}	

interaction ($P < 0.01$) for chlorophyll *a*, indicating that chlorophyll *a* levels developed differently over time at different depths.

While the development of periphyton DM was more or less similar in treatments 0.5F and 2F during the entire period of study (Figure 1A), significantly higher ($P < 0.01$) mean DM values were found in treatments F and 1.5F (Table 1). In treatment 1.5F, DM development showed a cyclic pattern with a peak at Week 4. In treatment F, DM reached the highest level at Week 2, thereafter stabilizing. There were significantly higher mean DM values ($P < 0.01$) at 30-60 cm substrate depths.

Periphyton mean chlorophyll *a* content was significantly lower ($P < 0.01$) in treatment 2F than in the other treatments. Chlorophyll *a* concentration increased steadily during the entire period of study in all treatments except treatment 2F in which it fell during the last week (Figure 1B). Chlorophyll *a* contents were significantly higher ($P < 0.0001$) at depth of 30-60 cm. Mean pheophytin *a* concentrations differed neither among fertilization levels nor among sampling dates ($P > 0.05$).

Ash percentage of periphyton DM in treatment 1.5F was significantly lower ($P <$ 0.0001) than in the other three treatments. However, the AFDM value was significantly higher ($P < 0.01$) in treatment 1.5F (Table 1). The AI was also higher ($P < 0.01$) in treatment 1.5 than in either treatment 0.5 or treatment F (Table 1).

Plankton biomass

Phytoplankton chlorophyll *a* concentrations at different fertilization levels and over different sampling dates are shown in Table 2 and Figure 1C, respectively. Fertilization level had a significant effect ($P < 0.05$) on chlorophyll *a* of phytoplankton. Chlorophyll *a* values reached a peak in Week 2 thereafter decreased and remained more or less stable during the remaining weeks of the experiment. The mean chlorophyll *a* value was significantly higher ($P < 0.05$) in treatment 2F, followed by treatments 1.5F, F and 0.5F, respectively.

The genera-wise abundance of plankton at different fertilization levels is presented in Table 3. Although plankton was generally more abundant at higher fertilization levels, differences were not statistically significant ($P > 0.05$). Twenty-five genera of phytoplankton belonging to Bacillariophyceae (5 genera), Chlorophyceae (11 genera), Cyanophyceae (5

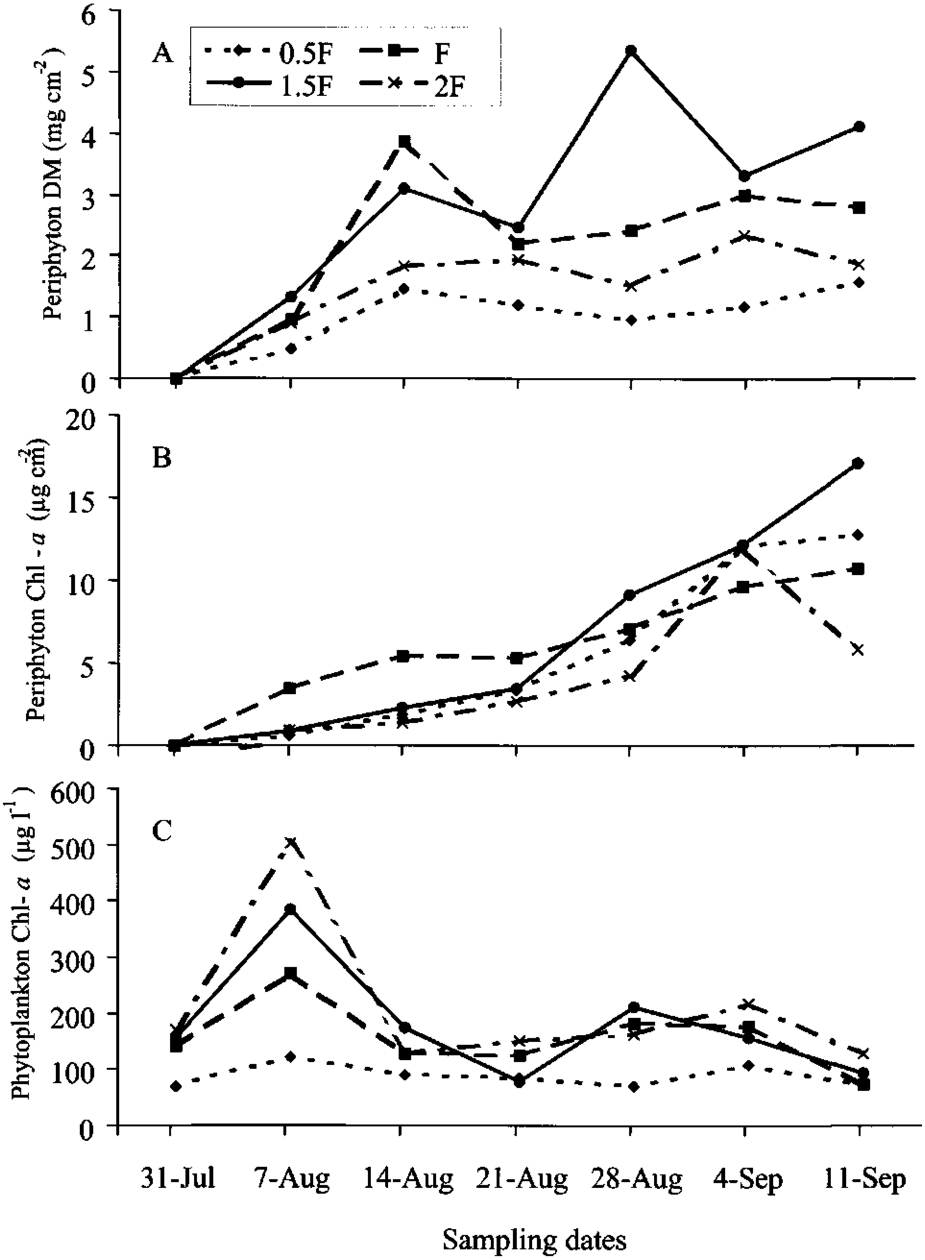

Figure 1. Periphyton and phytoplankton biomass under different fertilization levels during the experimental period. Values are means of four depths, three poles and three ponds for periphyton and only three ponds for phytoplankton per fertilization level. (A) Periphyton dry matter. (B) Periphyton chlorophyll *a* (C) Phytoplankton chlorophyll *a*.

genera) and Euglenophyceae (4 genera), and 4 genera of zooplankton (Crustacea, 1 genus; Rotifera, 3 genera) were found. Cyanophyceae was the most dominant group in treatments 0.5F, 1.5F and 2F, whereas Chlorophyceae was the dominant group in treatment F.

Water quality parameters

Weekly monitored water quality parameters, including chlorophyll *a*, are presented in Table 2. Fertilization level had a significant effect ($P < 0.05$) on total ammonia. Sampling date affected ($P < 0.01$) all weekly water quality parameters, except total alkalinity. In general, values of all weekly water quality parameters increased with increasing fertilization levels. Nitrate fluctuated between 1.4 and 6 mg l^{-1} with higher values during Week 4 of the experiment. Total ammonia values were significantly higher ($P < 0.05$) in treatments 1.5F and 2F than in either treatment 0.5 or treatment F. Phosphate values fluctuated between 0.17 and 2.75 mg l^{-1} in all fertilization treatments, peak concentrations occurring in Week 3.

Table 2

Summary of weekly water quality parameters. Figures are means of three ponds and seven sampling dates ($N = 21$). The range of observed values is given in parentheses. If main effects are significant, figures followed by different superscripts indicate differences at 0.05 level of significance based on Tukey test.

Parameters	**Fertilization level**			
	0.5F	**F**	**1.5F**	**2F**
Total alkalinity	61.26	71.62	76.05	85.14
(mg l^{-1})	(34-112)	(41-122)	(38-122)	(60-122)
Nitrate-N	2.89	3.36	3.72	3.45
(mg l^{-1})	(1.6-4.7)	(1.7-5.9)	(1.4-5.8)	(1.8-5.5)
Total ammonia	0.89^{b}	1.10^{b}	1.67^{a}	1.86^{a}
(mg l^{-1})	(0-2.33)	(0-2.48)	(0-2.75)	(0.21-2.75)
Phosphate-P	0.55	0.75	1.09	1.30
(mg l^{-1})	(0.21-0.92)	(0.17-1.99)	(0.18-2.75)	(0.16-2.75)
Chlorophyll *a*	87.3^{b}	156.7^{ab}	178.4^{ab}	207.8^{a}
(µg l^{-1})	(26.2-428.4)	(48.8-797.3)	(32.1-735.4)	(53.6-827.1)

Table 3

Abundance of plankton (cells or colonies l^{-1}) during last day of the experiment. Numbers are means of three ponds per fertilization level.

Group/Genera	**Fertilization level**			
	0.5F	**F**	**1.5F**	**2F**
Bacillariphyceae				
Cyclotella	6000	10667	11333	32667
Frustularia	2667	0	667	667
Melosira	0	4000	2000	0
Navicula	0	2667	0	2000
Surirella	4000	3333	4000	3333
Total	12667	20667	18000	38667
Chlorophyceae				
Ankistrodesmus	2000	0	1333	667
Ceratium	1333	0	0	2000
Chlorella	44667	75333	66667	82667
Closterium	0	0	9333	667
Crucigenia	4667	6667	0	667
Gloeocystis	0	1333	0	2000
Gonatozygon	667	0	0	3333
Oocystis	13333	42000	12000	11333
Pediastrum	0	667	2000	3333
Scenedesmus	667	8000	0	2000
Tetraedron	2667	5333	8000	667
Total	70000	139333	111333	109333
Cyanophyceae				
Anabaena	667	0	0	4667
Aphanocapsa	8000	3333	11333	40667
Chroococcus	8667	24667	11333	13333
Gomphosphaeria	1333	3333	12000	8000
Microcystis	68667	55333	174000	51333
Total	87333	86667	208667	118000
Euglenophyceae				
Euglena	12000	18667	6000	141333
Glenodinium	0	22000	0	0
Phacus	1333	667	667	667
Trachalomonas	0	3333	0	6000
Total	13333	44667	6667	148000
Crustacea				
Nauplius	0	1333	667	1333
Total	0	1333	667	1333
Rotifera				
Brachionus	2000	1333	2000	2667
Lecane	667	667	1333	1333
Keratella	4000	4000	2667	4667
Total	6667	6000	6000	9333
Grand total	190000	298667	351333	424667

Means (and ranges) daily water quality data according to fertilization levels are given in Table 4. Fertilization level had no significant effect ($P > 0.05$) on daily water quality parameters, but there were significant effects of sampling date ($P < 0.01$). Surface and bottom temperature varied between 27.9-33.1°C and 27.8-31.8°C, respectively. Although mean Secchi depth was higher in treatment 0.5F (51 cm) than in the other treatments (29-37 cm), differences were not statistically significant. Mean surface and bottom DO in different treatments varied between 5.33-6.95 and 2.61-3.17 mg l^{-1}, respectively. The pH levels were more or less similar in all the treatments, fluctuating between 6.25 and 10.3.

Table 4
Summary of daily water quality parameters. Figures are means of three ponds and 46 sampling dates ($N = 138$). The range of observed values is given in parentheses.

Parameters	**Fertilization level**			
	0.5F	**F**	**1.5F**	**2F**
Surface temperature (°C)	30.22 (28.3-32.9)	30.06 (28.3-32.7)	30.12 (28.1-33.0)	30.06 (27.9-33.1)
Bottom temperature (°C)	29.62 (28.0-31.5)	29.44 (28.0-31.4)	29.56 (27.9-31.6)	30.40 (27.8-31.8)
Secchi depth (cm)	51.43 (14-101)	30.95 (15.5-205)	37.09 (13-88)	28.51 (12-66)
Surface DO (mg l^{-1})	5.33 (1.5-16.8)	6.26 (1.6-16.7)	5.74 (1.6-16.4)	6.95 (2.4-18.2)
Bottom DO (mg l^{-1})	2.96 (0.6-8.5)	3.08 (0.5-8.0)	2.61 (0.1-7.2)	3.17 (0.6-8.1)
pH range	6.59-10.3	6.56-9.42	6.25-9.11	6.8-9.7

Discussion

Periphyton biomass

Except for treatment 1.5F, periphyton biomass, as measured by DM, more or less stabilized after Week 2 probably due to algal competition for substrate, nutrients and light, shading by plankton and the periphyton itself, and decreased productivity of older periphyton, and finally higher abundance. The DM contents of periphyton in treatment 2F were lower than those in treatments F and 1.5F during the entire period of experiment (Figure 1A) despite the fact that this treatment received the highest fertilizer dose. The quadratic relationships between periphyton DM and fertilization levels show that the DM increased linearly from fertilization level 0.5F to 1.5F and then collapsed at 2F (Figure 2A). This might be due to the higher planktonic abundance in treatment 2F (higher phytoplankton chlorophyll *a*; Table 2) which might impede sunlight penetration into the water column and hamper periphyton growth. Ocean coral reef algae must be grazed constantly and kept at a low biomass to maintain their high productivity (Hatcher 1983; Hay 1991). Huchette et al. (2000) reported that the periphyton communities grazed by tilapia were younger, healthier and more productive. Although fish were absent in this experiment, grazing by zooplankton, molluscs and other invertebrates (especially chironomid larvae) occurred possibly to a lower extent.

Although the ash content of the periphyton varied among different fertilization levels the reason for this is unknown. The lower ash content and higher organic matter of periphyton in treatment 1.5F indicated that it was superior as a food for fish. Huchette et al. (2000) reported similar periphyton ash contents derived from cages.

Chlorophyll *a* differed significantly among treatments as well as between depths, with higher values in the upper 0-60 cm depth. These results are in agreement with the findings of Konan-Brou and Guiral (1994), Keshavanath et al. (2001) and Azim et al. (2001c) who reported maximum periphyton biomass levels coinciding with the euphotic zone. The quadratic regression line comparing the periphyton chlorophyll *a* concentrations over different fertilization levels are shown in Figure 2B, indicating a similar trend to those of DM (Figure 2A).

The algal portion in the periphyton can be estimated from the relationship between ash-free dry matter and chlorophyll *a* concentrations. The periphyton AI values show that 1 mg chlorophyll *a* is equivalent to about 84, 124, 294 and 173 mg AFDM in treatment 0.5F, F, 1.5F and 2F, respectively. Generally, 65-85 mg algal organic dry matter contains 1 mg chlorophyll *a* (Reynolds, 1984; Dempster et al., 1993; APHA, 1992). This indicates that algae comprised 77-100%, 52-68%, 22-29% and 38-49% of the periphytic biomass in treatments 0.5F, F, 1.5F and 2F, respectively. Therefore, a considerable amount of the periphyton was not of an algal nature. Huchette et al. (2000) reported that AI values fluctuated between 150-

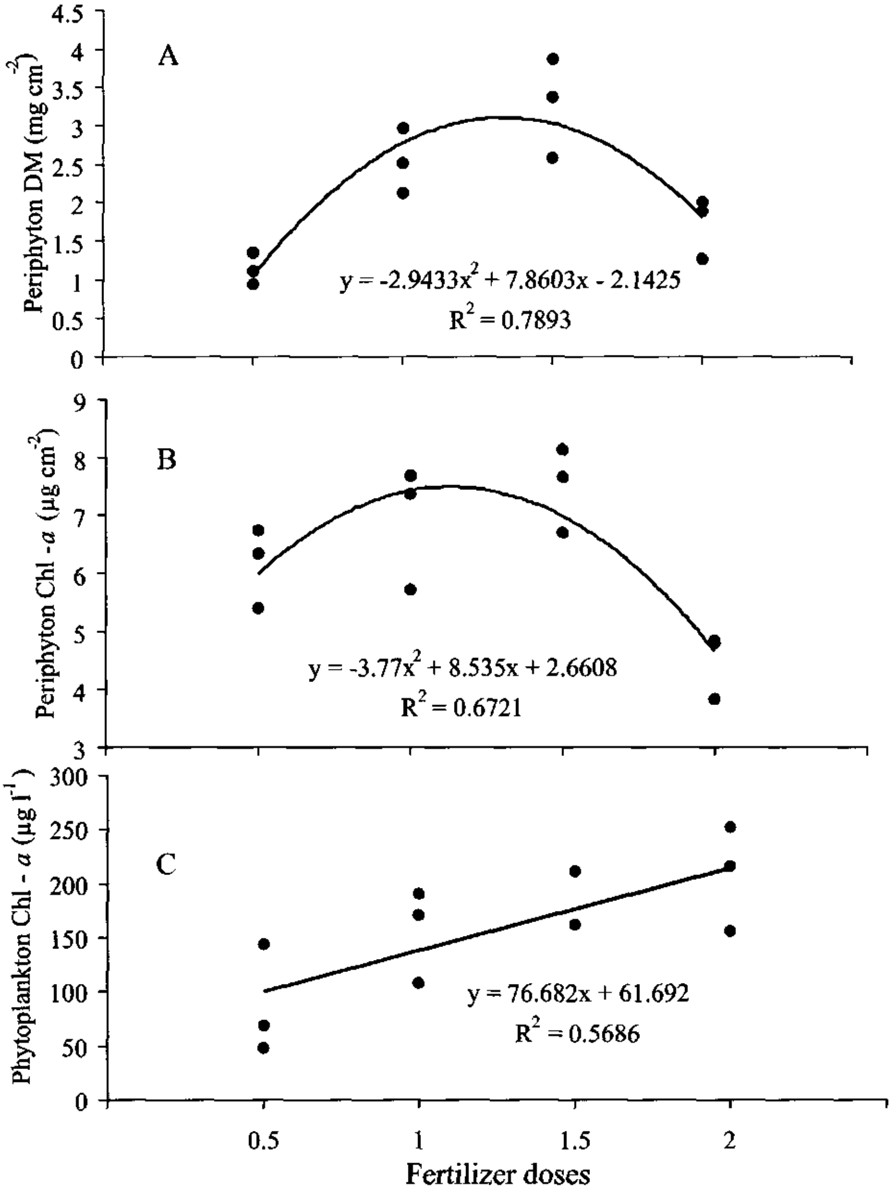

Figure 2. Relationship between biomass and fertilization level. Values are means of three poles, four depths and six sampling dates for periphyton and only six sampling dates for phytoplankton per fertilization level. (A) Periphyton dry matter (B) Periphyton chlorophyll *a* and (C) Phytoplankton chlorophyll *a*.

300 in ungrazed conditions and remained stable at around 300 in grazed conditions, suggesting a highly heterotrophic community. Azim et al. (2001c) estimated that AI values ranged from 190 to 350 depending on the substrate used.

The maximum increase in periphyton DM was 2.89 mg cm^{-2} (1.79 mg AFDM cm^{-2}) between Weeks 3 and 4 (Figure 1A), equivalent to 1.20 g C m^{-2} d^{-1} (assuming 47% C content in periphyton AFDM, Reynolds 1984; Azim et al. 2001c). The highest values for periphytic algae are those reported for benthic algal turfs on coral substrates (1-3 g C m^{-2} d^{-1}), the most productive natural ecosystems in tropical waters (e.g. Wanders, 1976; Polovina, 1984; Carpenter, 1986; Polunin 1988; Van Rooij et al., 1998).

Plankton biomass

Despite a peak in Week 2 (270-500 μg l^{-1}) in all treatments except treatment 0.5F, mean chlorophyll *a* concentrations in water were around 100-200 μg l^{-1} during the remaining weeks of the experiment. There was a significant positive relation between chlorophyll *a* and fertilization levels (Figure 2C) (R^2 = 0.57). A similar trend was detected for plankton abundance. It is well established that phytoplankton productivity is positively correlated with nutrient concentrations (Boyd, 1990), as is apparent in the present study. However, phytoplankton chlorophyll *a* stabilized after Week 3 whereas periphyton chlorophyll *a* increased steadily throughout the experimental period (Figure 1B, C). An apparent negative correlation between phytoplankton and periphyton chlorophyll *a* was not significant (r = -0.33).

Maximum phytoplankton productivity of experimental ponds in treatment 1.5F (Figure 1C) can be estimated from the increase in chlorophyll *a* concentration of pond water (226 μg l^{-1}) between Weeks 1 and 2. The increase was equivalent to an estimated production of 1.0 –1.3 g C m^{-2} d^{-1} (assumes 47% C content in phytoplankton DM, 1 mg Chl-*a* per 65-85 mg DM; Reynolds, 1984; Dempster et al., 1993; APHA, 1992). Ponds with phytoplankton blooms can produce 2-4 g C m^{-2} d^{-1} (Delincé, 1992). The highest phytoplankton productivity in ponds under treatment 2F was 1.37-1.88 g C m^{-2} d^{-1}.

Water quality parameters

Water temperature at both pond surface and bottom were within the optimal range for tropical fish culture. DO concentrations were generally suitable for fish culture throughout the experimental period, although exceptionally low DO values were recorded on a few occasions. Ammonia concentrations increased in treatments in which higher doses of fertilizer were used. Garg and Bhatnagar (1996) also observed that nitrate, phosphate and ammonia increased with increasing fertilizer dose.

The scope for periphyton-based aquaculture systems

From a theoretical point of view, the greatest advantage conferred by adding substrate to an aquaculture system is an increase in the energy and nutrient transfer efficiencies of the system due to the additive effect of the periphyton-based and phytoplankton-based components of production. Filter-feeding on small planktonic algae is unlikely to meet the energy demands of most herbivorous carps and tilapia species (Dempster et al., 1995). Herbivorous fish generally require larger-sized food sources such as benthic algae, algal-based detritus or higher aquatic plants that can be harvested more efficiently to supplement the intake of phytoplankton (Dempster et al., 1993; Yakupitiyage, 1993). Hard substrate, which is required for larger benthic algae, is generally absent in traditional fish ponds, and benthic algal mats usually do not develop owing to light limitation in highly eutrophic ponds with dense phytoplankton blooms.

In periphyton-based systems, algae growing on substrates, and the associated bacterial and zooplanktonic biomass, can be exploited directly by many herbivorous fish species (Prejs, 1984; Horn, 1989; Huchette et al., 2000), resulting in a higher fish yield than when an intermediate step is involved. However, such increases can only be realized if the combined periphyton and algal production is higher than that of phytoplankton, and if water quality is not negatively affected by the presence of the substrates. Based on the findings of the present study, it would seem that the combined production of phytoplankton and periphyton in tropical aquaculture ponds could achieve comparable production figures (1.0–1.3 g C m^{-2} d^{-1} from phytoplankton and 1.20 g C m^{-2} d^{-1} from periphyton in treatment 1.5). Productivity could be further increased when the plankton and periphyton community are grazed constantly by fish (Hatcher, 1983; Hay, 1991; Huchette et al., 2000). From the biomass increase in periphyton between Weeks 3 and 4 in treatment 1.5F, it is estimated that a maximum periphyton production of 2.56 g AFDM m^{-2} d^{-1} can be achieved. Based on an average FCR value of 1.22 on periphyton AFDM basis for tilapia and 70% utilization of total biomass (Azim et al., unpublished data), periphyton alone could contribute a fish production of 1.47 g fresh weight m^{-2} d^{-1}, equivalent to 5,365 kg ha^{-1} y^{-1}. Production is likely to be influenced by a range of factors, such as age, size, species and food and feeding habit of fish, the nutritional quality of the periphyton, grazing efficiency of fish, availability of other food sources in ponds, environmental parameters, etc. However, the estimated production is comparable to results from some earlier studies. A maximum production of 8,000 kg ha^{-1} y^{-1} of tilapia (*Sarotherodon melanotheron*) was achieved in acadja-enclos in the Ebrie Lagoon, Ivory Coast, with natural recruitment of fingerlings (Hem and Avit 1994). Azim et al. (2000a) recorded a total production of rohu (*Labeo rohita*) of 5,800 kg ha^{-1} y^{-1}, of which 2,520 kg ha^{-1} y^{-1} was estimated to have been contributed by periphyton grown on bamboo substrates. Using traditional stocking densities (1 juvenile m^{-2}), Wahab et al. (1999) reported an additional production of 954 kg ha^{-1} y^{-1} of kalbaush (*Labeo calbasu*) from ponds provided with substrate, above that from control ponds (no substrates). The low production from the trial was attributed to a combination of the low temperatures prevalent throughout the culture period and low stocking densities. No significant reductions in periphyton biomass were observed, suggesting that fish density could be increased in this system. In a polyculture trial with rohu (*Labeo rohita*) and catla (*Catla catla*), Azim et al. (2001b) reported an additional net yield of 4,000 kg ha^{-1} y^{-1} by doubling the pond surface area with bamboo substrate.

Conclusion

The fortnightly fertilizer dose with cow dung, urea and TSP at a rate of 4,500, 150 and 150 kg ha^{-1} (treatment 1.5F) is recommended for optimal periphyton growth, in view of not only the higher periphyton production but also the high quality of the periphyton. It was estimated that this fertilization level may result in a yield of as much as 5,000 kg fish ha^{-1} y^{-1} by providing only a substrate area equal to the pond surface. Important research questions remain as to estimate proximate composition of periphyton, to determine optimum substrate density and to select the fish species combinations that result in highest production or profit.

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PART II

Selection of suitable indigenous fish species by monoculture

Chapter 4

The potential of periphyton-based culture of two Indian major carps, rohu *Labeo rohita* (Hamilton) and gonia *Labeo gonius* (Linnaeus)

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Fish stocking and pond management

All ponds were stocked (10,000 juveniles ha^{-1}) with similar-sized juveniles of rohu (mean weight 9.96±0.04 g) for the first trial and gonia (mean weight 4.15±0.35 g) for the second trial. Throughout the entire experimental period, cow manure, urea and TSP were applied fortnightly to the ponds at rates of 4,500, 150 and 150 kg ha^{-1}, respectively.

Assessment of water quality, plankton and periphyton

Water quality measurements were made on a weekly basis between 0900-1000 h on each sampling day. Water temperature and dissolved oxygen (DO) were determined with a portable DO meter (YSI model 58). The pH of water was determined with a pH meter (Jenway model 3020). Chlorophyll *a* was determined spectrophotometrically after acetone extraction following Boyd (1979). Water samples were filtered before nutrient analysis. Analyses of NH_4-N, NO_3-N and PO_4-P were carried out using a Hach kit (model DR 2000) and following standard methods (APHA, 1992).

For plankton enumeration, 5 l of water were collected from different locations and depths of each pond fortnightly and filtered through a fine meshed (25 μ) phytoplankton net. The filtered samples were then carefully transferred to a measuring cylinder and made up to a standard volume of 100 ml with distilled water. Buffered formalin (5%) was added as a preservative and the samples 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). Plankton density was estimated using the following formula:

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

where N = number of plankton cells or units per litre of pond water; P = total number of plankton counted in 10 fields; C = volume of final concentrate of the sample (ml); L = volume of the pond water sample (litre). Identification of plankton to genus level was performed using keys from Prescott (1962), Belcher and Swale (1976), Palmer (1980) and Bellinger (1992).

Collection of periphyton samples commenced 7 days after juveniles were stocked and continued at fortnightly intervals throughout the trials. From each pond, three bamboo poles were selected randomly and periphyton samples taken carefully. A 2 x 2 cm^2 surface area of each substrate 30 cm below the water surface was removed carefully by scalpel blade. Pooled samples were resuspended in 50 ml of distilled water and preserved in 5% buffered formalin in sealed plastic vials. Periphyton was enumerated using an S-R cell according to the procedure for plankton given above. Periphyton numbers were estimated using the following formula:

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

where, N = number of periphyton cells or units cm^{-2} surface area; P = total number of periphyton units counted in 10 fields; C = volume of final concentrate of the sample (ml); S = area of scraped surface (cm^2). Identification of periphyton relied on the keys used for plankton cited above.

Fish harvesting

Ponds were drained by pump at the end of the experiment and all fish were harvested. Total bulk weight and number of fish from each pond were recorded. The average individual weight gain was calculated by deducting the average initial weight from the average final weight. Specific growth rate (SGR) was estimated as :

SGR (% bw d^{-1}) = [($\log_e$final weight – $\log_e$initial weight) x 100]/culture period (days).

Data analysis

For statistical analyses of data, one-way ANOVA and linear regression were applied using a statistical package, STATISTICA version 5 on a personal computer. Percentage data were converted to arcsine values prior to analysis. Comparisons between different parameters were made for substrate and control ponds.

Euglenophyceae in both the trials. Five genera of zooplankton were also recorded (Rotifera 3, Copepoda 1 and Cladocera 1).

Table 3

List of plankton and periphyton community recorded from the experimental ponds in both trials. The common algae and zooplankton collected as both planktonic and periphytic nature are shown in separate column.

Groups	Plankton	Common	Periphyton
Bacillariophyceae	*Diatoma*	*Cyclotella*	*Achnanthes*
	Mepsira	*Cymbella*	*Fragillaria*
	Tabellaria	*Nitzschia*	*Synedra*
		Navicula	
Chlorophyceae	*Actinastrum*	*Ankistrodesmus*	*Ceratium*
	Arthrodesmus	*Botryococcus*	*Chlamydomonas*
	Characium	*Chlorella*	*Coleochaete*
	Cosmarium	*Closterium*	*Draparnaldia*
	Gloeocystis	*Gonatozygon*	*Oedogonium*
	Pediastrum	*Oocystis*	*Rhizoclonium*
	Scenedesmus	*Ulothrix*	*Stigeoclonium*
	Spirogyra		*Zygnema*
	Spirulina		
	Volvox		
Cyanophyceae	*Aphanocapsa*	*Anabaena*	*Aphanigominon*
	Gomphosphaeria	*Chroococcus*	*Gloetrichia*
	Merismopedia	*Microcystis*	*Phormidium*
		Oscillatoria	
Euglenophyceae	*Trachalomonas*	*Euglena*	*Difflugia*
		Phacus	
Rhodophyceae	--	*Audouinella*	*Batrachosperium*
Rotifera	*Asplanchna*	*Brachionus*	--
	Hexarthra	*Filinia*	
	Polyarthra	*Keratella*	
Copepoda	*Diaptomus*	*Cyclops*	--
	Nauplius		
Cladocera	*Daphnia*	*Diaphanosoma*	--

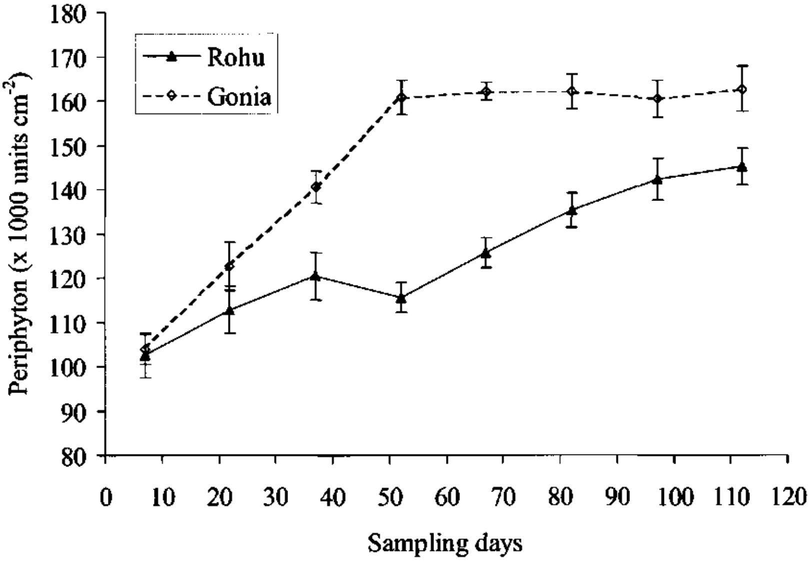

Figure 1. The mean numbers of periphyton grown on bamboo substrates in both rohu and gonia ponds during fortnightly samplings over experimental periods. Shown are the means (±S.D.) per species pond (five ponds per species, eight sampling dates).

