Desalination and Water Treatment
www.deswater.com
11 (2009) 264–274

Assessment of the water quality of aquatic resources using biological methods

Z. Dulić[a,*], V. Poleksić[a], B. Rašković[a], N. Lakić[a], Z. Marković[a], I. Živić[b], M. Stanković[a]

[a]*University of Belgrade, Faculty of Agriculture, Department of Animal Science, Nemanjina 6, 11080 Belgrade, Serbia*
Tel. +381 113168499; Fax. +381 113168499; email: zorkad@agrif.bg.ac.rs
[b]*University of Belgrade, Faculty of Biology, Department of Animal Morphology, Sistematics and Phylogeny, Studentski trg 16, 11000 Beograd, Serbia*

Received 28 January 2009; Accepted 24 August 2009

ABSTRACT

Assessment of water quality at Radmilovac estate near Belgrade that is used for irrigation of cultures and as a water supply for experimental fish farm and hatchery was performed by using aquatic invertebrates (zooplankton and macrozoobenthos) and fish gill histology as bioindicators. Two open wells and a stream were monitored during a three year investigation. A total of 25 and 31 species of zooplankton were found in open wells and 11 and 12 taxa of macrozoobenthos at two sites of stream Šugavac. The saprobity index (S) was used to evaluate the water quality of these four sites. Statistical analysis showed that site S2 was the most polluted, with Oligochaeta and Chironomidae larvae dominating. Overall, β mesosaprobic organisms have been prevailing at all four sites indicating that the water was polluted at moderate levels. All analyzed fish gills showed predominantly normal structure. Identified histopathological changes of gill structure indicated mild and reparable alterations. The results obtained imply that these water resources can be used as a supply for carp fish farm. We suggest that the type of biological water assessment depends on waterbodies characteristics.

Keywords: Water quality; Bioindicators; Zooplankton; Macrozoobenthos; Saprobity index; Gill histology

1. Introduction

In order to provide a complete array of information for the assessment of water quality, the best way is to compile a range of different physical, chemical and biological parameters [1,2]. However, such studies are usually time and money consuming. Using biological parameters together with physical and chemical information to assist in interpretation [3] can be reliably and relatively inexpensive compared to the cost of assessing different pollutants [2]. Biological methods are now extensively integrated into water quality monitoring programs worldwide. The advantage of monitoring with the use of bioindicators is that biological communities reflect overall ecological quality and integrate the effects of different stressors providing a broad measure of their impact and an ecological measurement of fluctuating environmental conditions.

A variety of aquatic organisms that are valuable indicators can be used for assessing water quality through analysis of their diversity, composition and abundance [4–10]. Change in characteristics of these biological communities can be assessed using the saprobity system [4,11–13]. This system uses the degree of organic pollution of an ecosystem as one of the main factor of water quality [11,14]. In general, there are four saprobity zones: polysaprobic—extremely polluted, alpha-mesosaprobic—heavy polluted, beta-mesosaprobic—moderately polluted, and oligosaprobic—slightly polluted. Each zone has optimal conditions for certain species and communities of organisms [14]. Zooplankton are small and rapidly reproducing organisms that respond quickly to

*Corresponding author.

doi: 10.5004/dwt.2009[illegible]

environmental changes and may be effective indicators of subtle alterations in water quality [7,16–19]. Local differences in zooplankton community presence do not generally result from dispersal limitation since most species have a wide geographic distribution [20,21]. Macrozoobenthos is the most commonly used group of invertebrates for the assessment of running waters [22] since they are highly sensitive to environmental changes [23,24]. They are fairly sedentary organisms with a relatively long life span and in close association with the sediment conditions. These characteristics favor them as useful bioindicator organisms [25,26].

Additionally, fish gills are also used as bioindicators, because they are highly susceptible to the changes in water quality due to their specific anatomy, in particular their large surface area that is in permanent contact with the water environment as well as the vital biological functions they perform (respiration, excretion, osmoregulation). The histopathological changes of gill tissue correspond to the intensity of pollution [27–29].

An investigation of the quality of aquatic resources at the Faculty of Agriculture School Estate "Radmilovac" near Belgrade was carried out using bioindicator organisms in order to assess water quality and possibilities of its usage for both carp farm and hatchery water supply and irrigation. Water quality of different waterbodies—different aquatic ecosystems: running water—small stream Šugavac and two open wells have been assessed. These are main aquatic resources available for irrigation and as water supply for the newly built carp farm and hatchery. In this paper our focus is on biological assessment methods such as the determination of saprobity indices using zooplankton and macrozoobenthos organisms as indicators, as well as some morphometric parameters of the gill tissue of experimental fish.

2. Material and methods

2.1. Study site

The research was carried out at the Faculty of Agriculture School Estate "Radmilovac" located in a region of undulating hills near Belgrade. It covers an area of 84 ha and is mainly agricultural land with orchards and vineyards. Many private houses and a hotel are interspaced throughout the school estate land. In 2006, an experimental fish farm and hatchery were built on this site in the vicinity of two open wells (Fig. 1). They started fully operating a year later. In order to assess the quality of water for supply of the carp fish farm, and hatchery samples were taken from four different sites during the three years period (2005–2007).

