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STABILIZING EFFECT OF WWTP DISCHARGE ON WATER QUALITY AND FISH ASSEMBLAGE STRUCTURE. A CASE STUDY

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Abstract
We examined the effluent from a municipal (Nyíregyháza, Hungary) wastewater treatment plant (WWTP) on hydrophysico-chemical properties and on diversity, community structure, and stability of fish assemblages at the recipient low flow channel system during a two-year period. The WWTP outflow increased significantly the nutrient concentrations (e.g. NO_2 and NO_3 concentrations increased to 4x and 8x respectively), and the regime (with the permanent ~0.23m^3/s load) at the recipient channel sections. The wastewater outflow not only altered, but stabilized the physico-chemical variables measured, and the water regime in the recipient channels. Thus the natural, periodic fluctuation of the environmental variables was diminished in the study period. The WWTP outflow caused significant changes in the fish fauna as well. High abundances and taxa richness were found in the stocks inhabiting the charged watercourse sections. At the same time, species composition and relative abundances of fish stocks proved to be more constant at the impaired sites. Our results show that the WWTP outflow caused altered, but significantly more stable

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environmental conditions. These alterations were favourable for the emergence of a more diverse and more stable fish community on the recipient channel sections. Hence, the dynamic variability in fish assemblage structure that is characteristic of natural lowland stream was not apparent in these perturbed, semi natural habitats

Key words: community variability, environmental stability, lowland watercourses, municipal discharge

1. Introduction

It has been long recognized that streams are influenced by the landscape through which they flow (Hynes 1975; Vannote et al., 1980), and thus watercourses crossing urban areas are affected by a multitude of human generated pressures (Johnson et al., 2006), such as organic pollution (Zamora-Muñoz and Alba-Tercedor 1996), nutrient enrichment (Paul and Meyer 2001), and alteration of hydromorphology (Stanner and Bordeaux 1995). One of the most important sources of disturbance in urbanized landscapes is the loading of wastewater into the receiving systems (e.g. rivers, lakes, dams, channels…). Growing urbanisation requires the expansion of the communal supplies, e.g. the development of sewage conduit and treatment systems. Therefore growing capacity of wastewater treatment plants (WWTP) affect the water quality and the water regime of the recipient systems simultaneously (Allan 2004; Brooks et al., 2006; Canobbio et al., 2009). Notwithstanding there is a lack of information how WWTP discharge affects the temporal dynamics of the physical and chemical water properties in the recipient watercourses. Responses of the aquatic fauna to WWTP discharge are relatively well documented, especially for macroinvertebrate and fish assemblages, which are frequently used organisms in bioassessment (Adams et al., 1992, Rosenberg and Resh 1993; Coimbra et al., 1996). Results show that the WWTP discharge may affect the biota at multiple organizational levels (Porter and Janz 2003). At the infraindividual level, it can cause metabolic changes by the alteration of the enzymatic expression or activity (Grung at al. 2007, Jeffries et al., 2008) such as altering the endocrine system (Xie et al., 2005, Pinto et al., 2009). These changes, (e.g. changes in sex ratio, or decreased mating capacity) may cause serious negative influences at the population level (Thorpe et al., 2009). Depauperated and uniformized assemblages were often found in stream sections receiving WWTP outflows, which could be characterized by the dominance of some tolerant taxa and the lack of sensitive species (Reash and Berra 1987; Nedeau et al., 2003; Roy et al., 2003).

There are several mechanisms that may drive variation in fish assemblages in natural streams, even on short time scales (Takács et al., 2012). Considerable change in assemblage structure may result from periodic fluctuation of environmental parameters such as water chemistry, flow regime, food availability and macrophytes cover (Moyle and Vondracek 1985; Taylor et al., 1996; Lusk et al., 2001; Taylor and Warren 2001; Bunn and Arthington 2002; Erős and Grossman 2005; Keaton et al., 2005) and the appearance and mortality of offspring (Gelwick 1990; Janáč and Jurajda 2005). Moreover, studies from primarily natural systems (Meffe and Minckley 1987, Matthews et al., 1988; Death 1995; Medeiros and Maltchik 2001) show that stable environmental conditions contribute to the establishment of temporally more stable assemblages. Tsai et al. (1991) showed that stabilized environmental conditions caused by WWTP loading may yield highly abundant assemblages.