Yield parameters of fish

Survival, growth and production data for rohu and gonia in ponds with and without substrate are summarized in Tables 4 and 5, respectively. Survival of rohu was 85 and 76% in the ponds with and without substrate, respectively, and differed significantly when arcsine transformed data were compared using ANOVA (F = 47.42; d.f. = 1,8; $P < 0.01$). Survival of gonia was same (96%) in both treatments (F = 0.06; d.f. = 1,8; $P > 0.05$). The individual mean weight of rohu at harvest was 232 g in ponds with substrate compared to 151 g in ponds without substrate (F = 15.38; d.f. = 1,8; $P < 0.01$) while SGR was 2.62 and 2.25% bw d^{-1} in ponds with and without substrate, respectively (F= 14.77; d.f. = 1,8; $P < 0.01$). The individual weight of gonia at harvest was 87 g and the SGR was 2.54% bw d^{-1}, and did not differ significantly in ponds with and without substrate (F = 0.78; d.f. = 1,8; $P > 0.05$ for individual weight at harvest, and F = 0.39; d.f. = 1,8; $P > 0.05$ for SGR).

Table 4
ANOVA and means of yield parameters of rohu in substrate and control ponds.

ANOVA Result	Survival (%)	Initial weight (g)	Individual weight at harvest (g)	Individual weight gain (g)	SGR (%)	Net yield (kg ha^{-1} 120 d^{-1})
F-ratio	47.42	0.57	15.38	15.37	14.77	23.15
P-level	0.000	0.471	0.004	0.004	0.005	0.001
d.f.	1, 8	1, 8	1, 8	1, 8	1, 8	1, 8
Treatment means						
With substrate	85	10	232	222	2.62	1901
Without substrate	76	10	151	141	2.25	1073

Table 5
ANOVA and means of yield parameters of gonia in substrate and control ponds.

ANOVA Result	Survival (%)	Initial weight (g)	Individual weight at harvest (g)	Individual weight gain (g)	SGR (%)	Net yield (kg ha^{-1} 120 d^{-1})
F-ratio	0.06	0.62	0.78	0.09	0.39	0.13
P-level	0.806	0.450	0.787	0.768	0.549	0.724
d.f.	1, 8	1, 8	1, 8	1, 8	1, 8	1, 8
Treatment means						
With substrate	96	4	87	83	2.54	794
Without substrate	96	4	86	82	2.54	788

Net productions of fish in the treatments with and without substrate were 1,900 and 1,073 kg ha^{-1} for rohu (Table 4), and 794 and 788 kg ha^{-1} for gonia (Table 5), respectively over the 120 days culture period. Rohu production was significantly higher in ponds with substrates than without substrate ($F = 23.15$; d.f. = 1,8; $P < 0.01$). There was no significant difference in gonia production between the two treatments ($F = 0.13$; d.f. = 1,8; $P > 0.05$).

Discussion

All water quality parameters were within the limits suitable for fish production. The pH values were slightly alkaline range in all the ponds indicating good pH conditions for biological production. Higher than usual nitrate nitrogen levels were recorded during the entire period of study in both the trials for reasons unknown. Nevertheless, nitrate can accumulate in production systems without affecting fish growth (Heinsbroek and Kamstra, 1990; Kamstra et al, 1996).

The plankton population indicates the productive status of a pond, representing both direct and indirect sources of food for the fish. Phytoplankton composition was representative of that found in Bangladesh fishponds (Mollah and Haque, 1978; Dewan et al., 1991; Wahab et al., 1999).

Of the 39 genera of periphyton that were identified, 23 were common as both plankton and periphyton and 16 were exclusively periphyton in nature (Table 3). The most dominant genera were *Stigeoclonium*, *Ankistrodesmus*, *Gonatozygon*, *Rhizoclonium*, *Botryococcus*, *Closterium*, *Navicula*, *Nitzschia*, and *Oscillatoria* colonizing the bamboo substrates in large numbers. Wahab et al. (1999) reported 53 genera of periphyton collected from scrap bamboo in fishponds in Bangladesh among which 12 genera rarely occurred. Huchette et al. (2000) identified about 32 species of diatom as periphyton along with other micro- and macroorganisms from both animal and plant kingdoms growing on artificial substrates in tilapia cages. In the present trials, a number of macrobenthic organisms, especially chironomid larvae, were often observed moving around the surface of the bamboo during periphyton sampling.

In rohu ponds, numbers of periphytic organisms were always lower than in gonia ponds (Figure 1), probably because of the rohu's reliance on periphyton and gonia's avoidance of periphyton for food. The periphyton numbers shown in Figure 1 peaked at week 8 and afterwards remained constant in gonia ponds perhaps because of the effects of self-shading on growth because it was ungrazed by fish. Figure 1 also shows that the numbers of periphyton increased throughout the entire period of study in the rohu trial, perhaps because the grazing stimulated growth and production, suggesting rohu density can be increased. 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). More detailed studies, quantifying the effect of grazing on periphyton biomass and production are needed.

While rohu were regularly observed grazing on the periphyton, gonia were never seen utilizing this source of food. Survival, growth and individual weight gain as well as net yield of rohu were significantly higher in the ponds where bamboo substrates had been introduced. This might be due to the increase in readily available food as periphyton grown on substrates. That did not occur with gonia although it grazed on periphyton in aquaria (Dr. S.M. Rahmatullah pers. comm.). This might be because of opportunistic behaviour of the fish species for which it preferred other food materials available in the ponds rather than periphyton. The net production of rohu in ponds with substrates was 77% higher (1,900 kg ha^{-1} 120 d^{-1}) than that of control ponds. Wahab et al. (1999) explored the possibility of periphyton-based culture with a native major carp kalbaush, *Labeo calbasu* and reported an approximate 80% increase in fish production in ponds provided with scrap bamboo as substrate in comparison to those without substrate. Ramesh et al. (1999) found 18-48% higher growth of rohu and 21-47% higher growth of common carp in ponds using sugarcane bagasse, paddy straw and *Eichhornea* as substrates than in control ponds.

In conclusion, provision of bamboo substrate increased production of rohu (77%) while the production of gonia is not enhanced by the provision of substrate for periphyton growth. It would be interesting to investigate the effect of substrate on the production of rohu and gonia in polyculture systems. Synergistic interactions among fish species are manifested by higher growth and yield in polyculture than in monoculture (Yashouv, 1971; Hepher et al., 1989). In addition, it might be interesting to look at other sources of cheap and locally available substrates that can yield similar results as bamboo. An economic evaluation of this new culture system is a prerequisite to the development of a sustainable periphyton-based aquaculture technology for resource-poor farmers.

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Chapter 5

The potential of periphyton-based culture of the native major carp kalbaush, *Labeo calbasu* (Hamilton)

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Abstract

The project evaluated the effect of installing scrap bamboo ('kanchi') as a substrate for periphyton on growth and production of the indigenous major carp kalbaush, *Labeo calbasu* (Hamilton). The impacts of fish grazing on the periphyton community were also assessed. Six ponds were used, three of which were provided with kanchi poles (700 per pond, spaced 30 cm apart). Ponds were limed and fertilized and stocked with *L. calbasu* juveniles (mean total length = 5.16 cm; mean weight = 2.10 g) at a rate of 10,000 juveniles ha^{-1} (75 fish per pond). There were no statistically significant differences in water quality between treatments, although differences in some genera of phytoplankton community composition were observed. Zooplankton numbers were the same in both treatments. While there was clear evidence that periphyton was being exploited by the fish, Chlorophyceae being most affected, grazing was insufficient to cause significant reductions in total periphyton densities. Fish survival and specific growth rates (SGRs) were significantly higher in ponds with substrates, production in treatments with and without scrap bamboo substrate being 713.90 and 399.11 kg ha^{-1}, respectively, over the 120-days period. However, production in both treatments was low in comparison to other studies, water temperatures being less than optimum for growth. It was concluded that kanchi and other locally available materials might be used to increase the production of some species of fish, although further evaluation of production economics is required.

Introduction

The traditional 'acadjas' of Côte Ivory Coast, West Africa, and 'kathas' of Bangladesh are brushpark-based fish attracting devices (FADs) used by fishermen (Welcomme, 1972; Wahab and Kibria, 1994). Dense masses of tree branches or bamboo are established in lakes, lagoons or rivers, and the fish attracted by the provision of shelter from predators and a suitable habitat for breeding in which natural food is also abundant. In recent years, these fisheries systems have served as models for novel periphyton-based aquaculture systems (Legendre et al., 1989; Konan et al., 1991; Hem and Avit, 1994; Konan-Brou and Guiral, 1994; Guiral et al., 1995; Sankare et al., 1997; Beveridge et al., 1998). Konan-Brou and Guiral (1994) reported that the proliferation of periphytic species algal concentrations on the submerged branches led to an eight-fold increase in algal standing crop within the 'acadja' compared with the lagoon waters. Furthermore, it is evident that food intake per unit time per fish can be many times higher when plant material is offered as periphyton rather than as phytoplankton (Dempster et al., 1993).

Periphyton-based aquaculture systems offer the possibility of increasing both areal primary production and food availability. In a resource constrained country such as Bangladesh, where more than 75% of households spend 90% of income on basic needs (BBS, 1995), many cannot afford to provide even rudimentary supplementary feeds for their fish ponds (O'Riordan, 1994). Alternative means of increasing fish production are essential. Recent trials with various types of bamboo carried out at the Bangladesh Agricultural University campus ponds, Mymensingh, and in the North-west of Bangladesh have shown significant increases in fish production (NFEP, 1997; Beveridge et al., 1998). The present investigation sets out to evaluate the impact of using scrap bamboo ('kanchi') as a substrate for periphyton growth in the culture of the major carp kalbaush, *Labeo calbasu* (Hamilton). The impacts of fish grazing on the periphyton community are also assessed.

Materials and methods

Study area

The 4-months experiment was carried out in six earthen ponds at the pond facilities of the Fisheries Faculty Field Laboratory, Bangladesh Agricultural University, Mymensingh. The ponds were rectangular in shape and the size (75 m^2), maximum depth (1.5 m), basin conformation and bottom soil type (loam) were very similar. The ponds were supplied by groundwater from an adjacent deep tube-well. All ponds were completely independent and fully exposed to prevailing sunlight. Pond embankments were covered with grass.

Experimental design

The experimental ponds were divided into two treatment groups of three replicates and arbitrarily numbered as ponds 1 - 6 for the purposes of the present study. Three ponds

(ponds 2, 4 and 5), chosen at random, were provided with kanchi (bamboo branches) substrates (treatment T_1), while the remaining three ponds (ponds 1, 3 and 6) without kanchi were regarded as the control (treatment T_2).

Pond preparation and stocking

Before the beginning of the experiment, ponds were drained, renovated and cleaned of aquatic vegetation and all small fish and other larger aquatic organisms eradicated. Kanchi sticks (mean length = 1.6 m; mean diameter = 1.52 cm) were installed vertically into the bottom muds of treatment T_1 ponds, the upper sections extending above the water surface. Seven hundred kanchi sticks were installed per pond (approximately 38 m^2 surface area), spaced 30 cm apart. To evaluate the effects of fish grazing on the periphyton community, three sticks in each pond were enclosed by fine mesh (1.5 mm) nylon net, thereby excluding fish. Five days later, ponds were limed (CaO) at a rate of 250 kg ha^{-1} and filled with water. Cow manure, urea and triple super phosphate (TSP) were applied at rates of 5,500, 125, and 125 kg ha^{-1}, respectively, four days after liming. Ponds were subsequently fertilized fortnightly with cow manure, urea and TSP at rates of 5,000, 50, and 50 kg ha^{-1}, respectively, throughout the study period.

Six days after the ponds had been filled with water, juveniles (mean total length = 5.16 cm; mean weight = 2.10 g) were released into the ponds at a rate of 10,000 juveniles ha^{-1} (75 fish $pond^{-1}$). Juveniles were collected from Purbadhala, Netrokona District, some 50 km from the experimental pond site. Stocking was carried out in the afternoon, care being taken to gradually acclimate the fish to pond conditions gradually.

Water quality monitoring

Physicochemical parameters, including temperature, transparency, dissolved oxygen (DO), pH, nitrogen (NH_4-N, NO_2-N, NO_3-N), phosphorus (PO_4-P) and chlorophyll *a* of pond water were monitored fortnightly between 09.00 and 10.00 h on each sampling day. Temperature was measured by thermometer (precision = 0.1 °C) and DO by a digital DO meter (YSI, model 58). Transparency was measured by Secchi disc and pH by a Jenway model 3020 pH meter. Analyses of NH_4-N, NO_2-N, NO_3-N were by Hach kit (model DR

2000). Phosphate-phosphorous concentrations were determined by spectrophotometer (Milton Roy Spectronic, model 1001 plus) according to the methods of Stirling (1985). Chlorophyll *a* was determined spectrophotometrically at 664 and 750 nm absorbance after acetone extraction (Boyd, 1979).

Plankton

Quantitative and qualitative samples of phytoplankton and zooplankton were taken fortnightly. A 10-l sample of pond water, comprising 10 1-l samples taken from five locations in each pond at mid-depth and surface, was passed through a plankton net (mesh size 45 μm). Samples were transferred to glass measuring cylinders in a fixed volume of water and left to settle until concentrated in a volume of 50 ml, excess water being carefully removed by siphon. Samples were then preserved in small, sealed plastic bottles containing 5% buffered formalin. Plankton were enumerated using a Sedgewick-Rafter counting cell (S-R cell). A sample (1 ml) was transferred to the counting chamber of the S-R cell (providing 1000 fields) and left to stand for 15 min to allow the plankton to settle. Using a binocular microscope (Swift M-4000), all cells/colony-forming units occurring in ten fields of the S-R cell selected at random were counted. Plankton density was estimated using the following formula:

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

where N = the number of plankton cells or units per litre of original water; P = the number of plankton counted in ten fields; C = the volume of final concentrate of the sample (ml); L = the volume (litres) of the pond water sample. Identification of plankton to genus level was carried out using keys from Ward and Whipple (1959), Prescott (1962) Belcher and Swale (1976), Palmer (1980) and Bellinger (1992).

Periphyton

Collection of periphyton samples commenced 15 days after stocking from both grazed and ungrazed kanchi substrates and continued at fortnightly intervals throughout the trial. From each pond, four kanchi sticks, two from ungrazed (covered by nylon net) and two from grazed conditions, were selected randomly and periphyton samples taken carefully. A 2x2 cm^2 surface area of the substrate 30 cm below the water surface was removed carefully using scalpel blades. Samples were resuspended in 20 ml distilled water and preserved in 5% buffered formalin in sealed plastic vials. Periphyton was enumerated using a Sedgewick-Rafter counting cell according to the procedure for phytoplankton given above. Periphyton numbers were estimated using the following formula:

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

where N = number of periphyton cells or units per cm^2 surface area; P = number of periphytic algae units counted in ten fields; C = volume of final concentrate of the sample (ml); S = area of scraped surface (cm). Identification of periphytic algae relied on the keys used for phytoplankton cited above.

Harvesting

At the end of the experiment, water was pumped out of the ponds, all fish recovered, weighed (Ohaus model CT1200; 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 day^{-1}) was estimated as:

$$SGR = [Log_e (final weight) - Log_e (initial weight) \times 100]/culture period (days).$$

Data analysis

For statistical analyses of data, a one-way ANOVA was carried out using STATGRAPHICS version 7 statistical package for the PC. Significance was assigned at the 0.05% level.

Results

Water quality parameters

Water quality results are summarised in Table 1. There were no significant differences ($F=1.27$, d.f. = 17, $P > 0.05$) in any water quality parameters among ponds. All parameters were within an acceptable range for fish culture in Bangladesh.

Plankton

Plankton mean abundance data are shown in Table 2. The fish pond phytoplankton communities were composed of four major groups: Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae.

The Bacillariophyceae comprised eight genera, of which four (*Actinella, Chodatella, Nostoc* and *Surirella*) were only occasionally observed in only one pond each. This was the least abundant group in both treatments with mean values of 8,361±786 units l^{-1} in T_1 and 10,900±1,710 units l^{-1} in T_2. When compared by ANOVA, there were no significant differences ($F = 0.93$, d.f. = 53, $P > 0.05$) in terms of numbers between treatments. While *Cyclotella* dominated in both treatments, there were no significant differences in abundance of any single genus of Bacillariophyceae between the two treatments.

In terms of genera present (25), the Chlorophyceae was the most dominant group of phytoplankton in both treatments. Mean abundance of Chlorophyceae in T_1 and T_2 was 158,243±16,397 units l^{-1} and 120,407±12,984 units l^{-1}, respectively, and these values were not significantly different ($F = 1.51$, d.f. = 53, $P > 0.05$). Ten genera were observed only occasionally. However, mean abundance of the most commonly observed genus *Ceratium* was significantly higher ($F = 4.10$, d.f. = 53, $P < 0.05$) in treatment T_1 (97,845±16,216 units l^{-1}) than in treatment T_2 (48,815±12,908 units l^{-1}). In contrast, the mean numbers of *Chlorella* ($F = 4.39$, d.f. = 53, $P < 0.05$) and *Actinastrum* ($F = 3.98$, d.f. = 53, $P < 0.05$) were significantly higher in T_2 (32,130±3,893 and 3,074±1,347 units l^{-1}, respectively) than in T_1 (22,111±2,700 and 993±466 units l^{-1}, respectively).

Table 1
Mean values (and range) of water quality observations of ponds with and without periphyton substrate.

Treatment	With substrate (T_1)			Without substrate (T_2)			Mean (SE)	
Pond	**2**	**4**	**5**	**1**	**3**	**6**	**T_1**	**T_2**
Temperature	29.5	29.4	29.5	29.5	29.4	29.3	29.45	29.44
(°C)	(23.7-32.0)	(24.8-32.2)	(24.0-32.1)	(23.7-32.7)	(23.6-32.0)	(23.7-32.0)	(0.89)	(0.89)
Secchi (cm)	30.4	31.5	42.6	22.7	26.7	39.3	34.90	30.91
	(18.0-45.0)	(17.0-62.0)	(21.0-71.0)	(16.0-45.0)	(17.0-54.5)	(24.0-57.0)	(4.96)	(3.79)
pH	6.89-8.45	6.60-8.70	6.15-8.52	7.13-8.40	6.34-8.50	6.56-8.38	--	--
DO (mg l^{-1})	4.2	4.4	4.1	4.7	4.2	4.1	4.18	4.29
	(3.0-6.2)	(3.3-5.1)	(3.2-4.2)	(4.1-5.6)	(2.6-5.8)	(3.4-4.9)	(0.15)	(0.18)
TAN	0.61	0.50	0.24	0.38	0.56	0.25	0.52	0.40
(mg l^{-1})	(0.19-1.72)	(0.00-0.97)	(0.05-0.59)	(0.06-0.72)	(0.17-1.29)	(0.07-0.63)	(0.14)	(0.06)
NO_2-N	9.0	7.0	8.0	6.3	9.0	6.0	8.0	7.0
(μg l^{-1})	(6.0-14.0)	(4.0-11.0)	(4.0-17.0)	(6.0-7.0)	(7.0-12.0)	(3.0-10.0)	(1.0)	(0.3)
NO_3-N	1.50	1.26	0.99	1.16	1.34	1.14	1.29	1.26
(mg l^{-1})	(0.90-2.50)	(0.60-1.80)	(0.80-1.50)	(0.60-1.80)	(0.80-1.90)	(0.70-1.80)	(0.11)	(0.08)
PO_4-P	0.28	0.24	0.27	0.22	0.24	0.27	0.28	0.25
(mg l^{-1})	(0.03-0.50)	(0.05-0.44)	(0.10-0.43)	(0.11-0.43)	(0.06-0.35)	(0.09-0.51)	(0.04)	(0.04)
Chl-*a*	157.0	242.0	180.1	289.5	262.1	183.4	165.7	220.4
(μg l^{-1})	(50-350)	(39-459)	(33-509)	(75-632)	(51-813)	(52-355)	(32.3)	(36.2)

Eight genera of Cyanophyceae were observed and ranked second in respect of abundance in both treatments. Mean abundance of Cyanophyceae in T_1 and T_2 were 90,701±13,970 and 116,685±18,960 colony forming units (cfu) l^{-1}, respectively and these were not significantly different ($F = 0.50$, d.f. = 53, $P > 0.05$). *Gloecapsa* and *Merismopedia* were rarely observed. Among this group, *Anabaena* numbers differed significantly ($F = 6.97$, d.f. = 53, $P < 0.05$), with a higher mean value (46,611±10,877 units l^{-1}) in T_2 than in T_1 (15,333±3,543 units l^{-1}). *Microcystis* was the most dominant genus of Cyanophyceae in treatment T_1 whereas *Anabaena* was the most abundant genus of the group in treatment T_2.

Table 2

Mean plankton abundance (units l^{-1}) of ponds with and without periphyton substrate.

Treatment	With Substrate (T_1)			Without Substrate (T_2)			Mean (±SE)		ANOVA
Pond	2	4	5	1	3	6	T_1	T_2	result
PHYTOPLANKTON									
Bacillariophyceae									
Actinella	--	--	--	167	--	--	--	--	--
Chodatella	389	--	--	--	--	--	--	--	--
Cyclotella	5278	3556	4438	12222	4333	3444	4424±531	6667±1648	NS
Fragillaria	833	778	1312	444	389	778	975±297	537±162	NS
Melosira	500	389	--	611	--	1056	296±180	556±400	NS
Navicula	1833	2889	2500	3812	2889	3167	2481±503	3289±469	NS
Nostoc	167	--	--	--	--	--	--	--	--
Surirella	--	222	--	--	--	--	--	--	--
Total	9000	7888	8250	16646	7611	8444	8361±786	10900±1710	NS
Chlorophyceae									
Actinastrum	2222	444	313	4556	4278	389	993±466	3074±1347	*
Ankistrodesmus	4222	3833	3562	5833	8111	2667	3873±871	5537±1543	NS
Botryococcus	6000	722	3375	7889	5722	4833	3365±983	6148±1210	NS
Ceratium	78444	84778	130313	6333	18667	121444	97845±16216	48815±12908	*
Characium	--	--	--	278	111	--	--	--	--
Centritractus	--	--	222	222	--	--	--	--	--
Chlorella	24611	20222	21500	40722	32500	23167	22111±2700	32130±3893	*
Chrysococcus	--	--	--	--	722	--	--	--	--
Closterium	3833	2111	1438	2944	2833	389	2461±663	2056±588	NS
Coelastrum	2778	667	1812	1278	2556	722	1752±885	1519±481	NS
Coelosphanium	--	--	250	--	--	--	--	--	--
Crucigenia	6000	2500	--	5556	5444	333	2833±1457	3778±1336	NS
Gonatozygon	5222	8611	6937	6111	4944	3889	6924±1278	4981±480	NS
Micrasterium	--	--	--	333	--	--	--	--	--
Oocystis	1722	4889	--	3278	2611	2444	1870±663	2778±991	NS
Pediastrum	3833	2278	2938	2389	2278	1500	3016±648	2056±359	NS
Phytococcus	--	889	--	--	1500	--	--	--	--
Scenedesmus	3333	1111	687	3389	3333	1222	1711±478	2648±567	NS
Spirogyra	--	--	--	1556	--	556	--	--	--
Staurastrum	--	--	--	111	--	--	--	--	--
Staurodesmus	--	722	--	--	2556	--	--	--	--
Tetradesmus	111	222	--	1611	611	--	111±64	741±425	NS
Tetraedron	2722	1778	875	1833	1111	1111	1792±476	1352±289	NS
Volvox	1722	1667	875	444	889	1889	1421±223	1074±331	NS

Table 2 Continued

Treatment	With Substrate (T_1)			Without Substrate (T_2)			Mean (±SE)		ANOVA
Pond	**2**	**4**	**5**	**1**	**3**	**6**	**T_1**	**T_2**	**result**
Zygnema	--	--	--	--	167	--	--	--	--
Total	146778	136444	145875	96056	98611	166556	158243±16397	120407±12984	NS
Cyanophyceae									
Anabaena	16778	19722	9500	66722	27500	45611	15333±3543	46611±10,877	*
Aphanocapsa	6278	4056	8125	11,667	7056	6167	6153±945	8296±1488	NS
Chroococcus	15500	4722	8313	13167	8389	9167	9512±2157	10241±1259	NS
Gloecapsa	--	--	--	8556	--	--	--	--	--
Gomphosphaeria	19944	1278	3000	1833	2500	3667	8074±5189	2667±903	NS
Merismopedia	--	--	--	--	167	--	--	--	--
Microcystis	56889	82000	9313	60333	38000	32389	49400±12435	43574±9589	NS
Oscillatoria	1056	2444	3187	1222	3500	2444	2229±741	2389±1050	NS
Total	116444	114222	41438	163500	87111	99444	90701±13970	116685±18960	NS
Euglenophyceae									
Euglena	10056	7389	7812	16333	8722	7556	8419±1064	10870±1788	NS
Phacus	3000	2778	2188	3111	1722	1056	2655±697	1963±448	NS
Trachalomonas	3611	3000	5062	3222	4889	1500	3891±734	3204±585	NS
Total	16667	13167	150622	22667	15028	10111	14965±1860	15935±90	NS
ZOOPLANKTON									
Crustacea									
Cyclops	2056	1611	1500	1889	1667	1611	1722±217	1722±238	NS
Daphnia	--	143	--	--	--	--	--	--	--
Diaphanosoma	1000	1500	187	1889	278	889	896±209	1019±297	NS
Diaptomus	--	--	750	111	--	444	--	--	--
Monostyla	--	--	--	167	--	--	--	--	--
Nauplius	4722	4444	3625	3778	3556	3389	4264±553	3574±389	NS
Total	7778	7698	6063	7833	5500	6333	7180±694	6556±746	NS
Rotifera									
Ascomorpha	--	333	--	--	--	--	--	--	--
Asplanchna	2000	3722	3375	3056	1500	4333	3032±512	2963±671	NS
Brachionus	3444	3611	4937	3167	4389	3722	3998±656	3759±719	NS
Filinia	1222	1000	1250	333	1222	1056	1157±312	871±255	NS
Lecane	389	444	--	--	56	444	278±142	167±100	NS
Keratella	1611	3389	3625	2444	2667	2833	2875±683	2641±463	NS
Polyarthra	2056	1444	625	250	1611	333	1375±567	731±321	NS
Trichocerca	2500	2000	4812	17,778	2722	4000	3104±593	2833±457	NS
Total	13222	15610	18250	11028	14167	16722	15694±1817	13972±1752	NS

*$P < 0.05$; NS, not significant.

Euglenophyceae ranked third in terms of abundance and only three genera, *Euglena, Phacus* and *Trachalomonas* were represented. No significant difference ($F = 0.40$, d.f. = 53, $P > 0.05$) in mean abundance of euglenoid phytoplankton between treatments T_1 (14,965±1,860 units l^{-1}) and T_2 (15,935±90 units l^{-1}) was observed.

The zooplankton community had representatives of five genera of Crustacea and eight genera of Rotifera. Among the crustaceans, *Cyclops* and *Diaphanasoma* were the most common, *Daphnia*, *Diaptomus* and *Monostyla* being found occasionally. Juvenile nauplii stages, however, accounted for more than 50% of zooplankton in both the treatments. Crustacean abundance in T_1 (7,180±694 organisms l^{-1}) and T_2 (6,556±746 organisms l^{-1}) were not significantly different ($F = 0.25$, d.f. = 53, $P > 0.05$). The abundance of rotifers in treatments T_1 (15,694±1,817 organisms l^{-1}) and T_2 (13,972±1,752 organisms l^{-1}) did not differ significantly ($F = 0.01$, d.f. = 53, $P > 0.05$). *Ascomorpha* was scarce, being found only in pond 4.

Periphyton

Mean (±S.E.) abundance of periphyton in different ponds under ungrazed and grazed conditions is shown in Table 3. The fish pond periphyton communities comprised five groups: Bacillariophyceae (12 genera), Chlorophyceae (25), Cyanophyceae (10), Euglenophyceae (4) and Rhodophyceae (2). The mean abundance of Bacillariophyceae was 45,456±2,112 and 41,571±1,965 cfu l^{-1} in ungrazed and grazed conditions, respectively. When compared using ANOVA, there was no significant difference ($F = 1.94$, d.f. = 47, $P > 0.05$) in numbers between treatments. There was also no variation in Bacillariophyceae at genus level between treatments. Chlorophyceae was the most dominant group in both ungrazed and grazed conditions in terms of abundance. Significantly higher ($F = 3.87$, d.f. = 47, $P < 0.05$) numbers (101,622±4,293 units l^{-1}) were found in ungrazed conditions than in grazed condition (91,305±3,585 units l^{-1}). Among the 25 genera, the mean abundance of *Cladophora*, *Gonatozygon* and *Stigeoclonium* were significantly higher ($F = 3.93$, 3.95 and 4.12 respectively, d.f. = 47, $P < 0.05$) on ungrazed substrates. Mean abundance of Cyanophyceae under ungrazed and grazed conditions was 52,948±2,271and 46,938±2,473 units l^{-1}, respectively. Although these values were not significantly different ($F = 2.94$, d.f. = 47, $P > 0.05$) from each other, *Chroococcus* and

Table 3

Mean abundance of periphyton (units cm^{-2}) on the substrate of different ponds under ungrazed and grazed conditions.

