

# Effect of Different Treatments on the Periphyton Quantity and Quality in Experimental Fishponds

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**Abstract**—Periphyton development and composition were studied in three different treatments: (i) two fishpond units of wetland-type wastewater treatment pond systems, (ii) two fishponds in combined intensive-extensive fish farming systems and (iii) three traditional polyculture fishponds. Results showed that amounts of periphyton developed in traditional polyculture fishponds (iii) were different compared to the other treatments (i and ii), where the main function of ponds was stated wastewater treatment. Negative correlation was also observable between water quality parameters and periphyton production. The lower trophity, halobity and saprobity level of ponds indicated higher amount of periphyton. The dry matter content of periphyton was significantly higher in the samples, which were developed in traditional polyculture fishponds ( $2.84 \pm 3.02 \text{ g m}^{-2} \text{ day}^{-1}$ , whereby the ash content in dry matter 74%), than samples taken from (i) ( $1.60 \pm 2.32 \text{ g m}^{-2} \text{ day}^{-1}$ , 61%) and (ii) fishponds ( $0.65 \pm 0.45 \text{ g m}^{-2} \text{ day}^{-1}$ , 81%).

**Keywords**—Artificial substrate, fishpond, periphyton, water quality

## I. INTRODUCTION

**P**ERIPHYTON is the complex of organisms found on submerged substrates that are of materials different from those of the water bottom and clearly distinguishable from them [1]. Periphyton is composed of algae, fungi, bacteria, and protozoa associated with substrates in aquatic habitats. Periphyton is often the dominant contributor to nutrient cycling in aquatic ecosystems and it is an excellent indicator for changes occurring in the aquatic environment [2]. The periphyton quantity and quality depends on abiotic and biotic factors (nutrients, light intensity and quality, temperature, water level, as well as the substrate type and the grazing activity of the fish and invertebrates). Nutrient availability is an important regulating factor for bacterial and algal production and growth. The bacteria can take up algal exudates and the algae may benefit from the regeneration of nutrients performed by the bacteria. Bacterial activity is high

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within periphyton [3], [4]. The autotrophic organisms of periphyton produce organic material and oxygen by using light energy and absorbing nutrients. The organic material produced in that way can provide valuable nutrition for the periphytic zoo-organisms and other heterotrophic communities in the water. The heterotrophic organisms also use drifting, produced, settling or settled organic material in their metabolic processes [5].

The use of periphyton in aquaculture improves both the water quality and aquatic production. The idea is originally derived from traditional fishing methods from tropical countries, such as the „acadjas” of Africa [6], the “samarahs” of Cambodia [7] and the “katha” fisheries of Bangladesh [8], where tree branches are placed in shallow open waters to attract fish and enhance productivity.

In our study, the periphyton appearing on artificial substrates in different types of experimental fishponds were examined. Since traditional periphyton based aquaculture does not exist yet in Hungary, detailed knowledge on the quantitative and qualitative changes of the periphyton may give possibilities to increase fish yield and improve water quality in fishponds, even under the temperate climate.

## II. MATERIALS AND METHODS

The experiment was carried out in seven experimental fishponds at the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), Szarvas, Hungary in 2007.

### A. Description of studied sites

W1 (0.25 ha) and W2 (0.12 ha) fishponds are parts of two wetland-type pond systems constructed for experimental wastewater treatment. These systems are comprised of four serially-connected ponds, two earthen fishponds (first and second units) and two macrophyte-covered earthen ponds (third and fourth units). The effluent water from an intensive African catfish farm was canalled into the first ponds. Our studies were carried out in the second pond units stocked with silver carp (*Hypophthalmichthys molitrix* V.) and common carp (*Cyprinus carpio* L.) at an initial stocking biomass of 800-1000 kg ha<sup>-1</sup>.