Water sources were two open wells, W1 and W2 and stream Šugavac at two sites, S1 and S2 (Fig. 1). Open wells are small reservoirs that accumulate surface waters. They have been used for decades in this area as a supply for irrigation. Every well has a surface area of 38.5 m^2, 6 m depth, and 231 m^3 in volume. The stream

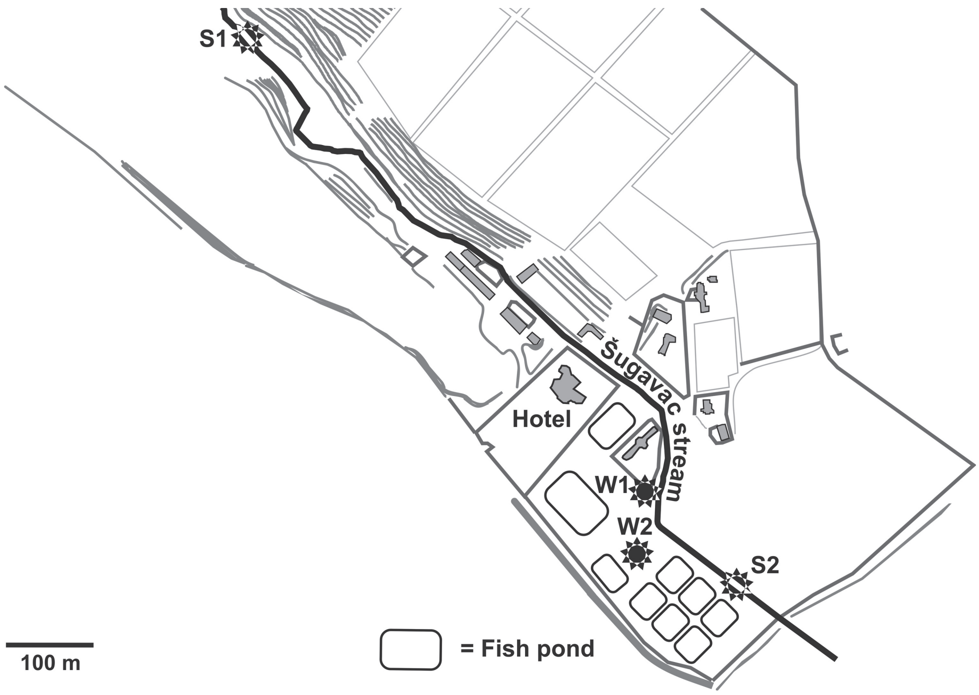

Fig. 1. Sampling sites at the school estate "Radmilovac".

Šugavac runs through the whole estate. The two sampling sites, "entrance" (S1) and "exit" (S2) are located above, and 400 m downstream, under the hotel. It is a relatively small stream, with average depth of 11 cm and width 45 cm. During hot summer months it can occasionally dry out.

2.2. Methods

The sampling of physical and chemical parameters, zooplankton and macrozoobenthos was carried out every other month, from May to September during three investigations years. An experiment lasting for 1 month was carried out with carp yearlings placed in cages in the open wells in June/July 2005, due to fish availability and the acclimatization period.

Physical and chemical parameters of water were measured at all four sites. The water temperature, dissolved oxygen, conductivity and pH were measured using a water field kit, MULTI 340 i/SET (WTW, Germany). In the open wells during the first investigation year (2005), measurements were performed 10 cm under the water surface and up to 6 m at 1 m intervals. Results of these measurements are published in a previous paper [31]. However, increased and frequent usage of water from wells for irrigation and in particular for filling fish ponds during the next 2 years (2006 and 2007) resulted in a very variable water level of the open wells so these parameters were measured only up to 1 m depth in the same intervals. Samples for qualitative and quantitative analysis of zooplankton were taken by pulling the plankton net No. 20 (mesh size 75 µm), through the upper layer of water in open wells [32]. The 100 mL samples were analyzed by subsampling technique using Sedgwick Rafter cell. Surber's net was used for the sampling of macrozoobenthos from the two sites of stream Šugavac. This type of mesh is specially used for sampling in running water and has a catchment area of 300 cm^2 and mesh size of 250 µm [32]. All samples were fixed immediately with 4% formaldehyde and later on analyzed. Zooplankton species were identified and relative abundance determined under light microscope Carl Zeiss Jena T125 (160×). In most cases identification was completed up to the species level. Some individuals were identified up to the genus level due to contractions occurring during preservation. Macrozoobenthos was identified up to family, genus or species level under a stereomicroscope Leica MZ 125 (100×).

Saprobiological analysis was applied by allocating identified zooplankton and macrozoobenthos taxa to their appropriate saprobic zones and saprobic value using a list of bioindicator organisms given by Wegl [33]. Using these individual species saprobic values (si), saprobic index (S) was calculated according to the Pantle–Buck method [34]:

$$S = \frac{\sum h \cdot s_i}{\sum h}$$

h – relative abundance of individual species
s_i – individual species saprobic value.

There saprobity index values for saprobity levels are: for oligosaprobity 0.51–1.50, β mesosaprbity 0.51–2.50, α mesosaprobity 2.51–3.50 and for polysaprobity 3.51–4.50 (Pantle Buck, 1955).

2.3. Experiment with carp yearlings in cages

Three cages (60 × 60 × 60 cm, 0.216 m^3 volume) were positioned in each well. In each cage 30 carp yearlings (average mass 56.9–59.9 g) were placed. Fish were taken from the fish farm "Mošorin" near Novi Sad, Serbia; acclimatization period was 3 weeks. Mortality during acclimatization was 4%. Prior to the start of the experiment gill samples were taken for histological analysis (control).

Three fishes from each cage were sampled at the beginning of the experiment, (Measurement I: June 21. 2005), and after a month's time another three fish were taken for sampling (Measurement II: July 20. 2005). These fish were sacrificed and their second left gill arch taken and fixed in 4% formaldehyde. For histology, standard technique of paraffin embedding and hematoxilin and eosin (HE) staining was used. Gills were examined, and microphotographs made using a Leica DM LS light microscope equipped with the DC 300 camera.

