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EVALUATION OF ORGANIC TILAPIA CULTURE IN PERIPHYTON-BASED PONDS

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Abstract

The introduction of hard surfaces in the water column to induce the growth of biofilms and periphyton on these surfaces is a method used to increase natural productivity of the water body and food for cultured aquatic organisms. In periphyton-based systems in Africa and Asia, substrate introduction and consequent periphyton development positively affected water quality and production of the target species. In Israel, this technology is being evaluated in the culture of organically produced tilapia. Among other restrictions imposed by organic standards, fish stocking densities must be low and only organic feeds and manures must be supplied. Organic pelleted feeds cost twice as much as regular aquaculture feeds. Since feed constitutes the major production expense, economic viability is hampered by using costly organic feeds. An experiment was performed at the Dor Aquaculture Station to explore methods of improving natural food production for tilapia and reducing added feeds. Submerged plastic surfaces equivalent to 40% of the pond surface area were immersed in polyculture ponds containing 85% hybrid tilapia *(Oreochromis niloticus* x *O. aureus)*, together with a reduction of 40% of the amount of pelleted feed. The treatment improved nitrification and saved 40% of the feed costs, with only a 10% reduction in the tilapia growth rate and yield. These results indicate that periphyton-based aquaculture is an appropriate technology for reducing production costs and allowing economically viable organic tilapia production.

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Introduction

Introduction of hard surfaces into the water column to induce the growth of biofilms and periphyton on these surfaces is used to increase the natural productivity in a water body and create food for cultured aquatic organisms. Periphyton-based systems have traditionally been used in Africa (Hem and Avit, 1994) and Asia (Wahab and Kibria, 1994) as a way to enhance fisheries in coastal lagoons. This technology was adapted for aquaculture in small lakes (Jamu et al., 2003) and ponds in the African rain forest where agricultural by-products to enhance the heterotrophic pathway are scarce or unavailable (Milstein, 1996; Sankare et al., 1997). Its application was recently expanded in Bangladesh and India, mainly in the polyculture of Indian carps, where introduction of the substrates had a positive effect on consequent periphyton development, production of the target species, and water quality (Beveridge et al., 1998; Wahab et al., 1999; Azim et al., 2001, 2002a; Keshavanath et al., 2001, 2002).

In Israel, this technology is being explored in organic tilapia culture. Among other restrictions imposed by organic standards (IFOAM, 2002, 2005; Naturland, 2004), fish must be stocked at low densities and only feeds and manures that comply with organic standards can be supplied to the fish. The cost of organic pelleted feeds doubles that of regular feeds. An experiment aimed at improving natural food production for tilapia while reducing added feed costs was performed in earthen ponds that met the requirements imposed by organic standards.

Materials and Methods

The experiment was conducted at the Dor Fish and Aquaculture Research Station in six earthen ponds of 300 m^2 area each, i.e., two treatments with three replicates each. A local well supplied water to fill the ponds and compensate for seepage and evaporation during the culture period, with no additional water from other sources. No spraying against weeds or insects was performed.

Fish were stocked on May 31, 2004, with the same polyculture composition in all ponds (Table 1). Fish were weighed fortnightly and the final harvest was on Oct. 31, 2004. The ponds were fertilized 5-6 times per week with dry chicken manure from organic henhouses at a rate of 20 kg/ha/day. Organic feed pellets were supplied to three ponds (treatment 'FEED'), initially at a rate of 2% of the tilapia biomass in the pond and gradually decreasing to 1%, to follow the organic criterion that at

Table 1. Fish stocking weight and density in 300 m^2 ponds.

	Weight (g)	*No./pond*	*No./ha*
Tilapia	90	360	12,000
Mullet	8.5	45	1,500
Grass carp	100	8	270
Hybrid carp[1]	133	7	230
Red drum & bass[2]	8.8	7	230
Common carp	600	5	170
Total		432	14,400

[1] silver carp (*Hypophthalmichthys molitrix*) x bighead carp (*Aristichthys nobilis*)
[2] predator species used to reduce wild spawning

least half of the food of the cultured fish should come from natural sources. Hard plastic surfaces equivalent to 40% of the pond surface (120 m^2) were introduced into the water column of the other three ponds (treatment 'PERIPHYTON") to promote periphyton growth, and only 60% of the amount of feed in the FEED treatment was given.