Conditions	Ungrazed			Grazed			Mean (±SE)		ANOVA
Pond	**2**	**4**	**5**	**2**	**4**	**5**	**Ungrazed**	**Grazed**	**Result**
Bacillariophyceae									
Actinella	--	3875	3571	--	3000	3214	2482± 413	2071±336	NS
Achnanthes	4625	5000	5143	4750	3937	4928	4922±467	4538±383	NS
Cyclotella	4750	3312	3143	3812	3062	3357	3735±295	3410±245	NS
Cocconeis	5062	--	4571	5750	--	5214	3211±618	3654±588	NS
Cymbella	--	3962	928	--	3375	--	1630±509	1125±365	NS
Fragillaria	6437	5750	5000	6062	4812	4571	5729±615	5148±352	NS
Gomphonema	3875	4375	3500	3937	4187	2500	3917±350	3541±411	NS
Melosira	500	4125	--	--	3125	--	1541±442	1042±361	NS
Microphora	--	1687	4000	--	1687	3571	1896±612	1753±528	NS
Navicula	6750	6187	4928	6500	6000	5071	5955±422	5857±4.94	NS
Nitzschia	9375	675	7857	8437	5937	6857	7994±736	7077±801	NS
Synedra	4687	2000	643	4437	2125	500	2443±526	2354±512	NS
Total	46061	47023	43284	43685	41247	39783	45456±2112	41571±1965	NS
Chlorophyceae									
Actinastrum	--	2062	1857	--	1750	1857	1306±486	1202±424	NS
Ankistrodesmus	6000	6187	7357	6125	6187	5286	6515±504	5866±470	NS
Ceratium	--	1437	285	--	1875	428	574±359	767±396	NS
Chaetophora	6437	6437	6214	6312	6187	6286	6363±540	6262±427	NS
Characium	--	1125	--	--	--	--	--	--	NS
Chlorella	6125	562	1357	5750	437	857	2681±698	2348±650	NS
Cladophora	7312	8875	8071	6625	6812	6571	8086±537	6670±567	*
Closterium	6687	7500	6214	5312	6667	5285	6800±500	5754±369	NS
Coleochaete	11875	10000	11214	11812	9562	10357	11029±1087	10577±1104	NS
Draparnaldia	4500	7125	7000	4437	5750	5643	6208±490	5276±288	NS
Geminella	--	--	--	--	--	500	--	--	NS
Gonatozygon	6312	6937	7571	5062	4812	6528	6940±572	5467±561	*
Microspora	1812	5750	3928	1062	4562	5286	3830±720	3637±652	NS
Microthamnion	--	1562	--	--	440	--	--	--	--
Mougeotia	6125	4625	6000	4812	5312	5714	5583±701	5279±677	NS
Oedogonium	6125	5125	4571	5562	4437	5571	5274±755	5190±363	NS
Oocystis	4812	4687	6571	5625	3812	3286	5356±605	4241±66	NS

Table 3 Continued

Conditions	Ungrazed			Grazed			Mean (±SE)		ANOVA
Pond	**2**	**4**	**5**	**2**	**4**	**5**	**Ungrazed**	**Grazed**	**Result**
Scenedesmus	812	--	--	812	--	--	--	--	--
Spirogyra	--	--	--	--	--	2214	--	--	--
Stigeoclonium	7875	8937	7571	6125	7562	6143	8127±490	6610±597	*
Tetraspora	--	2875	5285	--	2812	5214	2720±970	2675±889	NS
Triplocerus	--	--	2428	--	--	3143	--	--	--
Ulothrix	8375	8937	6571	7250	9375	5071	7961±622	7232±631	NS
Volvox	--	--	--	--	--	1071	--	--	--
Zygnema	4312	5562	3000	4062	5437	1071	4291±605	3524±479	NS
Total	95496	106307	103065	86745	93788	93383	101622±4293	91305±3585	*
Cyanophyceae									
Anabaena	4562	4187	4643	4750	3750	3875	4464±293	4125±298	NS
Aphanigominon	6625	6312	9000	6062	6500	7143	7312±785	6568±639	NS
Chroococcus	5375	5875	6928	4250	4875	5143	6059±460	4756±488	*
Gloetrichia	7812	8625	9428	8000	6812	8643	8622±439	7818±530	NS
Gomphosphaeria	--	--	--	--	812	--	--	--	--
Lyngbya	1562	3625	643	2187	3937	625	1943±496	2249±539	NS
Oscillatoria	8250	7812	9857	6437	6562	8571	8640±502	7190±645	*
Phacothamnion	--	625	--	--	312	--	--	--	--
Phormidium	8625	6687	7143	7437	7062	5571	7485±429	6690±536	NS
Rivularia	6375	7625	10643	6562	6937	8000	8214±737	7166±639	
Total	49186	51373	58285	45685	47559	47571	52948±2271	46938±2473	NS
Euglenophyceae									
Difflugia	11187	1812	3643	5437	1625	2714	5547±1365	3258±553	*
Euglena	--	2187	1000	--	1937	357	1062±252	764±249	NS
Phacus	4625	2625	2214	3625	2250	1714	3154±337	2530±294	*
Trachelomonas	--	--	500	--	--	643	--	--	--
Total	15812	6624	7357	9062	5812	5428	9931±1378	6767±626	*
Rhodophyceae									
Batrachosperium	3250	3000	2428	2437	2437	1000	2893±247	1958±286	*
Lemanea	3000	3937	2928	3437	3437	3643	3288±239	3505±268	NS
Total	6250	6937	5356	5874	5874	4643	6181±329	5463±343	NS

*$P < 0.05$; NS, not significant.

Oscillatoria were significantly more abundant (F = 3.98 and 3.90 respectively, d.f. = 47, $P < 0.05$) on ungrazed substrates. The mean abundance of Euglenophyceae in ungrazed and grazed conditions were 9,931±1,378 and 6,767±626 units l^{-1}, respectively. When compared using ANOVA, Euglenophyceae showed significant differences (F = 4.06, d.f. = 47, $P < 0.05$) between the two conditions. Among the four genera found, the numbers of *Difflugia* and *Phacus* were significantly higher (F = 3.91 and 3.97 respectively, d.f. = 47, $P < 0.05$) in ungrazed conditions. Rhodophyceae was the least abundant group (6,181±329 and 5,463±343 units l^{-1} in ungrazed and grazed conditions, respectively) and although total numbers did not vary significantly (F = 3.02, d.f. = 47, $P > 0.05$) between conditions, the mean abundance of *Batrachosperium* was significantly higher (F = 5.83, d.f. = 47, $P < 0.05$) in ungrazed conditions.

Growth and production performance of fish

Fish growth and production data are summarised in Table 4. Although the same numbers of fish were stocked in each pond, the numbers of fish harvested differed between ponds and treatments. Survival rates at harvest ranged from 87 to 90% in treatment T_1 and 72 to 77% in treatment T_2, highest survival being observed in pond 5 (T_1) and the lowest in pond 6 (T_2). Survival was significantly higher in treatment T_1 (88.7±0.9%) than in treatment T_2 (74.7±1.5%) when arcsine transformed data were compared by ANOVA (F = 67.85; d.f.= 5; $P < 0.01$).

Although there were no significant differences in total lengths or weights of fish at stocking, marked differences in fish growth rate between treatments, in terms of both total lengths and weights at harvest, were readily apparent. The total lengths of fish at harvest were significantly higher in T_1 (17.6±0.2 cm) than those of treatment T_2 (14.4±0.4 cm) when compared by ANOVA (F = 51.35; d.f. = 119; $P < 0.01$). The mean weight of fish at harvest (80.2±3.9 g in T_1; 53.5+5.3 g in T_2) was significantly higher in treatment T_1 (F = 48.70; d.f. = 119; $P < 0.01$), as was the mean weight gain per fish (78.1±2.3 in T_1; 51.5±3.1 g in T_2) (F = 48.01; d.f. = 119; $P < 0.01$).

Table 4

Yield parameters of fish from ponds with and without substrate.

Treatment	With substrate (T_1)			Without substrate (T_2)			Mean (±SE)		ANOVA
Pond	2	4	5	1	3	6	T_1	T_2	result
No. of fish at harvest	67	65	68	56	58	54	67.0±1.5	56.0±2.0	*
Survival (%)	89	87	90	75	77	72	88.7±0.9	74.7±1.5	*
Initial length (cm)	5.1	5.2	5.2	5.2	5.1	5.2	5.2±0.04	5.2±0.05	NS
Final length (cm)	17.7	17.8	17.3	13.6	14.8	14.9	17.6±0.2	14.4±0.4	*
Initial weight (g)	2.1	2.1	2.2	2.1	1.9	2.1	2.1±0.1	2.0±0.1	NS
Final weight (g)	83.1	81.9	75.8	48.8	52.6	59.3	80.2±3.9	53.5±5.3	*
Weight gain (g)	81.0	79.8	73.6	46.7	50.7	57.2	78.1±2.3	51.5±3.1	*
SGR (% bw d^{-1})	3.05	3.05	2.95	2.62	2.77	2.78	3.04	2.74	*
Net yield (kg ha^{-1} 120 d^{-1})	742.7	709.3	686.7	364.0	406.7	426.7	712.9±7.66	399.1±8.71	*

*$P < 0.01$; NS, not significant.

Growth and production data were extrapolated in order to express net yields on a per hectare basis over the 120-days culture period (Table 4). The higher net yield (712.90±7.66 kg ha^{-1}) was from treatment T_1, which had been provided with kanchi substrates, compared with a mean net yield of 399.11±8.71 kg ha^{-1} in ponds with no additional substrates. Analysis by ANOVA shows these differences to be highly significant (F = 162.46; d.f. = 5 ; $P < 0.01$).

Discussion

Water quality measurements made during the trial indicated that there were no statistically significant differences between treatments as a result of the presence of the scrap bamboo substrate. Nevertheless, there was a trend of higher Secchi disc values - a measure of the presence of suspended particles, including phytoplankton, in the water column - and lower chlorophyll *a* concentrations in water samples from ponds with kanchi substrates, indications that there may be a trade-off between phytoplankton and periphyton production.

While Chlorophyceae were the most abundant members of the phytoplankton community in both treatments, abundance was apparently higher in ponds provided with substrates. In contrast higher densities of Bacillariophyceae, Cyanophyceae and Euglenophyceae were observed in ponds without substrates. Zooplankton numbers were the same in both treatments.

There was clear evidence that the periphyton was being exploited by the fish. Although there were no statistically significant changes in community diversity at the genus level, significant decreases in the densities of eight genera were observed in grazed compared to ungrazed substrates. Overall, the Chlorophycae were more affected by grazing than the other groups (Table 3). However, grazing had not been sufficient to cause significant reductions in total periphyton densities. While zooplankton were rarely seen in the periphytic community, a number of macrobenthic organisms, especially chironomid larvae, were observed.

Survival and specific growth rates of fish were significantly higher in ponds with substrates. The higher survival in treatment T_1 might be due to not only for the availability of periphyton as additional food but also due to shelter or protection from predators (e.g. birds, snakes, frogs, etc.) by substrates. Pond-based, periphyton trials conducted with *Labeo rohita* in north-west Bangladesh also found higher mean survival of fish in treatment (91%) than in control (78%) ponds (NFEP, 1997). Production in treatments T_1 and T_2 was 712.90 and 399.11 kg ha^{-1}, respectively, over the 120-days period, which was low in comparison to other studies. Water temperatures declined from a maximum of 32.7°C at the start of the trial in August to values as low as 23.6°C in November, these temperatures being less than optimum (30 - 34°C) for growth of Indian major carps (Dewan et al., 1991). It is hypothesised that the increases in net yields of fish were largely as a result of the availability of easily grazed periphytic food growing on the kanchi substrates. The significant enhancement of production of *L. rohita* were reported by NFEP (1997) and Ramesh et al. (1999) through the addition of substrates.

It is concluded that kanchi (bamboo scrapings), which is ubiquitous throughout Bangladesh, not usually sold but used for household works such as fencing, fuel wood etc., can be used to increase the production of the native major carp *Labeo calbasu* in monoculture. Moreover, there are other locally available substrates that might also be used for this type of aquaculture. The potential of periphyton-based systems for polyculture and the optimization of stocking densities in relation to periphyton substrate remain to be determined. The practicalities and economics of periphyton-based aquaculture systems in Bangladesh also remain to be demonstrated.

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PART III

Evaluation of polyculture by exploring fish stocking densities, species ratios and combinations

Chapter 6

Optimization of stocking ratios of two Indian major carps, rohu (*Labeo rohita* Ham.) and catla (*Catla catla* Ham.) in a periphyton-based aquaculture system

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Abstract

Production of combinations of rohu (*Labeo rohita*) and catla (*Catla catla*) from periphyton-based aquaculture system was compared using twelve ponds and six stocking ratios: 100% rohu alone (treatment 100R), 80% rohu plus 20% catla (80R/20C), 60% rohu plus 40% catla (60R/40C), 40% rohu plus 60% catla (40R/60C), 20% rohu plus 80% catla (20R/80C) and 100% catla alone (100C). Ponds (75 m^2, depth 1.5 m) were stocked at a rate of 15,000 juveniles ha^{-1}. Bamboo poles (mean length = 2.0 m; mean diameter 5.5 cm; 9 poles m^{-2}) used as periphyton substrates were planted vertically into the pond bottoms and ponds were fertilized fortnightly with cow manure, urea and triple super phosphate (TSP) at rates of 4,500, 150 and 150 kg ha^{-1}, respectively. Phytoplankton biomass decreased with increasing catla biomass whereas periphyton biomass decreased with increasing biomass of rohu. Ash content of periphyton increased with increasing number of catla and decreasing number of rohu. Growth of catla was dependent ($P \leq 0.05$) on stocking density but that of rohu was independent ($P > 0.05$) of stocking density, possibly because of the reliance of the latter on periphyton for food. Highest fish yield was recorded in treatment 60R/40C (586 kg ha^{-1}), followed by treatments 40R/60C (459 kg ha^{-1}), 80R/20C (439 kg ha^{-1}), 20R/80C and 100R (both 225 kg ha^{-1}), and 100C (146 kg ha^{-1}), respectively, over the 70-days period. A stocking ratio of 60% rohu and 40% catla appears appropriate for periphyton-based production systems of Indian major carps, although the ratio is likely to be influenced by both stocking density and food supply.

Introduction

In recent years, a range of substrate-based aquaculture systems has been developed with the aim to increase the production of periphyton per unit fertilizer input, thereby increasing economic viability. Trials have demonstrated that fish production from ponds supplied with additional substrates for periphyton production is higher than that from substrate-free controls (Legendre et al., 1989; Konan et al., 1991; Hem and Avit 1994; Guiral et al., 1995; NFEP, 1997; Wahab et al., 1999; Azim et al., 2001). Laboratory-based grazing trials also indicated that algal-feeding species, such as tilapias, can ingest more plant based food per unit time when presented as periphyton than as plankton (Dempster et al., 1993). However, most field studies have concentrated on the potential of periphyton-based culture with selected fish species in monoculture.

As elsewhere in the region, the development of polyculture systems in Bangladesh has proceeded on an *ad hoc* basis and little effort was directed towards development of guidelines on appropriate species combinations and ratios (Wahab et al., 1995). It has long been apparent that higher growth rates and yields can be derived from polyculture than from monoculture as a result of both complementary and synergistic interactions among fish species (Yashouv, 1971; Hepher et al., 1989). By stocking different species with complementary feeding habits, feeding niches may be fully exploited. Synergies arise from two interrelated processes, the increase in available food resources and improvements in environmental conditions (Milstein, 1992).

To date, two Indian major carp species, the column feeder rohu, *Labeo rohita* and the bottom feeder kalbaush, *Labeo calbasu* have been shown to be suited for periphyton-based culture systems (NFEP, 1997; Wahab et al., 1999; Ramesh et al., 1999; Azim et al., 2001). Using traditional fish stocking densities (1 fish m^{-2}) and a substrate surface area of 50% of the pond water surface, grazing by kalbaush did not result in significant reductions in periphyton densities (Wahab et al., 1999) suggesting that fish density could be increased in this system. However, phytoplankton may remain under-grazed in periphyton-based monoculture ponds, thereby hindering sunlight penetration and adversely affecting periphyton growth. The indigenous surface feeding catla, *Catla catla*, with its complementary feeding habit to rohu, might be a suitable candidate species in periphyton-based systems, and could reduce the plankton community and increase the fish production.

In order to develop sustainable periphyton-based pond culture that can be used as a viable means of rural fish production for poverty alleviation and nutritional security, the optimal stocking combinations of the principal indigenous farmed species for polyculture must be known. As a first step, the present study was designed to compare yields from six stocking ratios of rohu and catla in substrate-based polyculture systems.

Materials and methods

Study area and experimental design

The 70-days experiment was carried out between September and December 1998 in 12 earthen ponds at the pond facilities of the Fisheries Faculty Field Laboratory, Bangladesh Agricultural University, Mymensingh. The ponds were rectangular and the sizes (7.5 m x 10 m), maximum depths (1.5 m) and basin shapes were similar. The ponds were separately supplied with groundwater from an adjacent deep tube-well and fully exposed to prevailing sunlight. Pond embankments were covered with grass.

Replicates of each of the six treatment groups were assigned randomly to the ponds. Ponds were stocked at a rate of 15,000 juveniles ha^{-1} at six different species ratios: 100% rohu (ponds 1, 7; treatment 100R), 80% rohu plus 20% catla (ponds 5, 9; 80R/20C), 60% rohu plus 40% catla (ponds 2, 6; 60R/40C), 40% rohu plus 60% catla (ponds 3, 12; 40R/60C), 20% rohu plus 80% catla (ponds 4, 11; 20R/80C) and 100% catla (ponds 8, 10; 100C).

Pond preparation and fish stocking

Prior to the trial, ponds were renovated, aquatic vegetation removed and all aquatic animals eradicated. On Day 1, bamboo substrates (mean length = 2.0 m; mean diameter = 5.5 cm) were planted vertically into the bottom mud, the upper portions extending above the water surface. Substrates were installed in a 40-m^2 area leaving a 2-m perimeter free of poles in each pond. These bamboo substrates (9 poles m^{-2}, 360 $pond^{-1}$) added an effective surface area of about 75 m^2 per pond.

Ponds were subsequently treated with lime (CaO, 250 kg ha^{-1}) and filled with water. On Day 5, cow manure, urea and triple super phosphate (TSP) were applied (4,500, 150, and 150 kg ha^{-1}, respectively). Ponds were then fertilized fortnightly at the same rates during the entire experimental period.

On Day 12, *Labeo rohita* (15 g) and *Catla catla* (20 g) juveniles were released into the ponds. Juveniles were collected from nearby hatcheries and stocking carried out in the afternoon, care being taken to gradually acclimatize the fish to pond conditions.

Water quality monitoring

From the first day of the trial, temperature and dissolved oxygen (YSI digital DO meter, model 58), Secchi depth (Secchi disc) and pH (Jenway pH meter, model 3020) were monitored daily, measurements being made between 0900 and 1000 h. From Day 3 after first fertilization, total alkalinity, chlorophyll *a*, ammonia (TAN), nitrate nitrogen (NO_3-N) and phosphate phosphorus (PO_4-P) were measured in all ponds on a weekly basis. Total alkalinity was determined by titrimetric method (Stirling, 1985). Chlorophyll *a* was determined spectrophotometrically after acetone extraction (Boyd, 1979). Water samples were filtered prior to nutrient analysis. Nutrient analyses were performed by Hach kit (model DR 2000) following standard methods (APHA, 1992).

Periphyton sampling and analysis

Periphyton collection was started ten days after stocking with juveniles and continued at monthly intervals. From each pond, three poles were selected by random number tables. Two samples of periphyton were taken at each of the four sampling point, every 30-cm along the length of the pole, starting at the water surface.

For the first sample of periphyton, 20 cm^2 (2.0 cm x 10.0 cm) surface area of substrate was carefully scraped with a sharp knife, care being taken not to remove any of the substrate itself, and the material transferred to pre-weighed and labeled pieces of aluminium foil. Samples were kept cool until returned to the laboratory. Samples were placed in a drying oven (Memmert, Model UM/BM 100-800) and dried at 105°C until constant weight (24 h), before being transferred to a dessicator until weighed (BDH, Model 100A; precision 0.0001 g). Dry samples from depths, poles and ponds per treatment were pooled, transferred to a muffle furnace and ashed at 450°C for 6 h and re-weighed. Dry matter (DM), ash free dry matter (AFDM) and ash content were determined by weight differences (APHA, 1992).

The second sample of 4 cm^2 (2.0 cm x 2.0 cm), taken at each sampling point along the pole, was used to determine chlorophyll *a* and pheophytin *a* concentrations. Material was immediately transferred to labeled tubes containing 10-ml 90% acetone, sealed and stored overnight in a refrigerator. The following morning, samples were homogenized for 30 sec using a tissue grinder, then refrigerated again for 4 h before being centrifuged at 2,000 to

3,000 rpm for 10 min. The supernatant was carefully transferred to 1 cm glass cuvettes and absorption measured at the optical density of 750 and 664 in a spectrophotometer (Milton Roy Spectronic, model 1001 plus). Samples were then acidified by addition of three drops of 0.1*N* HCl and 90 sec after acidification absorbance measured again at the optical density of 750 and 665 nm. Chlorophyll *a* and pheophytin *a* concentrations were determined following standard methods (APHA, 1992).

After collection of the periphyton samples, the poles were returned to their original position. Sampled poles were excluded from all subsequent samples.

Fish harvesting

At the end of the experiment, bamboo poles were removed, the ponds drained, and all fish were collected, weighed (Ohaus model C100R200; precision = 0.1 g) and total length measured (measuring board; precision = 1 mm) individually. Specific growth rate was estimated as:

SGR (% bw d^{-1}) = [ln (final weight) – ln (intial weight)]/culture period (days)x100.

Data analysis

Daily and weekly water quality parameters were compared by ANOVA in split-plot design with treatments (different stocking ratios) as the main factor and time as the sub-factor (Gomez and Gomez, 1984) using the SAS 6.11 program (SAS Institute Inc., Cary, NC 27513, USA). Periphyton biomass and pigment parameters for each pond, depth and sampling date (means of three poles per pond) were analyzed in a split-split-plot design with treatments as the main factor, depth as the first sub-factor and sampling date as the second sub-factor. Due to the small amount of dry matter, periphyton samples from poles, depths and ponds were pooled on each sampling day for ashing. Therefore, no ANOVA for ash percentage and AFDM could be done. A one-way ANOVA was followed for production parameters. If a main effect was significant, the ANOVA was followed by Tukey test.

Results

Daily water quality parameters

Mean values (and range) of daily water quality parameters of different treatment ponds are shown in Table 1. Treatments had no significant effect ($P > 0.05$), but time effects did result in significant differences ($P \leq 0.001$) in water quality parameters. Surface temperature was around 30°C at the start and reached a maximum (33°C) on Day 18, decreasing to 23.5°C during the final days of the experiment. Bottom temperatures showed similar trends as surface temperatures, but slightly higher temperatures were recorded in treatments 60R/40C (28.26°C) and 100C (28.30°C) than in the other treatments (28.05-28.15°C). The highest mean Secchi value (41.90 cm) was recorded in treatment 100C, followed by treatments 60R/40C (34.95 cm), 80R/20C (32.95 cm), 20R/80C (29.90 cm), 40R/60C (25.20 cm) and 100R (22.17 cm), respectively, although the differences were not statistically significant.

Surface and bottom DO ranged from 0.6-15.1 and 0.2-11.7 mg l^{-1} respectively, during the experimental period. Surface DO fell below 2.0 mg l^{-1} in all treatments on some occasions, except in treatment 60R/40C. Bottom DO dropped below 1.0 mg l^{-1} on occasion in all treatments except treatment 60R/40C.

Weekly water quality parameters

Mean values of weekly water quality parameters are shown in Table 2. There were no significant difference ($P > 0.05$) in weekly monitored water quality parameters among treatments. All weekly water quality parameters varied significantly ($P \leq 0.0001$) among sampling weeks but they did not follow any specific trends during the experimental period. Lowest mean value of total ammonia was recorded in treatment 60R/40C (1.03 mg l^{-1}) in comparison to other treatments (1.24-1.58 mg l^{-1}). The highest mean chlorophyll *a* concentration of the water (phytoplankton) was determined from ponds without catla and there was a quadratic relation between phytoplankton chlorophyll *a* and net yield of catla (Figure 1).

Table 1

Daily water quality parameters recorded from different stocking ratio treatments. Values are means of 70 sampling dates and two ponds ($N = 140$). Ranges are given in parentheses.

Parameters	Treatments/Stocking Ratio					
	100R	*80R/20C*	*60R/40C*	*40R/60C*	*20R/80C*	*100C*
Temperature	28.92	28.90	29.01	30.42	28.98	29.11
Surface (°C)	(23.4-33.4)	(23.0-33.7)	(23.6-33.7)	(23.3-33.5)	(23.3-33.8)	(23.0-33.6)
Temperature	28.06	28.12	28.26	28.15	28.14	28.30
Bottom (°C)	(23.0-33.2)	(22.9-32.7)	(23.2-33.7)	(23.1-32.9)	(23.0-32.8)	(23.0-33.4)
Secchi depth	22.17	32.95	34.95	25.20	28.90	41.90
(cm)	(15-62)	(17-72)	(17-69)	(15-40)	(16-52)	(15-66)
DO Surface	6.34	5.91	6.47	6.56	6.22	6.09
(mg l^{-1})	(0.9-12.6)	(1.0-12.8)	(2.1-14.9)	(1.7-13.1)	(0.6-13.4)	(1.1-15.1)
DO Bottom	4.05	3.57	4.44	4.00	3.71	3.52
(mg l^{-1})	(0.7-9.6)	(0.7-9.8)	(1.4-11.7)	(0.2-10.3)	(0.3-10.2)	(0.3-9.9)
pH range	7.05-10.14	6.45-9.43	7.00-9.90	6.15-9.65	6.19-9.33	6.46-9.20

Table 2

Weekly water quality parameters of different stocking ratio treatments. Values are means of 10 sampling dates and two ponds ($N = 20$). Ranges are given in parentheses.

Parameters	Treatments/Stocking Ratio					
	100R	**80R/20C**	**60R/40C**	**40R/60C**	**20R/80C**	**100C**
Total alkalinity	97.60	92.60	94.45	102.60	108.35	113.85
(mg l^{-1})	(63-137)	(56-130)	(68-125)	(67-140)	(47-160)	(62-165)
Nitrate nitrogen	4.28	3.75	4.06	4.43	3.71	4.12
(mg l^{-1})	(2.3-8.4)	(2.0-5.7)	(2.6-7.6)	(2.9-8.8)	(1.8-7.0)	(2.2-10.1)
Total ammonia	1.58	1.44	1.03	1.43	1.41	1.24
(mg l^{-1})	(0.22-2.75)	0.3-2.75	(0.30-0.16)	(0.34-2.75)	(0.02-2.75)	(0.18-2.58)
Orthophosphate	1.16	0.89	0.81	0.72	0.57	0.67
(mg l^{-1})	(0.23-2.75)	(0.12-2.20)	(0.29-1.76)	(0.19-1.95)	0.25-1.42)	(0.21-1.82)
Chlorophyll *a*	322.74	254.82	265.01	276.20	195.76	160.83
(μg l^{-1})	(120-910)	(97-656)	(75-871)	(67-603)	(44-506)	(54-421)

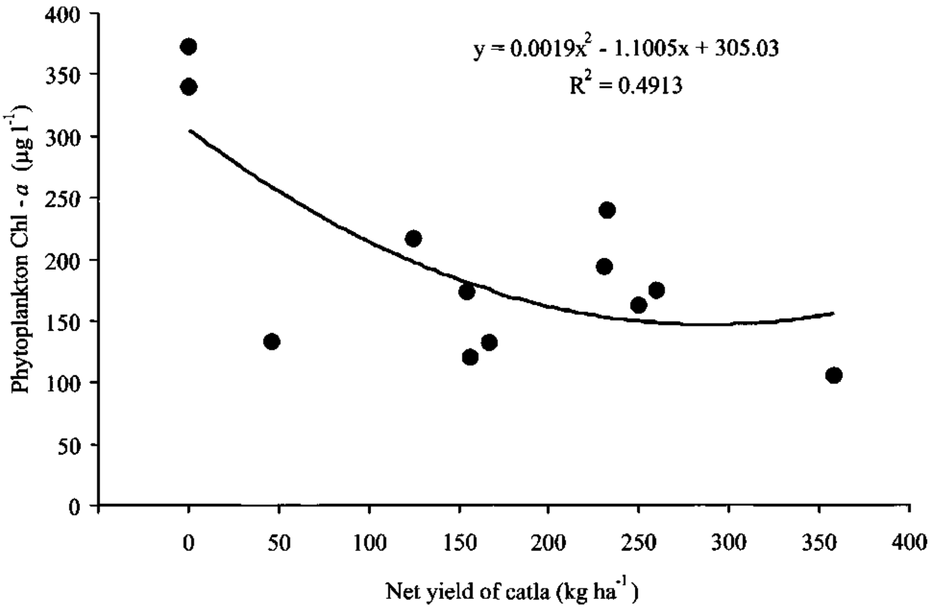

Figure 1. Relationship between chlorophyll *a* of phytoplankton collected from pond water on the last day of the experiment and net yields of catla obtained from different stocking ratio treatments. Relationships shown were obtained by quadratic regression with $N = 12$ data pairs for each treatment.

Periphyton biomass

Mean values (and S.D.) of periphyton dry matter (DM), ash free dry matter (AFDM), ash content and pigment concentrations are shown in Table 3. There was no significant variation ($P > 0.05$) in periphyton DM and chlorophyll *a* among different treatments, among different depths of the substrate or among different sampling times. Ash contents of periphyton dry matter ranged from 12 to 68% among the different stocking ratio treatments and increased as the stocking ratio of catla was increased (Figure 2). The highest chlorophyll *a* concentration (14.81 $\mu g\ cm^{-2}$) was recorded in ponds stocked with 100% catla and the lowest (6.52 $\mu g\ cm^{-2}$) were recorded in the ponds stocked with 100% rohu. Pheophytin *a* content of periphyton varied between sampling times ($P \leq 0.0001$), increasing over the experimental period, but did not vary significantly among treatments ($P > 0.05$).

Table 3

Means (and S.D.) of periphyton biomass and pigment parameters scraped from substrates in different stocking ratio treatments. Values are means of three sampling dates, four depths, three poles and two ponds ($N = 72$), except for ash and AFDM where samples from depths, poles and ponds were pooled (means of three dates, $N = 3$).