Although various effects of the WWTP effluent on aquatic community structure have been well explored, there are only few pieces of information about how the WWTP outflows influences the temporal dynamics of assemblages (i.e. stability vs. variability) in the impacted channel sections (Wakelin et al. 2008; Carey and Migliaccio 2009).

We hypothesized that continuous discharge would make the regime and the physico-chemical parameters more balanced in the recipient channel sections, mitigating the natural yearly trend (e.g. reducing the dilution effect of spring floods, etc.). We also predicted that taxa richness would be lower and both richness and assemblage structure would be temporally more constant at the impaired sections of the system than at the non-affected sites. Therefore the aims of our study were 1) to characterize the cleaning efficacy of the WWTP, consequently the organic load of the effluent water, 2) to explore the effects of the WWTP outfall on the values and on the variability of the studied hydrophysical and hydrochemical parameters in the recipient drainage system, and 3) to quantify the changes in the structure and in the variability of fish assemblages inhabiting the wastewater impacted channel sections.

2. Materials and methods

2.1. Study area and sampling procedure

The studied drainage system is situated to the Middle-Nyírség subregion (A: 1958 km^2) in NE Hungary (Fig. 1). The altitude of the catchment area ranges between 95 and 183 m. Cultivation and horticulture are the characteristic land use forms in this lowland area. The main watercourse (length: 91 km) of the catchment area is the Lónyay Main Canal (LMC), which flows and carries the water to the River Tisza.

The average runoff of the LMC is ~2 m^3/s with one peak in flow caused by snowmelt in early spring (Q_{max}: 10.6 m^3/s Q_{min}: 0.2 m^3/s). The largest joining canal to the LMC is the Érpataki-canal (EC; Q_{av}: 0.54 m^3/s, Q_{max}: 2.01 m^3/s, Q_{min}: 0.14 m^3/s,), which receives the effluent waters (~20000 m^3/day ≈0.23m^3/s) of the WWTP of Nyíregyháza, a town with ~120’000 inhabitants. Flow rate data show that more than 40% of the average output in the EC supplied by the WWTP discharge.

The water treatment consist of three processes with the primary action being mechanical filtering (upright step-screen, sand catcher, preliminary sedimentation basins). Following this, biological processes are performed using active sludge (denitrification basins, aearation basins, secondary sedimentation basins). The final process of the sewage treatment is the phosphorus precipitation by chemicals.

