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Aquat. Living Resour. 15 (2002) 231–241

Aquatic Living Resources

www.elsevier.com/locate/aquliv

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

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Received 5 April 2001; accepted 19 June 2002

Abstract

As a first step in assessing the viability of periphyton-based fish production in South Asian pond aquaculture systems, the effects of artificial substrates on development of periphyton and on water quality were evaluated. Earthen ponds (10 × 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 per m^2 pond surface). Higher periphyton biomass, in terms of dry matter (DM) (4.9 mg cm^{-2}) and chlorophyll *a* (11.5 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 (AFDM)) was 50% higher than that on the other two substrate types. Maximum periphyton productivities 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 few 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 2.2–2.8 g AFDM m^{-2} d^{-1}, periphyton alone can sustain an estimated fish production of 5000 kg ha^{-1} $year^{-1}$ through the addition of a substrate area equivalent to 100% of the pond surface area.

Résumé

Effets de substrats artificiels sur la productivité et la qualité de l'eau, et conséquences sur l'aquaculture en eau douce, basée sur le périphyton. Nous avons évalué les effets de différents substrats artificiels sur le développement du périphyton et sur la qualité de l'eau, en tant que première étape pour établir la viabilité, en Asie méridionale, de la production de poissons, basée sur le périphyton en bassin d'aquaculture. Des bassins en terre (10 × 7,5 m) ont été aménagés avec un substrat artificiel formé, soit de perches de bambou, soit de kanchi ou de branches d'arbre d'hizol (1,0 m^2 de substrat artificiel m^{-2} de surface de bassin). Des biomasses de périphyton étaient plus importantes, en terme de matière sèche (4,9 mg cm^{-2}) et de chlorophyll *a* (11,5 μg cm^{-2}) développées sur hizol et bambou, respectivement. Le périphyton était plus élevé sur hizol (41 %) que sur les deux autres types de substrat (29 %). Le contenu protéinique du périphyton se développant sur du bambou (38 % de la matière sèche libre de cendre) était de 50 % plus élevé que celui des deux autres types de substrat. Les maximums de productivité de périphyton 1,01, 1,38 et 1,03 g C m^{-2} j^{-1} ont été obtenus pour le bambou, l'hizol et le kanchi, respectivement. La composition taxonomique du périphyton a montré un rapide développement d'une communité relativement stable avec de petites différences entre les types de substrat. Au total, 56 genres de périphyton algal et 35 genres d'algues phytoplanctoniques ont été identifiés. Basé sur une estimation de la productivité du périphyton de 2,2 à 2,8 g de matière sèche libre de cendre m^{-2} j^{-1}, le périphyton peut soutenir, seul, une

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PII: S0990-7440(02)01179-8

production de poisson de 5000 kg ha^{-1} an^{-1} par l'addition d'une surface de substrat équivalente à 100 % de la surface de bassin.

Keywords: Periphyton; Productivity; Proximate composition; Tropical aquaculture

1. 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 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). Improving the conversion of nutrients into harvestable products, through adoption of periphyton-based production into existing pond systems, is one solution worth exploring.

Periphyton is defined here as the entire complex of all sessile biota attached to the substratum, plus associated detritus and micro-organisms. The idea is originally derived from traditional fishing methods, 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 tons ha^{-1} $year^{-1}$, eight 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; Keshavanath et al., 2001). 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.

A programme of systematic research on the potential of periphyton-based aquaculture systems has been initiated under the framework of an EC DG XII-funded regional project. 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.

2. Materials and methods

2.1. Pond facilities and design

The field trial was carried out in 12 earthen ponds (10×7.5 m, mean water depth 1.2 m) at the Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, over a 6 week period between May and July 1998. Three substrate types plus one control were evaluated in triplicate using a complete randomized design.

2.2. 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 in length 1 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 nine poles per m^2, yielding a total submerged substrate area of 74.2 m^2 per pond, approximating that of the pond 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.