Since all the gills examined in both wells, at the beginning and after 1 month experiment had similar histological structure, two morphometric parameters were measured on gill secondary lamellae: diffusion distance (DD) and lamellar thickness (LT), in order to determine whether there were differences between investigated parameters between two wells and between two measurements. DD and LT are measures of the diffusion distance between the water environment and fish blood/erythrocytes. Increase of this distance impairs the gill respiratory function [28,36]. For morphometric analysis measurements were carried out on histological slides of the gills from three fish using a Leica IM 1000 and Image J program [35]. Lamellar thickness was considered as the perpendicular distance across the lamella, from the outermost epithelium on each side of the lamellar cross-section; and diffusion distance as the minimum distance from a randomly selected point on the outer lamellar epithelium to the inside of the nearest blood lacuna [36]. For these analyses, only gill sections in which primary lamellae had similar proportions of cartilage in their centre were selected in order to maintain consistency of section angle through the secondary lamellae in relation to the gill arch and primary filament. Every secondary lamella was measured once, for each morphometric parameter. Range of measurements per one fish was from 113 to 177 (since sections had different number of secondary lamellae). In summary, total number of secondary lamellae measured was 2284 for both parameters. To avoid bias, measurements were performed according to the following scheme: in the basis of the first lamella, middle of the second, and tip of the third lamella. This scheme was applied for each triplicate of secondary lamellae for both morphometric parameters.

Statistical analysis of the results obtained in the experiment was carried out using statistical package STATISTICA v.6. All the results were statistically evaluated using ANOVA, LSD and T-test for parametric or Kruskal–Wallis and Mann–Whitney U-test for nonparametric statistical analysis depending on the coefficient of variation and the results of Levene's test for homogeneity of variances.

3. Results

3.1. Analysis of water quality according to physical and chemical parameters

Average values for all three investigation periods show that water temperature was the highest at W1 and lowest at W2 (Table 1). LSD test showed statistically significant difference between these two sites ($p = 0.02$). Among investigation years statistical analysis showed that there was significant difference between water temperature in the first and second year ($p = 0.015$) and very significant between second and third ($p = 0.009$). Dissolved oxygen was on average similar between W1 and W2 (8.78 and 8.27 mg/L) and statistically not significant ($p = 0.301$). Much lower was dissolved oxygen in S1 and S2 but similar in-between (4.63 and 5.38 mg/L). For this parameter, location W1 statistically very significantly differed from S1 and S2 ($p < 0.01$) and location W2 very significantly from S1 ($p < 0.01$) and significantly from S2 ($p < 0.05$). Concerning investigation years, the highest average value for dissolved oxygen was observed in the first year (10.053 mg/L). LSD test did show that the second and third year differed very significantly from the first year ($p < 0.01$). Electroconductivity of water showed statistically very significant differences between locations ($F = 34.253$, $p < 0.01$) as well as between years ($F = 14.386$, $p < 0.01$). Concerning this parameter all locations differed significantly from each other except between W1 and S2 (Fig. 1). Similar to dissolved oxygen, the second and third year differed very significantly from the first year. Additionally, there was significant differences between electroconductivity in the second and third year ($p = 0.025$). Factor year had a statistically very significant effect on dissolved oxygen ($F = 38.783$, $p = 0.000$) and electroconductivity ($F = 14.386$, $p = 0.000$) and significant effect on temperature ($F = 4.817$, $p = 0.018$). Interactions between years and locations showed very significantly differences only for dissolved oxygen ($F = 17.878$, $p = 0.000$). Due to problems with the pH meter, statistical analysis of this parameter was not possible since there were not enough measurements. Average values of this parameter at all four sites were in the range from 6.43 to 7.93.

Measurements of nitrogen content as well as other potential chemical contaminants, such as heavy metals, have shown that the surface and groundwater of the Radmilovac catchment area were not polluted [30].

3.2. Analysis of water quality using zooplankton and macrozoobenthos

In both wells zooplankton community generally had a low diversity observing individual investigating periods. Overall, summarized for the three years, W1 had 25 and W2 31 identified taxa (Table 2). In 2005, 2006, and 2007 year W1 had 13, 12, 8 and W2 20, 14, 13 identified taxa, respectively. Rotifers were the dominating group with 22 taxa in W1 and 23 taxa in W2 identified during the whole investigation period. Among this group of zooplankton organisms β mesosaprobity indicators prevailed. A gradual decline in species diversity as well as their frequencies was observed at these locations during three subsequent years but there was no statistical

Table 1
Descriptive statistics of physical and chemical water parameters of W1, W2, S1 and S2 sites for all three investigation periods.

Site	T_{water} (°C)		Dissolved O_2 (mg/L)		% of water saturation		pH		Electroconductivity (μS/cm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
W1	20.22	4.14	8.75	3.35	97.29	39.31	7.93	0.20	1086.00	81.07
W2	16.68	3.24	8.27	8.16	89.40	89.97	6.43	2.09	1323.11	86.57
S1	17.86	2.88	4.63	0.92	49.80	9.91	7.76	0.58	1164.78	40.23
S2	19.15	2.92	5.38	1.40	57.53	14.86	7.92	0.27	1103.63	93.13

Table 2
Occurrence of zooplankton taxa at locations W1 and W2.

Locations	W1				W2			
Inv. years	I	II	III	Sum	I	II	III	Sum
Taxa								
Rotatoria								
Anueropsis fissa (Gosse)						+		+
Ascomorpha sp.		+	+	+	+	+	+	+
Asplanchna sp.	+	+	+	+		+	+	+
Brachionus angularis (Gosse)		+	+	+	+			+
Brachionus calyciflorus Pallas	+	+	+	+	+	+	+	+
Brachionus quadridentatus (Hermann)	+			+				
Cephalodella sp.	+			+		+		+
Collurella sp.	+			+	+			+
Filinia sp.	+			+	+			+
Filinia longiseta (Ehrenberg)							+	+
Filinia terminalis (Plate)							+	+
Gastropus sp.						+		+
Keratella sp.	+			+	+			+
Keratella cochlearis (Gosse)	+			+	+			+
Keratella quadrata (O.F. Muller)	+	+		+	+		+	+
Keratella valga (Ehrenberg)					+			+
Lecane sp.	+			+	+			+
Lepadella sp.	+	+		+	+	+		+
Lepadella patella (O.F.M.)					+		+	+
Mytilina mucronata (O.F.M.)	+			+	+			+
Phylodina sp.		+		+	+	+		+
Polyarthra sp.	+	+	+	+	+		+	+
Polyarthra dolicoptera Idelson	+			+	+	+		+
Polyarthra vulgaris Carling		+		+		+	+	+
Pomfolix complanata Gosse		+		+				
Synchaeta sp.	+			+				
Testudinela sp.	+			+				
Trichocerca sp.	+			+				
Cladocera								
Alona sp.			+	+	+			+
Bosmina longirostris (O.F. Muller)	+	+	+	+	+		+	+
Chydorus sphaericus (O.F. Muller)						+	+	+
Daphnia longispina (O.F. Muller)							+	+
Moina brachiata Jurine						+		+
Moina sp.						+		+
Copepoda								
Acanthocyclops sp.					+			+
Cyclops sp.	+	+	+	+	+	+	+	+
Total No. of taxa	19	12	8	25	20	14	13	31