IE1 and IE2 fishponds (0.03 ha) were the extensive units of two combined intensive-extensive systems, where one cage per system was operated as the intensive unit (mean water depth 1 m). In the intensive units European catfish (*Silurus glanis* L.) were cultured and fed with pellet – initial stocking

biomass was 90 kg (10 m<sup>3</sup>) –, whereas in the extensive units common carp (*Cyprinus carpio* L.) and Nile tilapia (*Oreochromis niloticus* L.) were raised without any artificial feeding – initial stocking biomass was 30 kg. The periphyton appearance in the extensive units was investigated.

FP1, FP2 and FP3 were traditional polyculture fishponds with a surface area of about 0.15 ha. Common carp (*Cyprinus carpio* L.), hybrids of silver carp and bighead carp (*Hypophthalmichthys molitrix* V. x *Aristichthys nobilis* R.), grass carp (*Ctenopharyngodon idella* V.) and European catfish (*Silurus glanis* L.) were stocked in polyculture in the proportion of 67:22:9:2%, respectively.

### B. Water quality measurements

Water quality was checked three times a week at the outlets of the ponds for water temperature (TEMP), conductivity (Cond), pH and dissolved oxygen concentrations (DO) with portable meters (WTW, model Oxi 315i; YSI 556 Multi Probe System and Horiba U-10). The whole water column was sampled for water chemical measurements at periphyton sampling (biweekly in W and IE ponds, and every second months in FP ponds) of the ponds, and the samples were analysed for nutrient concentrations – ammonium-nitrogen (NH<sub>4</sub>-N), total organic and inorganic nitrogen (KN, TIN), total nitrogen (TN) and soluble reactive phosphorus (PO<sub>4</sub>-P), total phosphorous (TP), volatilised suspended solids (VSS), total suspended solids (TSS), biochemical oxygen demand (BOD<sub>5</sub>), chemical oxygen demand (COD<sub>Cr</sub>) according to Hungarian Standard Methods (MSZ). The chlorophyll-a (Chl-a) and pheophytin (Pheop) concentrations were determined by colorimetric analysis using a spectrophotometer after extraction with 90% ethanol [9].

### C. Periphyton measurements

The periphyton samples were collected from epiphytological habitats (plastic pipe substrates – diameter 1.8 cm – placed vertically in the ponds). The pipes were enclosed in small cages which were used to avoid periphyton consumption by fish. Samples were taken between June and November in 2007 periodically. The first sampling was done 14 days after submersion. From each pond, two pipes were selected per sampling and sub-samples of periphyton were taken at two depths (20 and 50 cm below the water surface) per pipe. The four sub-samples were mixed into two single samples (20 cm, 50 cm) which were analysed separately. Substrates were replaced after collecting the samples to allow further development and periodical sampling of periphyton.

The periphytic material was scraped with a scalpel from a known surface area – 2x113 cm<sup>2</sup> + 2x170 cm<sup>2</sup>– (wet mass). These samples were dried at 105°C until constant weight (24 h), and kept in a desiccator until weighed dry matter content (DM). Ash-free dry matter (AFDM) was determined after the samples ashed at 500 °C for 4 hours. Chl-a concentration was determined by colorimetric analysis using a

spectrophotometer after extraction with methanol [9].

### D. Data analysis

The data were analysed by SPSS using significant difference comparison (T-test) to determine the differences between treatments at 0.05 level of probability. The correlations among the water quality parameters and periphyton quantity parameters were determined using Pearson correlation.

## III. RESULTS AND DISCUSSION

### A. Water quality parameters

Comparing the measured water quality parameters of different ponds no significant differences were found (except FP1 and FP2 conductivity  $t=3.55$ ,  $p=0.024$ ). Water quality parameters of treatments are shown in Table I.