2.3. 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 to 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 3000, 100 and 100 kg ha^{-1}, respectively, was started immediately after pond filling and maintained throughout the experimental period.

2.4. Determination of periphyton biomass

Starting 1 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, 1998). From each pond, three poles were selected by random number tables and two 2×2 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 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 DM samples were too small to allow reliable determination of ash content, 2×2 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 Fish Culture and Fisheries Group of Wageningen University and Research Center (WUR), Netherlands.

The other sample was used to determine chlorophyll *a* and pheophytin *a* content following standard methods (APHA, 1998). Upon removal, the material was immediately transferred to labeled tubes containing 10 ml 90% acetone, which were 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 2000–3000 rpm. The supernatant was carefully transferred to 1 cm glass cuvettes and absorption measured at 750 and 664 nm. 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 s acidification. Chlorophyll *a* and pheophytin *a* concentrations and AI (AFDM/Chl-*a*) were calculated using the equations given in (APHA, 1998).

2.5. Study of taxonomic composition of periphyton and plankton

In addition to the two samples taken from four depths, an extra 2×2 cm^2 periphyton sample was collected from each sampled pole at a depth of 25 cm for determination of the periphyton community composition. Samples were collected on a weekly basis starting after 1 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 1000 squares, upon which the number of colonies (algae) or individuals (invertebrates) was 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 is the number of periphyton cells or units per cm^2 surface area; P, the number of periphytic units counted in ten fields; C, the volume of final concentrate of the sample (ml); and S, the 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 45 μm). The concentrated samples were preserved in small plastic bottles with 5% buffered formalin. Plankton numbers were estimated using an S-R cell. Concentrated sample (1 ml) 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 planktons 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 is the number of plankton cells or units per l of original water; P, the number of plankton counted in 10 fields; C, the volume of final concentrate of the sample (ml); and L, the volume (l) of the pond water sample. Identification of plankton to genus level was carried out using the keys mentioned above for periphyton.

2.6. Analysis of proximate composition and energy content of periphyton

Due to 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, a sample as small as 1 mg DM can be used to determine the proximate composition of the AFDM. On the last sampling day, 70% of the DM samples were used for determination of ash content. The remainder (2–8 mg) of the DM as well as ash fractions were used to determine the CHN content in a CHN analyzer. Each sample was used in triplicate for CHN analysis. The CHN content of the DM samples was corrected for ash fractions according to equation (1) in Gnaiger and Bitterlich (1984):

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

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

2.7. 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 09:00 and 10:00 hours 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 analysed using a HACH kit (DR 2000).

2.8. Statistical analyses

Daily and weekly water quality parameters were compared by split-plot ANOVA 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):

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

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

Periphyton DM and pigment parameters (means of three poles per pond) were analysed 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:

$$Y_{ijkl} = \mu + S_i + e_{ij} + D_k + e_{ijk} + T_l + (S \times T)_{il} + (D \times T)_{kl} + (S \times D \times T)_{ikl} + e_{ijkl}$$

in which Y_{ijkl} is the observed value; μ, the overall mean; S_i, the effect of substrate type ($i = 3$); e_{ij}, error 1 ($j = 3$ replicates); D_k, the effect of depth ($k = 4$); e_{ijk}, error 2; T_l, the effect of sampling date ($l = 6$); $(S \times T)_{il}$, the interaction of substrate type and sampling date; $(D \times T)_{kl}$, the interaction of depth and sampling date; $(S \times D \times W)_{ikl}$, the interaction of substrate type, depth and date; and e_{ijkl}, error 3.

Again, DM and chlorophyll *a* were analysed within each substrate type separately in a split-plot design with depth as the main factor and sampling dates 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 AI were not considered for ANOVA because of small sample numbers. Periphyton and plankton taxonomic data were analysed by Systat 5.0, using the non-parametric Kruskal–Wallis test. The assumption of normal distributions and homogeneity of the variances was checked before analyses. In the 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.