+ Occurrence of taxa.

significance between them (t = –0.933; p = 0.404). The saprobity index at both open wells had a trend that corresponded with the decrease in the number of species and frequencies, having a slight increase at both locations by the third investigation year (Fig. 3). At Well 1 the saprobity index ranged from 1.6 to 1.96 during the whole investigation period. Well 2 had a slightly wider saprobity index range, from 1.40 to 2.10. However, analysis of variance showed no statistically significant differences (p > 0.05) between years for this parameter at locations W1 and W2. On average, wells had a similar saprobity index namely 1.79 for W1 and 1.75 for W2 statistically not significant (t = 0.423, p = 0.678). In regard to monthly fluctuations, in July the saprobity index was slightly higher than during other months in W1 during the first and second year and in W2 during all investigation years (Fig. 2).

During the investigation period macrozoobenthos at both sites of stream Šugavac had a generally low diversity (Table 3). The decrease in the taxa diversity towards the 3rd investigation year (Table 3) showed a similar trend as zooplankton in wells (Table 2). However, S1 had 8, 7, 2 and S2 had 6, 7, 4 identified taxa, respectively. Concerning number of identifies taxa, no statistical difference was observed between these two locations during three investigation yeas (t = 0.189; $p = 0.859$). Identified taxa were mainly from two groups, Oligochaeta and Chironomidae, that are indicators of beta- and alpha-mesosaprobity. S1 was less polluted having a saprobity index ranging from 1.6 up to 2.3 during all three years that was justified by analysis of variance (F = 0.182, $p = 0.838$) and LSD test showing no statistical significance between years. S2 had smaller variations of this parameter in the same period, ranging from 2.28 to 2.56 (Fig. 2). Kruskal–Wallis ANOVA and Mann–Whitney U-test showed no differences between years. Differences between saprobity index of S1 and S2

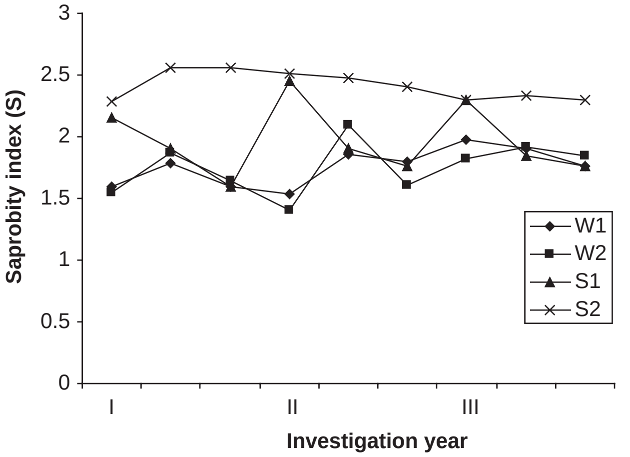

Fig. 2. Saprobity index of investigated wells during three investigation years.

was found to be significantly different indicated by Mann–Whitney U-test (Z = –2.791, $p = 0.005$). Comparing all four locations in-between showed that saprobity index of S2 was statistically very different from other three locations

Table 3
Occurrence of macrozoobenthos taxa at locations S1 and S2 + occurrence of taxa.

Locations	S1				S2			
Inv. years	I	II	III	Sum	I	II	III	Sum
Taxa								
Oligochaeta							+	+
Nedet. Oligochaeta	+	+		+	+	+		+
Hirudinae								
Hirudo medicinalis Linnaeus					+			+
Erpobdella testacea Savigny		+		+	+	+		+
Glossiphonia complanata Linnaeus		+		+		+		+
Nematomorpha								
Gordius aquaticus Linnaeus	+			+	+			+
Isopoda								
Aselluss aquaticus Linnaeus			+	+				
Gammaridae								
Gammarus balcanicus Schäferna	+	+		+		+	+	+
Gammaru fossarum Koch	+	+		+				
Heteroptera								
Nepa cinerea Linnaeus	+			+				
Odonata								
Onychogomphus sp.							+	+
Diptera								
Tipula sp.					+			+
Bezzia sp.	+	+		+		+		+
Pericoma sp.						+		+
Coleoptera								
Helodes minuta Linnaeus	+		+	+				
Chironomidae								
Undet. Chironomidae	+	+	+	+	+	+	+	+
Total No. of taxa	8	7	3	11	6	7	4	12

($p < 0.01$). Similar to these results, comparing saprobity index between all four locations in every investigation year separately showed that location S2 was statistically very significantly different from W1, W2 and S1 ($p \leq 0.01$).

3.3. Analysis of water quality using fish gills

Lesions found on the gills of carp yearlings are presented on Fig. 3.