TABLE I  
WATER QUALITY PARAMETERS IN THE TREATMENTS

Parameters		W (n=22)	IE (n=16)	FP (n=9)
Cond	μS cm <sup>-1</sup>	1014±	412±	394±
		135	17	20
BOD <sub>5</sub>	mg l <sup>-1</sup>	19.0±	27.3±	11.3±
		11.1	12.1	4.8
COD	mg l <sup>-1</sup>	100±	80.0±	52.4±
		43	37.9	21.9
NH <sub>4</sub> -N	mg l <sup>-1</sup>	4.57±	0.209±	0.058±
		3.58	0.124	0.040
TIN	mg l <sup>-1</sup>	7.55±	0.702±	0.096±
		4.09	0.504	0.058
KN	mg l <sup>-1</sup>	3.26±	3.40±	2.50±
		1.80	1.05	0.92
TN	mg l <sup>-1</sup>	10.8±	4.10±	2.60±
		3.8	1.37	0.89
PO <sub>4</sub> -P	mg l <sup>-1</sup>	1.25±	0.120±	0.068±
		0.59	0.070	0.050
TP	mg l <sup>-1</sup>	1.79±	0.432±	0.247±
		0.66	0.207	0.089
Chl-a	ug l <sup>-1</sup>	389±	595±	112±
		340	391	44
Pheop	ug l <sup>-1</sup>	575±	819±	165±
		452	457	46
DO	mg l <sup>-1</sup>	5.61±	8.60±	6.19±
		2.34 <sup>a</sup>	1.07 <sup>b</sup>	2.34 <sup>c</sup>
TEMP	°C	20.1±	21.8±	17.5±
		6.78 <sup>a</sup>	3.95 <sup>b</sup>	6.12 <sup>c</sup>
pH	-	8.60±	8.78±	n.d.
		0.93 <sup>a</sup>	0.34 <sup>b</sup>	

Values are means ± S.D. – n.d.: no data, <sup>a</sup>(n=16), <sup>b</sup>(n=15), <sup>c</sup>(n=12)

Three water quality parameters were used to classify the treatments (Table II) [9]. W and IE treatments were similar according to trophity and saprobity levels. Water condition (conductivity) was indicating high degree of halobity in W. According to the grades of trophity, intensity of primary production was high in these treatments. Water treatment

function of W and IE was shown by saprobity level that indicated the high organic content of inflow water and mass development of bacteria that were involved in decomposition processes. FP was found eu-politrophity and alpha-mesosaprobic according to this classification.

TABLE II  
CLASSIFICATION OF TREATMENTS

Treatments	Feeding	Halobity (Con)	Trophity (Chl-a)	Saprobity (COD <sub>Cr</sub> )
W	no	oligo-mesohalobity	politrophity	alpha-meso-polisaprobic
IE	no	beta-alpha oligohalobity	politrophity	alpha-meso-polisaprobic
FP	yes	beta-alpha oligohalobity	eu-politrophity	alpha-mesosaprobic

The treatments were also separated by significant differences of water chemical parameters (Table III). The lowest concentration of different water chemical parameters was found in the FP except DO and TSS concentrations.

TABLE III  
SIGNIFICANT DIFFERENCE BETWEEN WATER QUALITY PARAMETERS IN THE DIFFERENT TREATMENTS

Parameters	W-IE	W-FP	IE-FP
Cond	t=17.69 p<0.001	t=13.58 p<0.001	t=2.263 p=0.033
BOD <sub>5</sub>	t=-2.187 p=0.035	-	t=3.743 p=0.001
COD <sub>Cr</sub>	-	t=3.165 p=0.004	-
TIN	t=6.636 p<0.001	t=5.412 p<0.001	t=3.560 p=0.002
KN	-	-	t=2.140 p=0.043
TN	t=6.811 p<0.001	t=6.427 p<0.001	t=2.948 p=0.007
PO <sub>4</sub> -P	t=7.586 p<0.001	t=5.938 p<0.001	-
TP	t=7.883 p<0.001	t=6.878 p<0.001	t=2.525 p=0.019
Chl-a	-	-	t=2.971 p=0.008
Pheop	-	t=2.192 p=0.038	t=3.447 p=0.003
DO	t=-4.526 p<0.001	-	t=3.566 p=0.001

