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Comparison of Periphyton Grown on Different Substrates as Food for Organic Tilapia Culture

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Abstract

Regulations regarding organic tilapia culture result in increased feed costs. To reduce such costs, experiments were conducted to enhance the natural production of periphyton, on which tilapia feed. Strips of substrates of different textures and colors were placed in the water column of tilapia culture ponds to induce periphyton growth. Some strips were placed in cages to protect them from fish grazing and some where placed in the open pond, accessible to the fish. Periphyton development was evaluated by the contents of chlorophyll, dry matter (DM), and ash free dry matter (organic matter) and by an autotrophic index. The first experiment tested growth on substrates of different textures including natural (palm leaves) and artificial (agricultural nets, plastic surfaces) materials. The second experiment tested the effect of different colored nets. The differences between periphyton grown in cages and in the open pond indicate that tilapia grazed on the periphyton. Palm leaves decomposed too quickly to be of practical use in large-scale aquaculture. Periphytic material seemed to be more easily dislodged from smooth plastic substrates than from rough nets, changing the structure of the residual attached periphyton. Growth was greater on nets with a fine mesh (5.3-9.6 mg/cm^2 DM) than with a coarse mesh (3.7-4.0 mg/cm^2 DM) or on smooth plastic surfaces (1.4-2.6 mg/cm^2 DM). The color 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 dry matter (11.5 vs 7.9-8.2 mg/cm^2) and 50% more ash free dry matter (2.1 vs 1.4 mg/cm^2) than the blue and black substrates.

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Introduction

Organic fish culture is a relatively new and rapidly growing branch of aquaculture. Among restrictions imposed by organic standards (e.g., IFOAM, 2005; Naturland, 2004), fish must be stocked at low density and provided only certified organic feeds and manures that comply with organic standards. Organic feeds must contain at least 95% organically-certified components, a requirement that seriously limits the use of two main sources of protein in conventional aquaculture feeds: soy meal, because most soybeans are genetically modified, and fishmeal, since most fishmeal fisheries are not sustainable and have inappropriate records. The use of strictly organic products doubles feed costs, compromising the economic viability of organic fish culture.

To reduce production costs under the imposed restrictions, a series of experiments were conducted in earthen ponds aimed at enhancing natural food production for tilapia. Substrates were introduced into the water column in tilapia culture ponds for bio-films and periphyton to grow upon and upon which fish, in turn, could feed. Substrates to induce periphyton growth as food for cultured fish are used in subsistence farms in Asia and Africa where access to expensive feeds is limited and agricultural by-products to feed fish are scarce or unavailable (Milstein, 1996; Sankare et al, 1997). This technology is being tested in Israel in organic tilapia culture where fish are stocked at low densities that allow recovery of grazed periphyton. Such technology is in accordance with the organic philosophy of favoring the recycling of materials and enhancement of natural processes. Periphyton-based aquaculture could be more widely used in the near future. The FAO reported on the causes and consequences of increases in prices of general agricultural commodities in 2007 (Diouf, 2007). This trend is bound to strongly affect the cost of feeds for fish. Thus, a fish culture technology based on a cheap food source that does not compete with other uses will be advantageous not only in organic culture but also in conventional fish culture.

A 40% reduced feeding rate was reported in experiments on the use of plastic substrates in tilapia culture during the nursery stage (Milstein et al., 2006) and early and late growout (Milstein et al., 2005, 2008). Since the type of substrate has a strong effect on the density of the periphyton growing on it (Azim et al, 2005), as a further step in the development of this technology, periphyton growth on different types of substrates is herein examined. The materials chosen for the present experiments had different textures and colors, included natural and artificial materials that are waste products originating from other agricultural activities, and are available in large quantities in Israel. The use of such materials complies with organic regulations that encourage recycling of materials.

Materials and Methods

The two experiments were conducted in 300-m^2 earthen ponds at the Fish and Aquaculture Research Station Dor. Strips of substrates of different materials were hung vertically in the epilimnion of dry ponds so that they would not touch or shade each other. Some of each substrate was installed in 1-m^3 cages to prevent grazing of the tilapia on the periphyton while some was installed in the open pond to be accessible to the fish. The ponds were filled with water one week before stocking the fish. Sub-sets of substrates were removed by sampling to analyze chlorophyll and dry and organic matter attached on them. Removed substrates were not reused.