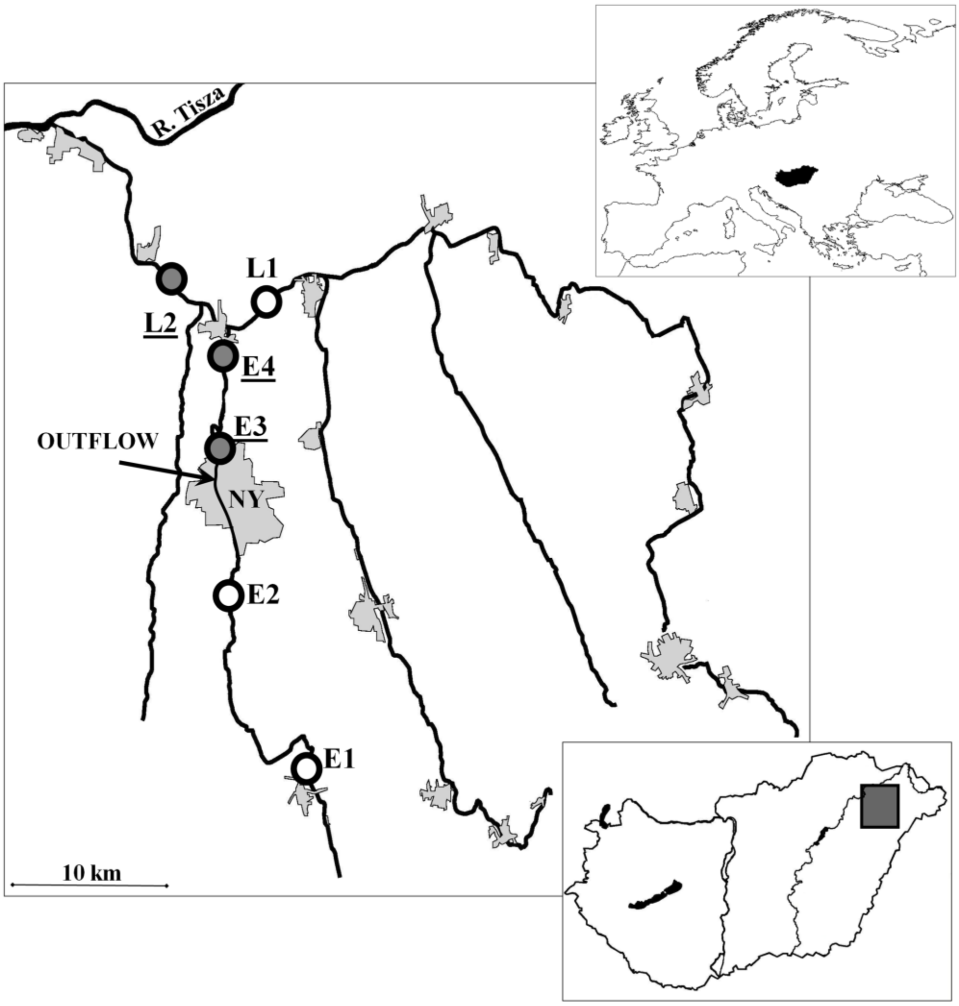

Fig. 1. Map of the studied area in Central-Europe, Hungary. (open circles: not impacted sites, filled circles: impaired sites) NY: City of Nyíregyháza, OUTFLOW: cleaned sewage outflow of the WWTP. Impaired sites are underlined

To assess the variation of water regime the daily recorded data (from the Upper-Tiszanian Environmental Protection, Nature Conservation and Water Authority) were analysed at four sites (at E1, E3, L1, L2), hydrophysico-chemical variables were examined in the outflowing water of the WWTP and at six sampling sites on the recipient watercourses. Of these six sites, four were designated on the EC; and two on the LMC, upstream and downstream from the EC mouth. Consequently, three of the sampling sites (E1, E2, L1) were unaffected, while the other three sites (E3, E4, L2) were influenced by the WWTP effluents (Figure 1). All the sections have sandy riverbeds and are highly modified by channelization. To characterize the sampling sites mean average depth, width, cover of macrophytes, altitude, slope and the distance from the main recipient (River Tisza) of the study sites are shown in Table 1.

Table 1. The mean average depth, width, macrophyte cover, altitude, slope and the spatial position of the study sites. Impaired sites are underlined

	Depth (m)	*Width (m)*	*Cover (%)*	*Altitude (m)*	*Slope (‰)*	*Distance from the R. Tisza (km)*
E1	0.6±0.2	2.9±0.2	35±22	115.0	0.5	52.4
E2	0.8±0.3	3.1±0.2	33±20	106.0	0.5	42.1
E3	0.8±0.4	5.5±0.1	28±18	97.0	1.0	31.1
E4	0.9±0.3	6.5±0.2	50±22	96.0	0.5	24.3
L1	1.1±0.2	10.2±0.3	48±20	95.0	0.2	27.1
L2	1.1±0.3	11.1±0.4	40±31	94.0	0.1	12.3

Since there is no other appreciable inflow on the studied watercourse sections beside the WWTP of Nyíregyháza, it is suggested that the changes in the hydrochemical variables on the two studied channels are caused by the WWTP outflow. – the westernmost joining canal to the LMC carried negligible amount of water in the study period.– Overall 32 measurements were made at biweekly intervals between 2005 and 2006.

A total of 12 environmental variables were measured at each site. Temperature, pH, conductivity, dissolved oxygen saturation (DOS) were measured in the field using a portable multiparameter water quality monitoring system (Multi 350i) (WTW Gmbh). The remaining parameters (NH_4, NO_2, NO_3, Kjeldahl-nitrogen, total-nitrogen, total-phosphorus, Biological Oxygen Demand -BOD_5-, Chemical Oxygen Demand –COD–) were determined in the accredited laboratory of Nyírségvíz Zrt. To evaluate the cleaning efficiency of the WWTP eight parameters (NH_4, NO_2, NO_3, Kjeldahl-nitrogen, total-nitrogen, total-phosphorus, BOD_5, COD) were also measured in the raw sewage as well.