3. Results

3.1. 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 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 3 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

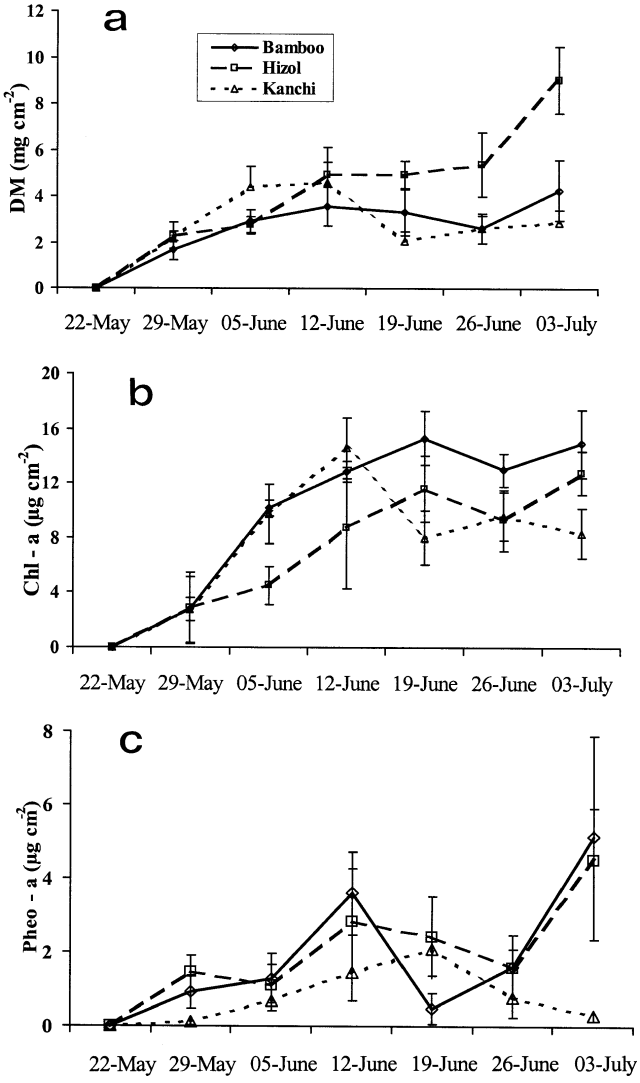

Fig. 1. DM contents (a), chlorophyll *a* (b) and pheophytin *a* (c) concentrations of periphyton grown on different substrates during the experimental period. Values (± S.E.) are means of four depths, three poles and three ponds per substrate type ($n = 36$).