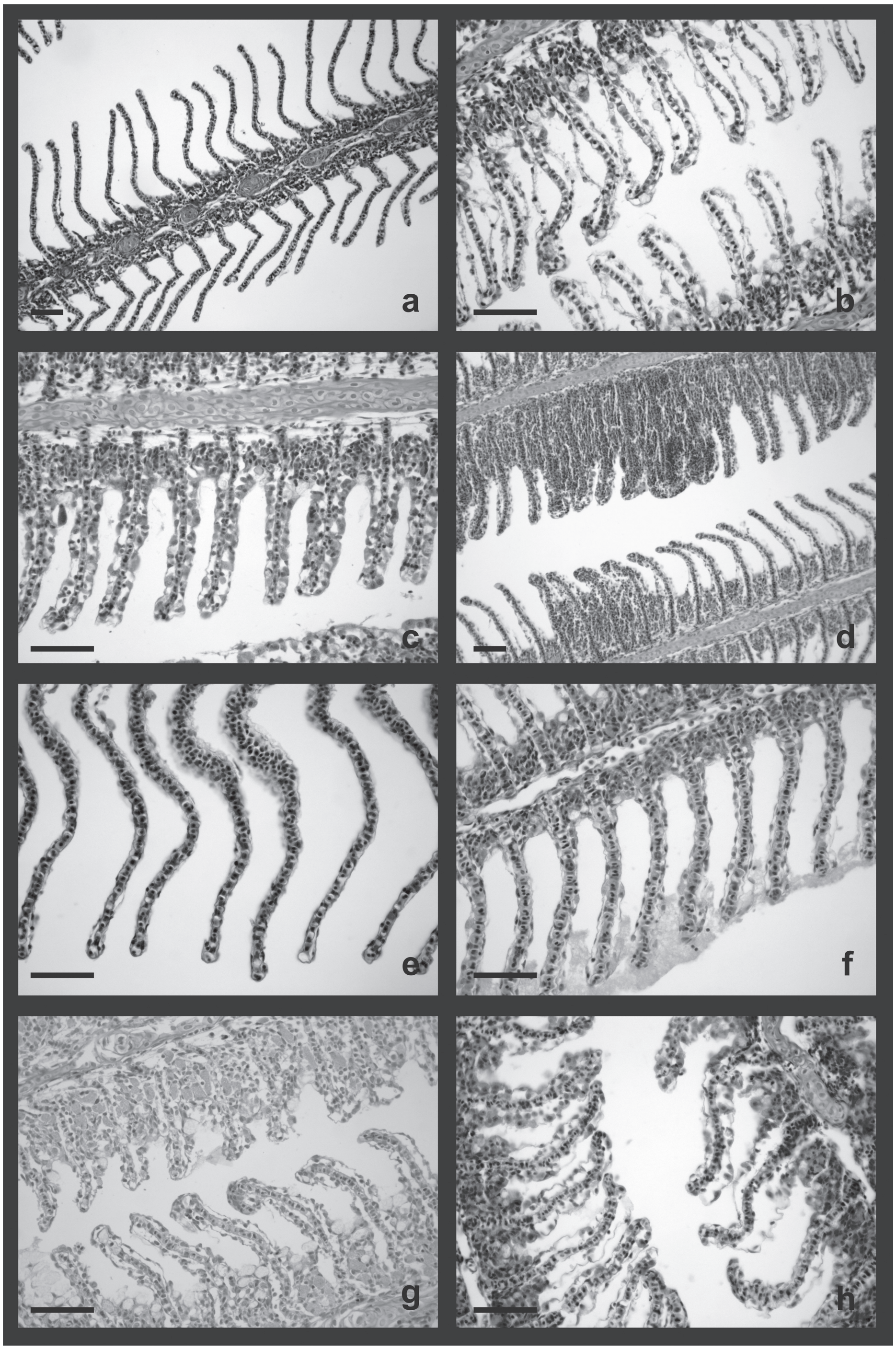

Fig. 3. Lesions found on the gills of carp yearlings (a) Normal gill structure (HE × 200); (b) Lifting of the secondary epithelium (HE × 400); (c) Hypertrophy of secondary epithelium (HE × 400); (d) Hyperplasia of primary epithelium (HE × 200); (e) Hyperemia of secondary lamellae (HE × 400); (f) Increased mucous secretion (HE × 400); (g) Proliferation of eosinophylic cells (HE × 400); (h) Wrinkled respiratory epithelium (HE × 400); Each bar represents 50 μm.

Besides normal gill morphology (Fig. 3,a) that prevailed, majority of gills examined had proliferated primary epithelium that filled up the space between secondary lamellae (Fig. 3,d), with hyperplasia of the eosinophylic undifferentiated cells (Fig. 3,g) (not found in well 2). Proliferation of mucous cells (Fig. 3,f), lifting of the respiratory, secondary epithelium (Fig. 3,b), wrinkled epithelium (Fig. 3,h), its hypertrophy (Fig. 3,c) and hyperemia of secondary lamellae (Fig. 3,e) are changes found on all the gills examined regardless of the well or period of sampling. Trichodinid parasites were found on all the gills examined except in well 2 at the beginning of the experiment.

Due to the fact that the histological analysis have unveiled that majority of the gills had similar alterations, a morphometrical examination was carried out: measurements of DD and LT followed by statistical analysis.

The results of descriptive statistics of morphological measurements are presented in Table 4. Based on the values of the coefficient of variation the following could be concluded: the least variability was found in lamellar thickness—measurement I, well 2;

most variability was in diffusion distance, measurement I, well 2. Data were more homogenous for variable LT than for variable DD. Data were homogenous (coefficient of variation less than 30%, C_V <30%) except for DD in the second measurement in well 2 (36%).

Statistical significance of the average values of the parameters measured between two wells and two measurements was assessed using a nonparametric Mann–Whitney U-test. Average values for LT and DD were lower in well 1. Their differences in the second measurement were higher than differences in first measurement. But, however all differences were not statistically significant ($p > 0.05$). It means that the measured parameters of the gills of fish placed in the wells did not differ between the wells. Between two measurements, there were no statistically significant changes in analyzed morphological parameters ($p > 0.05$), although in the well 1 the difference between two measurement had a trend towards statistical significance ($p = 0.06$). In well 1 average value of LT and DD decreased between two measurements, while in 2 it increased.