To determine the mean cleaning efficiency of the WWTP the following equation was used (Musatti et al., 2002):

$$CE=(\text{input}-\text{output})/\text{output}\cdot 100$$

where CE: Cleaning Efficacy (%), input: concentration of a certain parameter in the raw sewage, output: concentration of a certain parameter in the outflowing, cleaned water.

At each site (except the outflow canal) the fish were sampled according to the Hungarian monitoring protocols (NBmR protocol 2012). This protocol proposes single-pass electrofisher sampling of 150 m long stream sections. This methodology gives representative information about the species composition and the relative abundance of the fish assemblage (Sály et al., 2009). At each sampling site at each sampling occasion, fish sampling was conducted using an IUP-12 backpack electrofishing gear (350 V, 4–15 A, 40–120 W). Pulsating direct current with a frequency of 75–100 Hz and a voltage of 250–350 V was used. The 2 m long catcher rod had a ring shaped anode with a diameter of 30 cm and equipped with a net (mesh size 6 mm). After identification all the fishes were returned to the channel. To eliminate the bias due to the environmental changes all the sampling sites assigned on one stream were assessed on the same day. Samplings were carried out daytime, usually between 8 am and 18 pm, starting at the uppermost sites and proceeding downstream.

2.2. Data analysis

To assess the variation of water regime in the study period, the coefficient of variation (CV), which represents the ratio of the standard deviation to the mean, was used.

Hydrophysico-chemical data, fish stock sizes (catch per unit effort, CPUE: number of individuals/150m), taxa richness, rarefied species richness -expressed as the number of species expected for 100 individuals: ES_{100} (Heck et al., 1975) and Shannon–Weaver diversity values were displayed on boxplots and compared by one-way analysis of variance (ANOVA). Pairwise comparisons among group means were made using Tukey's range test using PAST statistical software (Hammer et al., 2001). Data were either [log(x+1] transformed prior to analysis when it was necessary to meet assumptions of normality and homoscedasticity. Rarefaction analysis was carried out by the EcoSim software (Gotelli and Entsminger 2001).

To characterize within-site temporal variation, pairwise similarity values were calculated between the hydrophysico-chemical, fish species composition and relative abundance datasets for each sampling site. To express similarities, the one-complement form of the classical Bray-Curtis distance index (i.e. 1 – Distance, as it is implemented in the software package PAST [Hammer et al., 2001]) for hydrophysico-chemical and fish relative abundance data, and Jaccard index (for fish presence-absence data) were used (Legendre and Legendre 1998). Similarity can range between 0 and 1, where the higher values show higher similarities. Temporal variability was then characterized by the overall means (±SD) of these values. The between sites variabilities were calculated by the same manner, and tested for significance by the nonparametric Kruskal-Wallis test. A Mantel test was used to test the congruence between the within site variability of the fish species composition and relative abundance data (Mantel 1967). Spearman rank correlation (R_S) was used to test: a) the relations between the fish relative abundance data and the distance from the main recipient, b) the relationship between temporal variability (i.e. mean similarities) of fish assemblages and spatial position of the sites along the longitudinal profile of the streams. For all statistical analyses 0.05 alpha level was used.

3. Results and Discussion

3.1. Regimes and other environmental variables of the studied canals

On the uppermost (E1) section of the EC runoff ranged between 0 and 0.61m^3/s (mean±SD: 0.13±0.1). At the impacted E3 site the regime increased and became more equable; it ranged between 0.16 and 2.01m^3/s (mean±SD: 0.57±0.3m^3/s). The runoff at the L1 site varied between 0.22 and 8.84m^3/s, (mean±SD: 1.06±0.98m^3/s), and ranged between 0.42 and 9.89m^3/s, (mean±SD: 1.63±1.1m^3/s) at the L2 site. The CV of runoffs in the not impacted E1-, and L1 sections were higher (0.791 and 0.941 respectively), than in the discharged sites, where the CV values were 0.531 and 0.719 on the E3 and L2 sites, respectively. The lowest variability was observed in the regime of the E3 site, which is right under the WWTP outflow. Since there is no other inflow between the two canal sections the remarkable decrease in the runoff variability between the E1 and E3 sites must be caused by the balancing effect of the sewage inflow. Similarly the more equable runoff of the EC balances the regime of the LMC on the lower (L2) section.