Texture experiment. In the texture experiment, periphyton growth was tested on eight substrates. Triplicates of each substrate were sampled three times during the fish culture season, at about 3-week intervals. The substrates included palm leaves, and 50 x 10 cm strips of smooth plastic or agricultural nets with different mesh sizes and types of thread (Table 1). The honeycomb structures (code RP) provided 5 cm wide strips but the difference in width did not affect comparison of results since periphyton was sampled from the same water depth for all substrates and measurements were standardized on a per cm^2 basis. Periphyton growing on the upper 5 cm of the substrates were analyzed for chloro-

Table 1. Substrates used in texture experiment.

Code[1]	*Substrate type*	*Texture*	*Color*
R90blue	Synthetic net of flat threads (raffia)	fine mesh	blue
R90	Synthetic net of flat threads (raffia)	fine mesh	black
M90	Synthetic net of cylindrical threads	fine mesh	black
R40	Synthetic net of flat threads (raffia)	coarse mesh	black
M40	Synthetic net of cylindrical threads	coarse mesh	black
RP	Rigid plastic surface[2]	smooth	black
FP	Flexible plastic surface[3]	smooth	white
D	Date palm leaf	smooth	pale green

[1] 90 and 40 refer to the percentage of shade provided by the agricultural net
[2] honeycomb-like structures used to prevent erosion on road sides
[3] empty sacks of industrial fish feed pellets

phyll. Periphyton growing on the 30 cm immediatelly below were analyzed for dry and organic matter.

Color experiment. To test the effect of substrate color on periphyton development, a second experiment was carried out the following year using nets of a single type that differed only in color. We learned from the first experiment that periphyton grown on smaller strips would provide enough material to obtain reliable measurements, facilitating laboratory analyses. Eight strips (20 x 2 cm) of white, blue, and black nets were installed in each location (cage and open pond) as above and removed 20 days after the fish were stocked. Half of the strips were used to determine chlorophyll and the other half dry and organic matter.

Fish stocking. In both experiments, hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) were stocked in polyculture with small quantities of silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), and, to control wild spawning of tilapia, the predator red drum (*Sciaenops ocellatus*). In the texture experiment, 360 hybrid tilapia (320 g), 10 silver carp (6 g), 15 grass carp (3 g), and 30 red drum (6 g) were stocked in each pond. In the color experiment, 300 tilapia (150 g), 7 silver carp (20 g), 10 grass carp (16 g), and 15 red-drum (4 g) were stocked in each pond. The fish were fed commercial organically-certified floating pellets containing 30% protein (Raanan Marketing Ltd., Israel).

Periphyton sampling and analysis. Periphyton on the smooth plastic substrates was scraped from the areas described above. Periphyton on nets and palm leaves were not separated from their substrates for chemical analysis since it was difficult to extract the material. In any case, the nets did not interfere with chlorophyll extraction (although a larger amount of methanol was needed to cover all the material) and the palm leaves were dry prior to the experiment and therefore contained a negligible amount of chlorophyll. Chlorophyll *a* was determined by the methanol extract technique. Dry matter (DM) was determined after drying at 105°C and organic matter (as ash free dry matter; AFDM) after burning at 550°C. To determine dry and organic matter of periphyton grown on nets and palm leaves, the dry and organic matter in blanks of each substrate were measured and deducted from the total (periphyton+substrate) measurement. To calculate perphyton concentration per area, it was assumed that the periphyton was collected from both sides of the sub-

strates. The autotrophic index (AI) was calculated according to the formula (APHA, 1992): AI = AFDM (µg/cm^2)/chlorophyll *a* (µg/cm^2).

Statistical analyses. Data were analyzed using ANOVA. Significant differences between levels of the main effects were tested with the Scheffe mean multi-comparison test using a significance level of $p<0.05$. Organic matter measured as a percentage of dry matter were normalized using the arcsine of the square root transformation. Analyses were run using the SAS statistical package.

Results

Texture experiment. At the time of the first periphyton sampling, 26 days after the substrates were placed under water, the palm leaves were covered with periphyton. The leaflets in the open water decomposed and were eaten by the fish, together with the periphyton growing on them, so that after another 22 days, leaflets remained in the cages but had completely disappeared in the open pond. By the next sampling, 20 days later, no leaflets remained in either the open pond or the cages. Besides the decomposition of this substrate, technical problems were encountered with its chemical analyses, so that our few successful measurements were not included in the statistical analyses.

