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UTILIZATION OF PERIPHYTIC NATURAL FOOD AS PARTIAL REPLACEMENT OF COMMERCIAL FOOD IN ORGANIC TILAPIA CULTURE - AN OVERVIEW

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ABSTRACT

This article summarizes the results obtained during five years of research at the Dor Fish and Aquaculture Research Station on partial replacement of commercial food by periphytic natural food in the culture of organic tilapia (hybrid *Oreochromis aureus* (Steindachner) x *Oreochromis niloticus* (L.)). Tilapia culture experiments were conducted in earth ponds with and without substrates, utilizing different substrates. Tilapias of sizes ranging from nursery to market-size fish were tested. Fish were stocked at densities common in organic fish culture, i.e. 5 tilapia/m^2 at the nursery stage, 1.2-1.4 tilapia/m^2 at the grow-out stage. Substrate experiments were carried out in 1 m^3 cages protected from fish grazing to test growth of periphyton on materials with different characteristics. The findings show that the inclusion of substrates in the water body at an amount equivalent to 40-50% of the pond water surface, allows the reduction of commercial food input by 30-40% without significantly hampering fish growth rate. It is recommended to use rough, rigid, white substrates, on which periphyton growth of 2 g dry matter/m^2/day has been measured. A figure and a table are provided as a tool to estimate periphyton contribution to the fish food ration, enabling the adjustment of the remaining daily food portion to be supplied as fish biomass increases during the culture period. Applying this technology will save food and money in the culture of organic tilapia, and it can also be appropriate in the conventional pond culture of tilapia as a method to reduce feed costs and increase sustainability.

RESUMEN: Acuacultura en humedales: uso de alimento natural perifítico como reemplazo parcial de ración comercial en el cultivo de tilapia orgánica.

Este artículo resume los resultados obtenidos en cinco años de investigación en la Dor Fish and Aquaculture Research Station, sobre el reemplazo parcial de ración comercial por alimento natural perifítico en la cría orgánica de tilapias (híbrido de *Oreochromis aureus* (Steindachner) x *Oreochromis niloticus* (L.)). Experimentos de cría de tilapias se llevaron a cabo en estanques de tierra con y sin substratos, utilizando diferentes substratos, con tilapias de diferentes tamaños (desde alevinos a tamaño comercial) a densidades practicadas en cría orgánica (5 alevinos/m^2, 1.2-1.4 juveniles/m^2). Experimentos para medir crecimiento de perifiton sobre substratos de diferentes características se llevaron a cabo en ausencia de peces en jaulas de 1 m^3. Los resultados indican que incluir substratos en el agua en una cantidad equivalente a 40-50% del área del estanque permite reducir la cantidad de ración en 30-40%

sin entorpecer significativamente la tasa de crecimiento de las tilapias. Recomendamos usar substratos rugosos, rígidos y blancos, sobre los cuales hemos medido un crecimiento de perifiton de 2 g/m^2/día de materia seca. Incluimos una figura y una tabla como herramienta para estimar la contribución del perifiton a la dieta de las tilapias y ajustar en consecuencia la cantidad de ración faltante para completar la demanda diaria a medida que la biomasa de peces aumenta durante el periodo de cría. Esta tecnología ahorrará ración y dinero en la cría de tilapia orgánica, y también puede ser apropiada como método de reducir costos en la cría convencional de tilapias en estanques.

REZUMAT: Acvacultura în zone umede: utilizarea de hrană naturală perifitică, ca substitut pentru hrana comercială în creşterea de tilapia organic - o vedere de ansamblu.