3.2. Cleaning efficiency of the WWTP

The cleaning efficiency of the WWTP was examined by using eight variables during the two years period (Table 2). The results show that the WWTP cleaning technology works with different efficacies. In the case of NH_4, Kjeldahl-Nitrogen, BOD_5, and COD the cleaning efficacy is over 90%. More than the 75% of the total phosphorus was eliminated from the system. Simultaneously more than 80% of the total nitrogen was denitrified and the most of the remaining parts were decomposed, increasing the nitrite and nitrate concentrations in the outflowing water. This can be the reason why the cleaning efficacies are negative in the case of nitrite, and nitrate ions.

Table 2. Cleaning efficiency of the WWTP

	Inflow (mg/l)	**Outflow** (mg/l)	**Cleaning efficiency** (%)
Ammonium	67.45±16.0	2.08±2.1	96.60±3.8
Nitrite	0.02±0.01	0.78±0.7	~ - 39000*
Nitrate	0.16±0.11	10.24±4.9	~ - 64000*
Kjeldahl Nitrogen	97.12±21.4	7.98±3.0	94.42±9.7
Total Nitrogen	98.71±17.6	19.05±5.1	80.25±6.0
Total Phosphorus	25.81±11.1	4.87±3.4	77.15±17.0
BOD_5	632.64±197.1	16.96±3.8	96.89±1.4
COD	990.45±324.5	59.10±8.9	93.46±2.3

3.3. Changes of the hydrophysico-chemical parameters in the recipient channels

The studied parameters in the outflowing wastewater show high level differentiation from the recipient in most cases (Fig. 2.). As can be seen, the studied parameters are consistently higher values (except for pH, which showed lower values) than those found in the recipient channels. Nevertheless, there are no differences in the pH on the lower sections. The base rock of the area is alkalic fluvial sand, therefore the sediment-water complex can buffer the low pH of the discharge.

Seven of the twelve studied hydrophysical and chemical parameters showed significant increase in the vicinity of the WWTP outflow (Fig. 2.). Mean values of ammonium, nitrite, nitrate, total-phosphorus, total-nitrogen, BOD concentrations and conductivity values were significantly higher at the three wastewater impacted sites (E3, E4, L2). Higher mean values were detected in case of temperature and DOS in the vicinity of the outflow (at the E3 site) but these differences were not significant. The differences between the E2 and the E3 sites in many variables (eg.: total-phosphorus, total-nitrogen, BOD, nitrite, nitrate) indicated that the wastewater input enriches considerably the concentration of organic pollutants in the recipient canal. The within and between sites similarities were displayed on the Table 3. The within site similarity values (diagonal) ranged between 0.809 and 0.963. The hydrophysical and chemical parameters in the WWTP effluent proved to be the most stable and significantly differed from all the others. Additionally, the within site similarity values were significantly higher in the impacted E3, E4, L2 sites than in the non-impacted sites. The between sites similarities ranged between 0.748 and 0.897.