Parameters	**Treatments/Stocking Ratio**					
	100R	**80R/20C**	**60R/40C**	**40R/60C**	**20R/80C**	**100C**
Dry matter (μg cm^{-2})	676	850	792	1129	1919	2409
	(400)	(1076)	(473)	(970)	(2067)	(2257)
AFDM (μg cm^{-2})	596	580	603	613	619	786
	(356)	(691)	(346)	(454)	(753)	(733)
Ash (%)	12	32	24	46	68	68
	(3)	(12)	(11)	(23)	(5)	(7)
Chlorophyll *a* (μg cm^{-2})	6.52	8.99	7.41	10.08	11.25	14.81
	(4.61)	(5.98)	(6.98)	(7.61)	(12.35)	(7.05)
Pheophytin *a* (μg cm^{-2})	2.75	1.69	3.96	3.02	2.98	6.60
	(5.18)	(2.17)	(5.88)	(5.11)	(5.17)	(10.83)

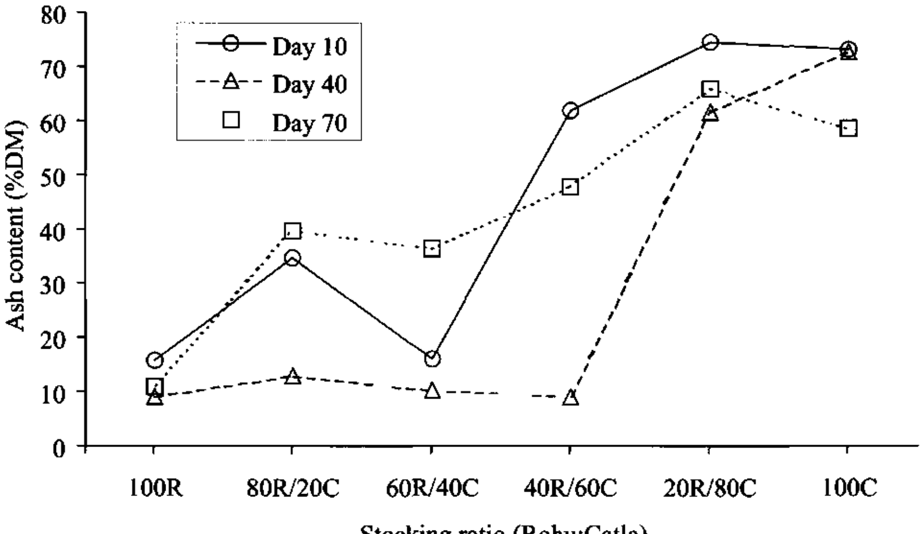

Figure 2. Ash percentage of periphyton dry matter in different stocking ratio treatments during different sampling days.

Survival, growth and yield of fish

Yield parameters of rohu and catla under six different stocking ratios are shown in Tables 4 and 5 respectively, and in Figure 3. Survival at the end of the experiment ranged from 66.37 to 95.45% for rohu and 45.56 to 86.85% for catla, depending on treatment. Mean survival of rohu (arcsine transformed) did not vary significantly with treatment (ANOVA, F = 1.68, d.f. = 5, $P > 0.05$) whereas that of catla varied significantly among treatments (F= 8.58, d.f. = 5, $P \leq 0.05$). Survival was significantly higher in treatment 80R/20C (86.85%) followed by treatments 40R/60C (72.05%), 100C (55.30%), 60R/40C (47.78%) and 20R/80C (45.56%), respectively (Tukey test, $P < 0.05$).

Table 4

Comparisons of means (and S.D.) of yield parameters of rohu in different stocking ratios by ANOVA. If main effects are significant, then means followed by the different superscript letter in each row are significantly different at 0.05 level based on Tukey test.

Yield	**Treatments/Stocking ratio**					**ANOVA**
Parameters	**100R**	**80R/20C**	**60R/40C**	**40R/60C**	**20R/80C**	**result**
Survival	66.37	72.78	90.44	75.56	95.45	NS
(%)	(5.00)	(22.78)	(13.52)	(12.56)	(0.00)	
Stocking	11.41	11.37	11.28	11.39	11.4	NS
length (cm)	(0.98)	(0.96)	(1.06)	(0.95)	(0.94)	
Harvesting	12.87^{b}	12.88^{b}	14.29^{a}	13.08^{b}	13.79^{a}	***
length (cm)	(1.15)	(0.98)	(1.37)	(1.27)	(1.31)	
Stocking	15.15	15.04	14.92	15.55	15.08	NS
weight (g)	(4.50)	(4.41)	(4.40)	(3.99)	(4.05)	
Harvesting	45.32^{b}	46.75^{b}	62.68^{a}	50.32^{b}	58.32^{a}	***
weight (g)	(11.39)	(11.54)	(15.96)	(15.60)	(18.86)	
SGR	1.57	1.65	2.06	1.62	1.92	NS
(% bw d^{-1})	(0.16)	(0.29)	(0.17)	(0.39)	(0.20)	
Yield	224.97^{ab}	227.75^{ab}	378.65^{a}	134.81^{b}	124.81^{b}	*
(kg ha^{-1} 70 d^{-1})	(17.85)	(47.64)	(14.84)	(97.00)	(24.00)	

*$P < 0.05$; ***$P < 0.001$; NS, not significant.

Specific growth rates of rohu did not vary significantly (ANOVA, F = 1.40, d.f. = 5, $P > 0.05$) with different stocking ratios, whereas those of catla were significantly affected (F = 10.90, d.f. = 5, $P \leq 0.05$) by increases in catla stocking densities. The highest growth rates of both rohu (2.06% bw d^{-1}) and catla (2.46% bw d^{-1}) were recorded in treatment 60R/40C.

Although there was no significant difference in total length of fish at stocking, marked differences in lengths at harvest due to different stocking combinations of both rohu (F = 32.46, d.f. = 511, $P < 0.001$) and catla (F = 81.63, d.f. = 383, $P \leq 0.001$) were apparent.

Table 5

Comparisons of means (and S.D.) of yield parameters of catla in different stocking ratios by ANOVA. If main effects are significant, then means followed by the different superscript letter in each row are significantly different at 0.05 level based on Tukey test.

Yield	Treatments/Stocking ratio					ANOVA
Parameters	80R/20C	60R/40C	40R/60C	20R/80C	100C	result
Survival	86.85^{a}	47.78^{b}	72.05^{ab}	45.56^{b}	55.30^{ab}	*
(%)	(12.16)	(1.57)	(6.24)	(3.14)	(6.88)	
Stocking length	12.09	12.02	12.15	12.22	12.16	NS
(cm)	(1.25)	(1.07)	(1.18)	(1.15)	(1.22)	
Harvesting	16.11^{a}	15.99^{a}	13.89^{b}	13.27^{c}	12.94^{c}	***
length (cm)	(1.13)	(1.68)	(1.50)	(1.23)	(0.94)	
Stocking weight	20.12	20.26	20.35	20.38	20.46	NS
(g)	(5.66)	(4.98)	(5.39)	(5.31)	(5.55)	
Harvesting	110.01^{a}	114.83^{a}	75.15^{b}	64.64^{c}	54.48^{d}	***
weight (g)	(24.92)	(30.90)	(25.53)	(17.94)	(9.75)	
SGR	2.43^{a}	2.46^{a}	1.87^{ab}	1.65^{b}	1.40^{b}	*
(% bw d^{-1})	(0.21)	(0.37)	(0.07)	(0.12)	(0.08)	
Yield	231.68	207.62	304.07	100.38	145.74	NS
(kg ha^{-1} 70 d^{-1})	(1.15)	(73.19)	(76.85)	(76.74)	(29.95)	

*$P < 0.05$; ***$P < 0.001$; NS, not significant.

The weight of fish at harvest for both rohu and catla varied significantly (rohu: F = 33.68, d.f. = 511, $P \leq 0.001$; catla: F = 103.34, d.f. = 383, $P \leq 0.001$) as a result of differences in stocking ratio. In general, the weights of both species at harvest decreased with increases in their own stocking density but the highest mean values were found in treatment 60R/40C.

Net yields of rohu were significantly different (F = 8.12, d.f. = 5, $P \leq 0.05$) with different stocking ratios, with the highest mean value (379 kg ha^{-1}) in treatment 60R/40C followed by treatments 80R/20C (228 kg ha^{-1}), 100R (225 kg ha^{-1}), 40R/60C (135 kg ha^{-1}) and 20R/80C (125 kg ha^{-1}), respectively. Although treatment effect on net catla yield was not significant (ANOVA, F = 3.42, d.f. = 5, $P > 0.05$), net catla yields were higher in treatments 80R/20C (232 kg ha^{-1}), 60R/40C (208 kg ha^{-1}) and 40R/60C (304 kg ha^{-1}), in which fewer catla were stocked, than in treatments 20R/80C (100 kg ha^{-1}) and 100C (146 kg ha^{-1}).

The combined net yields, however, from any combinations of rohu and catla were 50-300% higher than those from rohu or catla treatment alone. The relative contribution of both species on per hectare production extrapolated from the data of 75-m^2 pond over a period of 70 days is shown in Figure 3. There were significant variations (F = 7.53, d.f. = 5, $P \leq 0.05$) in total net yields among treatments. The highest total net yield of 586 kg ha^{-1} was found in treatment 60R/40C followed by 459 kg ha^{-1} (40R/60C), 439 kg ha^{-1} (80R/20C), 225 kg ha^{-1} (20R/80C and 100R) and 146 kg ha^{-1} (100C), respectively. The 60R/40C treatment resulted in a significantly higher production than either of the monoculture treatments (100R and 100C) and treatment 20R/80C (Tukey test; Figure 3).

Discussion

Water temperature at both pond surface and bottom were initially within the optimal range of carp culture, thereafter falling until the last days of the experiment. The bottom temperatures were higher in treatments 60R/40C and 100C. The Secchi depth was also higher in these two treatment ponds allowing greater light penetration through the water column. However, Secchi depth increased with increasing catla numbers due to lower abundance of phytoplankton, indicating that catla grazed more on plankton. Dewan et al. (1991) and Ahmed (1993) similarly observed an inverse relationship between Secchi depth value and chlorophyll *a* in ponds in Mymensingh, Bangladesh. DO concentrations were generally suitable for fish culture throughout the experimental period, although exceptionally low DO values were recorded on a few occasions.

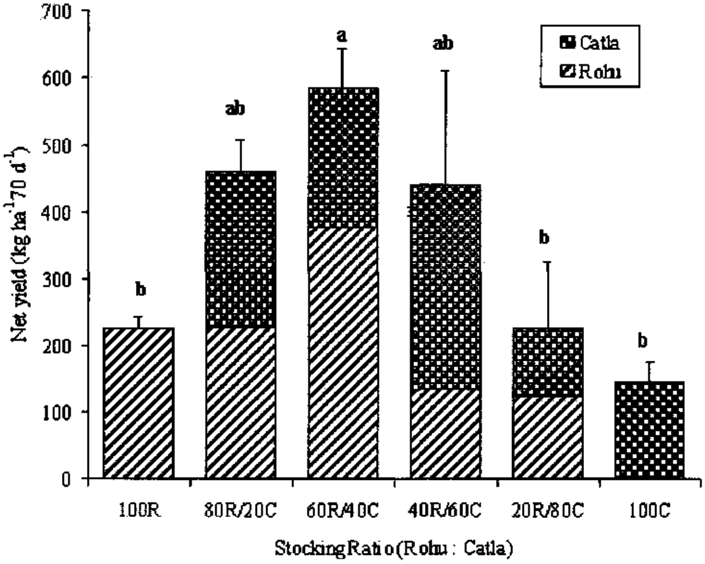

Figure 3. The relative contributions of rohu and catla to total net yield (+S.D.) in different stocking ratio treatments. Per hectare production was extrapolated from the data of 75-m^2 pond over a period of 70 days. The different superscript letters among different bars are significantly different at 0.05 level based on Tukey test.

Unusually high orthophosphate levels were recorded during the entire period of study. Phosphate-phosphorus levels decreased with increasing numbers of catla stocked despite the fact that identical fertilization rates were maintained in all treatments. This may have been due to a greater turnover of nutrients by phytoplankton rather than periphyton (Hwang et al., 1998) where the ratio of catla/rohu was higher. The chlorophyll *a* concentration of pond water decreased with increasing stocking density of catla, whereas the chlorophyll *a* content of periphyton scraped from the substrates decreased with increasing numbers of rohu stocked.

This further indicates preferential grazing on periphyton and plankton by rohu and catla, respectively.

Periphyton biomass as measured by DM, AFDM and pigment concentrations, did not significantly change with depth, in contradiction to the findings of Konan-Brou and Guiral (1994) and Keshavanath et al. (2001) who reported maximum periphytic biomass levels coinciding with photosynthetic compensation depth. In our study, there were no bottom-dwelling fish species that might graze below the compensation depth of the substrates. Ash content of periphyton decreased with increasing ratio of rohu/catla, indicating that periphyton quality improved under grazing. As the densities of catla increased and rohu decreased, less grazing occurred, periphyton grew to maximum density and more sediment particles were trapped. Pigment concentrations (chlorophyll *a* and pheophytin *a*) in periphyton scraped from the substrates decreased with increasing stocking density of rohu although they were not significantly different.

Survival and growth of rohu were independent of the density at which the species was stocked, whereas those of catla significantly decreased as stocking density of the species increased. Rohu appeared to rely on periphyton as a food source, irrespective of stocking density. The lower survival and growth of catla with increased stocking density may have been due to intraspecific competition for food. Rohu is known to be a predominantly column feeding fish, consuming phytoplankton and decaying suspended organic matter (Das and Moitra, 1955; Dewan et al., 1991). It also feeds on periphyton in ponds provided with substrates (NFEP, 1997; Ramesh et al., 1999; Azim et al., 2001). Aravindakshan et al. (1995) regarded both rohu and catla as surface dwellers. Ahmed (1993) described catla as a filter-feeding species, depending primarily on zooplankton during early life stages and subsequently switching to a combination of phytoplankton and zooplankton. In the present trial, a higher survival of rohu than of catla and a higher net yield of rohu in monoculture (treatment 100R) than net yield of catla in monoculture (treatment 100C) further indicated the preference for periphyton by rohu and avoidance of periphyton by catla. Indeed, rohu were regularly observed grazing on the periphyton. Regression analysis of chlorophyll *a* sampled on the last day of the experiment further revealed that periphyton chlorophyll *a* decreased with increasing biomass of rohu (Figure 4).

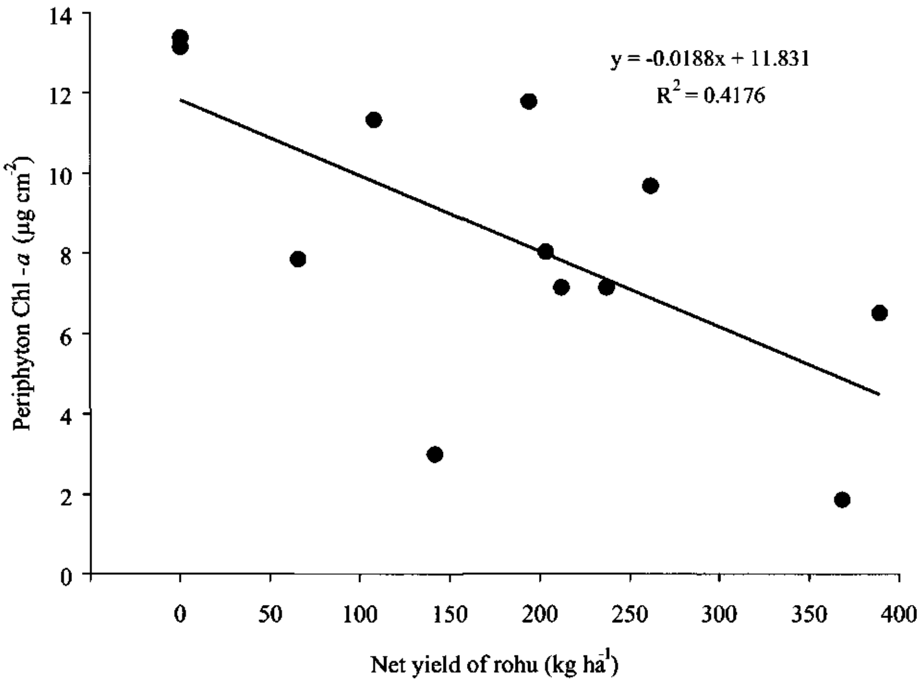

Figure 4. Relationship between chlorophyll *a* of periphyton scraped from bamboo substrates from different ponds and net yields of rohu obtained from different stocking ratio treatments. Relationships shown were obtained linear regression with $N = 12$ data pairs for each treatment.

Highest harvesting weight and greatest total yield was found in treatment 60R/40C. Although catla were not observed grazing on periphyton, higher net yields were apparent in treatments stocked with 20, 40 and 60% catla rather than treatments stocked with 80 and 100% catla. Possibly, at a 60R/40C ratio synergistic interactions exist between the two species, resulting in optimal utilization of food resources and improved environmental conditions. The lowest ammonia concentrations (1.03 mg l^{-1}) and highest bottom DO (4.44 mg l^{-1}) occurred in the same ponds. Ramesh et al. (1999) reported that ponds with substrates had lower total ammonia levels than control ponds and concluded that enhanced bacterial biofilms (periphyton) on the substrates might reduce ammonia levels through promotion of nitrification. Lower ammonia levels were also reported by Langis et al. (1988) in aquaria harbouring bacterial biofilms on glass panels. Milstein (1992) also explained the synergistic effect as the combined action of an increase of food resources and an improvement of environmental conditions.

Highest yield over the 70-days experimental period was 586 kg ha^{-1} of fish in treatment 60R/40C. The experiment started at the end of September when maximum water temperature was 31.1°C and finished early December when temperatures had fallen to 22.3°C. Optimum temperatures for Indian major carps are about 30-34°C (Dewan et al., 1991), and the best growing season of carps in South Asia is from April to October. Other reported yields include: 1,900 kg ha^{-1} of rohu in 120 days with bamboo substrate (Azim et al., 2001); a maximum tilapia biomass equivalent to 8 metric tonnes ha^{-1} y^{-1} in acadja-enclos in the Ebrie Lagoon, Ivory Coast, with natural recruitment of fingerlings (Hem and Avit, 1994); 1,235 kg ha^{-1} of rohu and common carp in 133 days with sugarcane baggase as substrate (Ramesh et al., 1999); and 713 kg ha^{-1} of kalbaush (*Labeo calbasu*) in 120 days in ponds with kanchi (scrap bamboo) as substrate (Wahab et al., 1999).

The growth and total yields of fish from any combination of rohu and catla treatments were higher (3-40% by individual weight and 50-300% by total yield) than those from rohu or catla treatment alone, indicating that polyculture of rohu and catla is superior to monoculture of either species in periphyton-based aquaculture systems. The present trial indicates that where a combination of 60% rohu and 40% catla is used, synergistic benefits compensate for any inter-specific or intra-specific dietary competition. The inclusion of bottom feeding carp species in the polyculture system may bring about yet further increases in yield.

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Chapter 7

Evaluation of polyculture of Indian major carps in periphyton-based ponds

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Abstract

Production of three Indian major carps, catla *Catla catla*, rohu *Labeo rohita* and kalbaush *Labeo calbasu* in a periphyton-based polyculture system was compared. Bamboo poles, approximating a submerged surface area equal to the total pond surface area, were used as substrates for periphyton and were planted vertically into the pond bottoms. Ponds were fertilized fortnightly with 4,500 kg cow manure, 150 kg urea and 150 kg triple super phosphate per hectare. Four stocking combinations were applied: 60% rohu plus 40% catla with a total stocking density of 10,000 ha^{-1} (treatment CR), CR plus 15% kalbaush (C15), CR plus 30% kalbaush (C30) and CR plus 45% kalbaush (C45). A treatment with 60% rohu plus 40% catla without bamboo substrate was used as control (CR0).

Treatments differed significantly in some water quality parameters (Secchi depth, total alkalinity, orthophosphate, total ammonia and chlorophyll *a*) and periphyton biomass (dry matter, ash free dry matter and ash content). The ash (15-19%), protein (23-26%) and energy (19-20 kJ g^{-1}) contents of the periphyton estimated can be considered as broadly appropriate to fish dietary needs. The relative contributions of algae to the periphytic biomass were 30-60% depending on the treatment. In total, 50 genera of algae, 13 genera of zooplankton and some macrobenthic invertebrates were identified from the periphyton samples.

Survival of rohu and catla was higher in ponds with bamboo poles than in the controls. Net fish yields of the three species were found to be higher in treatment C15. Highest total fish yield, over a 90 days culture period, was recorded in treatment C15 (2,306 kg ha^{-1}) followed by treatment C45 (1,914 kg ha^{-1}), treatment CR (1,652 kg ha^{-1}), treatment C30 (1,507 kg ha^{-1}) and treatment CR0 (577 kg ha^{-1}). Fish production from the periphyton-based system was 2.8 times higher than that of control. The addition of 15% kalbaush (i.e. a stocking ratio of 60:40:15 rohu:catla:kalbaush) at a total stocking density of 11,500 juveniles ha^{-1} resulted in a further 40% increase in production and is an appropriate combination in a periphyton-based polyculture system. The stable nitrogen and carbon isotopes ratio indicated that rohu grazed on periphyton whereas catla depended on planktonic food organisms.

Introduction

Many trials have demonstrated that fish production from ponds provided with artificial substrates for periphyton is higher than that from substrate-free controls (Hem and Avit, 1994; NFEP, 1997; Wahab et al., 1999; Ramesh et al., 1999; Azim et al., 2001a). Most studies have concentrated on the potential of periphyton-based culture of particular fish species grown in monoculture. In traditional extensive or semi-intensive fish culture ponds, higher growth rates and yields are derived from polyculture than from monoculture as the results of synergistic interactions among fish species and improvements of environmental conditions (Yashouv, 1971; Hepher et al., 1989; Milstein, 1992; Wahab et al., 1995). The traditional concept of polyculture has only recently been considered in relation to periphyton-based culture system.

A programme of systematic experiments has recently been initiated to investigate the potential of periphyton-based aquaculture of indigenous species in southern Asia. Two carp species, the column-feeder rohu, *Labeo rohita* and the bottom-feeder kalbaush, *Labeo calbasu* proved suitable for periphyton-based culture system (Wahab et al., 1999; Ramesh et al., 1999; Azim et al., 2001a). Since the experimental fish largely depended on periphyton in these monoculture ponds, planktonic food organisms remained undergrazed, and thereby hindered sunlight penetration and periphyton growth. The surface feeder catla, *Catla catla* showed better performance in this system when it was cultured with the periphyton-feeder rohu, and the stocking ratio of these two species was optimized (Azim et al., 2001b). The production difference between the substrate-based and substrate-free control system using the optimized stocking density of these two species is yet to be quantified. Moreover, it is worthwhile to investigate whether the periphyton-feeding, bottom dwelling, kalbaush further enhances fish production in this system.

The ultimate objective of this experiment was to develop a periphyton-based polyculture technique with indigenous carp species. Specific objectives were therefore (a) to establish the production difference between periphyton-based and traditional carp polyculture, and (b) to optimize the stocking density of kalbaush with catla and rohu in a periphyton-based polyculture system. Nutritional quality of periphyton and trophic levels of experimental animals were also described. Throughout this paper, the term "periphyton" is defined as the entire complex of all sessile biota attached to the substrates along with associated detritus and microorganisms.

Materials and methods

Study area and experimental design

The 90-days experiment was carried out between August and November 1999 in 10 earthen ponds at the pond facilities of the Fisheries Faculty Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh. All ponds were rectangular (7.5 m x 10 m) with a maximum depth of 1.5 m. The ponds were individually supplied with ground water from an adjacent deep tube-well and fully exposed to prevailing sunlight. Pond embankments were covered with grass.

The design was based on a previous experiment (Azim et al., 2001b) that resulted in an optimal stocking ratio of 60% rohu and 40% catla in a periphyton-based system. Four stocking combinations were compared in the present study: 60% rohu plus 40% catla with total stocking density of 10,000 ha^{-1} (treatment CR), CR plus 15% kalbaush (i.e. 1,500 juveniles ha^{-1}) (treatment C15), CR plus 30% kalbaush (treatment C30), and CR plus 45% kalbaush (treatment C45). Bamboo poles, used as substrates for periphyton, were planted vertically into the pond bottoms. As a control, CR without substrate was used (treatment CR0). Two replicates of each treatment were assigned randomly to the ponds.

Pond preparation and fish stocking

Prior to the trial, ponds were renovated, aquatic vegetation removed and all larger aquatic animals eradicated by frequent netting. As substrates, bamboo poles (mean length = 2.0 m; mean diameter = 5.5 cm) were staked into the bottom mud, the upper portions extending above the water surface (Day 1). Substrates (9 poles m^{-2}) were installed in a 40-m^2 area, leaving a 2-m wide perimeter free of poles in each pond. The poles added an effective submerged surface area of about 75 m^2 per pond.

Ponds were treated with lime (CaO, 250 kg ha^{-1}) and filled with water. On Day 7, semi-decomposed cow manure, urea and triple super phosphate (TSP) were applied at the rates of 4,500, 150, and 150 kg ha^{-1}, respectively. Ponds were then fertilized fortnightly at the same rates during the entire experimental period.

On Day 14, *Labeo rohita* (individual weight 4-5 g), *Catla catla* (6-7 g) and *Labeo calbasu* (4-6 g) juveniles were released into the ponds. Juveniles were collected from nearby hatcheries and stocked in the afternoon, care being taken to gradually acclimatize the fish to pond conditions.

Periphyton sampling and analysis

Periphyton collection started on Day 20 and continued at fortnightly intervals. From each pond, three bamboo poles were selected by random number tables. Two samples of periphyton were taken at three depths, 25, 50 and 75 cm along the length of the pole, starting at the water surface. After collection of the periphyton samples, the poles were returned to their original positions and marked so that sampled poles were always excluded from subsequent samples.

For the first sample, a 2.0 cm wide band was carefully removed with a scalpel blade from the entire circumference at each sampling point along the pole, care being taken not to remove any of the substrate itself. Samples from different depths and different poles were mixed thoroughly. One third of the mixed sample (pond-wise) was used to determine chlorophyll *a* and pheophytin *a* content. Material was immediately transferred to labeled tubes containing 10-ml 90% acetone, sealed and stored overnight in a refrigerator. The following morning, samples were homogenized for 30 s using a tissue grinder, then refrigerated again for 4 h before being centrifuged at 2,000 to 3,000 rpm for 10 min. Chlorophyll *a* and pheophytin *a* concentrations were then determined spectrophotometrically (Milton Roy Spectronic, model 1001 plus) following standard methods (APHA, 1992). The remaining sample was pooled treatment-wise, used to study the taxonomic composition of periphyton. Periphyton samples were collected and enumerated as described in Azim et al. (2001a).

For the second sample, a 5.0 cm band was scraped from the area immediately adjacent to the surface of the first sample. The material from all depths and poles were pooled and transferred to pre-weighed and labeled pieces of aluminium foil. Samples were kept cool until returned to the laboratory. Samples were placed in a drying oven (Memmert, Model UM/BM

100-800) and dried at 105°C until constant weight (24 h), before being transferred to a dessicator until weighed (BDH, Model 100A; precision 0.0001 g). Half of the dried sample was transferred to a muffle furnace and ashed at 550°C for 4 h and re-weighed. Dry matter (DM), ash free dry matter (AFDM) and ash contents were determined by weight differences. The autotrophic index (AI) was calculated using the following formula (APHA, 1992):

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

The remaining portion of the sample from all dates were pooled (pond-wise) and used for analysis of proximate composition, energy content and stable nitrogen and carbon isotopes in the laboratory of the Wageningen Institute of Animal Sciences (WIAS), Wageningen University and Research Centre (WUR), Netherlands. Nitrogen content was determined by Kjeldahl method. Protein content of DM was calculated using the nitrogen to protein conversion factor of 5.8 suggested by Gnaiger and Bitterlich (1984), who found this to be a more appropriate value for bacteria, algae and aquatic invertebrates than the 6.25 that is usually applied. Fat content was determined by Soxhlet apparatus. Energy content was determined by bomb calorimeter.

Water quality monitoring

From Day 14 onwards, the following variables were monitored daily (6 days per week) between 0900 and 1000 h: temperature, dissolved oxygen (YSI digital DO meter, model 58), Secchi depth (Secchi disc) and pH (a portable Hanna pH meter). Total alkalinity, chlorophyll *a*, total ammonia, nitrate nitrogen (NO_3-N) and phosphate phosphorus (PO_4-P) were measured weekly in all ponds. Total alkalinity was determined by titrimetric method (Stirling, 1985). Chlorophyll *a* was determined spectrophotometrically after acetone extraction (Boyd, 1979). Water samples were filtered (Whatman GF/C filter paper) prior to nutrient analysis. Nutrient analyses were performed by Hach Kit (model DR 2000).

Fish harvesting

At the end of the experiment, all bamboo poles were removed, the ponds drained, and all fish were collected and weighed on a balance (Ohaus model C100R200; precision 0.1 g). Specific growth rate (% day^{-1}) was estimated as:

SGR = [ln (final weight) – ln (intial weight)] /culture period (days) x 100.

Analysis of stable nitrogen and carbon isotope ratio

Samples analyzed for stable isotope compositions included all food sources within the ponds and the experimental fish. Plankton samples were collected monthly from the water column using a plankton net (45 μm) from all ponds. Collection of periphyton samples was described before. Bottom sediment was collected by installing a sediment trap in the bottom of each pond. Samples from all ponds were pooled before analysis. During stocking and harvesting, two individuals of each species per ponds were selected randomly for stable isotope analysis. Plankton, periphyton and sediment samples were oven dried at 105°C and fish samples at 70°C for 24 h. Whole body biomass of fish was used for analysis. Stable nitrogen and carbon isotope compositions were determined using an Isotope Ratio Mass Spectrophotometer (Finnigan MAT Delta C) in the IRMS Laboratory of the WIAS, WUR, Netherlands.

Statistical analysis

A one-way ANOVA was used for yield parameters. Survival of fish was analyzed using arcsine-transformed data. Daily and weekly water quality parameters and periphyton biomass were compared by split-plot ANOVA with treatments as the main factor and time as the sub-factor (Gomez and Gomez, 1984) using the SAS 6.12 program (SAS Institute Inc., Cary, NC 27513, USA). The pH values were transformed to hydrogen ion concentrations before statistical analysis. If a main effect was significant, the ANOVA was followed by Tukey test at $P < 0.05$ level of significance.

Results

Yield parameters

Effect of bamboo substrate

Yield parameters of rohu and catla are shown in Tables 1 and 2, respectively. Whereas there was no significant difference in initial total weight of fish between treatments at stocking, weights at harvest differed markedly. Specific growth rate (SGR) of rohu in ponds with bamboo poles was higher than in ponds without bamboo, but not significantly so. However, when comparing only CR0 and CR in a separate ANOVA, the difference was significant. The SGR of catla varied significantly between treatments, with higher mean values in ponds with bamboo poles than in the control. Survival of both rohu and catla were higher in ponds with bamboo poles as substrate (treatment CR) than in the control (CR0).

Net yield of rohu was 2.6 times higher in treatment CR than in CR0, whereas, net yield of catla was 3.2 times higher in CR than in CR0. The combined net yields of rohu and catla after 90 days were 1,652 in ponds with bamboo poles and 577 kg ha^{-1} in control ponds (Figure 1), an overall increase of 2.8 times.

Effect of inclusion of bottom feeder kalbaush

Individual weight of rohu at harvest was higher at the low stocking density of kalbaush (C15) than at the higher stocking densities (C30 and C45) or even without kalbaush (CR) (Table 1). The SGRs of rohu were not different among treatments. However, survival of rohu was more than 90% and not significantly different among different stocking densities of kalbaush. The net yield of rohu was highest in treatment C15 followed by treatments CR and C45 and lowest in C30.