Over 80% of the variability, as indicated by the coefficient of determination r^2, of chlorophyll *a*, DM, and AFDM was accounted for by the model (Table 2). Substrate type accounted for 6% of the variability of chlorophyll, with 60% more chlorophyll in periphyton grown on M90 than in periphyton grown on R40 or FP. Time accounted for 23% of the chlorophyll variability, with the highest level in late summer (first sampling) and less than half that level in autumn (second sampling). The substrate*time cross accounted for 67% of the chlorophyll variability since chlorophyll on periphyton from smooth plastic substrates (RP and FP) was very high (25-40 µg/cm^2) in late summer and very low (under 2 µg/cm^2) on other sampling days, while chlorophyll in periphyton from nets fluctuated 5-20 µg/cm^2 throughout the experiment (Fig. 1). The location of the substrates explained a further 1% of the chlorophyll variability, with 15% higher chlorophyll in the cages than in the open pond.

Type of substrate accounted for approximately 90% of the DM and AFDM variability. The amount of matter retained on the substrate was higher on the fine nets than on the coarse nets, and even lower on the smooth plastic substrates. On average, DM was 8% higher in the cages and decreased by about 10% from sampling to sampling while AFDM did not differ between locations. The decrease in dry matter with time was more pronounced in periphyton grown on smooth plastic substrates (RP and FP) than on nets (Fig. 2). On average, organic matter constituted about 20% of the dry matter, with lower values in late summer and the M90 net.

The autotrophic index was affected by substrate type, with heterotrophic periphyton (high AI) dominating in R90 and autotrophic communities (low AI) dominating in M90, M40, and R40. On average, AI was 35% higher in the pond than in the cages and highest on the second sampling day. Values were highest in periphyton grown on R90 on the first sampling day and in peripyton from the smooth plastic substrates on the second (Fig. 3).

Color experiment. The color of the substrate did not affect the chlorophyll content but did affect the dry matter contents; the white substrate had 40% more DM and 50% more AFDM than the blue and black substrates (Table 3). Organic matter constituted 18% of the dry matter, independent of substrate color or location. The autotrophic index was 46% higher in the white substrate than in the blue while the black substrate did not significantly differ from either. Location of the substrate did not produce a significant difference in any of the tested variables.

Discussion

A variety of substrates have been used in periphyton-based aquaculture including natural materials such as bamboo and tree branches, jute sticks, and bundles of sugarcane bagasse (Ramesh et al., 1999; Keshavanath et al., 2001; Azim et al., 2003), and artificial

Table 2. Texture experiment: ANOVA and Scheffe mean multi-comparisons of periphyton grown on substrates with different textures.

ANOVA model	*Chlorophyll a (µg/cm²)*	*Dry matter (mg/cm²)*	*Ash free dry matter (mg/cm²)*	*Organic matter (%)[1]*	*Autotrophic index*
Significance	***	***	***	***	***
Coefficient of determination (r^2)	0.83	0.92	0.81	0.54	0.71
Source of variance (significance; % of total sums of squares)					
Substrate	*** 6	*** 89	*** 92	*** 40	*** 26
Sampling time	*** 23	*** 2	* 2	*** 31	*** 8
Location	* 1	** 1	ns 0	ns 2	* 2
Substrate*time	*** 67	*** 6	ns 4	ns 19	*** 47
Substrate*location	ns 2	*** 2	ns 2	ns 8	*** 13
Time*location	ns 1	ns 0	ns 0	ns 0	** 4
Mean multi-comparisons by location					
Cage (n = 63)	12.6[a]	5.2[a]	1.1	20	132[b]
Pond (n = 62)	10.9[b]	4.8[b]	1.0	22	179[a]
Mean multi-comparisons by sampling time					
14 Aug 2006 (n = 42)	17.3[a]	5.6[a]	1.1	18[c]	138[b]
3 Sep 2006 (n = 41)	8.2[b]	5.0[b]	1.2	25[a]	218[a]
25 Sep 2006 (n = 42)	9.7[b]	4.4[c]	1.0	21[b]	111[b]
Mean multi-comparisons by substrate					
R90blue (n = 18)	14.0[ab]	9.6[a]	2.1[a]	22[a]	167[b]
R90 (n = 18)	11.9[ab]	8.5[a]	2.1[a]	25[a]	301[a]
M90 (n = 17)	14.5[a]	5.3[b]	0.9[b]	15[b]	63[b]
M40 (n = 18)	10.4[ab]	4.0[c]	0.8[b]	20[ab]	78[b]
R40 (n = 18)	9.0[b]	3.7[cd]	0.9[b]	24[a]	105[b]
RP (n = 18)	13.3[ab]	2.6[d]	0.5[bc]	21[a]	186[ab]
FP (n = 18)	9.2[b]	1.4[e]	0.2[c]	20[ab]	181[ab]