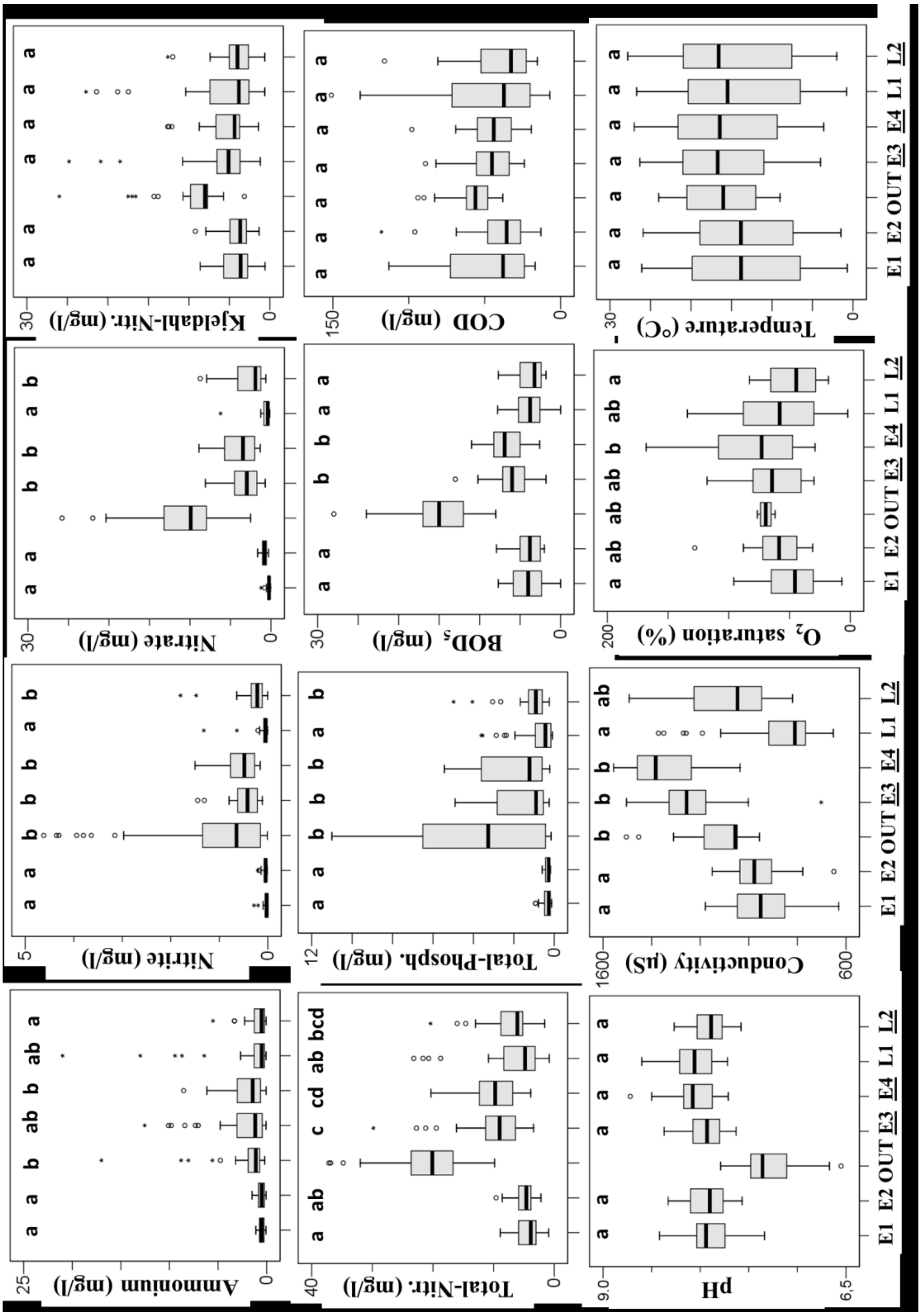

Fig. 2. Boxplots of the physico-chemical parameters. Boxplots with the same letters do not differ significantly based on ANOVA Tukey post hoc comparisons (p≥0.05). The box represents the 25% and 75% quartiles, the band in the box is the median. The whiskers represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are more than 1.5 times the interquartile range) are represented by circles beyond the whiskers. Impaired sites are underlined

The lowest average similarity (0.748) was detected between the E4 and L1 sites and the highest value (0.897) was revealed between the neighbouring E3 and E4 sites, situated to the vicinity of the WWTP outflow.

Table 3. Within and between sites similarities (1 – Bray-Curtis distance) of the physico-chemical parameters. Diagonal (bold): within site similarities of abundance data (mean±SD); above: p values of the Kruskal-Wallis tests (*=significant difference); below: similarity of two related sites (mean±SD). Impaired sites are underlined