### B. Periphyton quantity and quality

The average periphyton composition on each plastic pipes was calculated. Substrates, submersion time and depth were the same in the treatments. Comparing the measured parameters of periphyton production, there were no significant differences ( $p>0.05$ ) between W1 – W2, IE1 – IE2, and FP1 –

FP2 – FP3 ponds similarly to the water chemical parameters. Thus, the fishponds can be characterised by the amount of periphyton similarly to the water chemical parameters. The DM of periphyton was significantly higher in the samples, which were developed in FP ( $2.84\pm 3.02 \text{ g m}^{-2} \text{ day}^{-1}$ , whereby the ash matter was  $2.11\pm 2.18 \text{ g m}^{-2} \text{ day}^{-1}$ ) than samples were taken from W and IE (Table IV, Fig. 1). Results showed that amounts of periphyton developed in traditional polyculture fishponds were different compared to the other treatments, where the main function of ponds were wastewater treatment. Maximal periphyton biomass could be observed where the combination of light and nutrient are optimal [10]. The maximums of periphyton dry matter development were found in IE ( $0.654 \text{ g m}^{-2} \text{ day}^{-1}$  at 18.06.2007), in FP ( $0.327 \text{ g m}^{-2} \text{ day}^{-1}$  at 28.08.2007) and in W ( $0.114 \text{ g m}^{-2} \text{ day}^{-1}$  at 02.07.2007) in the summer months.

Higher ash ratio was observed in IE than in the other treatments (ash content in dry matter IE=81%, FP=74%, W=61%). The high amount of inorganic fraction of periphyton was caused by the using of paddle aerators for water-circulation and inorganic particles from the water column increased the ash content.

Mean periphyton Chl-a pigment varied between  $0.061\pm 0.080 \text{ mg m}^{-2} \text{ day}^{-1}$  and  $0.143\pm 0.168 \text{ mg m}^{-2} \text{ day}^{-1}$ . Relatively more Chl-a was present in W and IE than in FP. Significant difference was found only between W and FP regarding the dry matter of periphyton ( $n=16$ ,  $n=10$ ,  $t=-2.889$ ,  $p=0.008$ ) and the quantity of ash ( $t=-3.119$ ,  $p=0.005$ ).

TABLE IV  
COMPARISON OF PERIPHYTON IN DIFFERENT TREATMENTS

Parameters		W (n=16)	IE (n=16)	FP (n=10)
DM	$\text{g m}^{-2} \text{ day}^{-1}$	$0.649\pm 0.452$	$1.600\pm 2.32$	$2.836\pm 3.02$
Chl-a	$\text{mg m}^{-2} \text{ day}^{-1}$	$0.143\pm 0.168$	$0.110\pm 0.094$	$0.061\pm 0.080$
Ash	$\text{g m}^{-2} \text{ day}^{-1}$	$0.398\pm 0.344$	$1.290\pm 2.09$	$2.110\pm 2.18$
AFDM	$\text{g m}^{-2} \text{ day}^{-1}$	$0.251\pm 0.164$	$0.310\pm 0.265$	$0.726\pm 0.954$

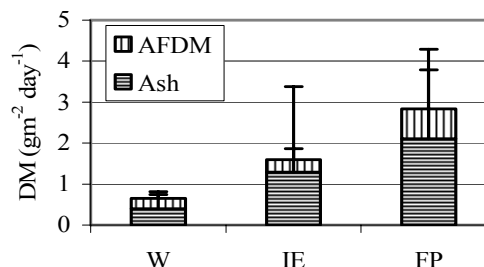


Fig. 1 Mean (+SD) periphyton dry matter in different treatments

### C. Influence of water chemical parameters at different sampling time

The periphyton dry matter and related water chemical parameters showed significant correlation in IE and W treatments. In every case the correlation was negative between the water chemical parameters and the periphyton dry matter (Pearson correlation,  $p < 0.05$ ,  $n=16$ ,  $W-BOD_5$ : -0.583,  $IE-COD$ : -0.577,  $KN$ : -0.621,  $TN$ : -0.581,  $TP$ : -0.530,  $Chl-a$ : -0.509,  $Pheop$ : -0.539).