* $p = 0.05$, ** $p = 0.01$, *** $p = 0.001$, ns = not significant
The same superscript in a column indicates no significant difference at the 0.05 level.
[1] Statistical tests are based on transformed data; means are untransformed.

materials such as PVC pipes, plastic sheets, and materials such as Aquamats™ (Bratvold and Browdy**,** 2001; Keshavanath et al, 2001). Substrate type has a strong effect on the density of the periphyton growing on it. Generally, bamboo and tree branches perform better in terms of fish production than plastic and PVC substrates. The reasons are unknown but may be attributed to leaching of nutrients, toxic substances from artificial substrates, or differences in surface roughness (van Dam et al., 2002). The materials chosen in the pre-

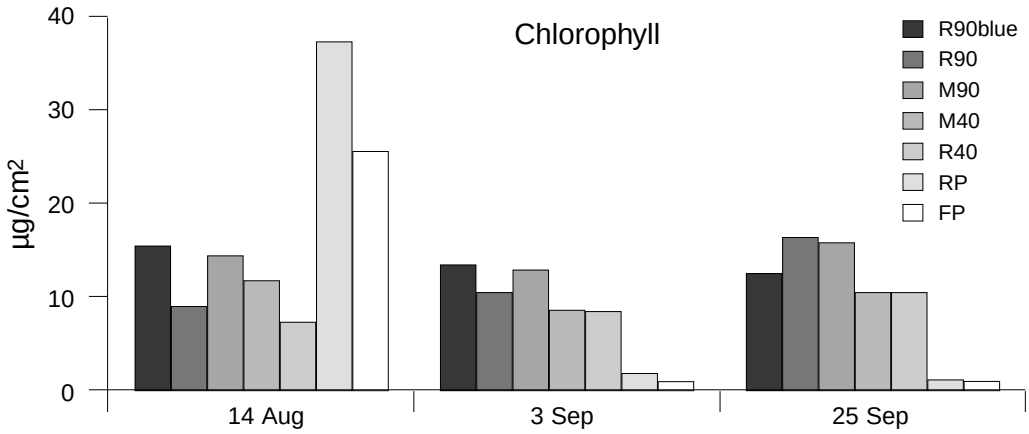

Fig. 1. Effect of substrate*time on chlorophyll *a* in periphyton grown on a variety of substrates with different textures.

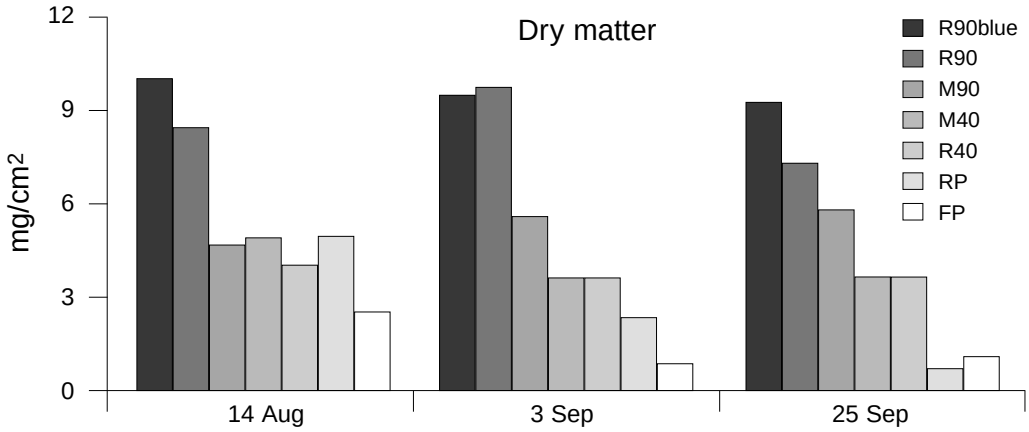

Fig. 2. Effect of substrate*time on dry matter in periphyton grown on a variety of substrates with different textures.