	E1	*E2*	*OUT*	*E3*	*E4*	*L1*	*L2*
E1	**0.895±0.06**	*<0.001**	*<0.001**	*<0.001**	*<0.001**	0.009*	1.000
E2	0.912±0.06	**0.907±0.05**	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**
OUT	0.888±0.07	0.901±0.06	**0.963±0.04**	*<0.001**	*<0.001**	*<0.001**	*<0.001**
E3	0.842±0.08	0.860±0.07	0.895±0.05	**0.919±0.07**	1.000	*<0.001**	*<0.001**
E4	0.813±0.09	0.832±0.07	0.874±0.05	0.915±0.06	**0.925±0.05**	*<0.001**	*<0.001**
L1	0.873±0.07	0.875±0.06	0.838±0.06	0.804±0.09	0.776±0.09	**0.871±0.09**	0.043*
L2	0.884±0.08	0.902±0.06	0.904±0.05	0.887±0.07	0.868±0.07	0.846±0.08	**0.896±0.06**

3.4. Fish assemblages

Altogether 14 800 specimens within 30 fish species were collected from the six sites during the two years period. Despite the relatively high species richness the assemblages were dominated by only few species (Table 4) at all sites. The most abundant species were the bleak (*Alburnus alburnus* Linneaus, 1758) with 51.39%, and the prussian carp (*Carassius gibelio* Bloch 1782) with 20.47% relative abundance. In addition, a further seven fish species occurred with over 1% relative abundance. From the dominant species only the relative abundance of bleak showed remarkable changes along the longitudinal profile of the river-system. Spearman rank correlation showed significant increase (R_s=-0.9, p=0.037) of bleak relative abundances in the lower sections. So it seems that it is the only species which occurence and relative abundance was not affected by the WWTP afflux.

Mean values of stock size, species richness, Shannon-Weaver diversities and the rarefied species richness (ES_{100}) are shown in Figure 3. The largest stock and coincidentlythe greatest SD values were found at the E4 section, which differed significantly from the other stock sizes, except for E3. Species richness showed the same pattern, but there were no significant differences between the wastewater impacted sites (E3, E4 and L2). For Shannon-Weaver diversity indices, and rarefied species richness (ES_{100}) the highest values were found at sites E3 and L2. These values tended to be higher in the impacted sites, however, they were not significantly different to the non-impacted sites.