Acest articol rezumă rezultatele obţinute în cinci ani de cercetare efectuată la Dor Fish and Aquaculture Research Station asupra înlocuirii parţiale a hranei comerciale cu hrană naturală perifitică în creşterea de tilapa organic (hibrid *Oreochromis aureus* (Steindachner) x *Oreochromis niloticus* (L.)). Experimentele de creştere a tilapiei s-au desfăşurat în iazuri nebetonate cu diferite tipuri de substrat şi fără substrat. Au fost testate exemplare de tilapia de mărimi diferite, de la alevini la peşti de dimensiune comercializabilă. Peştii au fost ţinuţi la densităţile recomandate în piscicultura organică, adică 5 tilapia/m^2 în bazinele de creştere, 1.2-1.4 tilapia/m^2, la vârstă adultă. Experimentele de substrat au avut loc în cuşti de 1 m^3, protejate de consumul de către ierbivori, pentru a testa creşterea perifitonului pe diferite materiale, cu diferite caracteristici. Rezultatele au arătat că introducerea substratelor în acvatoriu într-un raport de aprovimativ 40-50% faţă de luciul de apă, duce la reducerea raţiilor de hrană comercială cu aprovimativ 30-40% fără a afecta semnificativ rata de creştere a peştelui. Se recomandă folosirea de substrate aspre, rigide, de culoare albă, pe care perifitonul creşte până la 2 g masă uscată/m^2/zi. Pentru estimarea contribuţiei perifitonului, la raţia zilnică a peştelui, au fost prezentate o figură şi un tabel ce permit ajustarea porţiei zilnice rămase de hrană comercială, pentru a permite creşterea biomasei piscicole pe durata culturii. Prin aplicarea acestei tehnologii crescătorii de tilapia organic pot face economii de hrană şi de bani. De asemenea, tehnologia se pretează şi utilizării în iazuri piscicole convenţionale ca metodă de reducere a costurilor cu hrana şi pentru a creşte exploatarea durabilă a acestora.

INTRODUCTION

The introduction of hard surfaces into the water column of earthen fish ponds induces the growth of bio-films and periphyton on them. This improves the natural productivity of the water body, thus providing more food for cultured aquatic organisms able of using periphyton as food. Periphyton-based aquaculture systems function like artificial wetlands in which grazing pressure is increased according to the stocking density of the target organism. Stocking density has to be low enough to allow recovery of grazed periphyton and high enough to allow an economically viable aquaculture business. Thus, this technology is applicable in extensive and low density semi-intensive systems, including organic aquaculture (organic meaning those that comply with organic standards) in which low stocking density to ensure welfare of the target animals is a prerequisite.

The cost of food constitutes one of the most expensive components of the running costs of aquaculture production. This is even more pronounced in organic aquaculture due to the specific requirements, to use only organic food ingredients (IFOAM, 2009; Naturland, 2012). Thus, the cost of organic pelleted food is double the cost of regular commercial food used in aquaculture, hampering economic viability.

To cope with this problem in organic tilapia culture, two approaches were simultaneously researched. In the first approach, alternative relatively cheap, food pellet ingredients that comply with organic regulations and are available from organic sources in appropriate quantities, were tested as components of food pellets for organic tilapia culture. One such study researched mainly the effects of different levels of dietary salt supplementation on growth of tilapia hybrids as reported by Cnaani et al. (2010). The second approach in this respect included experiments in periphyton-based conditions, aimed at improving natural food production for tilapia in the ponds while concomitantly reducing the amounts of added food. This approach is in line with the organic culture philosophy and allows a reduction in production costs without negatively affecting fish growth.

Periphyton-based specific systems with no additional feeding have long been practiced in the African Continent (Hem and Avit, 1994) and Asia (Wahab and Kibria, 1994), mainly using bamboo and other locally available natural substrates. In those regions, a positive effect of substrate introduction and consequent periphyton development, on the production of the target species and on water quality has been observed (van Dam et al., 2002).

Bamboo is not readily available in Israel geographical area and the labor required to collect and install other possible natural substrates is prohibitively expensive. Therefore, synthetic substrates were used to evaluate this technology in the culture of organically produced tilapia. The present article summarizes the results obtained during five years of research; some of the data has already been published in detail (Milstein et al., 2005, 2008a, 2008b, 2009).

MATERIALS AND METHODS

Five specific experiments were carried out in 6-12 earthen ponds of a 300 m^2 area and water depth of 1 m at the Fish and Aquaculture Research Station Dor, with the tilapia hybrid *Oreochromis aureus* (Steindachner) x *Oreochromis niloticus* (L.). This type of hybrid is the major commercially cultured tilapia in organic and conventional farms in Israel geographic area. The different specific experiments tested tilapia performance in “periphyton + reduced feed” ponds (Periphyton) in relation to conventional (Control) ponds, for tilapias at different stocking sizes utilizing different substrates for periphyton development. The characteristics of the five tilapia culture specific experiments are shown in table number 1. In all done experiments three ponds were allocated to each treatment or control.