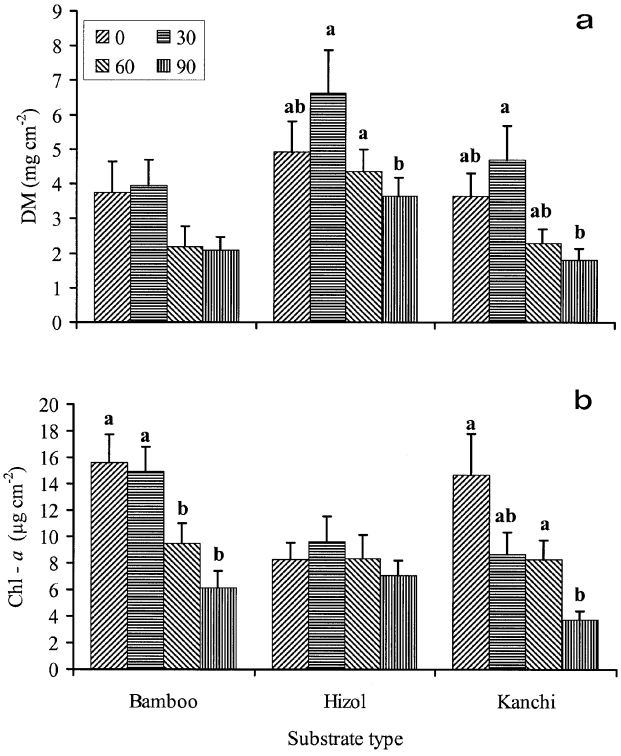

Fig. 2. DM contents (a) and chlorophyll *a* concentrations (b) of periphyton grown on different substrates along the different depths of the substrate from the water surface. Values are means (± S.E.) of three poles, three ponds and six sampling dates per substrate type ($n = 54$). If the main effects 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.

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}) (Fig. 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 (Fig. 1a). There were significant variations ($P < 0.01$) in DM contents among different depths of the hizol and kanchi substrates (Fig. 2a). The differences occurred between 30 and 90 cm deep 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 4 weeks for bamboo and hizol and during the first 3 weeks for kanchi; thereafter they levelled off (Fig. 1b). Mean chloro-

Table 1
Mean values (± S.E.) of periphyton biomass. Figures are means of samples from four depths, three poles, three ponds and six sampling dates ($n = 216$) for DM, chlorophyll *a* and pheophytin *a* and only six sampling dates ($n = 6$) for AFDM, Ash content and AI for each combination of substrate

Parameters	Substrate types		
	Bamboo	Hizol	Kanchi
DM (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
AFDM (mg cm^{-2})	2.17 ± 0.15	2.87 ± 0.23	2.22 ± 0.33
AI	189 ± 62	346 ± 62	251 ± 62

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 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)	Carbohydrate (% 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

phyll *a* during the second half of the experiment was higher on bamboo than on kanchi (interaction of time and substrate; Fig. 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 depths of 0 and 30 cm in comparison with 60 and 90 cm in bamboo substrate (Fig. 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 3 weeks for bamboo and hizol and during the first 4 weeks for kanchi; then they decreased (Table 1; Fig. 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 ($41 \pm 5\%$) was higher than on either bamboo or kanchi (29 ± 1–3%) during the entire experimental period (Table 1). The peak value was found during week 4 on bamboo and hizol substrates, whereas on kanchi 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) and decreased with time (Table 1). However, no statistical analysis was performed for ash, AFDM and AI because samples from replicated ponds were pooled during the laboratory analysis.

3.2. 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 g^{-1} 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.

3.3. 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. Chlorophyceae were most abundant (168–223×10^3 cells or colonies per cm^2) and most specious (29 genera, six rarely occurred) on all substrates, followed by Bacillariophyceae (97–156×10^3 cells or colonies per cm^2; 13 genera, three rarely occurred), Cyanophyceae (102–146×10^3 cells or colonies per cm^2; 10 genera) and Euglenophyceae (21–$29 \times$ cells or colonies per 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 *Nitjschia* 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). 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 (72–73×10^3 cells or colonies l^{-1}) whereas the Chlorophyceae were the most dominant group in hizol and control ponds (63–66×10^3 cells or colonies l^{-1}).

Table 3
Mean values of daily water quality parameters. Values are means of three replicates and 44 sampling dates (n = 132). The range of observed values is given in parentheses

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

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.

3.4. Water quality parameters

Means (and ranges) of daily monitored water quality data by substrate and control ponds are given in Table 3. 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 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.

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 4). 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 2 weeks. Nitrate fluctuated between 1 and 4 mg l^{-1} with higher values during the last 2 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 4 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.

4. Discussion

4.1. Periphyton productivity

Periphyton biomass as measured by DM, AFDM and pigment concentrations, differed significantly between depths with higher values in the upper 0–60 cm in 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 content of DM, 1 mg chl-a per 65–85 mg DM; Reynolds, 1984; Dempster et al., 1993). Whereas, from the biomass increase of the periphyton during the first week of the experiment, when clean substrates were first

Table 4
Mean values of weekly water quality parameters. Figures are means of three replicates and seven sampling dates (n = 21). The range of observed values is given in parentheses

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

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 trials, 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 4). 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 (Fig. 1a), possibly because of the inadvertent inclusion of hizol bark in the periphyton samples. 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 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 few differences between substrate types.