Individual weight at harvest and SGR of catla were higher in treatments C15 and C45 than treatments CR and C30 (Table 2). Survival was the same in all stocking combinations of kalbaush. Net yield was highest in treatment C15 and lowest in C30.

Table 1

Growth, survival and yield of rohu (*L. rohita*) in different treatments (CR0 = combination of catla-rohu without bamboo; CR = CR0 with bamboo; C15, C30 and C45 = CR plus 15, 30 and 45% kalbaush, respectively). Standard deviations are given in the parentheses. In each column, different letter superscripts indicate significant difference (ANOVA and Tukey test).

Treatments	Yield parameters				
	Individual stocking weight (g)	Individual harvesting weight (g)	SGR (% bw d^{-1})	Survival (%)	Yield (kg ha^{-1} 90 d^{-1})
CR0	5.2^{a} (0.04)	90.5^{d} (0.95)	3.18^{a} (0.02)	67^{b} (3)	331^{c} (13)
CR	4.7^{a} (0.16)	155.1^{b} (5.13)	3.81^{a} (0.00)	94^{a} (5)	851^{ab} (16)
C15	5.2^{a} (0.05)	181.8^{a} (0.69)	3.93^{a} (0.02)	91^{a} (3)	963^{a} (38)
C30	5.0^{a} (0.13)	132.3^{c} (17.17)	3.57^{a} (0.11)	92^{a} (5)	703^{b} (132)
C45	5.1^{a} (0.59)	154.3^{b} (7.16)	3.36^{a} (0.18)	93^{a} (3)	833^{ab} (15)
ANOVA result	NS	***	NS	*	***

* $P \leq 0.05$; *** $P \leq 0.001$; NS, not significant.

Individual weight at harvest, SGR and net yield of kalbaush were higher in treatment C15 than in C30 and C45. Survival ranged from 78-91% and did not vary among the different stocking densities.

The combined net yield of fish and relative contributions of different species are shown in Figure 1. A significantly higher combined fish yield, over a 90-days culture period, was recorded in treatment C15 (2,306 kg ha^{-1}) than in C45 (1,914 kg ha^{-1}), CR (1,652 kg ha^{-1}) and C30 (1,507 kg ha^{-1}).

Table 2

Growth, survival and yield of catla (*C. catla*) in different treatments (CR0 = combination of catla-rohu without bamboo; CR = CR0 with bamboo; C15, C30 and C45 = CR plus 15, 30 and 45% kalbaush, respectively). Standard deviations are given in the parentheses. In each column, different letter superscripts indicate significant difference (ANOVA and Tukey test).

Treatments	Yield parameters				
	Individual stocking weight (g)	Individual harvesting weight (g)	SGR (% bw d^{-1})	Survival (%)	Yield (kg ha^{-1} 90 d^{-1})
CR0	6.7^{a} (0.08)	99.6^{c} (3.24)	3.0^{c} (0.05)	68^{b} (12)	246^{d} (56)
CR	6.3^{a} (0.37)	225.0^{b} (11.37)	4.0^{b} (0.01)	92^{a} (2)	799^{b} (19)
C15	6.3^{a} (0.07)	310.6^{a} (7.33)	4.3^{a} (0.01)	90^{ab} (5)	1093^{a} (33)
C30	6.3^{a} (0.02)	206.7^{b} (4.53)	3.9^{b} (0.03)	82^{ab} (2)	650^{c} (5)
C45	6.2^{a} (0.02)	273.6^{a} (10.96)	4.2^{a} (0.05)	87^{ab} (0)	923^{b} (38)
ANOVA result	NS	***	***	*	***

* $P \leq 0.05$; *** $P \leq 0.001$; NS, not significant.

Table 3

Growth, survival and yield of kalbaush (*L. calbasu*) in different treatments (C15, C30 and C45 = Catla-rohu combination with bamboo plus 15, 30 and 45% kalbaush, respectively). Standard deviations are given in the parentheses. In each column, different letter superscripts indicate significant difference (ANOVA and Tukey test).

Treatments	Yield parameters				
	Individual stocking weight (g)	Individual harvesting weight (g)	SGR (% bw d^{-1})	Survival (%)	Yield (kg ha^{-1} 90 d^{-1})
C15	4.2^{a} (0.24)	191.0^{a} (12.41)	4.3^{a} (0.14)	91^{a} (13)	248^{a} (19)
C30	5.0^{a} (0.77)	61.9^{b} (3.08)	2.8^{b} (0.23)	89^{a} (9)	158^{b} (28)
C45	5.6^{a} (1.14)	51.9^{b} (0.64)	2.5^{b} (0.24)	78^{a} (2)	154^{b} (12)
ANOVA result	NS	***	**	NS	*

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS, not significant.

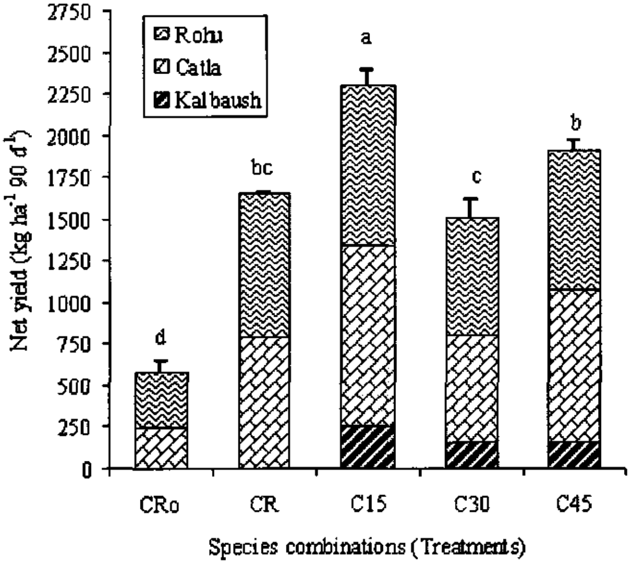

Figure 1. Net yield of fish and relative contributions of different species in five treatments. Standard deviations are derived from pond wise combined net yields (N = 2). Different letters among the bars indicate significant differences at $P > 0.05$ based on Tukey test.

Quantity and quality of periphyton

Periphyton biomass data over different sampling dates are shown in Figure 2. There were significant variations in periphyton dry matter (DM) and ash free dry matter (AFDM) per surface area among treatments, among sampling dates and among treatment-time interactions. Mean periphyton DM was higher in treatment CR (6.77 mg cm^{-2}) than in any of the treatments with kalbaush (3.3-4.8 mg cm^{-2}). Periphyton DM and AFDM increased over time in treatment CR, but not in the other treatments (Figure 2A, B). There were no treatment effects or treatment - time interactions on chlorophyll *a* and autotrophic index (AI) although a time effect was noted. Chlorophyll *a* concentrations also increased over time in treatments CR and C45, but not in treatment C30 (Figure 2C). Pheophytin *a* concentrations did not vary

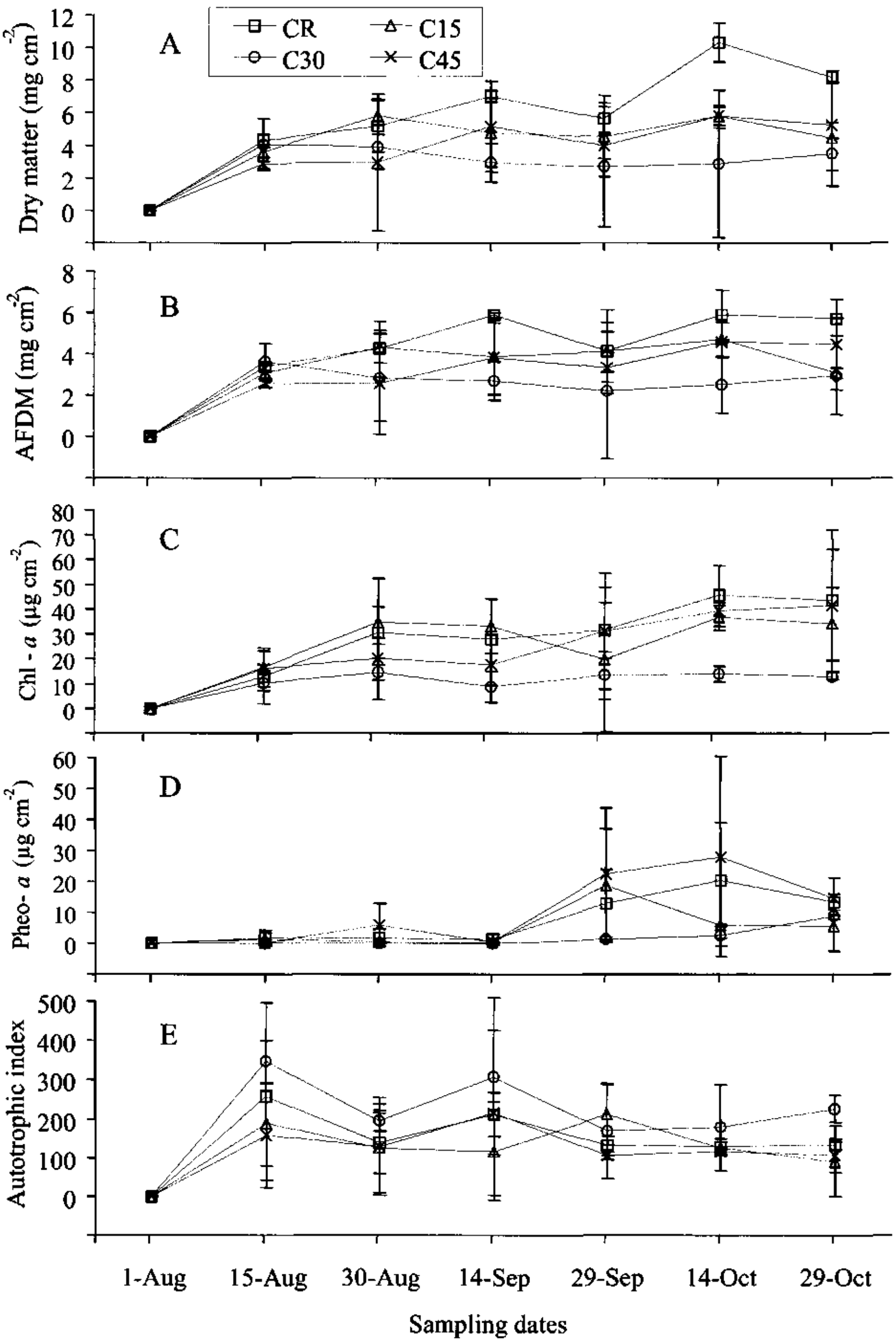

Figure 2. Periphyton biomass (A) Dry matter (B) Ash free dry matter (C) Chlorophyll *a* (D) Pheophytin *a* and (E) Autotrophic index. Values are means (±S.D.) of two ponds per treatment per sampling date.

among either treatments, or time or treatment-time interactions (Figure 2D). The AI values decreased over time in all treatments (Figure 2E).

Proximate compositions of periphyton are shown in Table 4. Protein content ranged from 23 to 26%. Ash content was 28% in treatment CR and 15-19% in the other treatments. Lipid content was 2.1-2.7%, whereas energy content was 19-20 kJ g^{-1}.

Table 4

Proximate composition of periphyton calculated on dry matter weight basis in different treatments (CR = catla-rohu combination with bamboo; C15, C30 and C45 = CR plus 15, 30 and 45% kalbaush, respectively).

Treatments	Parameters Nitrogen (%)	Protein (%)	Lipid (%)	Ash (%)	Energy (kJ g^{-1})
CR	4.14	24.0	2.1	28	20.2
C15	4.39	25.5	2.7	19	n.d.
C30	3.92	22.7	n.d.	15	n.d.
C45	4.53	26.3	n.d.	18	18.8

n.d., not determined because of insufficient materials.

The periphyton community consisted of five groups of phytoplankton and two groups of zooplankton (Table 5). Fifty genera of algal periphyton belonging to the Bacillariphyceae (12 genera), Chlorophyceae (24), Cyanophyceae (8), Euglenophyceae (4) and Rhodophyceae (2) were identified. Chlorophyceae was the most dominant group followed by Bacillariophyceae, Cyanophyceae and Euglenophyceae in all treatment ponds. Thirteen genera of zooplankton belonging to Crustacea (6) and Rotifera (7) were identified. Some macrobenthic invertebrates, especially chironomid larvae and oligochaetes were also recorded.

Table 5

Taxonomic composition of periphyton identified from periphyton samples scraped from bamboo substrates in different treatments (CR = catla-rohu combination with bamboo; C15, C30 and C45 = CR plus 15, 30 and 45% kalbaush, respectively). Values are means of six sampling dates and expressed in cells cm^{-2}.

Group/Genus	Treatments (Cells cm^{-2})			
	CR	C15	C30	C45
Bacillariophyceae				
Actinella	4522	2266	2820	1965
Achnanthes	2931	2622	1646	0
Cyclotella	5245	3425	770	5055
Cocconeis	294	0	0	1412
Cymbella	6373	0	4784	0
Fragillaria	371	0	4332	789
Gomphonema	4120	2772	4511	0
Melosira	0	9883	4791	8289
Microphora	8311	4705	4640	11543
Navicula	0	3935	6353	0
Nitzschia	8765	2251	4870	0
Synedra	0	9975	0	11407
Total	40931	41833	39518	40461
Chlorophyceae				
Actinastrum	4639	2202	0	5460
Ankistrodesmus	2888	4004	11466	0
Chaetophora	650	3785	3238	0
Chlorella	6770	1537	0	10674
Cladophora	1029	0	2463	1865
Closterium	0	2102	0	2565
Coleochaete	3964	3550	5316	0
Cylindrocapsa	4624	8521	3176	8383
Draparnaldia	1516	1976	2092	3765
Geminella	4046	5362	554	5239
Gonatozygon	7174	5293	5513	5215
Microspora	5869	3010	9784	2938
Microthamnion	0	2495	257	864
Mougeotia	1364	2974	0	0
Oedogonium	4348	2787	1867	1268
Oocystis	0	749	4490	1981
Scenedesmus	4627	1025	0	4444
Spirogyra	1751	2373	2584	789
Stigeoclonium	8479	10935	7848	9243
Tetraspora	2220	2465	4901	3596
Triplocerus	2570	0	0	0

Table 5 continued

Group/Genus	**Treatments (Cells cm^{-2})**			
	CR	**C15**	**C30**	**C45**
Ulothrix	5091	7238	9503	10383
Volvox	2059	0	1393	1151
Zygnema	9923	5256	5216	4212
Total	80117	79639	74251	77703
Cyanophyceae				
Aphanigominon	3093	1865	1157	2271
Chroococcus	3835	3168	4365	3752
Gomphosphaeria	0	946	1926	997
Lyngbya	5157	1857	4285	0
Oscillatoria	4999	9990	5760	9404
Phacothamnion	0	2374	0	0
Phormidium	3898	3385	6227	5429
Rivularia	12404	9198	11641	10486
Total	33385	32783	35362	32338
Euglenophyceae				
Difflugia	3694	3924	2269	6279
Phacus	777	1548	1026	1583
Euglena	2217	1363	2070	902
Trachelomonas	1747	610	1067	1573
Total	8435	7444	6434	10337
Rhodophyceae				
Batrachosperium	0	439	0	1828
Lemanea	333	659	0	288
Total	333	1098	0	2116
Crustacea				
Cyclops	0	171	175	457
Daphnia	147	136	0	288
Diaphanosoma	202	220	95	410
Diaptomus	0	328	0	0
Monostyla	467	244	0	0
Nauplius	0	108	231	0
Total	815	1207	501	1154
Rotifera				
Asplanchna	777	0	0	758
Brachionus	670	0	0	182
Filinia	0	0	281	288
Lecane	147	0	95	0
Keratella	186	0	251	0
Polyarthra	93	816	484	391
Trichocerca	0	108	218	867
Total	1872	925	1330	2485
Benthos	1858	790	570	1722

Water quality parameters

Mean values (and range) of daily and weekly monitored water quality parameters of different treatments are shown in Table 6. All water quality parameters varied significantly among sampling dates. Treatments had no significant effect on water temperature, which was around 30°C during the study. There were treatment effects on Secchi depths, the highest mean value (36 cm) recorded in treatment CR0. Dissolved oxygen did not vary among the treatments, ranging 2.39-10.36 mg l^{-1} during the study. There was no difference in pH among the treatments, the values ranged 5.6-9.9.

Total alkalinity varied among treatments, lower in treatments CR0 and C45 than other treatments. Orthophosphate was lower in treatment CR than in treatment CR0. Nitrate did not vary among the treatments. Total ammonia was higher in treatment C15 than CR0. Chlorophyll *a* concentration of pond water was lower (102 $\mu g\ l^{-1}$) in the control than in the treatments with bamboo poles (165-254 $\mu g\ l^{-1}$).

Stable isotope composition

The outcomes of stable nitrogen and carbon isotope analysis of different food sources within the system and experimental fish are shown in Figure 3. The stable nitrogen isotope ratio of rohu at harvest in substrate ponds was similar to that of periphyton although its ratio at stocking and at harvesting in control ponds were relatively higher. Stable carbon isotope ratio also varied among rohu at stocking time, harvesting time in substrate ponds and harvesting time in control ponds. The nitrogen stable isotope ratio in catla biomass decreased during the culture period. The same trend is reflected for stable carbon isotope ratios. Nitrogen isotope ratio of kalbaush did not vary during the culture period but carbon isotope decreased at harvest by comparison with stocking.

Table 6

Mean values of water quality parameters in different treatments (CR0 = combination of catla-rohu without bamboo; CR = CR0 with bamboo; C15, C30 and C45 = CR plus 15, 30 and 45% kalbaush, respectively). Values are means of two ponds and 76 sampling dates for daily (N = 152) and two ponds and 13 sampling dates for weekly monitored water quality parameters (N = 26). If main effects are significant, then means followed by the different superscript letter in each row are significantly different at 0.05 level based on Tukey test.

Parameters	**Treatments**				
	CR0	**CR**	**C15**	**C30**	**C45**
Daily					
Temperature	29.9	29.8	29.8	29.8	29.8
(°C)	(27.5-32.0)	(27.1-31.9)	(27.4-32.4)	(27.2-32.4)	(27.5-32.7)
Secchi (cm)	36^{a}	30^{ab}	28^{b}	26^{b}	30^{ab}
	(26-48)	(17-41)	(17-44)	(17-41)	(12-41)
Dissolved	5.1	4.9	5.0	5.3	5.0
oxygen (mg l^{-1})	(2.4-8.9)	(2.7-9.9)	(2.9-9.7)	(3.0-10.4)	(2.7-8.6)
pH	7.5	7.5	7.5	7.6	7.6
	(5.8-9.1)	(5.8-9.6)	(5.6-9.5)	(6.3-9.4)	(6.2-9.9)
Weekly					
Total alkalinity	96^{b}	105^{a}	107^{a}	110^{a}	98^{b}
(mg l^{-1})	(37-122)	(70-151)	(67-130)	(79-132)	(75-131)
Orthophosphate	1.87^{a}	1.62^{b}	1.73^{ab}	1.73^{ab}	1.68^{ab}
(mg l^{-1})	(0.35-2.71)	(0.25-2.71)	(0.37-2.73)	(0.18-2.71)	(0.23-2.68)
Nitrate (mg l^{-1})	2.62	2.69	2.48	2.78	2.78
	(1.2-4.8)	(1.1-4.3)	(0.7-4.2)	(1.1-5.6)	(1.2-4.7)
Total ammonia	0.79	1.35	1.39	1.36	1.09
(mg l^{-1})	(0-2.69)	(0.08-2.71)	(0.12-2.72)	(0.03-2.71)	(0.05-2.63)
Chlorophyll *a*	102	243	254	248	165
(μg l^{-1})	(18-322)	(40-850)	(31-728)	(95-455)	(32-762)

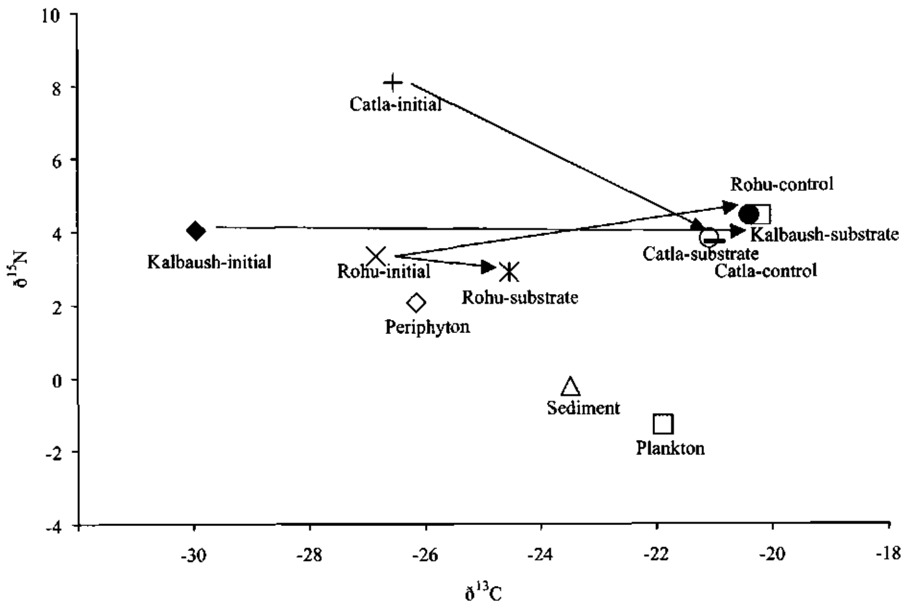

Figure 3. Stable nitrogen and carbon isotope ratios of different food sources and experimental fish species. The arrows indicate the trends of stable isotope ratio from initial stage (at stock) to final stage (at harvest).

Discussion

Effect of bamboo poles as periphyton substrate

Survival, growth rate, individual weight at harvest as well as net yield of both rohu and catla were significantly higher in the ponds where bamboo substrates had been planted. This was most likely due to the increase in periphyton food growing on the bamboo poles and perhaps to the better shelter for fish from predators (snakes, frogs, birds, etc.). The net yield of catla was higher (3.2 times) than that of rohu (2.6 times) in ponds provided with bamboo, although catla had negative selection for periphyton food. In the periphyton-based system, rohu largely depended on periphyton for food, whereas catla filtered the planktonic organisms, possibly allowing more light penetration into the water column for periphyton growth. In ponds without substrate, the species seemed to have competed with each other for the same planktonic food materials, although their feeding niches are different. The competition for phytoplankton between the two species being further demonstrated by the higher Secchi value and lower chlorophyll *a* concentrations in pond water without bamboo poles (Table 6). Lower orthophosphate in treatment CR than in CR0 indicated less water movement possibly because the bamboo poles decreased wind effect on bottom. However, addition of the bottom dwelling kalbaush to ponds with bamboo poles increased orthophosphate, pointing that this fish disrupted the bottom mud.

From a theoretical point of view, the biggest advantage to be gained from the added substrates is an increase in the energy- and nutrient- transfer efficiency of the system due to a shift from phytoplankton- to periphyton-based production. Miller and Falace (2000) suggested two mechanisms for increasing fish production in artificial reefs-based systems : 1) the additional shelter provided by the substrate allows more of the resources to flow into fish biomass, and 2) the new primary production and attached benthic secondary production fostered by the artificial substrate support a new food web, part of which will end up in fish biomass. Filter-feeding on small planktonic algae is unlikely to fully cover the energy demands of most herbivorous carp and tilapia species (Dempster et al., 1995). Besides phytoplankton, these fish generally require larger-sized food sources such as benthic algae, algal detritus or plant fodder, that can be harvested more efficiently (Dempster et al., 1993, Yakupitiyage, 1993). Hard substrate that is required for larger benthic algae is generally absent in traditional fish ponds and benthic algal mats usually do not develop due to light limitation in highly eutrophic ponds with dense phytoplankton blooms. However, a large number of benthic algae (50 genera) was identified in the present study.

In the present study, the combined average net production of the two species in ponds with substrates was 2.8 times higher than that of control ponds. Therefore, the periphyton-based fish production technology offers considerable potential in a resource-constrained country such as Bangladesh. Wahab et al. (1999) explored the possibility of periphyton-based monoculture with kalbaush, *L. calbasu* and reported an approximate 1.8 times higher fish production in ponds provided with scrap bamboo as substrate than in those without. The similar enhancements of production were reported by Ramesh et al. (1999) in a polyculture trial with rohu and common carp (*Cyprinus carpio*) using sugarcane bagasse and Azim et al. (2001a) in a monoculture with rohu and through the addition of bamboo substrates.

Effect of inclusion of kalbaush

Production of rohu and catla benefited from the presence of additional 15% kalbaush above the fixed stocking ratio of 60 rohu/40 catla at 10,000 juveniles ha^{-1}. Total biomass of rohu was 13% higher in treatment C15 compared to treatment CR without kalbaush. Catla biomass was 38% higher in treatment C15 than in the other treatments. The highest production of kalbaush was reached in treatment C15 while enhancing production of rohu and catla as well. The overall growth and production of fish were lower in treatment C30 than treatment C45 but the reasons are unknown. However, with only two replicates, within-treatment variability was high and therefore, three or more replicates are suggested for this type of production trial in earthen ponds in order to draw reliable conclusions.

The total yield of the three species in treatment C15 (2,306 kg ha^{-1}) was 40, 53 and 20% higher as compared to treatments CR, C30 and C45, respectively. This might be due to the synergistic interactions among the three species, resulting in optimal utilization of food resources and improved environmental conditions in the combination of C15. Milstein (1992) explained the synergistic effect as the combined action of an increase of food resources and an improvement of environmental conditions. The lower Secchi value and higher nutrients and chlorophyll *a* concentrations of pond water and better quality of periphyton in treatment C15 indicated a higher food abundance and better environmental conditions than in treatment CR without kalbaush. The reason for reduced production of kalbaush as well as rohu and catla in higher kalbaush densities might be due to intra- and inter-specific competition for food and space. Kalbaush showed severe territorial competition and less food ingestion when four individuals were stocked together rather than singly in a grazing trial on periphyton in aquaria (S.M. Rahmatullah, pers. comm.; BAU, Mymensingh). Feeding and growth may be influenced by density-dependent behaviour such as social interactions, the development of hierarchies and establishment of territorial borders (Jobling, 1985) and /or stress-responses associated with high stocking densities (Vijayan and Leatherland, 1988). According to Milstein (1992), antagonistic interactions may occur when the stocking rates of various fish species are unbalanced. Reported yields in periphyton-based systems include: a maximum tilapia biomass equivalent to 8 tonnes ha^{-1} y^{-1} in acadja-enclos in the Ebrie Lagoon, Ivory Coast, with natural recruitment of juveniles (Hem and Avit, 1994); 1,235 kg ha^{-1} of rohu and common carp in 133 days with sugarcane baggase as substrate (Ramesh et al., 1999); 713 kg ha^{-1} of kalbaush in 120 days with scrap bamboo as substrate (Wahab et al., 1999); 1,900 kg ha^{-1} of rohu in 120 days with bamboo substrate (Azim et al., 2001a); and 586 kg ha^{-1} of rohu and catla in 70 days with bamboo substrates (Azim et al., 2001b). Yields were highly variable with substrate types, species cultured, environmental factors etc. The present experiment showed a higher net yield than those in the single or two species polyculture systems listed above. A combination of rohu:catla:kalbaush at 60: 40:15 (i.e. 12:8:3) at the total stocking density of 11,500 juveniles ha^{-1} may be used, wherein synergistic benefits compensate for any inter-specific or intra-specific dietary competition. However, this ratio may be influenced by changes in stocking density of fish, nutrient status and quality of pond water, supply of inputs and overall management.

Periphyton as natural fish food

The higher standing biomass in terms of periphyton DM, AFDM and chlorophyll *a* contents was sustained over the entire culture cycle. The autotrophic index (AI) ranged between 130 and 225 in the present experiment. According to APHA (1992), AI values between 100-200 are considered as algae dominating periphytic matter. Azim et al. (2001c) reported AI values ranging from 190-350 in ungrazed conditions depending on substrate types. Algae typically grow on the outer surfaces of substrate where there is sufficient sunlight and are continuously harvested by fish grazing the outer surface of the substrate. Assuming 1 mg chlorophyll *a* per 65-85 mg AFDM in algae (Dempster et al., 1993; Reynolds, 1984), the relative contributions of algae to the periphytic biomass were 43-56% in CR, 50-65% in C15, 29-38% in C30 and 49-64% in C45. Thus, around 50% of the periphyton biomass is not algal. However, the ratio of algal chlorophyll *a* to biomass varies among algal species (Dempster et al., 1993; Reynolds, 1984). A large number of the diatoms and euglenoid algae identified in this experiment are not chlorophyll-rich. In addition, algal periphyton might be converted into dead organic matter because of self-shading after a certain period of time. A considerable population of zooplankton and macro-invertebrates also contributed to the AFDM in this experiment.

The ash content of periphyton DM was 15-19% in all treatments with three species combinations. In addition, the protein (23-26%) and energy (19-20 kJ g^{-1}) contents from the periphyton estimated in the present experiment can be considered as broadly appropriate to fish dietary needs and compare well with some other plant-based materials used in aquaculture (Hepher, 1988; Yakupitiyage, 1993; Dempster et al., 1995). Periphyton was well exploited by fish species combination and considerably higher production was achieved in this experiment than reported elsewhere.

The standing biomass of periphyton during the experimental period was 2.8-4.87 mg AFDM cm^{-2} depending on treatment. On average, periphyton contributed a standing biomass of 17.77 g C m^{-2} (assuming 47% C content in periphyton AFDM; Reynolds, 1984; Dempster et al., 1993; Azim et al., 2001c). Again, on average, standing chlorophyll *a* content of pond water (phytoplankton) was 203 $\mu g\ l^{-1}$, equivalent to 6.20-8.11 g C m^{-2} (assuming 47% C content in phytoplankton DM, 1 mg Chl-*a* per 65-85 mg DM; Reynolds, 1984; Dempster et al., 1993). Thus, the available food biomass is approximately doubled as a result of the periphyton-bearing substrate.

Food preferences of experimental fish

Efforts have been made to investigate the food preferences of different species in this experiment by analyzing stable nitrogen and carbon isotope ratios (Gu et al., 1996; Harvey and Kitchell, 2000). There were clear differences in both nitrogen and carbon isotope ratios among different natural food sources within the ponds. All three species of fish showed higher stable isotope ratios at stocking which might be due to the fact that juveniles were fed artificial feed from animal origin in hatcheries. The similar stable nitrogen and carbon isotope ratios of rohu in the substrate ponds and periphyton indicated that rohu mostly relied on periphyton for food in the system. The stable carbon isotope ratios in catla biomass were similar in both substrate and control ponds and decreased towards the ratios of plankton biomass from its initial ratio indicating the preference of catla for plankton rather than periphyton as a source of food. Nitrogen isotope ratio of kalbaush did not vary during the culture period but carbon isotope was lower and more similar to plankton at harvest.