Small cages (1 m^3) with a mesh size of 1 cm^2 were constructed and placed in the periphyton ponds to allow evaluation of periphyton development without interference by fish grazing.

Water quality was monitored 1-2 times a week during early morning (about 6:00) for dissolved oxygen, ammonium and nitrite levels, pH, and temperature. Four times during the experiment, periphyton was sampled for microscopic inspection, chlorophyll *a*, and organic matter. At the same time, water was sampled for nitrates, phosphates, and chlorophyll *a*. During each sampling period, periphyton was taken from plastic strips installed vertically in the upper 60 cm of the water column in the open pond (3 strips) and in the small cage (3 strips) to compare the growth of periphyton in the open ponds exposed to fish grazing and in the small cages that were not exposed to fish grazing. The periphyton growing on a 150 cm^2 surface were collected to measure dry and organic matter (weight of matter after drying at 105°C and burning at 550°C). For chlorophyll determination, periphyton on 15 cm^2 was sufficient. The periphyton samples were collected from the entire surface of the plastic strip.

Data were analyzed through 2-way-ANOVA by treatment, time, and treatment*time interaction. Significant differences between treatments and time were tested with the Duncan mean multi-comparison test. Water quality parameters were analyzed using factor analysis to identify ecological processes in the pond responsible for the variability of water quality data (Milstein, 1993). From the several available techniques to extract factors, principal components calculated from the correlation matrix among variables were used. The first factor extracted from that matrix is the linear combination of the original variables, which accounts for as much of the variation contained in the samples as possible. The second factor is the second such function that accounts for most of the remaining variability, and so on. The factors are independent of one another, have no units, and are standardized variables (normal distribution, mean = 0, variance = 1). The coefficients of the linear functions defining the factors were used to interpret their meaning, using the sign and relative size of the coefficients as an indication of the weight to be placed upon each variable.

Results

Water quality was good in all ponds, with significant differences over time for temperature, pH, nitrite, and nitrate (Table 2). Treatment was a secondary significant source of variability for pH, which was higher in the FEED treatment, and for nitrite and nitrate which were higher in PERIPHYTON treatment.

Table 3 presents the results of factor analysis on water quality data and ANOVA results on the extracted factors. The first factor (Factor1) accounted for 36% of the overall data variability, showing a strong positive correlation between nitrite and nitrate, a strong negative correlation between them and ammonium (high coefficients), and a weaker correlation with chlorophyll, transparency, and phosphate (mid-size coefficients). The strongly correlated variables indicate nitrification, a process that reduces ammonium in the water to nitrite and nitrate. Nitrification was stronger when the phytoplankton biomass (indicated by chlorophyll *a*) and water transparency (Secchi) were low and the phosphates were high, that is when the microbial biomass, not the phytoplankton biomass, was high and the main cause of water turbidity. The ANOVA model attributed 79% of the nitrification variability ($r^2 = 0.79$) to this factor, most of which (71%) was due to changes over time following the pattern indicated in the mean multi-comparison section of the table. A secondary source of variability was due to treatment (15%), with higher nitrification in the PERIPHYTON ponds than in the FEED ponds.

The second factor (Factor2) accounted for a further 23% of the overall data variability,

Table 2. Results of ANOVA and Duncan mean multi-comparisons of water quality parameters.

	Temperature (°C)	*DO (mg/l)*	*pH*	*Secchi (cm)*	*Chlorophyll a (µg/l)*	*NH_4 (mg/l)*	*NO_2 (mg/l)*	*NO_3 (mg/l)*	*PO_4 (mg/l)*
ANOVA models									
Significance	***	ns	***	ns	ns	ns	***	***	ns
r^2	0.99	0.47	0.94	0.36	0.29	0.45	0.93	0.79	0.50
Variance source	*Sig. %*	*Sig. %*	*Sig. %*	*Sig. %*	*Sig. %*	*Sig. %*	*Sig. %*	*Sig. %*	*Sig. %*
Treatment	ns 0	ns -	*** 9	ns -	ns -	ns -	*** 11	* 8	ns -
Time	*** 99	* -	*** 86	ns -	ns -	ns -	*** 56	*** 80	* -
Treatment*time	ns 1	ns -	* 5	ns -	ns -	ns -	*** 33	ns 12	ns -
Mean multi-comparisons by treatment									
Feed	27.8 a	6.8 a	7.4 a_	40 a	141 a	0.25 a	0.08 _b	12.0 _b	0.11 a
Periphyton	27.8 a	7.7 a	7.3 _b	36 a	123 a	0.08 a	0.12 a_	14.1 a_	0.10 a
Mean multi-comparisons by time									
Jun 29	28.5 _b_	8.7 a_	7.4 _b_	47 a_	154 a	0.33 a_	0.06 _b	10.2 _b	0.07 _b
Aug 2	29.3 a__	7.9 a_	7.2 __c	34 ab	121 a	0.08 ab	0.08 _b	17.2 a_	0.23 a_
Aug 23	28.4 _b_	5.4 _b	7.2 __c	40 ab	123 a	0.25 ab	0.07 _b	9.5 _b	0.03 _b
Oct 4	25.2 __c	6.8 ab	7.7 a__	31 _b	130 a	0.00 _b	0.18 a_	15.2 a_	0.09 _b

Significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant
r^2 = coefficient of determination
% = percentage of total sums of squares
Mean multi-comparisons: same letters in a column indicate no significant difference at the 0.05 level. a>b>c.

showing a positive correlation between dissolved oxygen and chlorophyll, and a negative correlation between them and water transparency (Secchi). This represents phytoplankton biomass and photosynthesis, since a higher phytoplankton biomass results in a higher chlorophyll concentration, lower water transparency, and higher oxygen level. The ANOVA model was not significant, indicating that phytoplankton biomass and photosynthesis did not significantly change with time and treatment.

Periphyton strips had large amounts of calcium deposits near the pond surface that decreased with water depth. Chironomid larvae were more abundant near the bottom, decreasing toward the pond surface. Table 4 presents the ANOVA results of the chemical analyses of periphyton. Only a relatively small portion of the variability was accounted for by the model (about 35% for chlorophyll and organic matter and almost 60% for dry matter), with no significant differences due to sampling place (cage or open pond) and most of the variability explained by time (51% for chlorophyll, 82% for dry matter) or place*time interaction (60% for organic matter). The multi-comparison test showed that the calcium deposits were more abundant on June 29 and August 2, as reflected by the dry matter content. In the periphyton ponds, chlorophyll was lower in the open pond than in the cages from Aug 2, onwards, while dry and organic matter were lower in the open pond from August 23, onwards (Fig. 1), indicating increased fish grazing pressure on the periphyton when the fish were larger.

Microscopic observations of the periphyton showed the presence of filamentous algae, accumulation of algal cells around detritus particles, and a variety of free and colonial algal forms. The dominant groups included Chlorophyta of mobile (Chlamidomonacea) and static (e.g., Chlorella, Scenedesmus, Coelastrum, Crucigenia) species, diatoms (mainly small species of Pennales), and Cyanophyta (Oscillatoria filaments and colonies of Chroococcus). There were also dinoflagellates, protozoa (free and sessile forms such as Vorticella), rotifers of several species, and benthic organisms (nematodes, ostracodes). No qualitative differences were observed in periphyton composition between depths in the pond (near-surface, about 30 cm, and around 60 cm) or between strips located in the open pond or in the small cages protected from fish grazing. With time, the dominance of Cyanophyta colonies and ciliates increased, while the dominance of Chlorophyta and diatom species increased during the first month and decreased thereafter.

The results of fish performance are summarized in Table 5. Since two predator species (red drum and bass) were used to reduce wild spawning of the fish, the data for these species were pooled. Survival of all species was high and equal in both treatments. Tilapia weight at harvest, yield, and growth rate were about 10% lower in the PERIPHYTON treatment than in the control (FEED treatment); for the other species there were no differences between treatments. Tilapia weight logarithmically increased in all ponds (Fig. 2). When the feeding rate was 1.5-2% of the tilapia biomass (until Aug 10), the growth rate was around 2.5 g/day in the FEED treatment and almost 2 g/day (except for one higher point) in the PERIPHYTON treatment (Fig. 3). When the feeding rate was decreased to 1-1.2%, the tilapia growth rate decreased to about 1 g/day in both treatments. Food conversion ratio for all fish and for tilapia was about 30% lower in the periphyton ponds, while total yield did not significantly differ between treatments (Table 5).