The treatments consisted of the addition of underwater surfaces equivalent to 30-50% of the pond surface area, while simultaneously reducing the amount of pelleted food supplied to the fish by 30-40%. The substrates used and their location in the water column varied in each experiment. As an example, figure number 1 shows one of the experimental ponds with substrates located in the epilimnion before the pond was completely filled with water. In the control ponds no underwater substrates were added and the full amounts of organically certified floating food pellets were supplied. The food amounts supplied in the control ponds were lower than the quantities used in conventional pond culture, because in organic culture a considerable part of the growth of the organisms must originate from natural foods (Naturland, 2012). Except for the nursery experiment (experiment 2) in which only tilapia was stocked, in all other experiments a polyculture system was used.

Table 1: Characteristics of the pond experiments and results (average values); # when differences between treatments were significant, the values shown represent averages in Periphyton / Control ponds, * plastic strips forming "honeycomb" used to avoid erosion in road side slopes, ** used in agriculture, *** plastic bags originally containing fish food, **** shown in figure 1, ***** arrow indicates decreasing feeding rate during culture period.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Number of ponds	6	6	6	12	6
Culture duration (days)	135	101	87	116	142
SUBSTRATES					
Materials	plastic sheets	strips *	strips *	shadow nets ** and sheets ***	plastic nets ****
Amount (area relative to pond surface area)	40%	50%	50%	30%	38%
Placement	epilimnion	water column	water column	epilimnion	epilimnion
Texture	smooth	smooth	smooth	rough and smooth	rough
Color	transparent	black	black	white	white
Rigidity	rigid	rigid	rigid	flexible	rigid
FEED					
Protein (%)	32	35	30	30	35
Feeding rate in CONTROL ponds (% of tilapia biomass) *****	2% → 1%	5% → 2%	2%	2% → 1.5%	1.5%
Feeding rate in PERIPHYTON ponds (% of control ponds)	60%	60%	60%	66%	70%
TILAPIA#					
In the polyculture (%)	85	100	90	91	92
Stocking weight (g)	90	2.8	330	180	113
Stocking density (fish/m^2)	1.2	5	1.2	1.1	1.4
Stocking biomass (g/m^2)	108	14	396	193	158
Survival (%)	97	70	94	53	84
Harvesting weight (g)	329/356	80	510	500	290
Harvesting biomass (g/m^2)	380/413	300	576	235	330

Table 1: continued.

Growth rate (g/day)	1.77/1.97	0.75	2.00/2.35	3.16	1.21
Growth rate in Periphyton ponds relative to control ponds	-10%	same	-10%	same	same
Wild tilapia spawning relative to tilapia harvested biomass	2%	3%	15%	53%	40%
Stocking density of the predator fish red drum (fish/m^2)	0.02	0	0.10	0.05	0.05
FCR	0.5/0.8	1.1/2.0	2.4/3.6	2.7/4.0	1.1/1.6
FCR improvement in Periphyton ponds relative to control ponds	30%	45%	33%	32%	32%
Reported in	Milstein et al., 2005	Milstein et al., 2008a	Milstein et al., 2009	herein	herein

The treatments consisted of the addition of underwater surfaces equivalent to 30-50% of the pond surface area while simultaneously reducing the amount of pelleted food supplied to the fish by 30-40%. The substrates which were used and their location in the water column varied in each experiment. As an example, figure number 1 shows one of the experimental ponds with substrates located in the epilimnion before the pond was completely filled with water. In the control ponds no underwater substrates were added and the full amounts of organically certified floating food pellets were supplied. The food amounts supplied in the control ponds were lower than the quantities used in conventional pond culture, because in organic culture a considerable part of the growth of the organisms must originate from natural foods (Naturland, 2012). Except for the nursery experiment (experiment 2) in which only tilapia was stocked, in all other experiments a polyculture system was used. Fish stocked consisted of 85-92% hybrid tilapia, an omnivorous fish able to graze on hard surfaces, in combination with small quantities of the plant eating grass carp (*Ctenopharyngodon idella* (Valenciennes)), the phytoplankton filter feeder silver carp (*Hypophthalmychthys molitrix* (Cuvier and Valenciennes)) and the predator red drum (*Sciaenops ocellatus* (L.)). The latter was stocked to control wild spawning of tilapia. In each specific experiment the initial stocking weight of the tilapia individuals varied (from fingerlings to advanced juveniles), but the fish in all ponds in the same experiment had the same initial weight and density. Experiments lasted 3-5 months.