4. Discussion

All monitored physical and chemical factors varied throughout the whole investigation period at all locations and investigation years. Water temperature of sites differed significantly among years, indicating that the 2006 year differed from the years 2005 and 2007 having the lowest average value. However, between locations this parameter was rather consistent, except for W2 that had the lowest water temperature and significantly differed from W1. Dissolved oxygen and electroconductivity were the most variable parameters concerning locations and years. Both sites of stream Šugavac differed significantly from open wells concerning dissolved oxygen that could be due to a bigger organic load exerted into this small waterbody, decreasing the oxygen level. There was a big inconsistency in this parameter at W2 between years with extremely low dissolved oxygen (under 1 mg/L) during year 2006. Such low concentration of dissolved O2 was probably caused by overgrowth of *Lemna* sp. that occasionally covered the whole water surface of the wells. This could have been one of the reasons for a gradual decrease of zooplankton diversity and water deterioration during three subsequent years [7]. All locations differed significantly in-between concerning electroconductivity except W1 and S2 probably due to their very close positions (Fig. 1). At all sites, pH value of water was mostly neutral. This was expected since moderate organic pollution usually doesn't dramatically affect the pH of a waterbody. Beside this, neutral pH of water is optimal for most aquatic invertebrates (including zooplankton and macrozoobethos) and for common carp and however didn't affect water quality of investigated locations. The origin of nitrogen in Šugavac stream and periodic increase

Table 4
Descriptive statistics of lamellar thickness and diffusion distance.

Morpho metric parameter	Measurement	Well	Mean	Median	Min.	Max.	SD	SEM	CV
Lamellar thickness (LT)	I	1	15.160	14.095	12.430	18.955	3.390	1.957	22.36
		2	16.560	17.090	15.430	17.160	0.979	0.565	5.91
	II	1	12.750	12.105	11.400	14.745	1.763	1.018	13.83
		2	17.675	15.905	11.500	22.620	4.340	2.506	24.55
Diffusion distance (DD)	I	1	4.755	4.255	3.890	6.120	1.196	0.691	25.155
		2	4.788	4.910	4.450	5.005	0.297	0.171	6.199
	II	1	3.665	3.915	2.860	4.220	0.714	0.412	19.472

may be connected to agricultural and urban runoff and uncontrolled sewage from the houses in the top of the Radmilovac hills [30].

The gradual decrease in species diversity observed at all four locations was probably due to the gradual increase in the water exploitation from the wells, for the maintenance of the newly built fish farm during 2nd and 3rd year, provoking disturbance in the zooplankton communities [37]. Among Rotifers, the dominating group of zooplankton, three species of Brachiunus (*B. angularis, B. calysiflorus and B. quadridentatus*) where present in both wells (Table 2). These species have a high saprobic value (2.5) meaning that they are associated with moderate to high organic pollution [6,7,38] and can reach remarkable densities in eutrophic waters [39,40). According to an extended scale of saprobity levels [41,42], the saprobity index ranging from 2.3 to 2.7 belong to the β to α mesosaprobity level. Other species of Rotifers, Cladocera and Copepoda found at W1 and W2 were mainly indicators of β mesosaprobity. However, just a few identified species were oligosaprobic, *Filinia terminalis*, *Keratella valga* and *K. quadrata*, *Polyarhtra dolichoptera* and *Anueropsis fissa* present in low frequencies. Saprobity index of W1 and W2 was similar in-between as well as between investigation periods. Occasional slightly higher saprobity index of wells observed in July was probably a consequence of more intensive pollution from the neighboring hotel and private houses during summer months.

Initial low taxa diversity and dominance of certain groups of macrozoobenthos at the two locations of stream Šugavac can be an indication of initial pollution. In small water ecosystems inflow of wastewater rich in decomposing organic matter generates decline of species diversity and replacement of pollutant-sensitive species with pollution-tolerant organisms, such as oligochaetes and chironomid larvae [43]. During all investigation periods, a high abundance and dominance of Oligochaeta and Chironomidae has been observed specially at S2, indicating low water quality [44–46]. These two groups have high saprobity values, 2.6 and 2.3, belonging to α and β mesosaprobity zones. The presence of family Gammaridae in freshwaters is generally associated with relatively clean water, springs and creeks [47] but since these organisms are mainly mesotrophic detritophagues they can feed on particulate organic mater [48,49]. The abundance of one species of this family, *Gammarus balcanicus* that is oligosaprobic at location S1 indicates that this part of the stream Šugavac (Fig. 1) was much less polluted than S2. This was expected since it is less affected by negative anthropogenic loads due to its location above the potential polluters. The site of Šugavac that is 400 m downstream, S2, was much more polluted and its saprobity index differed significantly not only from S1, but as well from both open wells. Kljujev and Raičević [50] and WATERWEB annual reports confirmed presence of total coliform bacteria in all investigated water sources and fecal coliform bacteria in all sources except in W1 [30,50].

In this study, normal gill morphology was prevalent. However, a number of alterations, mostly moderate have been noticed: proliferation of the primary epithelium filling up the space between secondary lamellae, often accompanied with undifferentiated cells hyperplasia ensuring the probable replacement of differentiated cells. Increased mucous production is one of the first reactions of the gill tissue to irritants present in the aquatic environment even in small concentration. Lifting of the respiratory, secondary epithelium, wrinkled epithelium, and hypertrophy of this, normally very thin epithelium are known defense mechanisms that increase a water–blood diffusion distance. Hyperemia of secondary lamellae represents a very first circulatory alteration that occurs in slightly deteriorated environment. All the lesions of the gills examined belong to reparable changes and characterize a reaction of the gill tissue that corresponds to mild water quality deterioration [27,28]. Irreparable changes such as complete fusion of the secondary lamellae, stasis in blood vessels, or necrotic changes were not found on the gills examined indicating that, according to gill histology, the investigated water from wells was not heavily polluted by toxicants or organic wastes. As already mentioned, a detailed chemical analysis of wells water didn't detect pollution by heavy metals, nitrogen, phosphorus, and pesticides [30]. Finally, as shown by the results of statistical analysis of morphometric measurements there were no differences in lesion severity between the wells and between two measurements, and therefore no significant differences in water quality between two wells.