Discussion

In conventional fishponds, the dominant autotrophic organisms are planktonic algae. The activity of planktonic algae occurs in the upper water layers while heterotrophic activity takes place mainly on the pond bottom. In periphyton-based aquaculture ponds, the addition of rigid surfaces into the oxygenated water column allows the development of attached autotrophic and heterotrophic populations, besides phytoplankton and bottom micro-organisms (Milstein, 2005). In conventional fishponds, the principal surface area for

Table 3. Results of factor analysis of water quality parameters, ANOVA, and Duncan mean multi-comparisons of the extracted factors. Factor coefficients in bold were used for interpretation.

	Factor1		*Factor2*	
DO	0.30		**0.63**	
NH_4	**-0.79**		-0.19	
NO_2	**0.70**		0.03	
NO_3	**0.88**		-0.18	
PO_4	0.43		-0.33	
Chlorophyll *a*	-0.45		**0.78**	
Secchi	-0.43		**-0.67**	
Variance explained	36%		23%	
Interpretation	Nitrification		Phytoplankton biomass & photosynthesis	
ANOVA models				
Significance	***		ns	
r^2	0.79		0.14	
Variance source	*Sig.*	*%*	*Sig.*	*%*
Treatment	**	15	ns	-
Time	***	71	ns	-
Treatment*time	ns	14	ns	-
Mean multi-comparisons by treatment				
Feed	_b		a	
Periphyton	a_		a	
Mean multi-comparisons by time				
Jun 29	_b		a	
Aug 2	a_		a	
Aug 23	_b		a	
Oct 4	a_		a	

Significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant
r^2 = coefficient of determination
% = percentage of total sums of squares
Mean multi-comparisons: same letters in a column indicate no significant difference at the 0.05 level. a>b>c.

nitrification is the sediment, where oxygen availability is a limiting factor. Placing substrates in the water column where oxygen is more available enhances nitrification (van Dam et al., 2002). In several studies in tanks (Langis et al., 1988; Ramesh et al., 1999; Bratvold and Browdy, 2001; Thompson et al., 2002) and fishponds (Azim et al., 2002b, 2004), lower ammonia concentrations in the water column were recorded in the presence of periphyton compared with controls that did not have substrates. This was attributed to the

Table 4. Results of ANOVA and Duncan mean multi-comparisons of parameters in periphyton ponds.

	Chlorophyll (µg/cm²)		*Dry matter (mg/cm²)*		*Organic matter (mg/cm²)*	
ANOVA models						
Significance	***		***		***	
r^2	0.33		0.58		0.38	
Variance source	*Sig.*	*%*	*Sig.*	*%*	*Sig.*	*%*
Place	ns	11	ns	0	ns	5
Time	***	51	***	82	***	35
Place*time	*	38	**	18	**	60
Mean multi-comparisons by place						
Pond	3.85	a	5.34	a	1.29	a
Cage	4.60	a	5.27	a	1.60	a
Mean multi-comparisons by time						
Jun 29	4.76	a_	9.31	a__	1.67	a_
Aug 2	5.12	a_	5.67	_b_	1.07	_b
Aug 23	3.02	_b	3.83	__c	1.87	a_
Oct 4	4.00	ab	2.65	__c	1.16	_b

Significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant
r^2 = coefficient of determination
% = percentage of total sums of squares
Mean multi-comparisons: same letters in a column indicate no significant difference at the 0.05 level. a>b>c.

stimulating effect of periphyton on nitrification. In the tank studies, the increased underwater surface for periphyton growth was 1-6 times larger than the tank water surface, while in the pond studies it varied 50-100% of the pond water surface. In the present study, the underwater surface was only 40% of the pond water surface, yet nitrification in the periphyton ponds was enhanced, as indicated by factor analysis, although differences in ammonia concentrations between treatments were insignificant.

Most of the pond studies on periphyton-based aquaculture were performed in extensive and semi-intensive ponds with an underwater surface for periphyton growth similar to that of the pond surface and using fertilization but no addition of feed. An exception is the work of Azim et al. (2004), who tested Indian carp polyculture production with underwater periphyton surfaces equal to 50, 75, and 100% of the pond water surface and no additional feed. They obtained combined fish production increases of 114, 168, and 209%, respectively, compared to control ponds without periphyton substrates. Based on their results, and taking into account the fact that feed was added in the present experiment, the amount of substrates used in our study was 40% of the surface area. Extrapolating the data of Azim et al. (2004) to the surface area utilized in our study, the addition of 40%