Substrate experiments

Three experiments were carried out in 1 m^3 cage placed in the tilapia culture pond experiments (Fig. 1), to test the growth of periphyton on materials with different characteristics. Strips of substrates were vertically placed in the epilimnion without touching or shading each other. All the strips used during each experiment were installed simultaneously. Sub-sets of substrates were removed at set sampling times to analyze chlorophyll and dry and organic matter attached on them. Each sub-set contained triplicates of each substrate tested. The removed substrates were not reused. Periphyton of all substrates was sampled from the same water depth and measurements were all standardized on a cm^2 basis.

Figure 1: Experimental systems; substrates for periphyton growth in the tilapia culture experiments and the cage in which the substrate experiments were performed; picture taken before the pond was completely filled with water.

In the first experiment, periphyton growth on eight substrates with different textures was tested, including plastic smooth surface sheets and agricultural nets of different mesh (fine and coarse mesh) and type of threads (round or flat) as rough substrates. In the second experiment, the effect of substrate colour on periphyton development was tested using nets of the same type, differing only in their colour (white, black or blue). In the third experiment, the growth rate of periphyton development on a white rigid rough plastic substrate was measured through sampling at short intervals during a 3 week test period.

In all experiments, periphyton was collected to determine chlorophyll-a (methanol extract technique), dry matter (DM) and organic matter as ash free dry matter (AFDM) (weight of matter remaining after drying at 105°C and after burning at 550°C, respectively). The periphyton on the plastic smooth substrates was scraped from a predetermined set area. Periphyton growing on the rough nets was not separated from the substrate for the chemical analyses. For dry and organic matter determinations, blanks of each net type were measured and reduced from the periphyton + substrate measurements.

Statistical analyses

Data were analysed using ANOVA. Differences between treatment levels were tested with the Scheffe mean multi-comparison tests, using a significance level of $P < 0.05$. The analyses were run using the SAS statistical package.

RESULTS

Tilapia culture experiments

Table 1 summarizes the results of the five fish culture experiments. Overall average experimental values are given when no significant differences between periphyton ponds and control ponds occurred, while averages in both treatments are provided when significant differences did occur. In each experiment there were no significant survival differences between treatments. In the periphyton ponds, providing 40% less food did not negatively affect fingerling performance in the nursery (experiment 2). In early juveniles grow-out from 90 g to 350 g and advanced grow-out from 320 g to 520 g, providing 40% less food led to a reduction of only 10% in tilapia's growth rate in relation to the control ponds (experiment 1 and experiment 3). This growth rate reduction did not result in significant differences between treatments in tilapia harvest weight and biomass when the culture period was short (87 days, experiment 3), while it did differ by 10% when the tilapia culture period was significantly longer (135 days; experiment 1).

In the last two experiments (4 and 5), large quantities of tilapia wild spawning occurred. This is problematic in a research experiment, but since wild spawning amounts were similar in all ponds of each experiment, comparisons between treatments can still be considered valid. In these experiments, substrate material was placed only in the epilimnion and more food was supplied to the periphyton ponds (food saving was reduced from 40% to 34% and 30% in experiment 4 and experiment 5, respectively). Under these conditions, even after a long culture period tilapia growth rate was not reduced and their performance was similar in periphyton ponds and in control ponds. In all the experiments similar or only 10% reduced tilapia performance together with the 30%-40% decrease in food amounts supplied to the periphyton ponds led to at least 30% improved food conversion ratio (FCR) in the studied periphyton ponds (45% in the nursery; experiment 2).

Substrate experiments

Results of the first substrate experiment, testing periphyton growth on eight substrates of different texture, and of the second experiment, testing the effect of the colour of the substrate on periphyton development on it, were reported in detail by Milstein et al. (2008b).

In the first experiment the amount of periphytic matter (measured as DM and AFDM) on fine nets more than doubled that on coarse nets (both rough substrates), which in turn about doubled the amount that developed on smooth plastic substrates. Chlorophyll was 60% higher on the fine mesh round thread net substrate compared with the coarse mesh flat thread net and the white flexible smooth surface plastic sheets, while other rough and smooth substrates were intermediate and not significantly different from either.