Rohu is known to be a predominantly column-feeding fish, consuming phytoplankton and decaying suspended organic matter in traditional fishponds (Das and Moitra, 1955; Dewan et al., 1991), whereas, it feeds on periphyton in ponds provided with substrates (NFEP, 1997; Ramesh et al., 1999; Azim et al., 2001a). Ahmed (1993) reported catla as a filter-feeding species, depending primarily on zooplankton during early life stages and subsequently switching to a combination of phytoplankton and zooplankton. However, visual observation confirmed the periphyton feeding by rohu, whereas, catla were never seen utilizing this food source in the present study. Kumar and Siddiqui (1989) regarded kalbaush as a bottom dwelling fish and reported that the food of the fish consisted of decayed organic matter, molluscs, diatoms, plant matter, green algae, blue-green algae and zooplankton. Juveniles showed a positive selection for zooplankton and the adults showed a negative selection for the zooplankton and a positive selection for decayed organic matter and molluscs. Wahab et al. (1999) found 1.8 times higher fish production in ponds provided with scrap bamboo as substrates than control ponds and reported kalbaush to be a periphyton feeder, contradicting the stable isotope result of the present study. However, each fish species has its own food selectivity within the plankton or periphyton that has not been considered in the isotope analysis. Gut content analysis of fish in combination with stable isotope analysis of the fish and specific food materials may give more definitive results.

Conclusions

Periphyton grown on bamboo poles is an excellent natural food for certain fish species and supports enhanced fish production. Fish production in the periphyton-based system using a rohu:catla ratio of 60:40 and a total stocking density of 10,000 juveniles ha^{-1} was 2.8 times higher than that of the control without bamboo poles. Addition of 15% kalbaush proved an appropriate combination (rohu:catla:kalbaush at 60:40:15) that further enhanced yield by 40% in a periphyton-based polyculture system. Future research should compare the production and profitability of traditional systems against periphyton-based aquaculture systems.

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PART IV

Comparison of fish production, nutrient efficiency and economics between traditional and periphyton-based production systems

Chapter 8

A comparison of fertilization, feeding and three periphyton substrates for increasing fish production in freshwater pond aquaculture in Bangladesh

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Abstract

A polyculture trial was conducted in earthen ponds in Bangladesh to compare traditional aquaculture techniques (fertilization only or fertilization plus feeding) with a combination of the same techniques and periphyton substrates. Three substrates (bamboo, jute stick and bamboo side shoot) were tested. Rohu (*Labeo rohita*), catla (*Catla catla*) and kalbaush (*Labeo calbasu*) were stocked at a 60:40:15 stocking ratio and at a total stocking density of 11,500 in fifteen earthen ponds with five treatments: standard fertilization as input (Control), control plus supplemental feeding with rice bran and oil cake (Feed), control plus bamboo substrate (Bamboo), control plus jute stick substrate (Jutestick) and control plus bamboo side shoot substrate (Kanchi). Water quality, plankton and periphyton were monitored throughout the experiment.

Significantly higher ammonia concentrations were recorded in Control and Feed treatments. The chlorophyll *a* concentration of pond water was significantly higher in the Feed treatment. Dry matter content and mean abundance of the periphyton communities were higher in the first and the last month of the experiment without significant difference among the three substrate types. Periphyton chlorophyll *a* concentrations per unit surface area did not vary significantly among different substrate types but increased throughout the experimental period and decreased with increasing water depth.

Specific growth rates of rohu and catla were higher in Substrate and Feed treatments than in the Control treatment. Combined net yields of fish in Control, Feed, Bamboo, Jutestick and Kanchi treatments were 1,226, 1,960, 2,098, 2,048 and 2,032 kg ha^{-1} 135 d^{-1}, respectively. Production in substrate systems was significantly higher (ANOVA, $P < 0.001$) than in the Feed and Control treatments. Nitrogen retention in fish was 1.6 times higher in substrate systems and 1.3 times higher in the Feed than in the Control treatment. Net profit margin was highest in Jutestick treatment (46%). The substrate-based systems performed better than the conventional systems, both from ecological and economical points of view.

Introduction

Asian aquaculture accounts for more than 90% of the total world aquaculture production, the major portion coming from ponds and rice fields (FAO, 2000). Pond production systems in Asia are becoming increasingly reliant on external resources to supplement or stimulate autochthonous fish food production, thus excluding poorer sectors of the community from participation (O'Riordan, 1992; Beveridge and Phillips, 1993; NACA, 1995). Bangladesh has emerged as one of the leading nations in freshwater aquaculture production during recent years. Inland fisheries contribute 1.19 million metric tonnes (80% of total production) of which 48% comes from aquaculture. This sub-sector contributes 5% to GDP and engages about 1.2 million people full-time and 12 million people part-time. Since last ten years, aquaculture production is growing by approximately 14% per annum, comparable to China and India (FRSS, 2001).

Fish represents 63% of total animal protein intake in Bangladesh. Despite the growth of the aquaculture sector, fish production still needs to double to satisfy the minimum protein requirement for human consumption. However, inland fisheries production is declining due to construction activities, over-fishing, irrigation and reclamation of land for human settlement. These developments also cause siltation, soil erosion, pollution from industrial and municipal wastes, and destruction of mangrove forests. There is, therefore, a strong need to develop aquaculture techniques that enhance fish production in closed water bodies.

More than 75% of households in Bangladesh spend 90% of their income on basic needs (BBS, 1995). Consequently, most households cannot afford to provide even rudimentary supplemental feeds for their fish ponds (O'Riordan, 1992). Alternative means of increasing fish production are essential if aquaculture production by resource-poor farmers is to grow further. Periphyton-based aquaculture may be one such option. The term 'periphyton' is applied to the complex of sessile biota attached to submerged substrata such as stones and sticks, and includes not only algae and invertebrates but also associated detritus and micro-organisms. The feasibility of periphyton-based systems has been explored in brackishwater fish ponds in West Africa (Welcome, 1972; Hem and Avit, 1994; Konan-Brou and Guiral, 1994) and was found to enhance primary production and food availability and increase fish production.

In this paper, a system for periphyton-based aquaculture developed in Bangladesh is presented. A programme of trials focusing on the selection of suitable species for periphyton-based aquaculture (Wahab et al., 1999; Azim et al., 2001a), the selection of locally available substrates (Azim et al., 2001b), the optimization of fertilizer dose for maximum periphyton growth (Azim et al., 2001c) and the determination of optimum stocking ratios of species in periphyton-based polyculture systems (Azim et al., 2001d,e) has been conducted. A three-species polyculture with rohu (*Labeo rohita*), catla (*Catla catla*) and kalbaush (*Labeo* *calbasu*) in combination with bamboo poles has been found to give highest yields. Traditionally, farmers only fertilize their ponds or apply a combination of fertilizers and feed. The main objective of the present study was to compare the newly derived periphyton technology with the traditional management techniques used in Bangladesh. Because bamboo is a relatively expensive material that has many uses, two alternative substrate materials were also tested.

Using the previously optimized three species polyculture system, the specific objectives of the experiment were: 1) to assess the effect of three locally available substrates on water quality, food quantity and quality and on fish production in comparison with traditional non-fed, and supplemental feed-driven ponds; 2) to compare the efficiency of nitrogen conversion into fish biomass; and 3) to compare the economics of the different production systems.

Materials and methods

Pond facilities and design

The experiment was carried out in 15 earthen fishponds (10 m × 7.5 m, mean water depth 1.2 m) at the Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh for a period of 135 days between April and September, 2000. The ponds were rain-fed and fully exposed to prevailing sunlight. Pond embankments were covered with grass.

Three indigenous major carps, rohu (*Labeo rohita*), catla (*Catla catla*) and kalbaush (*Labeo calbasu*) were stocked at the ratio of 60:40:15, respectively with total stocking density of 11,500 juveniles ha^{-1} (Azim et al. 2001d,e), in all treatments, each with three replicate ponds. Treatments were: standard fertilization only as input (treatment Control), control plus commonly practiced supplemental feeding with rice bran and mustard oil cake (treatment Feed), control plus bamboo, *Bambusa* sp., poles as substrate (treatment Bamboo), control plus jute, *Corchorus* sp., stick as substrate (treatment Jutestick) and control plus bamboo side shoot, locally called kanchi, as substrate (treatment Kanchi). Of the several types of locally available types of bamboo, *Bambusa* sp. was chosen, as it is less useful for house building but yielded good results in previous experiments. Jute stick is an agricultural by-product that remains after removing the fiber as the main product. This substrate has not been tested in ponds but gave promising results in tank experiments (Dr. S.M. Rahmatullah, BAU, pers. comm.). Kanchi was selected because of its wide availability and reasonable results in previous experiments. Moreover, farmers can collect this substrate from the homestead garden, virtually at no cost.

A fortnightly fertilization regime with cow manure, urea and triple super phosphate (TSP) at the rates of 4,500, 150 and 150 kg ha^{-1} was applied to all ponds. The fourth fertilizer application was cancelled because of a phytoplankton bloom and subsequently the fertilization rates were reduced to 1,000, 100 and 100 kg ha^{-1} of cow manure, urea and TSP, respectively. Rice bran and mustard oil cake (2:1) (derived as by-products of agriculture) were applied to the Feed treatment at the rate of 3% of the total fish biomass stocked and adjusted fortnightly by weighing at least 20% of all fish. The oil cake was soaked with water overnight and mixed with rice bran to make a dough that was broadcasted evenly over the pond surfaces.

Pond preparation and fish stocking

Maintaining a substrate-free perimeter, an effective area of 8×5 m^2 was planted with approximately 2 m length substrates on 13 April, 2000. Bamboo poles (mean diameter 5.6 cm) were driven vertically into the pond bottom, the upper portion extending above the water surface, at a density of 9 poles per m^2, yielding a total submerged substrate area of 76 m^2 per pond, approximating that of the pond water surface area (75 m^2). Similar substrate areas were obtained for the other two substrates by planting 45 jute sticks (mean diameter 1.12 cm) and 36 kanchi poles (mean diameter 1.4 cm) per m^2. Quicklime (CaO) was applied to the pond at the rate of 250 kg ha^{-1} immediately after substrate installation. Fertilization started 10 days later, on 23 April.

On 1 May, *Labeo rohita* (average individual weight 26-29 g), *Catla catla* (28-34 g) and *Labeo calbasu* (24-28 g) juveniles at the ratio of 60:40:15, respectively, and a total stocking density of 11,500 fish ha^{-1} were released into the ponds. Juveniles were collected from nearby hatcheries and stocking was carried out in the afternoon, care being taken to gradually acclimatize the fish to pond conditions.

Water quality monitoring

Weekly starting on 1 May, temperature, dissolved oxygen (YSI digital DO meter, model 58), Secchi depth (Secchi disc) and pH (a portable Hanna pH meter) were monitored between 0900 and 1000 h. Total alkalinity was determined by titrimetric method (Stirling, 1985). Nutrients (TAN, NO_3-N and PO_4-P) analyses were performed by Hach kit (model DR 2000).

Plankton and periphyton sampling and analysis

Water samples for phytoplankton chlorophyll *a* analyses were collected weekly. A known amount of water was filtered through Whatman GF/C filters. The filtered water samples were used for nutrient analyses and filter papers were used to determine chlorophyll *a* concentrations following Boyd (1979). Monthly taxonomic study of plankton commenced on 1 June. Plankton samples were collected and enumerated as described in Azim et al. (2001a).

Periphyton collection started on 22 May and was repeated monthly. From each pond, three poles were selected using a random number table. Two samples of periphyton were taken at three depths, 0, 25 and 50 cm along the length of the pole, starting at the water surface. Samples from three poles, three depths and three ponds were pooled for dry matter and ash contents analysis. Pooled samples of three poles were used for chlorophyll *a* and taxonomic study. Samples were scraped and analyzed as described in Azim et al. (2001e).

On the last day of the experiment, plankton, periphyton and sediment samples were collected for analysis of nitrogen content (Kjeldahl method), fat content (Soxhlet apparatus) and energy content (bomb calorimeter) in the Fish Culture and Fisheries Group, Wageningen University. Plankton and periphyton samples could only be collected on the last day because the amounts needed for proximate analysis would have depleted the standing stock of these natural feeds when sampled more often. Protein content of dry matter was calculated using the nitrogen to protein conversion factor of 5.8 for bacteria, algae and aquatic invertebrates suggested by Gnaiger and Bitterlich (1984). At harvest, two fish per species were taken randomly from each pond, and dried and analyzed for nitrogen content. Cow manure, urea, rice bran and oil cake were also analyzed for nitrogen content.

Fish harvesting

On 15 September, all substrates were removed, the ponds drained, and all fish were collected. By species, total bulk weight and number collected from each pond were recorded. Specific growth rate (% bw d^{-1}) was estimated as:

SGR = [ln (final weight) – ln (intial weight)] /culture period (days) x 100.

Net fish yield was calculated as:

NFY = [total weight of fish at harvest - total weight of fish at stocking].

Statistical analysis

A one-way ANOVA was used for fish yield parameters. Water quality parameters, plankton biomass and periphyton dry matter contents were compared by split-plot ANOVA with treatments as the main factor and time as the sub-factor (Gomez and Gomez, 1984). Periphyton chlorophyll *a* and taxonomic composition were compared using a split-split plot ANOVA with treatments as the main factor, depth as the first sub-factor and time as the second sub-factor. The assumptions of normal distributions and homogeneity of the variances were checked before analyses. In case of significant deviations from normality or heterogeneous variances, log transformed data were used for ANOVA. Survival of fish was analyzed using arcsine-transformed data. The pH values were transformed to hydrogen ion concentrations before statistical analysis. If a main effect was significant, the ANOVA was followed by a Tukey-HSD test. All statistical tests were done at a 5% probability level. All calculations were done using SAS 6.12 (SAS Institute Inc., Cary, NC 27513, USA).

Results

Water quality

Water quality results are summarized in Table 1. All parameters were within an acceptable range for carp culture. There were no significant differences in any water quality parameter among different treatment ponds, except for total ammonia concentrations which were significantly higher in Control and Feed than in Substrate treatments.

Table 1
Mean water quality parameter values recorded from different treatment ponds. Values are means of 20 sampling dates and three ponds ($N = 60$) per treatment. Parameter ranges during the entire culture period are given in parentheses. If main effects are significant in a split-plot ANOVA at 0.05 level, then means in the same row are followed by different superscript letters.

Main Effects	Control	Feed	Bamboo	Jute stick	Kanchi
Temperature	29.60	29.70	29.54	29.67	29.55
(°C)	(26.4-31.1)	(26.4-31.7)	(26.5-31.2)	(26.5-31.3)	(26.4-31.5)
Secchi (cm)	26.32	25.15	25.50	33.26	31.36
	(18-45)	(16-38)	(15-45)	(22-60.5)	(16.5-78)
Dissolved	6.38	5.67	5.74	5.60	5.75
oxygen (mg l^{-1})	(4.2-9.7)	(3.9-8.3)	(4.1-8.0)	(3.9-7.6)	(3.6-9.4)
pH	7.45	7.39	7.38	7.38	7.33
	(7-8.1)	(7-7.9)	(7-7.8)	(7-8.2)	(7-7.7)
Total alkalinity	95.76	90.37	94.39	88.69	98.65
(mg l^{-1})	(60-145)	(45-153)	(46-161)	(46-157)	(43-161)
Orthophosphate	3.98	4.59	4.42	4.21	4.91
(mg l^{-1})	(2.3-9.5)	(1.98-7.5)	(1.76-9.12)	(1.98-8.50)	(2.0-8.2)
Nitrate (mg l^{-1})	1.22	1.38	1.38	1.34	1.35
	(0.6-2.3)	(0.8-2.6)	(0.8-2.6)	(0.6-2.5)	(0.6-2.1)
Total ammonia	0.95^{ab}	1.13^{a}	0.56^{c}	0.82^{bc}	0.67^{bc}
(mg l^{-1})	(0.02-2.92)	(0.10-3.92)	(0.02-2.61)	(0.10-3.65)	(0.01-2.75)

Plankton and periphyton biomass and composition

Mean chlorophyll *a* concentrations of the water samples were 355, 410, 376, 215 and 371 μg l^{-1} in Control, Feed, Bamboo, Jutestick and Kanchi treatments, respectively, and varied significantly among sampling dates (Figure 1). It was also significantly higher in Feed than in Jutestick ponds. There were no significant differences among other treatments.

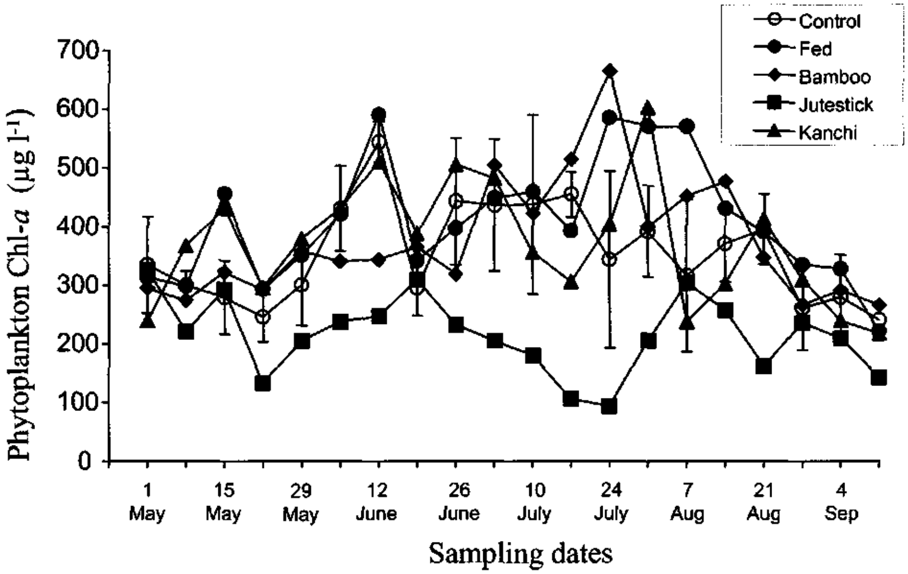

Figure 1. Phytoplankton chlorophyll *a* concentrations of different treatments throughout the experimental period. Values are means (±S.E.) of three ponds ($N = 3$) per sampling date in each treatment.

There was a significant effect of sampling date on periphyton DM with higher mean values on the first and last sampling dates than on the second (Figure 2A). Mean periphyton dry matter values were highest in Kanchi (3.02 mg cm^{-2}), followed by Bamboo (2.62 mg cm^{-2}) and Jutestick treatment (2.54 mg cm^{-2}), but the differences were not significant. Periphyton chlorophyll *a* concentrations per unit surface area did not vary significantly among the three substrates, but did vary with sampling date and depth. Highest chlorophyll *a* concentration was recorded on the last sampling date followed by the second, third and first sampling dates, respectively (Figure 2B). Mean chlorophyll *a* concentrations on Bamboo, Jutestick and Kanchi substrates were 9.87, 10.49 and 11.55 μg cm^{-2}, respectively and decreased with increasing water depth (Figure 3).

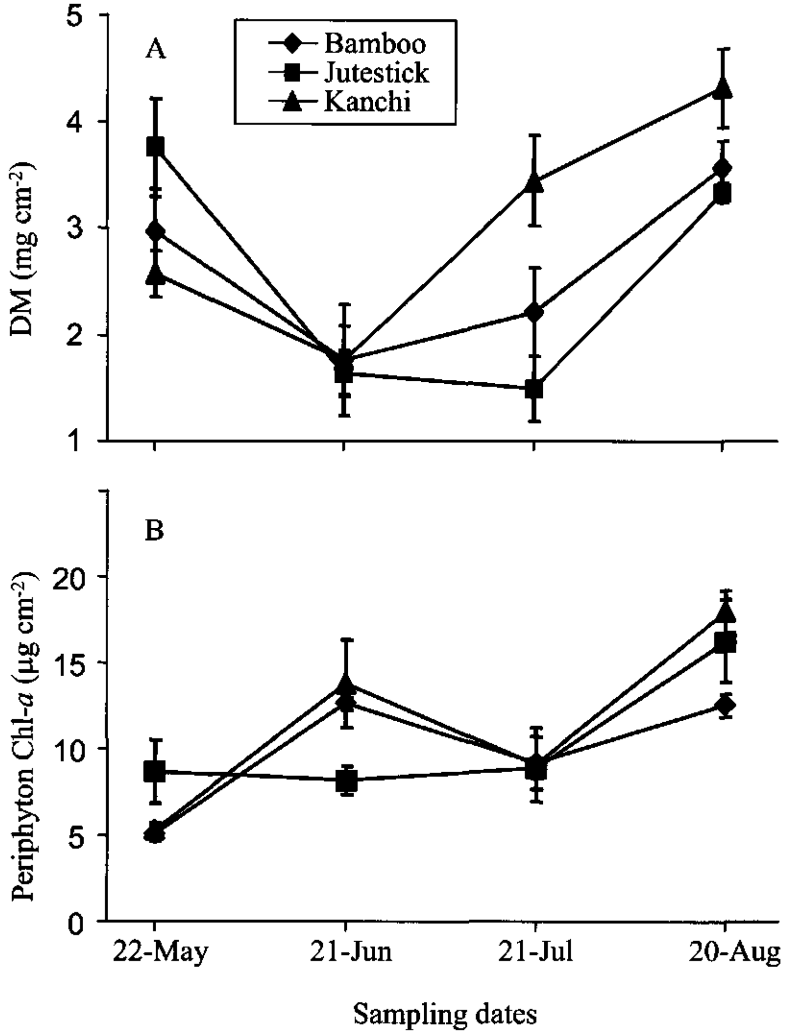

Figure 2. Quantity of periphyton dry matter (A) and chlorophyll *a* (B) per unit surface area of different substrates over the experimental period. Values are means (±S.E.) of three depths and three ponds (N = 9) per sampling date in each treatment.

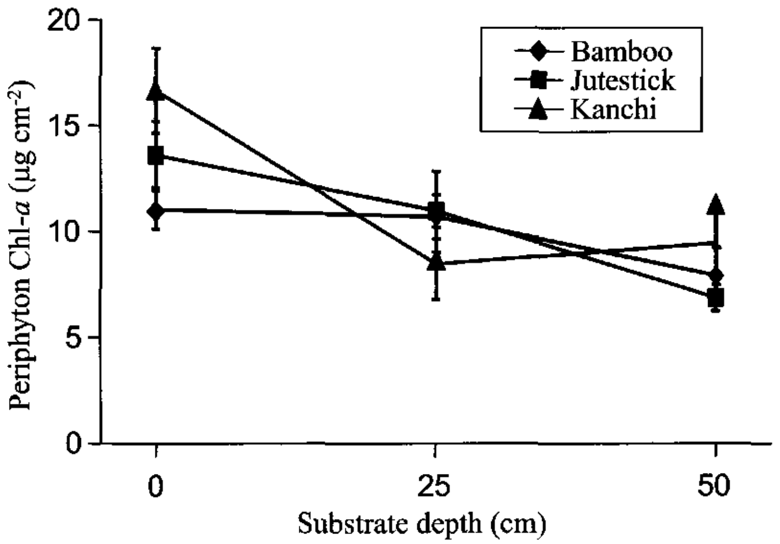

Figure 3. Quantity of periphyton chlorophyll *a* per surface area of different substrates along the substrate depths. Values are means (±S.E.) of three ponds and four sampling dates (N = 12) per depth in each treatment.

Both phytoplankton and periphyton communities were composed of four groups of algae: Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae, and two groups of zooplankton : Crustacea and Rotifera. For the phytoplankton, the Chlorophyceae was the dominant group in terms of number of cells or colony-forming units, followed by Cyanophyceae, Euglenophyceae and Bacillariophyceae in all treatments (Figure 4A). There was no significant difference in abundance in any group of phytoplankton or zooplankton among the treatments. Abundance increased significantly over the experimental period.

For the periphyton, the mean abundance of different groups varied neither among substrate types nor among different depths, but it was different among sampling dates. A slightly higher number of Bacillariophyceae and Chlorophyceae was apparent in the Jutestick treatment but it was not statistically significant (Figure 4B). Periphyton communities were higher in numbers on the first and last sampling dates than on the second and third.

Bamboo and Jutestick, while Kanchi and Control treatments had significantly lower productions.

Table 2

Proximate composition of natural and supplemental feeds calculated on moisture-free dry weight basis.

Food sources/ Treatments	Nitrogen (%)	Protein (%)	Lipid (%)	Ash (%)	Energy (kJ g^{-1})
Plankton					
Control	8.37	48.57	2.42	8.37	22.61
Feed	6.96	40.36	3.53	10.36	21.08
Bamboo	4.60	26.68	4.43	24.45	17.74
Jutestick	8.63	50.08	4.39	9.74	23.07
Kanchi	6.75	39.15	4.66	19.15	20.22
Periphyton					
Bamboo	5.58	32.34	3.47	19.32	18.52
Jutestick	2.19	12.69	2.75	31.12	13.62
Kanchi	5.10	29.56	2.93	13.57	19.40
Sediment					
Control	0.31	1.79	0.03	93.06	-
Feed	0.29	1.70	0.04	93.11	-
Bamboo	0.28	1.63	0.04	93.23	-
Jutestick	0.25	1.46	0.04	93.48	-
Kanchi	0.20	1.14	0.03	95.04	-
Rice bran					
Feed	2.70	16.90	1.01	18.87	16.21
Oil cake					
Feed	6.06	35.17	11.04	9.46	21.25

Table 3

Comparison of means of yield parameters of three species in different treatments. Mean values followed by different superscript letter in each row indicate where main effects are significant ($P > 0.05$) based on Tukey test.

Species/Yield parameters	**Control**	**Feed**	**Bamboo**	**Jutestick**	**Kanchi**	**ANOVA result**
Rohu						
Survival (%)	71	78	82	80	78	NS
Individual stocking weight (g)	28	26	28	27	29	NS
Individual harvesting weight (g)	181^b	255^a	260^a	247^{ab}	260^a	*
SGR (% bw d^{-1})	1.38^b	1.69^a	1.65^a	1.64^a	1.62^a	**
Gross yield (kg ha^{-1} 135 d^{-1})	772^c	1192^b	1279^a	1185^b	1218^{ab}	***
Net yield (kg ha^{-1} 135 d^{-1})	604^c	1036^b	1111^a	1023^b	1044^{ab}	***
Catla						
Survival (%)	80	80	83	80	87	NS
Individual stocking weight (g)	31	35	34	28	29	NS
Individual harvesting weight (g)	180^b	255^a	265^a	280^a	259^a	**
SGR (% bw d^{-1})	1.32^c	1.47^{bc}	1.52^{ab}	1.71^a	1.62^{ab}	***
Gross yield (kg ha^{-1} 135 d^{-1})	575^c	816^b	879^a	897^a	901^a	***
Net yield (kg ha^{-1} 135 d^{-1})	451^c	676^b	743^a	785^a	785^a	***
Kalbaush						
Survival (%)	82	91	91	91	82	NS
Individual stocking weight (g)	24	26	24	28	27	NS
Individual harvesting weight (g)	168	210	205	207	198	NS
SGR (% bw d^{-1})	1.44	1.55	1.59	1.48	1.48	NS
Gross yield (kg ha^{-1} 135 d^{-1})	207^c	287^a	280^a	282^a	243^b	***
Net yield (kg ha^{-1} 135 d^{-1})	171^c	248^a	244^a	240^a	203^b	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

The combined net yields (kg ha^{-1} 135 d^{-1}) and the relative contributions of the three species are shown in Figure 5. The highest total net yield of 2,098 kg ha^{-1} was found in the Bamboo treatment, followed in diminishing order by 2,048 kg ha^{-1} (Jutestick), 2,032 kg ha^{-1} (Kanchi), 1,960 kg ha^{-1} (Feed) and 1,226 kg ha^{-1} (Control). The Control treatment yielded a significantly lower production than either the Feed or Substrate treatments (Tukey test). The total net yields from Feed, Bamboo, Jutestick and Kanchi treatments were 60, 71, 67, and 66% higher, respectively, than that from the Control treatment.

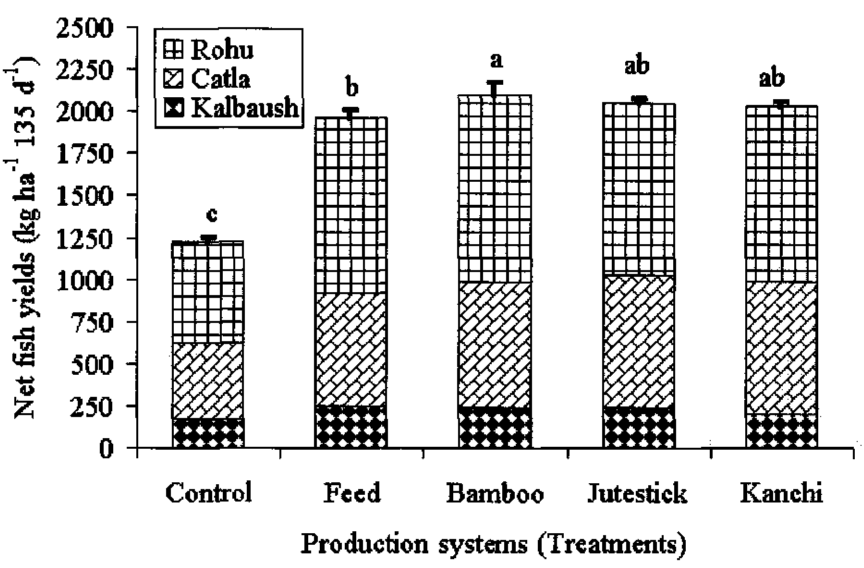

Figure 5. Net yield of fish and relative contributions of different species in five treatments. Standard deviations are derived from pond-wise combined net yields ($N = 3$).

Nitrogen conversion efficiency

A comparison of nitrogen input-output relationship among different culture systems is shown in Table 4. Fertilization with inorganic and organic manure was considered as the source of nitrogen in all systems. In addition, feed applied as rice bran and mustard oil cake was also considered to contribute to the nitrogen input in Feed treatment. Nitrogen in fish biomass was considered as output nutrient.

The nitrogen retentions in fish in the Substrate treatments (8.2-8.7%) were 54-64% higher than in the Control treatment (5.3%). The conversion rate was 6% in the Feed treatment. However, the contribution of supplemental feed to nitrogen retention in fish could be estimated by deducting the contribution of fertilizer input (Control treatment) from Feed treatment. Total nitrogen output in Feed treatment was 368 g of which fertilizers contributed 241 g N. Hence, supplemental feed (1490 g) contributed 127 g nitrogen resulting in a conversion efficiency of 8.5%.