The present study has confirmed that the selection of a biomonitoring method and its effectiveness depends primarily on the physical and chemical characteristics of the waterbody [1,11]. In fact, in the shallow stream Šugavac the use of macrozoobenthos organisms as indicators of water quality was the best option. Zooplankton was assessed in open wells, since they prefer low flow areas such as backwaters, pools and other standing waters [51] where they can easily reproduce [52] and where populations can attain significant abundance. Experimental fish were placed in cages in open wells to study their gills. It was noted that following microbiological investigation, both the water from Šugavac and the well 2 were polluted by coliform fecal bacteria, and therefore could not be used for irrigation of vegetables without previous treatment [50]. Finally, the study has demonstrated that the aquatic resources of Radmilovac catchment can be used for carp farm and hatchery water supply. Moreover, in order to preserve water resources at Radmilovac and prevent possible pollution and/or water shortage, a recirculation system has been established in the hatchery during 2008.

Acknowledgements

The study was supported by three Projects "Water Resources Strategies and Drought Alleviation in Western Balkan Agriculture—WATERWEB" FP 6 INCO-West Balkan (FP6 INCO-WB), Reinforcement of Sustainable Aquaculture ROSA (FP7 REGPOT No. 205135) and biotechnical project of Serbian Ministry of Science "Improvement of semi-intensive production of common carp (*Cyprinus carpio*) in sustainable aquaculture" (No. TP 20047).

References

[1] P.D. Abel, Water Pollution Biology, Taylor and Frencis Ltd, Gunpower Square, London, 1996.

[2] J.I. Georgudaki, V. Kantzarisa, P. Kathariosa, P. Kaspirisa, Th. Georgiadis and B. Montesantouc, An application of different bioindicators for assessing water quality: A case study in the rivers Alfeios and Pineios (Peloponnisos Greece), Ecol. Indic., 2 (2003) 345–60.

[3] R.A. van Dam, C. Camilleri and C.M. Finlayson, The potential of rapid assessment Techniques as early warning indicators of wetland degradation: A review, Environ. Toxicol. Water Qual., 13 (1998) 297–312.

[4] P. Madoni, Ciliated protozoan communities and saprobic evaluation of water quality in the hilly zone of some tributaries of the Po River (north Italy), Hydrobiologia, 541 (2005) 55–69.

[5] M.E. Carew, V.P. Reneel, L. Cox and A.A. Hoffmann, The response of Chironomidae to sediment pollution and other environmental characteristics in urban wetlands, Freshwater Biol., 52 (2007) 2444–2462.

[6] D.N. Saksena, Rotifers as Indicators of Water Quality, Acta Hydrochimica et Hydrobiologica., 15(5) (2006) 481–485.

[7] J.L. Attayde and R.L. Bozelli, Assessing the indicator properties of zooplankton assemblage to disturbance gradients by canonical correspondence analysis, Can. J. Fish. Aquat. Sci., 55 (1998) 1789–97.

[8] C. W. C. Branco, M.-I. A. Rocha, G. F. S. Pinto, G. A. Gômara and R. De Filippo, Limnological features of Funil Reservoir (R.J., Brazil) and indicator properties of rotifers and cladocerans of the zooplankton community, Lakes & Reservoirs: Research and Management, 7 (2002) 87–92.

[9] A. Lehmann and J.-B. Lachavanne, Changes in the water quality of Lake Geneva indicated by submerged macrophytes, Freshwater Biol., 42 (1999) 457–66.

[10] J.E. Gannon and R.S. Stemberger, Zooplankton (Especially Crustaceans and Rotifers) as Indicators of Water Quality, T. Am. Microsc. Soc., 97 (1978) 16–35.

[11] V. Sládecek, System of Water Quality from the Biological Point of View, Limnology Report; No. 7, Lubrecht & Cramer Ltd. 973.

[12] S. Cadjo, A. Miletic and A. Djurkovic, Zooplankton of the Potpec reservoir and the saprobiological analysis of water quality, Desalination, 213 (2007) 24–8.

[13] K. Kaatra, Saprobiological Evaluations of the Silvola Reservoir, Acta Hydrochimica et Hydrobiologica, 6 (4) (2006) 321–28.

[14] I.F. Spellerberg, Monitoring Ecological Change, Cambridge University Press, Cambridge, 2005.

[15] V. Sládecek, Rotifers as indicators of water quality, Hydrobiologia, 100 (1983) 1, 169–201.

[16] L. Hakkari, Zooplankton species as indicators of environment, Aqua. Fennica, P (1972) 46–54.

[17] P.V. Cairns, B.R. McCormick and A. Niederlehner, A proposed framework for developing indicators of ecosystem health, Hydrobiology, 263 (1993) 1–44.

[18] R.M. Pontin and J.M. Langley, The use of rotifer communities to provide a preliminary national classification of small water bodies in England, Hydrobiology, 255/256 (1993) 411–9.

[19] H.Y. Zakaria, M.H. Ahemed and R. Flower, Environmental assessment of spatial distribution of zooplankton community in Lake Manzalah Egypt, Acta Adriat., 48 (2007) 161–72.

[20] J.C.H. Carter, M.J. Dadswell, J.C. Roff and W.G. Sprules, Distribution and zoogeography of planktonic crustaceans and dipterans in glaciated eastern, North America, Can. J. Zool., 58 (1980) 1355–87.

[21] J.B. Shurin, J.E. Havel, M.A. Leibold and B.P. Alloul, Local and regional zooplankton species richness: a scale-independent test for saturation, Ecology, 81 (2000) 3062–73.

[22] L. Triest, P. Kaur, S. Heylen and N. De Pauw, Comparative monitoring of diatoms, macroinvertebrates and macrophytes in the Woluwe River (Brussels, Belgium), Aquat. Ecol., 35 (2001) 183–94.

[23] D.M. Rosenberg and V.H. Resh, Freshwater Biomonitoring and Benthic Macroinvertebrates, Chapman and Hall, New York, 1993.