The second experiment showed that the colour of the substrate did not affect the chlorophyll content of periphyton but did affect its dry and organic matter content. The white substrate had 40% more DM and 50% more AFDM than the blue and black substrates.

In the third experiment, linear growth of periphyton on a white rigid rough plastic net substrate during 22 days was observed (Fig. 2). The regression lines of the chlorophyll, DM and AFDM calculated on the time scale (number of days submerged) were:

Chlorophyll (mg/m^2) = 2.97 day - 5.99
DM (g/m^2) = 1.98 day + 5.24
AFDM (g/m^2) = 0.31 day + 7.64

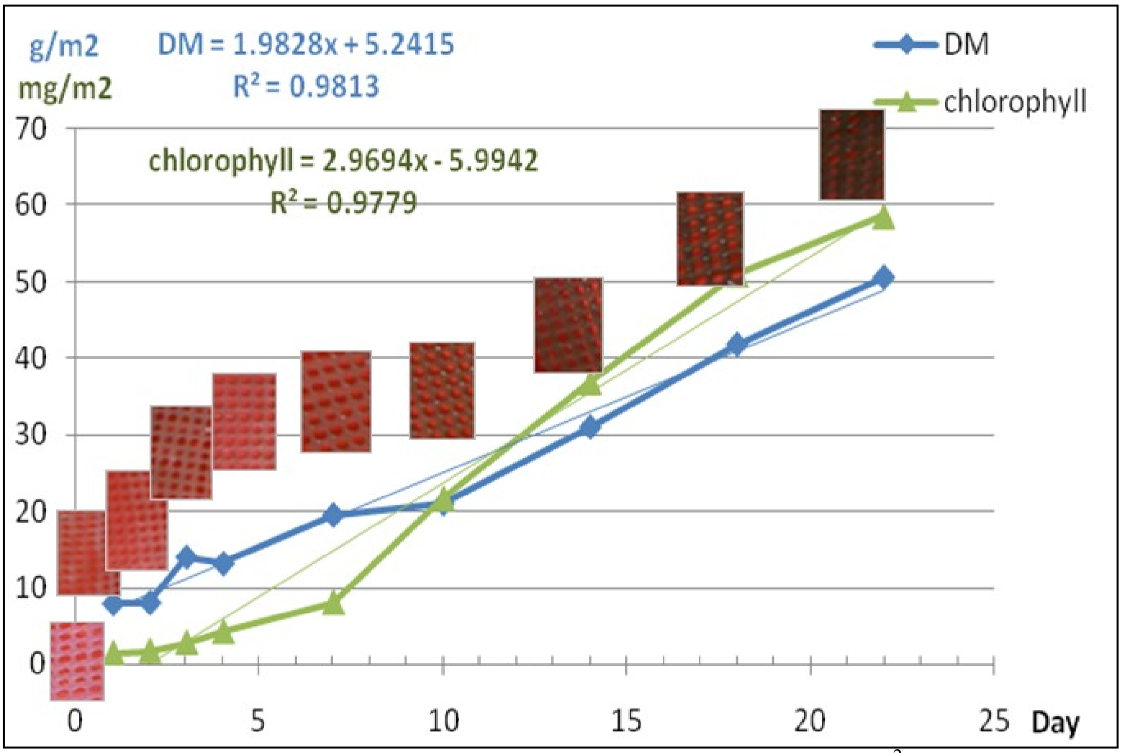

Figure 2: Substrate experiment 3. Increase of periphyton chlorophyll (mg/m^2) and dry matter (DM, g/m^2) in time; thick lines join the measurements, thin lines are the regression lines; the rectangles are photographs of the nets taken before starting the experiment (day 0) and at sampling days with periphyton grown on them.

The first two regressions had coefficients of determination r^2 = 0.98, and that of AFDM r^2 = 0.63. The equations show that periphyton increased daily by 3 mg chlorophyll, 2 g DM and 0.3 g AFDM per square meter of substrate.