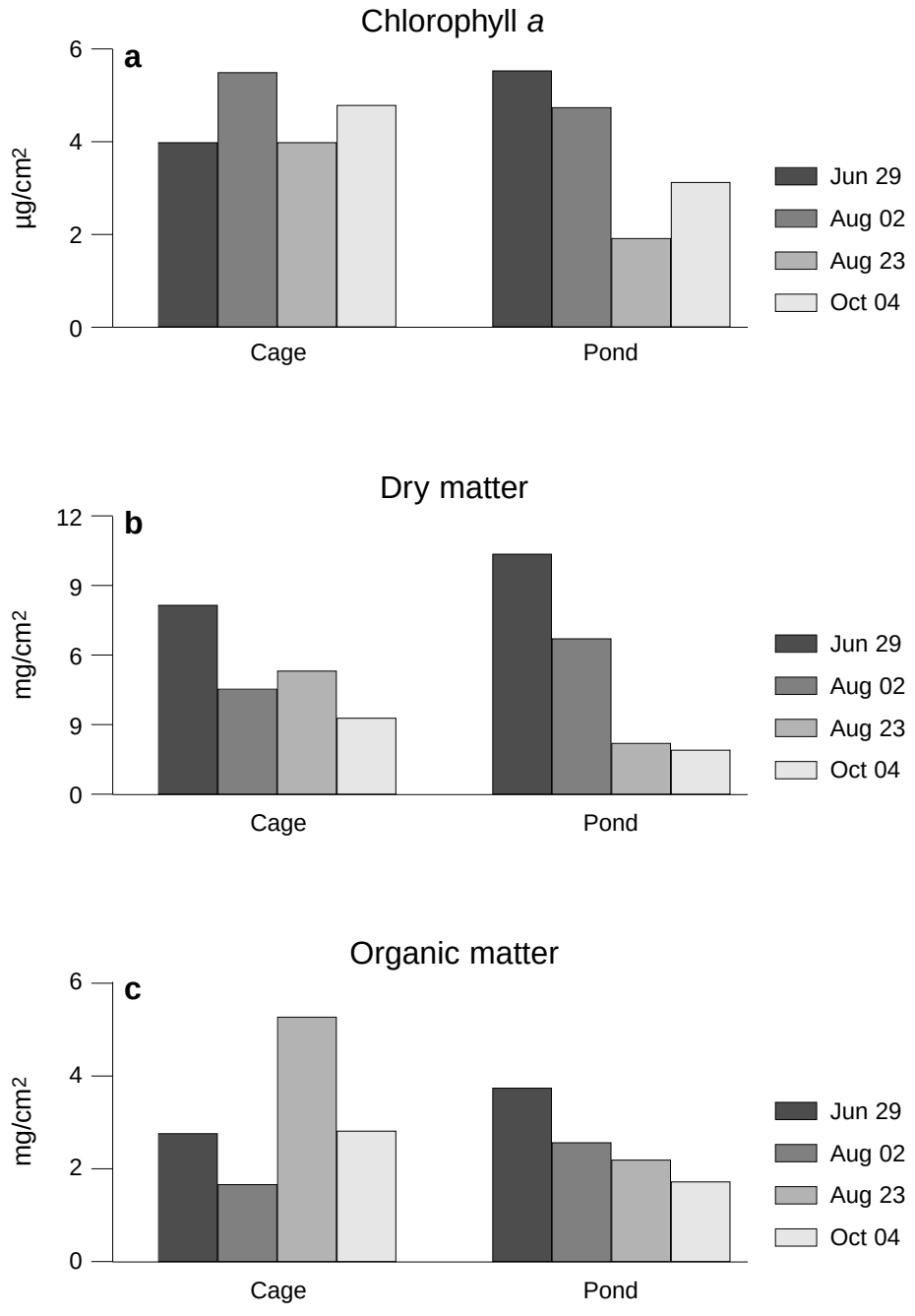

Fig. 1. Chlorophyll (a), dry matter (b), and organic matter (c) on plastic strips in the PERIPHYTON treatment. Some strips were exposed (pond) and some were not exposed (cage) to fish grazing. Data are shown by date and represent the place*time interaction of ANOVA in Table 4.

Table 5. ANOVA and Duncan mean multi-comparisons of fish production parameters.