[24] J. Skriver, N. Friberg and J. Kirkegaard, Biological assessment of running waters in Denmark: Introduction of the Danish Stream Fauna Index DSFI, Verh. Internat. Verein. Limnol., 27 (2000) 1822–1830.

[25] J.L. Wilhm, Biological indicators of pollution, In: B.A. Whitton, (ed.), River ecology Studies in ecology Vol 2. Blackwell, London, 1975.

[26] J.M. Hellawell, Biological indicators of fresh-water pollution and environmental management, Elsevier, London, 1986.

[27] Z. Svobodova, R. Lloyd, J. Máchová and B. Vykusová, Water quality and fish health EIFAC Technical Paper. No.54, 1993.

[28] V. Poleksić and V. Mitrović-Tutundžić, Fish gills as a monitor of sublethal and chronic effects of pollution, In: R. Muller, R. Lloyd (ed) Sublethal and chronic effects of pollution on freshwater fish, FAO Fishing News Books, 1994.

[29] B. Jezierska and M. Witeska, Fresen, The effects of metals on fish gill function—gas and ion exchange. Environ. Bull., 13 (2004) 1370–8.

[30] www.waterweb.dk.

[31] Z. Marković, I. Živić, V. Poleksić, Z. Dulić and M. Stanković, Biological Characteristics of Two Small Aquatic Ecosystems-Uncovered Wells on the Experimental School Estate of the

Faculty of Agriculture University of Belgrade, Proceedings Conference on Water Observation and Information System for Decision Support “BALWOIS 2006”, Ohrid, 2006.
[32] American Public Health Association (Standard Methods for the Examination of Water and Wastewater. American Water Works Association, Water Environment Federation. Managing editor Mary Ann H. Franson. Washington, DC, 1998.
[33] R. Wegl, Wasser und Abwasser, Beitrage zur Gewasserforschung 12 Band 26, Ortendorfer J.L. Hofrat, W. Wien, 1983.
[34] E. Pantle and H. Buck, Die biologische Uberwachung der Gewaser und die Darstellung der Ergnisse, Gas-u Wasserfach, 96 (1955).
[35] M.D. Abramoff, P.J. Magelhaes and S.J. Ram, Image Processing with Image, J. Biophot. Inter., 11 (2004) 36–42.
[36] H.M. Lease, J.A. Hansen, H.L. Bergman and J.S. Meyerc, Structural changes in gills of Lost River suckers exposed to elevated pH and ammonia concentrations, Comp. Biochem. Phys. C, 134 (2003) 491–500.
[37] D.F. Maley, P.S.S. Chang and D.W. Schindler, Decline of zooplankton population following eutrophication of Lake 227 Experimental Lakes Area Ontario: 1969–1975, Can. Tech. Rep. Fish. aquat. Sci., 1619 (1988) 29.
[38] B. Brznis B and B. Pejler, Rotifer occurrence in relation to oxygen content, Hydrobiology, 183 (1989) 165–72.
[39] M. Doohan, Ecological aspects of used water treatment: Vol. 1. Rotifera. The organisms and theirecology, Academic Press, London, 1975.
[40] P.L. Starkweather, Sensory potential and feeding in rotifers: structural and behavioral aspects of diet selection in ciliated zooplankton. In: Lenz, P.H.; Hartline, J.E.; Purcell, J.E.; Macmillan, D.L. (eds.). Zooplankton: sensory ecology and physiology.Gordon and Breach Publishers, Amsterdam, 1996, 255–66.
[41] LAWA, Die GewaË sserguË terkarte der Bundesrepublik Deutschland. LaË nderarbeitsgemmenschaft Wasser, 1985, Berlin.
[42] DIN 38410, 1990. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung: Biologisch-oË kologische GewaË sseruntersuchung (Gruppe M): Bestimmung des Saprobienindex (M2).
[43] D.G. Farara and A.G. Burt, Environmental assessment of Detroit River sediments and benthic macroinvertebrate communities–1991, Report prepared for the Ontario Ministry of Environment and Energy by Beak Consultants Limited, Brampton Ontario, Volume I, 1993.
[44] W.L. Hilsenhoff, Rapid field assessment of organic pollution with a family-level biotic index, Am. Benthol. Soc., 7 (1998) 65–8.
[45] J.L. Plafkin, M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes, Rapid Bioassessment Protocols for use in Streams and Rivers: Benthic Macroinvertebrates and Fish. US Environmental Protection Agency EPA 440/4–89/001, 8 chapters, Appendices A-D, 1989.
[46] R.W. Bode, M.A. Novak and L.E. Abele, Quality Assurance Work Plan for Biological Stream Monitoring in New York State, NYS Department of Environmental Conservation Albany NY, 1996.
[47] G.S. Karaman and S. Pinkster, Freshwater Gammarus species from Europe North Africa and adjacent regions of Asia (Crustaces—Amphipoda) 1 Gammarus pulex-group and related species, Bijdr. Dierk., 47 (1977) 1–97.
[48] H.J. De Lange, W. Noordovena, A.J. Murkc, M. Lürlinga and E.T.H.M. Peetersa, Behavioural responses of Gammarus pulex (Crustacea Amphipoda) to low concentrations of pharmaceuticals, Aquat. Toxicol., 78 (2006) 209–16.
[49] S. Trajkovic, S. Brankovic and M. Gocic, Analysis of Biological Water Quality Parameters of the River Nisava Upstream of Water Treatment Plant Mediana Conference on Water Observation and Information System for Decision Support “BALWOIS 2008”, Ohrid, 2008.
[50] I. Kljujev and V. Raićević, Dynamics of number coliform bacteria in waters from experimental fields, Proceedings Conference on Water Observation and Information System for Decision Support “BALWOIS 2006”, Ohrid, 2006.
[51] W. Richardson, Micro-crustaceans in flowing water: Experimental analysis of washout times and a field test. Freshwater Biol., 28 (1992) 217–30.
[52] P. Vila, The occurrence and significance of Cladocera (Crustacea) in some streams of Central Indiana, USA. Hydrobiologia 171 (1989) 210–4.