Table 4

Comparison of nitrogen retention efficiencies in different treatments. Calculations are based on 75 m^2 ponds where 146.25 kg cowdung (0.8% N) and 7.875 kg urea (42.69% N) were applied to all treatment ponds during the 135-days culture period. In addition, 26 kg rice bran (2.7% N) and 13 kg mustard oil cake (6.06% N) were used in Feed treatment.

	Control	**Feed**	**Bamboo**	**Jutestick**	**Kanchi**
Input N (in g)					
Cowdung	1170	1170	1170	1170	1170
Urea	3360	3360	3360	3360	3360
Rice bran		702			
Oil cake		788			
Total	4530	6110	4530	4530	4530
Output N (g)					
Rohu	127	188	203	186	192
Catla	81	132	144	147	148
Kalbaush	33	48	47	39	32
Total	241	368	394	372	372
% Retention in fish	5.32	6.11	8.70	8.21	8.21

Cost-benefit analysis

Key financial characteristics of the treatments are presented in Table 5. In all treatments, the main items for expenditure were land rental value, juveniles, fertilizer (especially TSP) and fish stocking and harvesting costs. The supplemental feed and substrates were also expensive inputs. Considering the nine months per year culture period in Bangladesh, it was assumed that bamboo can be used for four years, kanchi for two years and jutestick for one year.

In Bangladesh, the market prices of indigenous fish species are higher than those of exotic species such as tilapia, Chinese carps, African catfish, and hybrid catfish. Experimental fish were sold at 80 Bangladesh taka (BDT) kg^{-1} for fish from Feed and Substrate treatments and 65 BDT kg^{-1} from Control treatment, price being determined by fish size. Net profit margin was higher in Jutestick (46%) followed by Control (35%), Kanchi (27%), Bamboo (14%) and Feed (9%) treatments.

Discussion

Feeding and provision of substrates resulted in 59% and 66-71% greater fish production, respectively, than in the control system. The highest net yield over the 135-day trial was more than 2,000 kg ha^{-1} of fish in substrate-based systems compared to 1,226 kg ha^{-1} in the substrate-free control. These results are in the same range as other fish yields reported with periphyton: 80% increased production of kalbaush (713 kg ha^{-1} 120 d^{-1}) with kanchi substrate (Wahab et al., 1999); 65% higher production of rohu and common carp (*Cyprinus carpio*) (1,235 kg ha^{-1} 133 d^{-1}) in a polyculture system with sugarcane baggase as substrate in India (Ramesh et al. 1999); 77% higher production of rohu (1,900 kg ha^{-1} 120 d^{-1}) in a monoculture system with bamboo substrate (Azim et al., 2001a); and 180% increased production in a polyculture system with rohu and catla (1,652 kg ha^{-1} 90 d^{-1}) compared to substrate-free control (Azim et al., 2001e). Despite some variability due to differences in fish age, size and species and differences in substrate types and density, there is no doubt that fish production was consistently higher in the periphyton systems than in the substrate-free controls.

While the results of the current experiment clearly confirmed previous results on the effects of periphyton, the mechanisms of enhanced fish production in the substrate-based system are still not fully elucidated. It seems likely that the increased fish production partly results from the additional food that the periphyton provides (Miller and Falace, 2000). There

Table 5

Comparison of economics among Control, Feed and Substrate-based aquaculture systems based on 75 m^2 pond and 135 days culture period. Currencies are given in Bangladesh Taka, BDT (1 US$ = 50 BDT).

Item	Amount and rate	Control	Feed	Bamboo	Jutestick	Kanchi
Financial Input						
Land rental cost	75 m^2, @ 15,000 ha^{-1} y^{-1}	56	56	56	56	56
Carp juveniles	86 @ 2	172	172	172	172	172
Lime	2 kg @ 6	12	12	12	12	12
Cowdung	146.25 kg @ 0.35	51	51	51	51	51
Urea	7.875 kg @ 6	47	47	47	47	47
TSP	7.875 kg @ 15	118	118	118	118	118
Rice bran	26 kg @ 6	-	156	-	-	-
Mustard oil cake	13 kg @ 9	-	117	-	-	-
Bamboo	120 culms @ 35, for 135 x 8 days	-	-	525	-	-
Jutestick	1800 @ 0.25, for 135 x 2 days		-	-	225	-
Kanchi	1440 @ 1, for 135 x 4 days	-	-	-	-	360
Transport costs		-	15	15	15	15
Labor						
Pond cleaning, fish stocking and harvesting	1 man-day @ 75	75	75	75	75	75
Substrate installation and removal	2 man-days @ 75	-	-	150	150	150
Feed application	5 man-days for 135 days @ 75	-	375	-	-	-
Total		531	1194	1221	921	1056
Interests on inputs	Annually 15%	29	66	68	51	59
Total financial inputs		560	1260	1289	972	1115
Financial returns						
Sale proceeds	Fish	758	1377	1463	1418	1417
Net margin		198	117	174	446	302
Net profit margin (%)		35	9	14	46	27

were no differences in abundance of plankton among treatments, suggesting that periphyton did not have a negative impact on phytoplankton production. Nevertheless, there were higher chlorophyll *a* concentrations in the Feed treatment water samples, probably caused by the supplemental feed which fertilized the ponds and enhanced phytoplankton production. However, the nitrogen content of plankton in the Bamboo and Kanchi treatments was lower than in the Control, indicating a negative effect of substrates on plankton nutritional quality. The jutestick substrate showed a different effect, with a much lower nitrogen and higher ash content in the periphyton and a much higher nitrogen content in the plankton. Although this may be a real effect of the different structure of the soft jutestick, it could also be an artifact resulting from the inadvertent removal soft surface material from the jutestick substrate when taking periphyton samples. Moreover, the samples for proximate analysis of the natural food sources were collected only on the last day of the experiment, which might not be representative of the whole culture cycle. In general, the proximate composition of periphyton was comparable to that of the plankton, the latter being the common food source in traditional ponds. Therefore, periphyton can be considered an appropriate natural food (Hepher, 1988; Yakupitiyage, 1993; Dempster et al., 1995).

Another reason for the increased fish production in systems with substrates could be the positive effect of periphyton on water quality. Lower total ammonia concentrations in the Substrate treatments were recorded compared to Feed and Control treatments. This might be due to higher nitrification rates in Substrate treatments. Langis et al. (1988) and Ramesh et al. (1999) reported that the bacterial biofilms (periphyton) on the substrates reduced ammonia levels through promotion of nitrification. Algal periphyton assimilates ammonia and traps organic matter from the water column and thus, as algal density increases, ammonia concentration declines (Hargreaves, 1998). In traditional ponds, the principal surface area for nitrification is the sediment, where oxygen availability is the limiting factor. By placing substrates in the water column where oxygen is more available, nitrification is enhanced. Although the ammonia concentrations in this experiment never exceeded the acceptable range for aquaculture, periphyton may be advantageous at higher fish stocking densities.

The nitrogen retention in fish in the Substrate treatments (all higher than 8%) were 54-64% higher than in the Control treatment (5.3%). Edwards (1993) reported nitrogen conversion efficiencies of 5% in ponds fertilized with 300 kg DM ha^{-1} d^{-1} buffalo manure; 6% in ponds fertilized with 100 kg DM ha^{-1} d^{-1} buffalo manure plus 4.4 kg N ha^{-1} d^{-1} urea and 0.4 kg P ha^{-1} d^{-1} TSP; and 15% in ponds fertilized with 8.5 kg DM ha^{-1} d^{-1} chicken manure plus 4.0 kg N ha^{-1} d^{-1} urea and 1.0 kg P ha^{-1} d^{-1}. Schroeder et al. (1990) found 15.9 and 10.7% of total nitrogen inputs recovered in fish biomass in chemically, and chemically plus organically manured fishponds, respectively. In more intensive feed-driven systems, 15-30% of the nitrogen added as feed is recovered by the fish (Acosta-Nassar et al., 1994; Gross et al., 2000). Our results are comparable to other pond systems and although periphyton considerably increases nitrogen retention efficiency, the efficiency remains low. This is caused by the extra step involved in the conversion of nutrients to fish through natural food production in fertilized ponds (Edwards, 1993). Supplemental feed, resulting in a nitrogen conversion efficiency of 8.5%, is also inefficient. This indicates that the cultured species preferred natural food rather than artificial feed, especially when natural food is abundant in the system.

Efforts have been made to analyze the economics of the traditional non-fed, semi-intensive feed-driven, and novel substrate-based fish production systems in the present study. This type of analysis might be fully realistic when it would be performed based on on-farm trials and for an annual production cycle. The fertilizer TSP was one of the major input costs. The dependency on TSP could be avoided or reduced if cheaper alternative fertilizers can be found, specially when considering the unusual high concentrations of orthophosphate (4-5 mg l^{-1}) measured in all treatment ponds.

The net margins were 198, 117, 174, 446 and 302 BDT in Control, Feed, Bamboo, Jutestick and Kanchi treatments, respectively, over a 135 days period and 75 m^2 pond area. In fact, most of the farmers use their own resources (land, labor, substrates, even rice bran and oil cake) within their household facilities. In that case, the net margins will be much higher than those reported here. Net profit relative to investment was highest in the Jutestick treatment, mainly because of the lower cost of substrates compared to the Bamboo and Kanchi treatments. It, therefore, can be concluded that the substrate-based polyculture systems perform better than the traditional non-fed or fed systems, not only from ecological and economical points of view but also considering that more fish is produced to satisfy the increasing demand.

A major issue that needs attention is the sustainability of the periphyton-based production systems. The demand for substrate materials may have negative environmental and social impacts on other resource users. This needs to be carefully assessed during the design and planning of aquacultural development efforts.

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Chapter 9

General Discussion

Introduction

The overall objective of this Ph.D. project was to determine the technical and economical potential of periphyton-based aquaculture systems in Bangladesh with the ultimate goal to increase the viability of low-input aquaculture. Wise and sustainable use of natural resources to enhance the production of low-input aquaculture systems can help to increase the income and food security of people in rural areas (NACA, 2000). Although fish pond production can be an important component of the rural economy, lack of access to feeds and fertilizers excludes many poorer families from adopting fish farming as part of their rural livelihood. This project, therefore, set out to address one of the key constraints preventing the poor from participating in fish culture: limited access to resources, such as nutrients, to be used as pond inputs. Therefore, the research concentrated also on maximizing nutrient retention into fish.

This thesis describes a systematic approach to assessing the technical viability of periphyton-based aquaculture in South Asia. Using fish production as the key criterion for evaluating the periphyton-based system, a range of environmental parameters and quality and quantity of plankton and periphyton were also assessed. While the results generally confirmed the positive effects of this novel culture system on fish production, they also raised some new research issues: the dietary requirement of experimental animals, factors influencing periphyton production, the nutritional status of periphyton and possible mechanisms underlying increased fish production in periphyton systems. The main objective of this final discussion chapter is to review these issues. In addition, the applicability of the periphyton technology, its dissemination and some possible impacts are highlighted. Finally, recommendations for future research are made.

Periphyton-sampling methods, substrate evaluation and biomass production

Most of the methods of periphyton sampling available in the literature were developed for open water habitats (Aloi, 1990; Seaman, 2000). Since the present project was concerned with closed water aquaculture systems, the available periphyton-sampling methods and parameters had to be modified. Throughout the thesis, the techniques used evolved gradually based on insights gained during previous experiments. Therefore, the techniques followed were not the same in all experiments.

In terms of periphyton production and quality, bamboo (*Bambusa* sp.) proved a better periphyton substrate than hizol branch (*Barringtonia* sp.), bamboo side-shoot or jute stick (*Corchorus* sp.). Economically, jute stick may give better returns.

The periphyton community was highly diverse, forming a complete ecosystem consisting of organic matter, inorganic nutrients, and autotrophic as well as heterotrophic organisms. Less than 50 percent of periphytic dry matter was composed of algae. More than 50 genera of algae were identified of which 16-30 were unique to the periphyton and did not appear in the phytoplankton. Some genera of zooplankton and invertebrates were also reported. Other components of the periphyton assemblage still need to be quantified.

Periphyton productivity was estimated at about 1.2 g C m^{-2} d^{-1} (2.5 g m^{-2} d^{-1} ash-free dry matter), which theoretically can support a fish production of 5,000 kg ha^{-1} y^{-1} (Chapters 2 and 3). Periphyton production effectively doubled autotrophic carbon production (on average, phytoplankton production was around 1.3 g C m^{-2} d^{-1}), no trade-off in production between autotrophic communities being observed. However, periphyton productivity data in aquaculture ponds are not available in the literatures. The present periphyton productivity figures are comparable to those reported for benthic algal mats on coral substrate, which range from about 1 to 3 g C m^{-2} d^{-1} (Carpenter, 1986; Polunin, 1988; Klumpp and Polunin, 1989; Van Rooij et al., 1998), showing spatial and temporal variations.

Although the quality varied, the quantity of periphyton biomass per unit surface area did not vary significantly among substrate types in the absence (Chapter 2) or presence of fish (Chapter 8). This might be due to the nutrient-rich environments in which the substrate acted merely as a platform for the periphyton (Moss, 1998). On the other hand, periphyton biomass in open water habitats strongly depended on substrate types (Blinn et al., 1980; Hansson, 1992; Vymazal and Richardson, 1995). The amount of periphyton biomass per surface area was found to be highly variable between experiments and was influenced by water depth (Konan-Brou and Guiral, 1994; Light and Beardall, 1998; Keshavanath et al., 2001a), nutrient availability (Elwood et al., 1981; Fairchild et al., 1985), grazing pressure (Hatcher and Larkum, 1983; Hansson et al., 1987; Hay, 1991; Huchette et al., 2000) and seasonality, including environmental parameters (Hatcher and Larkum, 1983; Carpenter, 1986; Bothwell, 1988; Arfi et al., 1997; Ledger and Hildrew et al., 1998).

In general, periphyton biomass, both in terms of dry matter and chlorophyll *a* concentrations per surface area, decreased with increasing water depth and was higher in the upper 0-60 cm water layer. This confirmed the dependency of algal periphyton on light.

Konan-Brou and Guiral (1994), Arfi et al. (1997) and Keshavanath et al. (2001a) reported the highest periphyton biomass around the compensation depth (40-70 cm).

While phytoplankton productivity in ponds and lakes is linearly correlated with nutrient concentrations (Boyd, 1990), the relationship was quadratic in periphyton-based ponds and affected by interactions with the phytoplankton (Chapter 3). Although fertilizer dose was optimized for periphyton-based earthen ponds, it did not give good results during the last experiment where it resulted in a phytoplankton bloom and had to be reduced (Chapter 8). This may have been due to the residual effects of fertilization (Knud-Hansen, 1992) as the same experimental ponds had been fertilized for the previous three years. In addition, fertilization rates were adjusted to maximize periphyton production in the absence of fish (Chapter 3) while it is known that grazing may affect periphyton productivity (Hatcher, 1983; Hay, 1991; Huchette et al., 2000). Therefore, it is recommended that fertilization be readjusted for the optimized fish stocking density. It should also be realized that the fertilization schedule is site-specific and will have to be adjusted when applied in other locations.

Periphyton dry matter decreased with increasing grazing pressure (Chapters 6 and 7). Without fish, periphyton biomass reached its peak in Weeks 3 or 4 and thereafter it levelled off (Chapter 2), whereas it was more or less stable in the presence of fish (Chapter 7). Periphytic algae needs to be grazed constantly and kept at low biomass in order to maintain high productivity, because increased standing biomass in absence of grazers may lead to self-shading and death of the algae, with consequent sloughing and dislodgment of the community (Hatcher and Larkum, 1983; Hay, 1991; Huchette et al., 2000). Grazing by fish damaged macrophytes and released nutrients into the water column, accelerating eutrophication (Hansson et al., 1987). Although the same might happen when fish graze on periphyton, it was not distinguishable in the highly fertilized environments of the present experiments.

The overall lowest biomass in terms of dry matter and ash-free dry matter reported in Chapter 6 was due to the prevailing low temperature during the experiment. Low temperatures are known to affect periphyton biomass, with biomass reported to be higher in summer and lower in winter (Hatcher and Larkum, 1983; Lane, 1991; Vymazal and Richardson, 1995).

Nutritional requirement of experimental fish and periphyton as fish food

One of the potential advantages of culturing omnivorous or herbivorous fish species in a semi-intensive system is the lower dependence upon supplementary feed and the possibility of eliminating the use of fish meal and animal proteins in supplementary feeds (Tacon and De Silva, 1997). When farming fish from low trophic levels, the food chain is short and the transfer of photosynthetic products into fish production is more efficient. Aquaculture of especially carnivorous species adversely affects the wild feed fish stocks because of the strong dependence on fishmeal as a feed (Naylor et. al., 2000). 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. The present project explored the alternative option of growing periphyton as a natural fish feed for Indian major carps.

There is little information available on the dietary requirements of the Indian carp species, endemic to the Indian subcontinent, used in this study. The protein requirement of rohu is reported to be 25-35 percent (Akand et al., 1991; Nandeesha et al., 1994; Hossain et al., 1997; Ashraf and Fairgrieve, 1998) depending on the age of the fish. Jafri and Farooq Anwar (1995) tested apparent protein digestibility of some low-cost feedstuffs for rohu and catla fingerlings and reported that protein from plant sources showed higher digestibility (81-94 percent) than that from fishmeal and slaughter house offal (74-76 percent). There is a dearth of information on dietary nutritional requirements of catla and kalbaush. However, the Indian major carps are mainly cultured in extensive or semi-intensive systems in the south Asian region and are mostly dependent on endogenous food supply. Rohu and catla are reported to be planktivorous (Dewan et al., 1991; Ahmed, 1993), whereas kalbaush is reported to feed on decayed organic matter along with plankton (Kumar and Siddiqui, 1989) in traditional pond culture. In-depth studies on feeding morphology of these species and digestibility of periphyton by target fish species remains to be accomplished.

The quality of periphyton varied substantially with substrate type, fertilization level, environmental conditions, grazing pressure and taxonomic composition (this thesis; Paine and Vadas, 1969; Hepher, 1988; Makarevich et al., 1993; Napolitano et al., 1996; Ledger and Hildrew, 1998; Huchette et al., 2000; Keshavanath et al., 2001a). The ash content of periphyton with the optimum fish species combination (Chapters 7 and 8) was 19 percent on bamboo, 31 percent on jute stick and 14 percent on kanchi. Protein content was 26-32 percent on bamboo, 13 percent on jute stick and 30 percent on kanchi on a dry matter basis. Lipid content was around 3 percent in all substrates. The energy content of periphyton was the same on bamboo and kanchi (19 kJ g^{-1} DM), but lower on jute stick (14 kJ g^{-1}).

Montgomery and Gerking (1980) reported 8-10 percent protein (dry weight basis), 2-5 percent lipid, 25-38 percent ash and 10-14 kJ g^{-1} energy in periphyton grown on granite boulders in coastal waters. Coral reef periphyton contained 2.5 percent N corresponding to 15 percent protein (Polunin, 1988). Lane (1991) reported 60-66 percent ash, 5.3-8.6 percent protein, 10-46 percent lipid and 3.0-4.3 percent carbohydrate (ash free dry matter basis) in periphyton grown on glass slides. Mixed culture biofilms collected from the biofilters in a recirculation aquaculture system contained 58 percent protein and 5 percent carbohydrate (Nielsen et al., 1997). In lacustrine environment, as low as 2-3 percent protein in periphyton grown on stones was reported (Ledger and Hildrew, 1998). Keshavanath et al. (2001a) reported 37-58 percent ash contents in periphyton dry matter in cement tanks depending on substrates type and grazing pressure. In general, ash content was higher in the absence of grazing by fish and increased with time, which was in agreement with the findings of Makrevich et al. (1993) and Huchette et al. (2000).

The nutritional value of plankton, which is the prevailing fish food in ponds, was also determined (Chapter 8) and was slightly higher (8-24 percent ash, 27-50 percent protein, 2-5 percent lipid and 18-23 kJ g^{-1} energy depending on the systems) than for periphyton. Hepher (1988, citing other literature) reported 18-31 percent protein, 4-10 percent lipid and 27-48 percent ash (dry matter basis) for planktonic algae in ponds depending on the taxonomic group.

In general, the nutritive value of periphyton reported in this thesis can be regarded as appropriate for the dietary needs of the experimental fish, although it contained more ash than the recommended levels (below 12 percent in formulated diets; De Silva and Andersen, 1995). Nevertheless, the ash content of periphyton was more or less similar to that of plankton, suggesting that herbivorous fish should be able to digest periphyton with little difficulty (Yakupitiyage, 1993).

The required protein:energy (P:E)-ratio for maximum fish growth is about 22.9 mg/kJ (Hepher, 1988). In the present investigation, the P:E-ratios for periphyton were 18.2-22.5 grown on bamboo, 12.4 on jute stick and 21.1 mg/kJ on kanchi (assuming 75 percent metabolizable energy; Hepher, 1988). For plankton, P:E-ratios ranged from 20.0 to 37.0 mg/kJ. Both periphyton and plankton (except periphyton on jute stick) thus conformed to the standards for complete diets. The higher growth and production of fish in the periphyton-based systems without any supplemental feed further confirmed the excellent nutritional quality of the periphyton. However, the fatty acid and amino acid compositions of periphyton were not determined in this study. Factors influencing periphyton quality in aquaculture ponds are to be further elucidated.

Effects of substrate on fish production

Selection of fish species by monoculture trials

Three indigenous major carps (rohu, *Labeo rohita*; gonia, *L. gonius*; and kalbaush, *L. calbasu*) were tested separately in monoculture. For rohu and gonia, bamboo substrates were used to promote periphyton production, resulting in a submerged surface area approximately equal to the pond water surface area (Chapter 4). For kalbaush, bamboo side shoots (kanchi) were used, resulting in a 50 percent increase in surface area (Chapter 5). In both cases, an 80 percent increase in rohu and kalbaush production was achieved in ponds provided with substrate compared to the substrate-free controls. In contrast, the growth and production of gonia did not vary significantly between the substrate and control ponds. The results indicated two key points: fish species used must be able to exploit periphyton; and substrate density should be sufficient to produce enough periphyton to guarantee enhanced growth of fish. In Chapter 5, it was reported that periphyton grazing by fish was insufficient to cause significant reductions in total periphyton densities. Grazing itself might have an effect on periphyton production (Hatcher, 1983; Hay, 1991; Huchette et al., 2000). This was not measured in this analysis.

Development of three species polyculture technique

Considering the unutilized or underutilized fish food (especially plankton) in periphyton-based monoculture systems, the possibility of increasing fish production in periphyton-based systems by practicing polyculture was explored with species of different feeding habits as in traditional pond culture practiced in South Asia and China. The indigenous surface feeder catla (*Catla catla*), with feeding habits complementary to those of rohu, was selected as a suitable candidate for exploiting the plankton community and increasing fish production in this system (Chapter 6). Total yields from any combination of rohu and catla were 50-300 percent higher than those from rohu or catla stocked separately, indicating that polyculture of rohu and catla is far superior to monoculture of either species in the periphyton-based system. When a combination of 60 percent rohu and 40 percent catla was used, the fish production was the highest and synergistic benefits compensated for any inter-specific or intra-specific dietary competition. Synergism in polyculture with compatible species combinations has been widely reported (Hepher et al., 1989; Milstein, 1992; Wahab et al., 1995).

Using the 60:40 ratio, another trial with and without bamboo substrates was carried out to verify the effect of substrates with this species combination (Chapter 7). Total net fish production in ponds with substrates was 180 percent higher than that in control ponds. In the same experiment, the impacts of including the bottom-dwelling kalbaush (*Labeo calbasu*) that also preferentially feeds on periphyton were investigated. It was found that the addition of 15 percent kalbaush to the optimum mix of rohu and catla increased the synergistic interactions among species, further increasing total production by 40 percent. Therefore, a stocking ratio of rohu, catla and kalbaush at 60:40:15, with a total stocking density of 11,500 juveniles per hectare was found to be an appropriate combination in a periphyton-based polyculture system. The production difference of this three-species combination in ponds with and without periphyton substrate was determined in the subsequent experiment.

In the final experiment (Chapter 8), a 70 percent increase in fish production was recorded in periphyton-based ponds provided with bamboo substrate and using the three-species combination in comparison to substrate-free control ponds. However, based on the differences in production between two-species (180 percent) and three-species (70 percent) polyculture, it is questionable whether three-species polyculture is better than two-species culture. The combined maximum yield of the three species was 2,098 kg ha^{-1} over a 135 days period, which was lower than that of the previous experiment (2,306 kg ha^{-1} over 90 days culture period; Chapter 7), although the same management protocols were maintained. Therefore, a conclusion cannot be drawn because there were significant temporal variations in fish production between the experiments. An experiment comparing two-species and three-species culture with and without substrate should be done to answer this question. It is also difficult to distinguish between the contribution of substrates and synergisms among species to fish production in periphyton-based polyculture.

In comparison to control ponds without substrate, the reported effects of artificial substrates on fish production in experimental ponds were (a) no significant increase (Shrestha and Knud-Hansen, 1994; Faruk-ul-Islam, 1996), (b) 50 percent increase (Shankar et al., 1998; Ramesh et al., 1999), (c) 75 percent increase (Keshavanath, 2001b) or (d) 200 percent increase (NFEP, 1997). This study confirmed the higher range of production increases due to the provision of substrate for periphyton growth. The combined average net fish production using the three-species periphyton-based polyculture technology ranged from 4,100 kg ha^{-1} y^{-1} (assuming 9 months culture season in a year; Chapter 8) to 6,900 kg ha^{-1} y^{-1} (Chapter 7), which is 60-170 percent higher than the average pond production in Bangladesh of 2,550 kg ha^{-1} y^{-1} (FRSS, 2001). Therefore, periphyton-based fish production technology offers considerable potential in this country.

Comparison of traditional and periphyton-based aquaculture systems

A comparison of fish production between traditional non-fed (control) and periphyton-based systems has been made in the chapters 4-7. In Chapter 8, the use of three different periphyton substrates was compared with traditional feeding practices, both from ecological and economical points of view. The traditional fed system resulted in similar increments of fish production over the non-fed fertilized system as the substrate-based systems did except the bamboo system. Legendre et al. (1989) cultured *Tilapia guineensis* and *Sarotherodon melanotheron* using pelleted feed (30 percent protein) at a daily rate of 5 percent of total biomass and compared the production to the traditional acadja systems. Supplemental feed gave disappointing results and, therefore, the traditional acadja system was recommended.

Chlorophyll *a* and total ammonia concentrations of pond water were higher in the fed system, but still within the range acceptable for fish culture. Lower nitrogen retention in fish in the fed system indicated more waste production than in the substrate systems. The cost-benefit analysis of different systems did not reflect the real situations of the farmers in Bangladesh, but it explored the possibility of scaling up the periphyton technique on commercial basis.

Possible mechanisms for enhanced fish production in the periphyton-based systems

More research is still needed to elucidate the mechanisms of enhanced fish production in substrate-based systems. This is best done by testing each mechanism in isolation. However, since the possible reasons have been discussed in detail in the respective chapters, the important points are summarized below.

Provision of additional food and shelter

In substrate ponds, periphyton served as an additional food source without reducing the production of other natural food in the system, as long as appropriate stocking and species combinations were maintained. It was estimated that pond productivity could be doubled by placing substrates equivalent to 100 percent of the pond surface area in the ponds (Chapter 2). Apart from serving as an additional source of food, substrates also provide shelter and protection from predators, such as birds, frogs, or snakes. From a theoretical point of view, the biggest advantage of the added substrates is an increase in the energy- and nutrient-transfer efficiency of the system due to the additional periphyton-based production (Miller and Falace, 2000). In traditional fishponds, the pond bottom is the only substrate on which some larger benthic algae can grow. But benthic algal mats seldom develop in highly eutrophic ponds due to shading by plankton blooms. In periphyton-based systems, attached algae along with zooplankton and invertebrates can easily colonize the substrate in the water column. Fish can then graze on these concentrated food items more efficiently than filter feeding on planktonic foods only (Dempster et al., 1993).

Opportunity for higher nutrient efficiency

Although the overall nitrogen conversion efficiency was low, it was 1.6 times higher in periphyton-based systems than in controls (Chapter 8). In periphyton-based systems, more nutrients are passed to higher trophic levels rather than accumulating in the system as in feed-driven ponds. The overall nitrogen turnover of the primary trophic level can be estimated from the data given in Chapters 2 and 8. The average periphyton productivity was 3.5 g DM m^{-2} d^{-1} (Chapter 2), equivalent to 0.122 g N m^{-2} d^{-1}, which resulted in 1,235 g N in a 75 m^2 pond during 135 days period. The average phytoplankton production was 2.55 g DM m^{-2} d^{-1}, equivalent to 0.166 g N m^{-2} d^{-1} resulting in 1,681 g N in 75 m^2 pond for 135 days (adjusted from an increment of 268 μg l^{-1} chlorophyll *a* in a week, Chapter 2; assumes 1.5 percent chlorophyll *a*, APHA, 1998; and 6.5 percent N in phytoplankton DM, Chapter 8). Total nitrogen input was 4,530 g in the control and three periphyton systems, whereas, it was 6,110 g in the fed system (Chapter 8). As a result, nitrogen retentions in algae in control, fed and substrate systems were 37, 28 and 64 percent, respectively. Nitrogen retention in higher trophic levels that can be harvested by the fish species is also higher in periphyton-based systems. Thus, the periphyton-based system offers the potential for increasing nutrient efficiency, reducing the accumulation of wastes in the system, and improving the overall water quality.

Create better environment through improving water quality

In none of the experiments, adverse effects on water quality parameters due to added substrates were observed. On the contrary, water quality improved in the periphyton systems. Suspended solids were trapped in the periphyton mat, which also took up ammonia and nitrate, produced oxygen, broke down organic matter and increased nitrification. In traditional aquaculture ponds, nitrification occurs mostly at the sediment surface and is limited not only by the available surface area but also by oxygen. In addition, heterotrophic bacteria may limit the population density of oxygen-requiring nitrifying bacteria on the pond bottom. Thus, ammonia toxicity is an important constraint in intensifying aquaculture in pond systems (Hargreaves, 1998). In substrate-based ponds, nitrifying bacteria can colonize the surface of the substrates that are located in the well-oxygenated water column rather than at the anoxic water-sediment interface. Therefore, periphytic biofilms on the substrate act as biofilters (Langis et al., 1988; Shankar and Mohan, 2001), keeping the potentially harmful ammonia in the ponds at low levels.