	Harvest weight (g)	*Yield (kg/0.1 ha)*	*Survival#* *(%)*	*Growth (g/day)*	*Wild spawn (kg)*
ANOVA models for tilapia					
Significance	**	**	ns	**	ns
r^2	0.77	0.83	0.15	0.82	0.42
Mean multi-comparisons by treatment for tilapia					
Periphyton	329 _b	275 _b	96 a	1.77 _b	2.8 a
Feed	356 a_	310 a_	97 a	1.97 a_	1.0 a
ANOVA models for mullet					
Significance	ns	ns	ns	ns	
r^2	0.44	0.31	0.26	0.43	
Mean multi-comparisons by treatment for mullet					
Periphyton	294 a	41.0 a	96 a	2.1 a	
Feed	245 a	35.4 a	99 a	1.7 a	
ANOVA models for grass carp					
Significance	ns	ns	ns	ns	
r^2	0.02	0.02	0.55	0.02	
Mean multi-comparisons by treatment for grass carp					
Periphyton	300 a	5.03 a	96 a	1.50 a	
Feed	323 a	5.63 a	85 a	1.67 a	
ANOVA models for hybrid carp					
Significance	ns	ns		ns	
r^2	0.15	0.06		0.15	
Mean multi-comparisons by treatment for hybrid carp					
Periphyton	1257 a	26.2 a	100	8.53 a	
Feed	1392 a	27.8 a	100	9.53 a	
ANOVA models for red drum and bass					
Significance	ns	ns	ns	ns	
r^2	0.53	0.07	0.16	0.53	
Mean multi-comparisons by treatment for red drum and bass					
Periphyton	69 a	1.1 a	76 a	0.54 a	
Feed	102 a	1.3 a	62 a	0.85 a	
ANOVA models for common carp					
Significance	ns	ns	ns	ns	
r^2	0.02	0.31	0.50	0.05	
Mean multi-comparisons by treatment for common carp					
Periphyton	2144 a	27.7 a	100 a	11.9 a	
Feed	2199 a	24.5 a	87 a	12.6 a	

Table 5. Con't.

	Total yield (kg/0.1 ha)	*FCR (all fish)* #	*FCR (tilapia only)* #
ANOVA models			
Significance	ns	*	*
r^2	0.53	0.77	0.77
Mean multi-comparisons by treatment			
Periphyton	375 a	0.39 _b	0.53 _b
Feed	404 a	0.63 a_	0.82 a_

Significance levels: * = 0.05, ** = 0.01, *** = 0.001, ns = not significant
r^2 = coefficient of determination
Multi-comparison test performed on transformed data.
Mean multi-comparisons: same letters in a column indicate no significant difference at the 0.05 level. a>b>c.

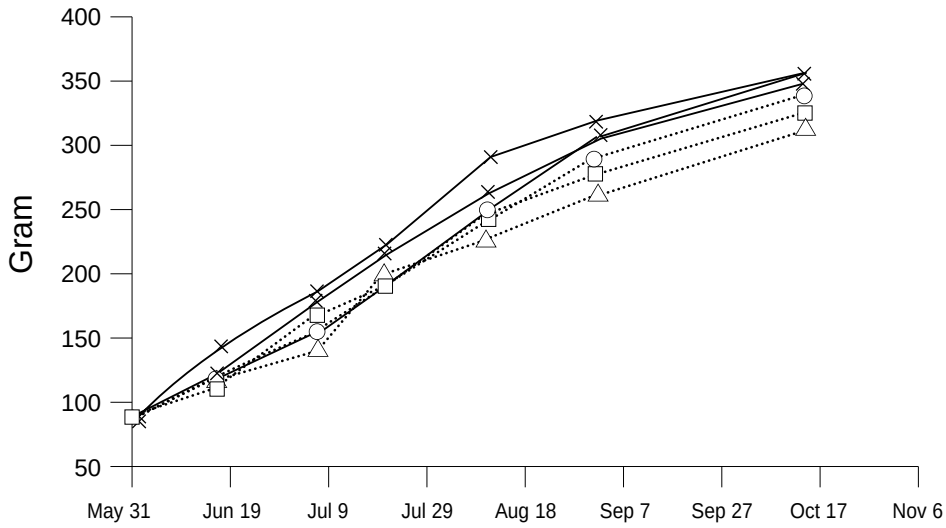

Fig. 2. Tilapia weight in each pond throughout the culture season. Thin lines - ponds without underwater substrates that received 100% feed ratio. Dashed lines - ponds with underwater substrates that received 60% of the feed ratio.