DISCUSSION

Manipulation of natural food webs is a method to increase the productivity and efficiency of aquaculture production that conforms to the criteria of ecological and organic aquaculture. The provision of substrates to increase periphyton development on them as natural food for herbivorous and omnivorous aquaculture organisms has been tested with positive results in a range of species, culture systems and environments (van Dam et al., 2002; Azim et al. (eds), 2005; Azim and Little, 2006). Much of the periphyton-based fish culture research has been carried out using natural substrates (mainly bamboo), comparing the growth of the target organism with and without substrates, when commercial food was not supplied (Ramesh et al., 1999; Keshavanath et al., 2001; Milstein et al., 2003; Rai et al., 2008). Studies conducted in fish ponds comparing the effect of food supply versus periphyton, found that the provision of substrates can reduce the need for artificial food and can be an alternative to commercial food in the culture of herbivorous fish and prawn (Azim et al., 2002a; Keshavanath et al., 2002, 2004; Uddin et al., 2008, 2009; Garcia et al., 2011). This approach can be an ideal alternative in resource-limited regions in Asia, Africa and Latin America, where small-scale rural tilapia culture is commonly practiced (El-Sayed, 2006).

Under Israel national territory conditions, in which land is limited and costs (mainly of labor) are rather high, extensive aquaculture practice is not economically viable. The fish densities needed to attain an economically viable production surpass the natural food production capacity of earthen ponds. Under these conditions, food addition is required even at the reduced stocking densities demanded by organic regulations. Thus, the experiments presented herein were directed to partial (not total) replacement of commercial food by periphytic natural food. This, together with the use of artificial substrates that are easy to install, save labor and/or are cheap and available in large amounts at any farm.

The results obtained in the present experiments are that at least under the low tilapia density required in organic aquaculture, the use of substrates in the water body in an amount equivalent to 40-50% to the pond surface allowed a 30-40% reduction in food, while either none or only slightly negative effects were observed on the tilapia performance. Since the price of food ingredients is increasing worldwide with all indications that it will continue to increase, the implementation of periphyton-based aquaculture will save both food and money in tilapia organic culture. The partial substitution of food by periphyton allowed a sustainable more intensive fish production and can also be appropriate in conventional tilapia culture.

Another advantage of the periphyton technology is the reduction of economical losses when something might go wrong. For unknown reasons, tilapia culture experiment 4 experienced high mortality levels in all ponds, which at harvest was found to be around 50%. Thus, the amount of food given was in fact double than planned, which should have reduced competition for food and might account, at least in part, for the lack of differences between treatments. Still, the periphyton ponds received 34% less food than the control ponds, which in this case can be considered as a 34% reduction of economic losses.

Another example of reduction of economical losses in periphyton ponds is related to wild spawning. In organic cultures, hormones are not used for sex reversal, as a result large amounts of tilapia wild spawning might occur. To cope with this problem a predator fish can be stocked. If large amounts of wild spawning occur in spite of the predator fish presence in the pond, tilapia biomass will be higher than expected, hence feeding rate will be lower than planned, competition for food will increase and tilapia performance will be reduced. This occurred in experiment 5, where the losses related to low tilapia performance were similar in all ponds. However, the food for tilapia supplied by the periphyton that developed on the provided substrates compensated for 30% artificial food reduction, which can be considered as a 30% reduction of economic losses.

The third substrate experiment was done to evaluate the potential of periphyton supply to fish and to estimate the amount of substrate required to have effect on tilapia growth. Thus, periphyton growth rate was measured at short intervals in the absence of grazing fish. This was done in near surface waters where most of periphyton development takes place (Azim et al., 2001, 2002b). The few experiments found in literature that measured periphyton growth in the absence of grazing fish were based on combined samples or samples integrated through depths and/or time, measured at weekly intervals (Azim et al., 2001, 2002a, 2003; Keshavanath et al., 2001; Milstein et al., 2008b). Direct comparisons with our data are thus not possible.

The measured periphyton growth of about 2 g DM/m^2/day in summer in the third substrate experiment provides a rough estimation of the amount of substrate required to supply food at different rates (Fig. 3) and different biomass of tilapias (Tab. 2). Thus, to supply food at a rate of 0.5% of tilapia biomass per day (about a quarter of the daily fish requirements) 2.5 m^2 of substrate per tilapia kg in the pond are required. At this feeding rate this amounts to 250 m^2 of underwater surfaces feed 100 kg of tilapia. Since the surface of substrates installed in a pond is constant while tilapia biomass will change with fish growth (Tab. 2) and (Fig. 3), can be used to estimate periphyton contribution to fish ratio and adjust accordingly the remaining feed portion to be supplied as fish biomass increases during the culture period.