sent experiments represent an array from natural and artificial origins, have different surface roughness, and include nets which generally are not used in such studies. In addition to roughness, the color of the nets was examined to determine whether it has an effect on periphyton development, as shown in vegetable production (Shahak, 2000; Oren-Shamir et al., 2001). Indeed, in both experiments, the submerged white net substrate resulted in 50% more organic matter than in periphyton grown on blue or black nets, with no significant difference between the black and blue.

To explore the grazing effect of fish on periphyton, substrates placed in a cage protected from the fish were compared to substrates placed in the open pond where fish were pre-

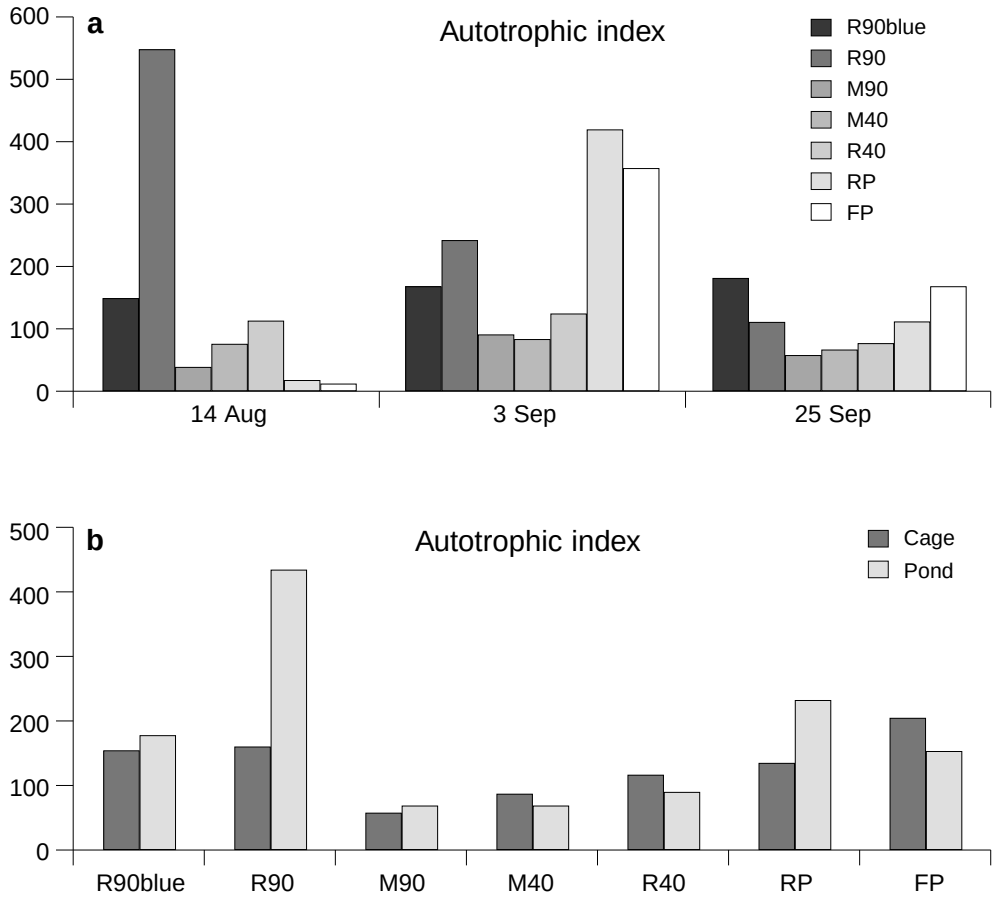

Fig. 3. Effect of (a) substrate*time and (b) substrate*location on autotrophic index in periphyton grown on a variety of substrates with different textures.