The significant difference of dry matter and quantity of ash in the different treatments was tested with two samples t-probe. Significant difference between the treatments at the same sampling date was observed in 50, 50 and 33% of W-IE, W-FP and IE-FP comparisons. Results of two sampling dates are shown here only for detailed discussion. In Table V is showed the third sampling date, where the higher periphyton production was found in W than in IE (DM:  $1.033 \pm 0.009 \text{ g m}^{-2} \text{ day}^{-1}$  vs.  $0.726 \pm 0.063 \text{ g m}^{-2} \text{ day}^{-1}$ ). The values of conductivity and the  $NH_4-N$  concentration were showed significant difference and negative correlation.

TABLE V  
SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENTS AT THE THIRD SAMPLING (16.07.2007)

Parameters		W	IE	Sig. diff. (n=2)
DM	$\text{g m}^{-2} \text{ day}^{-1}$	$1.033 \pm 0.009$	$0.726 \pm 0.063$	$t=6.801$ $p=0.021$
Ash	$\text{g m}^{-2} \text{ day}^{-1}$	$0.767 \pm 0.0389$	$0.057 \pm 0.0213$	$t=6.280$ $p=0.024$
Cond	$\mu\text{S cm}^{-1}$	$1081 \pm 33.9$	$406 \pm 1.41$	$t=28.10$ $p=0.001$
$NH_4-N$	$\text{mg l}^{-1}$	$0.0525 \pm 0.07$	$0.290 \pm 0.0233$	$t=-4.561$ $p=0.045$

The eighth sampling dates are showed in Table VI. Higher periphyton production was found in FP than in IE ponds (DM:  $5.49 \pm 1.00 \text{ g m}^{-2} \text{ day}^{-1}$  vs.  $0.745 \pm 0.490 \text{ g m}^{-2} \text{ day}^{-1}$ ). Significant difference was showed to the assay in seven water parameters ( $BOD_5$ ,  $COD_{Cr}$ ,  $NH_4-N$ ,  $TIN$ ,  $TN$ ,  $PO_4-P$ ,  $TP$ ). In these cases, the negative correlations were also observable between water quality parameters and periphyton production. The lower trophity, halobity and saprobity level indicated higher amount of periphyton. These results were close to the third sampling date.

TABLE VI  
SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENTS AT THE EIGHTH SAMPLING (24.09.2007)

Parameters		IE	FP	Sig. diff. (n=2)
DM	$\text{g m}^{-2} \text{ day}^{-1}$	$0.745 \pm 0.490$	$5.49 \pm 1.00$	$t=-6.00$ $p=0.027$
Ash	$\text{g m}^{-2} \text{ day}^{-1}$	$0.448 \pm 0.348$	$4.48 \pm 1.23$	$t=-4.43$ $p=0.047$
$BOD_5$	$\text{mg l}^{-1}$	$22.5 \pm 3.53$	$5.66 \pm 1.15$	$t=8.20$ $p=0.004$
$COD_{Cr}$	$\text{mg l}^{-1}$	$97.0 \pm 21.2$	$50.3 \pm 2.31$	$t=4.12$ $p=0.026$
$NH_4-N$	$\text{mg l}^{-1}$	$0.294 \pm 0.046$	$0.034 \pm 0.011$	$t=10.1$ $p=0.002$
$TIN$	$\text{mg l}^{-1}$	$0.884 \pm 0.365$	$0.061 \pm 0.037$	$t=4.23$ $p=0.024$
$TN$	$\text{mg l}^{-1}$	$4.35 \pm 0.156$	$3.71 \pm 0.090$	$t=6.04$ $p=0.009$
$PO_4-P$	$\text{mg l}^{-1}$	$0.111 \pm 0.007$	$0.010 \pm 0.003$	$t=23.9$ $p=0.000$
$TP$	$\text{mg l}^{-1}$	$0.391 \pm 0.088$	$0.177 \pm 0.029$	$t=4.15$ $p=0.025$

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