From the point of view of the organic fish farmer, a 10% saving in the artificial food costs, which can be reached with the addition of substrates, would already be an important achievement. Purchasing some materials (like those used in the tilapia culture experiments 2 and 3) to be used specifically as periphyton substrate may not be economically practical. On the other hand, recycled substrate materials can be very cheap and can include discarded plastic irrigation pipes, empty plastic bottles or old leftover plastic sheeting. The use of discarded agriculture shade nets, plastic feed sacks and other such materials (as in experiment 4) requires anchoring them in place when exposed to wind, otherwise it is not appropriate for re-use in the next culture cycle. Some labor is required to install the substrates, yet, if they are strong enough and can be reused in the following culture cycles they do not have to be removed from the pond. Substrates can be tied to poles stacked into the pond bottom (in shallow ponds) or hung from ropes fastened to the banks (in shallow or deep ponds). Considering that most periphyton development occurs in the epilimnion, the proper vertically installation of the substrates, only in the upper half meter of the water column would save material and money. Between the substrates there should be enough space for the fish to swim. Based on the growth results, it is recommended to use rough, rigid, white substrates.

Table 2: Underwater substrate area (m^2) required to supplement feed of different tilapia biomass (kg) at different daily feeding rates (% of fish biomass/day), estimated from the DM equation of the third substrate experiment.

	Feeding rate (% of biomass/day)		
Tilapia biomass (kg)	0.2%	0.5%	1%
50	50	125	250
100	100	250	500
250	250	625	1250
500	500	1250	2500
750	750	1875	3750
1000	1000	2500	5000

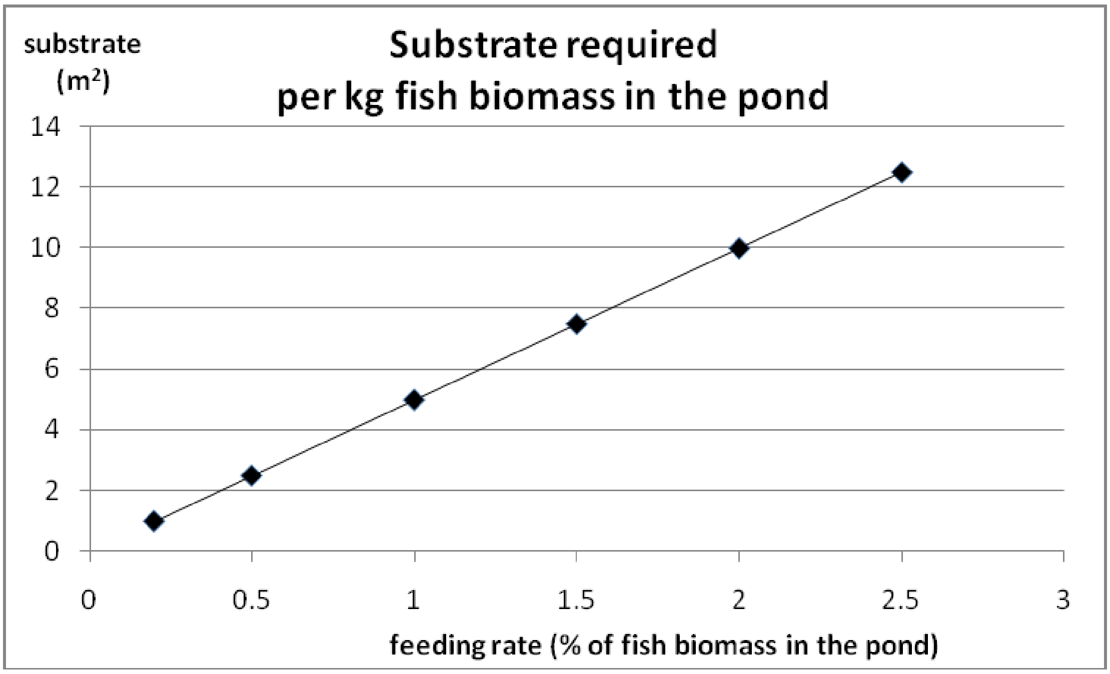

Figure 3: Substrate required per kg fish biomass in the pond under different feeding rates, estimated from the DM equation of the third substrate experiment.

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