Ammonia concentrations were lower in periphyton systems than in control systems, although they did not reach critical levels in the latter (Chapter 8). Similarly, dissolved oxygen (DO) concentrations never became critical in ponds without substrate. This might have been due to the optimum fish stocking combinations and densities. Periphyton systems may have a higher carrying capacity compared to ponds without substrate, where higher ammonia and lower DO levels might become a problem at higher stocking densities. This should be confirmed experimentally by comparing highly fertilized and/or fed systems with and without substrates. The success of the system also depends on the quality of the substrates as some artificial substrates may even cause deterioration of water quality in fishponds. Keshavanath et al. (2001a) reported 100 percent fish mortality in ponds provided with sugarcane bagasse as substrate due to critically low DO concentrations (1.63 - 1.86 mg

l^{-1}). There is also growing evidence that periphyton can act as an antibiotic against a variety of fouling bacteria (Boyd et al., 1998) or as a probiotic/vaccine to animals that feed on it (Azad et al., 1999).

Optimise the synergistic effects

The benefits of traditional polyculture can also be enjoyed in periphyton-based aquaculture systems. The provision of substrates may enhance the synergism among fish species. The net fish yields at different stocking ratios of rohu and catla were 50-300 percent higher than in monoculture with either species (Chapter 6). The bottom feeding kalbaush further enhanced overall production by 40 percent (Chapter 7). Based on the production data, stable isotope analysis (Chapter 7) and, more importantly, visual observation, it can be concluded that rohu is predominantly a periphyton-feeding fish, whereas catla prefers filter-feeding on plankton. Since rohu depended on periphyton while catla utilized the plankton, there was little or no dietary competition. More sunlight penetrated into the water column which further enhanced both phytoplankton and periphyton production. Mineralization may also be better in periphyton-based systems, leading to higher nutrient availability in the system and enhanced autotrophic production (Shankar and Mohan, 2001).

Although the production of kalbaush was greater in both periphyton-based mono- and polyculture systems, this species could not be confirmed as a periphyton feeder based on stable isotope analysis. Nevertheless, in polyculture ponds, the bottom dwelling activities of kalbaush had a synergistic effect by stimulating the release of nutrients from the bottom to the water column (Chapter 7). However, the higher yields in polyculture attributed to the complementary fish species interactions and the improvements of the environmental conditions have been well documented by Yashouv (1971), Hepher et al. (1989) and Milstein (1992).

Applicability of the new aquaculture technology and its socio-economic impacts

The sustainability of improving livelihoods through modification of traditional farming practices should be evaluated carefully. Changes in existing practices may lead to increased risk, undesirable changes in the stock and flow of resources or have negative environmental effects. Bunting et al. (2001) undertook a system-based assessment of the technology to assess the possible impact of promoting periphyton-based aquaculture, focusing on its potential role in the livelihoods of rural farming households in Bangladesh. Although it was not possible to isolate the relative contribution of periphyton to increased production, farmers perceived several benefits of adding branches to their fishponds. They reported seeing fish feeding on periphyton and rubbing against branches, possibly to dislodge parasites. They believed that the addition of substrate reduced poaching, improved fish health, reduced predation and increased production. Substrate management appeared to represent an important area and warranted further investigation. As households have limited access to wood material, the optimal use of substrate introduced to ponds is paramount. It is also laborious to remove the substrate before netting the ponds for fish harvest. Partial harvesting of fish from substrate-installed ponds is impractical and may interfere with regular fish consumption of households. It is a social custom for farmers to catch some fish and offer it to visiting relatives or friends. Sometimes, people bring fish as a gift when visiting relatives or neighbors. However, netting might not be a problem if the substrates are biodegradable like jute sticks.

There is a strong competition in using resources among other household activities. Bamboo is used as house building material (Dunham, 1994), raw material in cottage industry (Kabir et al., 1993), household fuel energy (Hossain, 1988), raw material in paper and hardboard industry (Razzaque and Khan, 1978), tubewell strainer in irrigation (Rab et al., 1980) and also as brush shelter in fisheries (Ahmed and Humbrey, 1999). Nevertheless, the lack of substrates should not be considered a constraint since the production is considerably higher in these systems. Bamboo may be planted in the homestead especially for use in periphyton ponds. Bamboo was considered a "wonder grass" in Asia (Hem and Avit, 1994) and can be grown easily on fallow land, even without any nursing. It is a self-sustaining plant and can be productive for hundreds of years. Although jute stick and kanchi are by-products of other agricultural activities, they are also used as materials for fencing and for cooking purposes. Other possible substrates in Bangladesh are the branches of the Gab tree (river ebony, *Diospyros peregrina*), Mango tree (*Mangifera indica*), Babla tree (acacia, *Acacia nilotica*), Mander tree (coral tree, *Erythrina indica*), Shaora tree (*Balanostreblus illicifolius*), Dhainche (*Sesbania aculeata*), Jhau (Conifer, *Casuarina equisetiafolia*), Epil epil (*Leucaena lencocephala*), Java plum (*Syzygium cumini*), Wax jambu (*Eugenia alba*), etc. These substrates should be tested for any adverse effects on water quality and for their ability to sustain a periphyton assemblage.

Being technically simple and making use of local resources (materials and manpower), substrate-based aquaculture is sustainable and feasible in South Asia. The most important strength of this system is that it is not a fixed technology, but a flexible package that can be adapted to the needs, capacity and resources of the users. It can generate income, nutrition and social benefits as required. Apart from benefiting the poor, the increases in production can also be of interest for commercial application. However, the consequences of a large-scale adoption of this technology should be considered. There will be a greater demand for substrate materials for aquaculture, which may lead to increases in the price of these materials, and there may be increased employment opportunities in producing and supplying them. Some agricultural by-products may gain importance as resources for periphyton-based aquaculture. An example is jute stick, the by-product of jute fibre. Once called "the golden fibre of Bangladesh", jute fibre lost the battle with synthetic fibres two decades ago. If the use of jute stick in aquaculture were economically viable, it could re-gain importance as a major resource in aquaculture. Any increased production of fish through introduction and adoption of this new technology is unlikely to cause a major problem with fish prices. The demand for fish and market prices in Bangladesh are very high and are expected to remain high because of the rapidly growing population (Hambrey et al., 2001).

Conclusions and future perspectives

This study demonstrated that pond fish production can be significantly increased with the introduction of substrates, without the need for supplemental feeding. In monoculture trials, fish production increased by 80 percent. In polyculture, the increase in production was 70-180 percent. Economic analysis indicated that the cost of bamboo was high and that either reduced densities of bamboo or cheaper substrates such as jute stick or kanchi could improve the economic viability of the technology.

Although the underlying biological mechanisms of the periphyton-based aquaculture system were not fully elucidated, fish production was consistently increased due to the addition of substrates. In this sense, the results are conclusive. However, a number of issues should be carefully explored before a wider implementation of this new approach can be initiated. Preliminary economical analysis provided only very general indications as to economic viability of the technology. It remains to be fully assessed whether the technology is appropriate under all circumstances, from the commercial level to resource-poor and marginal farmers. This can be best addressed through carrying out adaptive (on-farm) and farming-systems action research involving considerable numbers of participating farmers in a range of locations and culture systems. On-farm optimization trials should emphasize environmental and socio-economic implications.

There still remains a large knowledge gap with regard to the attached food organisms and their succession, their nutritive value, biofilter properties, etc. More insight into the whole system may be obtained by doing fundamental biological research under controlled conditions. The results of the present study strongly indicate the need for a better understanding of the dynamics and the productivity of periphyton, especially under grazing pressure. Modelling of periphyton-based systems will be very useful to determine the optimum combinations of substrate and fish stocking densities, to select the herbivore and omnivore fishes in relation to the nutritive value of periphyton and to optimize the manuring and fertilization rates in periphyton-based aquaculture systems. Of special interest for future research are the exploration of the bio-filter properties and the probiotic effects of periphyton.

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Summary

The potential of periphyton-based aquaculture production systems

This project developed a low-cost fish culture technology for resource-poor farmers in South Asia. The technology is based on increasing the production of natural fish food attached to surface areas (periphyton) by introducing artificial substrates in pond aquaculture systems. The general objectives were to reduce the dependency on external resources and to increase nutrient and energy utilization, thereby improving simultaneously aquaculture sustainability and accessibility for poor farmers. The project comprised four distinct but inter-related specific objectives, each worked out in one or two chapters.

First, periphyton production on different substrates was assessed. The suitability of periphyton as food for fish, in terms of its nutritional quality, was determined. The fertilizer dose for maximizing periphyton production was determined. Secondly, some suitable indigenous fish species were selected for periphyton-based systems by monoculture. Thirdly, a periphyton-based polyculture technology was developed by exploring fish production under different stocking densities, ratios and species combinations. Finally, ecological and economical implications were considered to evaluate the periphyton-based aquaculture systems.

Under the first objective (Part I), two separate experiments were carried out. The first experiment determined the production and the quality of periphyton grown on different artificial substrates (Chapter 2). Three locally available substrates, bamboo (*Bambusa* sp.), bamboo side shoot (locally called kanchi) and hizol tree (*Barringtonia* sp.) branch were evaluated: periphyton dry matter was higher (4.89 mg cm^{-2}) on hizol, and periphyton chlorophyll *a* was higher (11.51 g cm^{-2}) on bamboo substrates. Ash contents of periphyton were 41% on hizol and 29% on other two substrates. Protein content of periphyton, determined stoichiometrically, was 38% of ash free dry matter (AFDM) on bamboo substrate, which was 50% higher than on other two substrates. The energy contents of periphyton AFDM ranged between 19 and 20 kJ g^{-1}. The periphyton productivity ranged from 2.17-2.83

g AFDM m^{-2} d^{-1} (1.0-1.4 g C m^{-2} d^{-1}), which was sufficient to support an estimated fish production of 5,000 kg ha^{-1} y^{-1}. The second experiment optimized the fertilization rate in relation to maximum periphyton production (Chapter 3). The normal fertilizer dose in Bangladesh is cow manure, urea and triple super phosphate (TSP) at rates of 3,000, 100 and 100 kg ha^{-1}, respectively, applied fortnightly. Fertilizer doses of 0.5, 1.0, 1.5 and 2.0 times the normal dose were tested of which the 1.5 times dose resulted in the highest periphyton production, quantitatively and qualitatively. The highest periphyton dry matter (3.27 mg cm^{-2}) and chlorophyll *a* (7.5 μg cm^{-2}) biomass developed in this treatment. Total ammonia and chlorophyll *a* of water increased with increasing fertilization rate.

To meet the second objective (Part II), the culture potential of three indigenous major carp species in periphyton-based systems was assessed. Rohu (*Labeo rohita*) and gonia (*L. gonius*) were cultured in separate experiments in ponds provided with bamboo substrate and in ponds without substrate (Chapter 4). Juveniles were stocked at a density of 10,000 fish ha^{-1}. Net yield of rohu was 77% higher in ponds provided with substrate (1,900 kg ha^{-1} 120 d^{-1}) than in control ponds (1,075 kg ha^{-1} 120 d^{-1}). The yield parameters of gonia did not vary significantly between the substrate and control ponds. The periphyton biomass in rohu ponds was always lower than in gonia ponds but increased steadily throughout the study period, whereas in gonia ponds the periphyton biomass reached a plateau in Week 8. Thirty-five genera of phytoplankton and eleven genera of zooplankton were identified in the water column and thirty-four genera of algae and five genera of zooplankton were found on the substrates. Sixteen genera were only found in periphyton, while most other algae species were found both in the water column and on the substrates.

The other experiment under the second objective was designed to evaluate the effect of kanchi as a substrate for periphyton on growth and production of the indigenous carp kalbaush (*L. calbasu*) (Chapter 5). Kanchi substrate was installed in three ponds and three substrate-free ponds acted as controls. Ponds were stocked with juveniles at a density of 10,000 fish ha^{-1}. To evaluate the effect of fish grazing on the periphyton community, three substrates in each pond were enclosed by a fine mesh nylon net, thereby excluding fish. Fish survival, growth rate and net production were significantly higher with substrate than in the control treatment, and the production difference between treatments with and without substrate was 80% (713 and 399 kg ha^{-1}, respectively) over the 120-days period. While there was clear evidence that the fish were exploiting the periphyton, grazing pressure was insufficient to significantly reduce total periphyton densities, suggesting that the fish density could be increased. In total, 44 genera of phytoplankton and 14 genera of zooplankton from pond water samples and 54 genera of algae from periphyton were identified, of which 29 were found only in the periphyton.

Based on the previous results, a periphyton-based polyculture technology was developed by exploring the different stocking densities and species combinations as third objective (Part III). In addition, environmental conditions including various water quality parameters, plankton and periphyton biomass were monitored to ensure that the new aquaculture approach did not negatively affect water quality.

In Chapter 6, the stocking ratio of rohu and catla in the periphyton-based production system with bamboo substrates was optimized. The phytoplankton biomass decreased with increasing stocking density of catla, whereas periphyton biomass decreased with stocking density of rohu, indicating the preferential feeding of rohu and catla on periphyton and plankton, respectively. Periphyton ash content decreased with increasing ratio of rohu:catla, indicating that periphyton quality improved under grazing. The net yields from any combinations of the two species in polyculture were 50-300% higher than those from monoculture of either species and the highest fish yield was recorded in the treatment comprising 60% rohu and 40% catla.

Chapter 7 compared the production of 60% rohu and 40% catla between substrate and substrate free controls, and in addition, described the effect of inclusion of the bottom-dwelling kalbaush in this system. Survival of rohu and catla was higher in substrate ponds than in the controls. Fish production from the periphyton-based system was 2.8 times higher (1,652 kg ha^{-1}) than that of the control (577 kg ha^{-1}) over a 90 days culture period. The addition of 15% kalbaush (i.e. a stocking ratio of 12:8:3 rohu:catla: kalbaush) with a total stocking density of 11,500 juveniles ha^{-1} resulted in a further 40% increase in production (2,306 kg ha^{-1}). Food abundance and environmental conditions were the best in the treatment with 15% kalbaush as indicated by the observed lower Secchi disk values and higher nutrient and chlorophyll *a* concentrations in the water column. The nutritional quality of the peripyton (ash 15-19%, protein 23-26% and energy 19-20 kJ g^{-1} dry matter) was appropriate for the species cultured. In total, 50 genera of algae and 13 genera of zooplankton were identified in this experiment.

Under the fourth objective (part IV), the newly evolved periphyton-based technology was compared with the traditional management techniques (only fertilization, fertilization plus feed) used in Bangladesh (Chapter 8). Since bamboo is a relatively expensive substrate and has various competiting uses, two other commonly available substrate materials were also examined: jutestick (*Corchorus* sp.) and kanchi. Five triplicate treatments were tried in fifteen earthen ponds: standard fertilization (control), control plus supplemental feeding with rice bran and mustard oil cake (feed), control plus bamboo substrate (bamboo), control plus jute stick substrate (jutestick) and control plus bamboo side shoot substrate (kanchi). The comparison was based on water quality parameters, quantity and quality of the natural food available, conversion of N-input into fish, fish production and economical performance. Significantly higher ammonia concentrations were recorded in the control and feed treatments. The chlorophyll *a* concentration of pond water was significantly higher in the feed treatment. Specific growth rates of rohu and catla were higher in substrate and feed treatments compared with the controls. Feeding and provision of substrates resulted in 59% and 66-71% greater fish production, respectively, than in the control system. The highest net yield over the 135-days trial was more than 2,000 kg ha^{-1} of fish in substrate-based systems compared to 1,226 kg ha^{-1} in the substrate-free control ponds. Nitrogen retention in fish was 1.6 times higher in substrate systems and 1.3 times higher in the feed than in the control treatment. Net profit margin was highest in the jutestick system (46%). The substrate-based systems performed better than the traditional systems, not only from an ecological or economical point of view but also in terms of productivity, which is important considering the presently unsatisfied demand for fish in Bangladesh.

In the final discussion (Chapter 9), some issues related to the applicability of the novel technology, such as the environmental impact and resource management and development aspects were discussed and some new ideas for future research were presented.

Samenvatting

De mogelijkheden van visproductiesystemen gebaseerd op perifyton

In dit project werd een goedkope visteelttechnologie ontwikkeld die binnen het bereik ligt van kleinschalige Zuid-Aziatische boerenbedrijven. De technologie bestaat uit het verhogen van de productie van natuurlijk, op oppervlakken groeiend voedsel voor vissen (perifyton) door het plaatsen van kunstmatige substraten in visvijvers. De hoofddoelstellingen waren een vermindering van de afhankelijkheid van externe inputs en een verhoging van de nutriënten- en energiebenutting, en daarmee een verhoogde duurzaamheid en toegankelijkheid van aquacultuur voor arme boeren. Het project omvatte vier verschillende, maar met elkaar verweven sub-doelstellingen, die elk werden uitgewerkt in een á twee hoofdstukken. Ten eerste werd de perifytonproductie op verschillende substraten onderzocht en werd de voedingswaarde, en daarmee de geschiktheid van peripfyton als voer voor vissen, bepaald. Ook werd de bemestingsdosis voor maximale perifytonproductie bepaald. Ten tweede werden enkele locale vissoorten gekozen voor monocultuur in systemen met perifyton. Ten derde werd een polycultuurtechnologie met perifyton ontwikkeld op basis van productieproeven met verschillende totale bezettingsdichtheden, soortenverhoudingen en –combinaties. Tot slot werden perifytonsystemen geëvalueerd op basis van ecologische en economische criteria.

Twee experimenten werden uitgevoerd met betrekking tot de eerste sub-doelstelling (Deel I). Het eerste experiment bepaalde de productie en samenstelling van perifyton gekweekt op verschillende substraattypes (Hoofdstuk 2). Drie lokaal beschikbare materialen, bamboe (*Bambusa* sp.), bamboe zijscheuten (lokale naam: Kanchi) en takken van de hizol-boom (*Barringtonia* sp.) werden getest. Op basis van droge stof was de perifytondichtheid het hoogst op hizol (4.89 mg cm^{-2}), maar de chlorofyl-a concentratie was het hoogst op bamboe (11.51 g cm^{-2}). Perifyton op hizol bevatte 41% as, vergeleken met 29% op de andere substraten. Het eiwitgehalte van perifyton werd stochiometrisch bepaald en was 38% van de as-vrij-droge-stof (AVDS) op bamboe substraat, 50% hoger dan op de andere substraten.

Perifyton AVDS had een energie-inhoud van 19–20 kJ g^{-1}. De productiviteit van perifyton fluctueerde tussen 2.17 en 2.83 g AVDS m^{-2} d^{-1} (1.0 – 1.4 g C m^{-2} d^{-1}), wat voldoende is voor een productie van 5000 kg vis ha^{-1} $jaar^{-1}$. In een tweede experiment werd de bemestingsdosis voor maximale perifytonproductie bepaald (Hoofdstuk 3). De standaardbemesting in Bangladesh bestaat uit 3000 kg koemest, 100 kg ureum en 100 kg tripel superfosfaat per hectare, toegepast om de 14 dagen. Proeven met 0.5, 1.0, 1.5 en 2.0 maal de standaard bemestingshoeveelheid toonden aan dat 1.5 maal de standaarddosis zowel kwantitatief als kwalitatief de hoogste perifytonproductie opleverde: 3.27 mg perifyton per cm^2 (op droge stof basis) en 7.5 μg chlorofyl a per cm^2. In de waterkolom namen de ammonium en chlorofyl-a concentraties toe met oplopende bemestingsdosis.

In relatie tot de tweede sub-doelstelling (Deel II) werd de geschiktheid voor teelt in perifytonsystemen van drie lokale karpersoorten onderzocht. Rohu (*Labeo rohita*) en gonia (*L. gonius*) werden elk afzonderlijk getest in vijvers met of zonder bamboesubstraat (Hoofdstuk 4). Pootvissen werden uitgezet in een dichtheid van 10000 vissen per ha. De netto opbrengst van rohu was 77% hoger in vijvers uitgerust met substraat (1900 kg ha^{-1} 120 d^{-1}) dan in vijvers zonder substraat (1075 kg ha^{-1} 120 d^{-1}). Bij gonia werden geen significante verschillen in opbrengst vastgesteld tussen vijvers met en zonder substraat. De perifytondichtheid op de substraten in de vijvers met rohu was altijd lager dan in de vijvers met gonia maar nam voortdurend toe gedurende de proefperiode, terwijl in de goniavijvers de perifytonbiomassa een plateau bereikte in week 8. In de waterkolom werden 35 genera fytoplankton en 11 genera zoöplankton geïdentificeerd, vergeleken met 34 genera algen en 5 genera zoöplankton in het perifyton. Zestien genera waren specifiek voor het perifyton, terwijl de meeste andere algensoorten zowel in de waterkolom als in het perifyton voorkwamen.

Het twee experiment onder de tweede sub-doelstelling was opgezet om het effect van perifyton, groeiend op kanchi substraat, op de groei en productie van de locale karpersoort kalbauch (*L. calbasu*) te onderzoeken (Hoofdstuk 5). In drie vijvers werd kanchi substraat geïnstalleerd en 3 vijvers zonder substraat deden dienst als controle-behandeling. De vijvers werden bezet met 10,000 pootvissen per hectare. Om het effect van begrazing door vissen op de perifytongemeenschap te schatten werden in elke vijver met substraat drie kanchi-stokken omringd door een fijnmazig nylon net, waardoor de vissen er niet bij konden. De overleving, groeisnelheid en netto productie van vis waren significant hoger met perifyton. Het productieverschil tussen de behandeling met en zonder substraat was 80% (respectievelijk

713 en 399 kg ha^{-1}) na 120 dagen. Alhoewel werd aangetoond dat de kalbaush het perifyton benutte was de begrazingsdruk te laag om de dichtheid van perifyton significant te verminderen. Dit was een aanwijzing dat de visbezettingsdichtheid kon worden verhoogd. In totaal werden 44 fytoplanktongenera en 14 zoöplankton genera geïdentificeerd in de waterkolom tegen 54 algengenera in het perifyton, waarvan 29 genera specifiek waren voor het perifyton.

Uitgaande van deze resultaten richtte het onderzoek zich vervolgens op verschillende bezettingsdichtheden en soortcombinaties voor het ontwikkelen van een perifytonsysteem voor polycultuur (Deel III). Tegelijkertijd werden de omgevingsfactoren, zoals waterkwaliteitsparameters, fyto- en zoöplanktonbiomassa en perifyton bemonsterd om negatieve effecten van de nieuwe aanpak op de waterkwaliteit te voorkomen.

In hoofdstuk 6 werd de verhouding in bezettingsdichtheid tussen rohu en catla geoptimaliseerd in vijvers met bamboesubstraat. De fytoplanktonbiomassa verminderde bij een verhoogde bezettingsdichtheid van catla, terwijl de perifytonbiomassa afnam bij een verhoogde bezettingsdichtheid van rohu. Dit wijst erop dat catla de voorkeur gaf aan fytoplankton terwijl rohu perifyton prefereerde. Het as-gehalte in perifyton verminderde bij een toenemende verhouding rohu:catla, wat erop duidde dat de kwaliteit van perifyton verbeterde onder toenemende begrazingsdruk. De netto opbrengst van rohu of catla was altijd 50 tot 300% hoger dan de opbrengst van rohu of catla in monocultuur, onafhankelijk van de onderlinge verhouding rohu:catla. De beste opbrengst werd behaald bij een rohu:catla verhouding van 60:40.

In hoofdstuk 7 werd de productie van deze 60:40 rohu:catla verhouding vergeleken tussen vijvers met en zonder bamboesubstraat, en werd bovendien het effect op de productie van de toevoeging van een derde soort, de bodembewoner calbaush (*L. calbasu*), bepaald. De overleving van rohu en catla was hoger in de vijvers met substraat dan in de controlevijvers. De visproductie was 2.8 keer hoger met perifyton (1652 kg ha^{-1}) dan in de substraatvrije controlevijvers (577 kg ha^{-1}) gedurende een teeltperiode van 90 dagen. Toevoeging van 15% calcaush (een rohu:catla:calbaush verhouding van 12:8:3) bij een totale bezettingsdichtheid van 11500 pootvissen ha^{-1} leidde tot een extra productieverhoging van 40% (2306 kg ha^{-1}). De voedselhoeveelheid en de milieuomstandigheden waren het best in de combinatie met 15% calbaush, wat bleek uit lagere Secchi waarden en hogere nutriënten- en chlorofyl-a concentraties in de waterkolom. De voedingswaarde van het perifyton, dat 15-19% ruwe as en 23-26% eiwit bevatte en een energieinhoud had van 19-20 kJ g^{-1} droge stof, was voldoende voor de geteelde soorten. In totaal werden 50 genera algen en 13 genera zoöplankton geïdentificeerd in dit experiment.

Met betrekking tot de vierde sub-doelstelling (Deel IV) werd de nieuw ontwikkelde perifytontechnologie vergeleken met de conventionele beheerssystemen voor vijvers (alleen bemesting, en de combinatie van bemesting en bijvoedering) in Bangladesh (Hoofdstuk 8). Omdat bamboe relatief duur is en verschillende andere toepassingen kent werden ook twee andere algemeen beschikbare materialen voor substraat getest: jute-stokken (*Corchorus* sp.) en kanchi. Vijf behandelingen werden getest in 15 vijvers, met 3 herhalingen elk: standaard bemesting (controlebehandeling), controlebehandeling met rijstslijpsel en mosterdzaadschroot als bijvoedering (bijvoedering), controlebehandeling gecombineerd met bamboesubstraat (bamboe), controlebehandeling met jutestokken als substraat (jute) en controlebehandeling met bamboe zijscheuten als substraat (kanchi). Waterkwaliteitsparameters, de hoeveelheid en samenstelling van het natuurlijke voedsel, de omzettings-efficiëntie van de stikstofinput in visbiomassa, de visproductie en economische parameters van de behandelingen werden vergeleken. Significant hogere ammoniaconcentraties werden gemeten in de controle- en bijvoederingbehandelingen. Het chlorofylgehalte in de waterkolom was significant hoger in de bijvoederingbehandeling. De specifieke groeisnelheid van rohu en catla was hoger in de bijvoedering- en substraatbehandelingen dan in de controlebehandeling. Bijvoedering en substraten leidden tot een respectievelijk 59% en 66-71% hogere productie dan de controlebehandeling. De hoogste productie na 135 dagen was meer dan 2000 kg ha^{-1} in de behandelingen met substraat, tegen 1226 kg ha^{-1} in de controlebehandeling. De stikstofretentie in de behandelingen met substraat was 1.6 keer hoger en met bijvoedering 1.3 maal hoger dan in de controlebehandeling. De netto winst was het hoogst in de jutebehandeling (46%). De systemen met substraat leverden betere resultaten dan de conventionele systemen, niet alleen in ecologisch en economisch opzicht, maar ook wat betreft productiviteit. Dit laatste is belangrijk, omdat op dit moment de vraag naar vis in Bangladesh het aanbod overtreft.

In de algemene discussie (Hoofdstuk 9) wordt de toepasbaarheid van de nieuw ontwikkelde perifytontechnologie besproken en worden nieuwe ideeën voor vervolgonderzoek gepresenteerd.

Completed PhD Education Programme **Graduate School WIAS**

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Group	Fish Culture and Fisheries
Period	February 1998 until December 2001
Supervisors	Prof. Dr. E.A. Huisman, Prof. Dr. J.A.J. Verreth
Daily advisor	Dr. M.C.J. Verdegem

The Basic Package (minimum 3-7 cp)	year	cp*
❍ WIAS Common Course "Science and Society" (mandatory)	1999	2.0
❍ WIAS Course "Philosophy of Science and Ethics" (mandatory)	2001	1.0
SUBTOTAL		3.0
International Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
❍ International Conferences (minimum 2 cp)		
AQUA 2000, Nice, France, 2-6 May	2000	1.0
Aquaculture 2001, Orlando, USA, 21-25 January	2001	1.0
Final workshop of EC-PAISA project, Dhaka, Bangladesh, 29-31 January	2001	0.6
XXVIII SIL Congress, Melbourne, Australia, 4-10 February	2001	1.4
Aquaculture Canada 2001, Halifax, Canada, 6-9 May	2001	0.8
❍ WIAS Seminars Plus (minimum 0.5 cp)		
Organic Farming	2000	0.3
Potential use of Stable Isotopes	2001	0.3
❍ Presentations (minimum 4 original presentations of which at least 1 oral)		
Oral presentation, AQUA 2000, Nice, France	2000	0.5
Oral presentation, Aquaculture 2001, Orlando, USA	2001	0.5
Two oral and 1 poster presentations, Final workshop of EC-PAISA, Dhaka, Bangladesh	2001	1.5
Oral presentation, XXVIII SIL Congress, Melbourne, Australia	2001	0.5
Oral presentation, Aquaculture Canada 2001, Halifax, Canada	2001	0.5
SUBTOTAL		8.9
In-Depth Studies (disciplinary and interdisciplinary courses, minimum 4 cp)	year	cp
❍ Aquaculture Production Systems	2000	2.0
❍ Design and Analysis of Experiments	2000	3.0
❍ Stable Isotopes in Studies of Nutrient Dynamics	2000	1.0
❍ Statistical Methods	2001	3.0
SUBTOTAL		9.0
Professional Skills (support courses, minimum 2 cp)	year	cp
❍ Introduction of Agricultural Research and Farming System Research Management with particular reference to Fisheries	1998	0.8
❍ Bateriological Diagnostic Techniques for Aquatic Animals	1998	1.0
❍ National Fish Disease Reporting System	1998	0.4
❍ Application of GIS in Agricultural Development Planning	1999	0.4
❍ Techniques for Scientific Writing	2000	0.6
❍ Design of Animal Experiments	2000	1.0
SUBTOTAL		3.4
TOTAL		24.3

* 1 cp (credit point) is approximately equivalent to a study load of 40 hours.

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Curriculum Vitae

Mohd. Ekram-Ul-Azim was born on 10 November, 1970 in the village Refaitpur (Joardderpara) of the district Kushtia, Bangladesh. He is the youngest son of Azimuddin Malitha (Late) and Nigara Khatun. He completed his primary and secondary education in his village school. His higher secondary education was completed at the Bheramara College, Kushtia. He enrolled in the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh in 1990 and obtained B.Sc. Fisheries (Honours) degree in 1995. In 1997, he finalized Master of Science (M S) in Fisheries Biology and Limnology degree from the same university. He obtained first class in both the degrees.

Mr. Azim worked in several research projects namely, IFS funded "Development of four species polyculture systems in Bangladesh", USAID funded "Aquaculture research for sustainable development", ODA funded "Fisheries dynamics of modified floodplains in southern Asia", WB funded "Agricultural research management", in both the Faculty of Fisheries, BAU, and Bangladesh Fisheries Research Institute, Mymensingh. He started his Ph.D. work in February 1998 under the framework of an EC-funded collaborative INCO-DC programme (PAISA, grant IC18 CT97 0196). Wageningen University also awarded him the Sandwich Scholarship. He has followed considerable number of academic and professional courses organized by the Wageningen University. He has also attended five international conferences and presented papers in relation to his Ph.D. project.

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Photos on the cover page:

Front: 1. Some periphyton species; 2. Indigenous Catla, Rohu and Kalbaush;
3. Experimental ponds at BAU.

Back: 1. Showing periphyton colonized on bamboo; 2. Katha fishery in Bangladesh.