4.2. 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 in fish nutrition terms (Yakupitiyage, 1993). Huchette et al. (2000) reported similar periphyton ash contents derived from cages. 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 compare well with some other vegetative materials used in aquaculture (Hepher, 1988; Yakupitiyage, 1993; Dempster et al., 1995). In our other experiments, ash (12–68%) and protein (22–26% DM) contents were found to be highly variable based on fertilization level (Azim et al., 2001b) and grazing pressure by stocking density and combination of fish (Azim et al., 2001c; Azim et al., 2002a). 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 DM basis for planktonic algae in ponds. 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 a higher amount of algae colonized the bamboo substrate than the hizol and kanchi (APHA, 1998). 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 AIs fluctuating between 150 and 300 in ungrazed conditions and remaining stable at around 300 in grazed conditions, suggesting a highly heterotrophic community. 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 to 85 mg algal AFDM (Dempster et al., 1993; Reynolds, 1984; APHA, 1998), 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. This confirms the importance of periphyton mats for attracting heterotrophs and trapping organic matter.

4.3. Effects of artificial substrates on water quality

Water temperature at both pond surface and bottom was within the optimal range for 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. 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 3). This may be an indication of reduced water mixing due to the presence of the substrates, but the difference seems not important. However, diel

fluctuations of DO were not measured in the present experiment which could give a better picture. Because, night-time oxygen concentrations seemed to go beyond the acceptable range. 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 3 weeks of the experiment, despite the fact that the 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.

4.4. The potential of periphyton-based aquaculture systems

Filter-feeding on small planktonic algae may not fully meet the energy requirements of most herbivorous fish tilapia species (Dempster et al., 1995). Herbivorous fish and 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). Benthic algal mats rarely develop on pond bottoms in highly eutrophic ponds due to light limitation. They need some hard substrate in the euphotic layer of the ponds to grow which is generally 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 reef-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 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 to 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 consumed 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., 2002b). Based on a productivity estimate of 0.59–0.83 g protein m^{-2} d^{-1} (calculated from 2.17 to 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 4500–6300 kg ha^{-1} $year^{-1}$ from periphyton alone. Although this is a rather bold extrapolation for a complex pond ecosystem, this figure is indeed comparable to the results from other studies. A maximum production of 8000 kg ha^{-1} $year^{-1}$ 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 3390 kg ha^{-1} $year^{-1}$ in a polyculture with rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*) of which sugarcane baggase (used as substrate) contributed about 1343 kg ha^{-1} $year^{-1}$. In a monoculture trial, Azim et al. (2001a) recorded a total production of rohu of 5800 kg ha^{-1} $year^{-1}$, of which 2520 kg ha^{-1} $year^{-1}$ was contributed by periphyton grown on bamboo substrates. In another polyculture trial with rohu and catla (*Catla catla*), Azim et al. (2002) reported a net yield of 6700 kg ha^{-1} $year^{-1}$ in a periphyton-based system compared to a net yield of 2340 kg ha^{-1} $year^{-1}$ in the control system without substrate. According to them, an increased production of 4360 kg ha^{-1} $year^{-1}$ in the substrate-based system was achieved not only because of added substrate but also from synergistic interactions of the two 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.

5. Conclusion

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 5000 kg ha^{-1} $year^{-1}$. 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.

Acknowledgements

This research was funded through the European Community (Grant INCO-DC IC18 CT97 0196), and consists of a co-operation between partners in South Asia (India and Bangladesh) and Europe (UK and The Netherlands). We thank Professor E.A. Huisman for commenting on the draft manuscript.

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