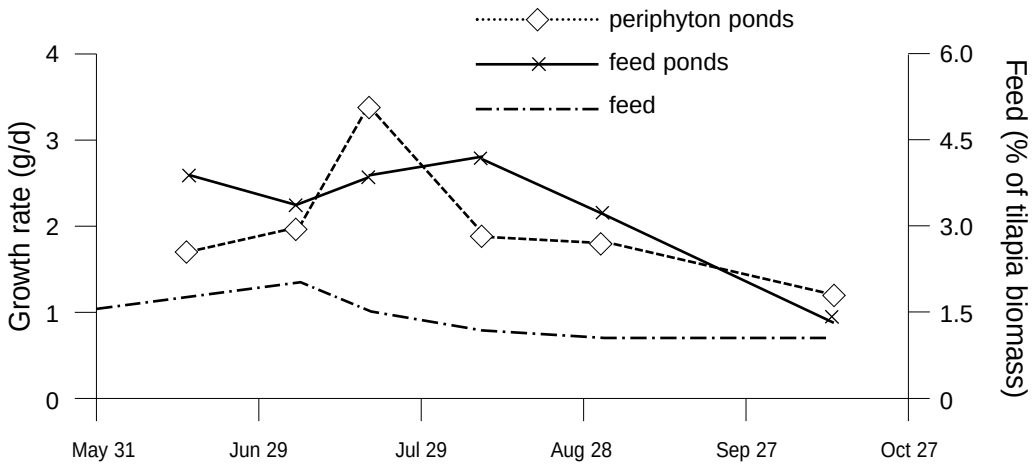

Fig. 3. Tilapia growth rates (means of three ponds per treatment) and feeding rates throughout the culture season.

underwater substrate area would produce a 10% increase in fish yield. Accordingly, if in the present experiment all ponds had been supplied the same amount of pelleted feeds, 10% higher yields would have been obtained in the periphyton ponds. This may account for the finding that a reduction in 40% of the feed in the periphyton ponds resulted in only 10% lower fish yields.

Following rules for organic tilapia farming in Israel, the feeding rate in the study (1-2% of the biomass per day) was notably lower than in conventional aquaculture (2-3%). This feeding rate was chosen since, according to organic standards, it is expected that at least 50% of the fish yield would be obtained via natural food (Naturland, 2004, section III-4.3). Although the fish density was about half that practiced in conventional ponds, the tilapia growth rate was low after Aug 10 when the daily feeding rate was less than 1.5% of the tilapia biomass, with a stronger negative effect in the FEED treatment. The decrease was related to the lower feeding rate and not to a lower temperature, since the temperature was consistently above 25°C.

Feed costs constitute one of the most expensive components in the running costs of aquaculture production and even more so in organic aquaculture due to specific requirements regarding use of only organic ingredients. Enhancement of natural food through the use of substrates in the ponds is an inexpensive alternative. Substrates can be very inexpensive and can include discarded plastic irrigation pipes, empty plastic bottles, or old leftover plastic sheeting such as used in the present experiment. Some labor is required to install the substrates yet, if they are reused in the succeeding culture cycle, they need not be removed from the pond. Even if new materials are used, 40% coverage will require an initial investment of roughly US$90 per 1000 m^2 of pond. Based on the encouraging results of the present study, an additional study is underway in which a more sophisticated substrate structure is being utilized and a comprehensive economic evaluation will be done.

In conclusion, the use of underwater substrates to allow periphyton development on these surfaces as a method of increasing natural food resources for tilapia is an appropriate technology for organic tilapia culture. Such a system allows a decrease in feed

inputs and reduction of costs. The addition of underwater substrates equivalent to 40% of the pond surface coupled with a 40% reduction of the amount of feed supplied to polyculture ponds containing 85% hybrid tilapia and small amounts of other fish species improved the tilapia feed conversion ratio by 30% while reducing the tilapia harvest weight, growth rate, and yield by only 10%. The relationship between substrates for periphyton and the reduction of feed requires optimization. For the tested stocking density (1.2 tilapia /m^2; 1.5 total fish/m^2), the daily feeding rate should be at least 1.5% of the tilapia biomass to maintain a good growth rate (at least 2 g/day during the grow-out phase). These findings are important for the establishment of organic standards of tilapia pond culture, some of which are still in the draft stage.

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