sent. The lack of a significant difference in chlorophyll between substrates in the cages and in the open pond in the color experiment indicates either that the fish did not graze on the substrates, or that periphytic algal reproduction compensated for fish grazing in the open pond. On the other hand, the significant difference in chlorophyll and dry matter between locations in the texture experiment, where larger tilapia were stocked at higher density and grazing pressure should have been stronger, indicates that fish grazing did indeed occur. This is supported by marks made by grazing fish on the smooth plastic substrates (on rough nets such marks are impossible to recognize). Other studies have also reported no significant difference in quantity of periphyton on protected substrates and substrates exposed to fish, in spite of clear indications of fish grazing. In an earlier study using smooth plastic sheets as a periphyton substrate, there were similar non significant ‘cage vs. pond’ chlorophyll and dry matter effects although location*time interactions showed clear grazing patterns towards the end of the experiment when the fish were large, i.e., chlorophyll and dry matter dropped with time in the open pond but not in the cage (Milstein et al., 2005). In a

Table 3. Color experiment: ANOVA and Scheffe mean multi-comparisons of periphyton grown on substrates with different colors.

ANOVA model	*Chlorophyll a (µg/cm²)*	*Dry matter (mg/cm²)*	*Ash free dry matter (mg/cm²)*	*Organic matter (%)[1]*	*Autotrophic index*
Significance	ns	***	**	ns	*
Coefficient of determination (r^2)	0.31	0.91	0.59	0.02	0.39
Source of variance (significance; % of total sums of squares)					
Color	ns	*** 100	*** 100	ns	* 98
Location	ns	ns 0	ns 0	ns	ns 2
Mean multi-comparisons by location					
Cage	6.6	9.4	1.7	18	256
Pond	6.0	9.3	1.6	17	269
Mean multi-comparisons by color					
White (n = 8)	6.6	11.5[a]	2.1[a]	18	320[a]
Blue (n = 8)	6.5	8.2[b]	1.4[b]	17	219[b]
Black (n = 6)	5.7	7.9[b]	1.4[b]	18	243[ab]

* $p = 0.05$, ** $p = 0.01$, *** $p = 0.001$, ns = not significant
The same superscript in a column indicates no significant difference at the 0.05 level.
[1] Statistical tests are based on transformed data; means are untransformed.

study of cage-cultured sea bream (*Sparus aurata*) juveniles, although there were no significant differences in chlorophyll, dry, or organic matter contents of periphyton growing on glass fiber or mosquito nets in cages with or without fish, the fish grew, indicating that they ate the periphyton since no other feed was provided (Richard et al., 2007).

Periphyton developed on the surfaces of palm leaves but the leaves decomposed quickly and lasted only a couple of months. Palm leaves might be an appropriate periphyton substrate but in large-scale aquaculture, where labor is expensive and palm leaves are transported from distant plantations, the costs involved in frequent installation and replacement of palm leaves might be too high for a substrate that does not last at least a few years.

Among the tested artificial substrates, the periphytic material seemed to more easily dislodge from the smooth plastic substrates than from the rough nets, a phenomenon that changed the structure of the residual attached periphyton (AI). During the first part of the texture experiment, periphyton developed on all substrates with more autotrophic populations (more chlorophyll) on the smooth plastic substrates. When the periphytic layer on those substrates became too thick, the external mostly autotrophic part was dislodged, leaving behind the heterotrophic inner layer. Thus, in the autumn, samples contained less periphyton, both in terms of dry matter and chlorophyll. The significant drop of periphyton quantity and the change from autotrophic to heterotrophic dominance was recorded in both the cage and the pond. Thus, fish grazing does not appear to account for it. In addition, these characteristics were found only on the smooth plastic substrates and not on the rough nets. Although dislodgement may have

also occurred from the nets, their rough surfaces should have facilitated periphyton attachment around the threads and reduced the intensity of dislodgement so that periphyton growth quickly masked its effect.

Periphyton growth was greater on the fine mesh nets than on the coarse mesh nets which in turn were higher than on the smooth plastic substrates. The dry and organic matter levels on the latter were in the same order of magnitude as those recorded on similar black (unpublished data) and transparent substrates (Milstein et al., 2005). In those cases the inclusion of underwater substrates with a surface area equivalent to 40% and 50% of the pond surface, combined with a 40% reduction of feed, resulted in only 10% lower fish yield than in ponds without substrates that received the full feed amount. Hence, substitution of smooth plastic substrates by nets should provide more natural food to fish. These findings remain to be tested on a larger scale.

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