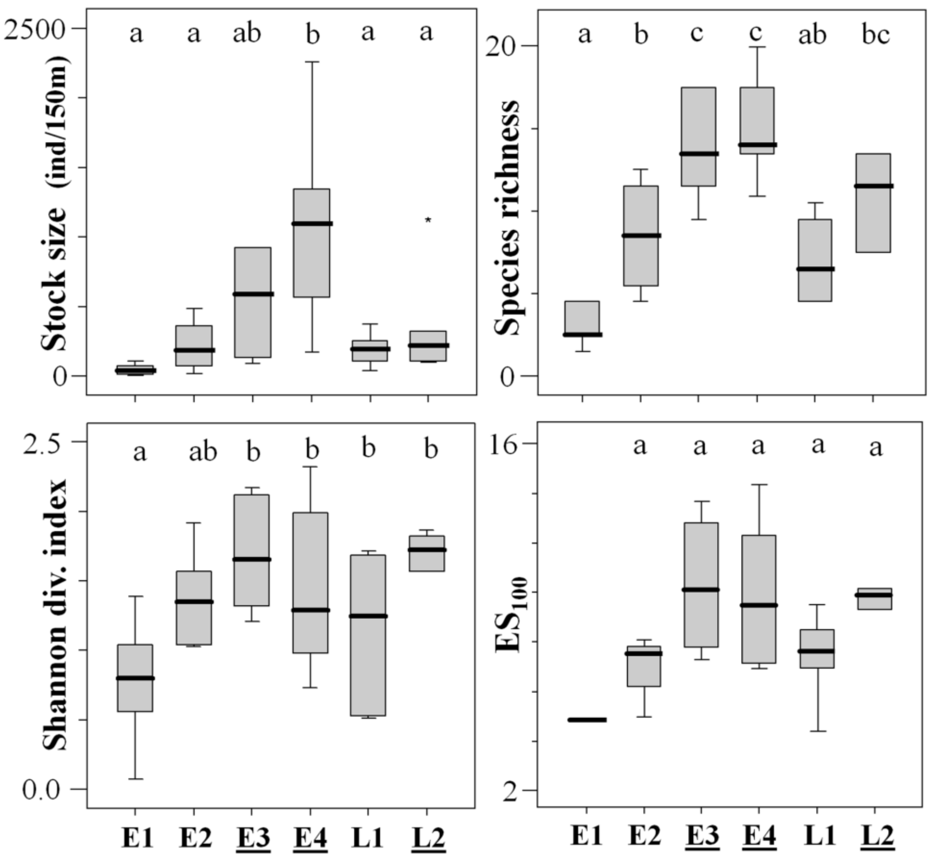

Fig. 3. Comparison of the studied parameters in the case of fish assemblages. Boxplots with the same letters do not differ significantly based on ANOVA Tukey post hoc comparisons ($p \geq 0.05$). The box represents the 25% and 75% quartiles, the band in the box is the median. The whiskers represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are more than 1.5 times the interquartile range) are represented by circles beyond the whiskers. Impaired sites are underlined

Within site similarity values of the species composition data ranged between 0.312 and 0.595 (Table 5). The most constant assemblages were found at the E3, and E4 sections. The lowest between sites similarity (0.172) was detected between the E1 and L1 sites, whereas the highest value (0.614) was observed between E3 and E4 sites.

Table 4. Fish fauna of the studied sites (bold: "frequent" species with more than 1% relative abundance). Impaired sites are underlined

Nº	Family	Species	E1	E2	E3	E4	L1	L2	∑%
1.	CYPRINIDAE	*Abramis ballerus* Linnaeus, 1758	-	-	2	1	-	-	0.02%
2.		*Abramis bjoerkna* Linnaeus, 1758	-	-	11	10	-	1	0.15%
3.		*Abramis brama* Linnaeus, 1758	-	5	62	40	4	2	0.76%
4.		***Alburnus alburnus*** Linnaeus, 1758	-	333	1252	4250	604	1166	**51.39%**
5.		*Aspius aspius* Linnaeus, 1758	-	1	2	3	-	-	0.04%
6.		*Carassius carassius* Linnaeus, 1758	1	-	-	2	-	-	0.02%
7.		***Carassius gibelio*** Bloch, 1782	205	151	942	981	199	552	**20.47%**
8.		*Cobitis elongatoides* Bačescu and Maier, 1969	45	22	30	38	-	2	0.93%
9.		*Ctenopharyngodon idella* Valenciennes, 1844	-	51	-	-	-	-	0.34%
10.		*Cyprinus carpio* Linnaeus, 1758	-	8	32	5	-	-	0.30%
11.		*Gobio gobio* Linnaeus, 1758	-	3	-	-	-	-	0.02%
12.		*Leucaspius delineatus* Heckel, 1873	1	-	34	2	2	2	0.28%
13.		***Leuciscus idus Linnaeus, 1758***	-	373	167	116	54	60	**5.20%**
14.		*Misgurnus fossilis Linnaeus, 1758*	1	28	-	11	3	6	0.33%
15.		***Pseudorasbora parva*** Temminck and Schlegel, 1842	10	6	111	47	4	2	**1.22%**
16.		***Rhodeus sericeus*** Pallas, 1776	6	264	143	196	23	11	**4.34%**
17.		*Romanogobio vladykovi* Lukasch, 1933	-	-	2	2	-	-	0.03%
18.		***Rutilus rutilus*** Linnaeus, 1758	2	46	124	441	147	192	**6.43%**
19.		*Scardinius erythrophthalmus* Linnaeus, 1758	1	-	9	10	1	3	0.16%
20.		***Squalius cephalus*** Linnaeus, 1758	-	2	61	314	2	2	**2.57%**
21.		*Tinca tinca* Linnaeus, 1758	-	-	-	1	2	1	0.03%
22.		*Vimba vimba* Linnaeus, 1758	-	-	1	1	-	-	0.01%
23.	ESOCIDAE	***Esox lucius* Walbum, 1792**	9	7	36	49	33	23	**1.06%**
24.	PERCIDAE	*Gymnocephalus cernuus* Linnaeus, 1758	-	-	1	-	-	-	0.01%
25.		*Lepomis gibbosus* Linnaeus, 1758	-	6	-	3	-	1	0.07%
26.		*Perca fluviatilis* Linnaeus, 1758	-	-	6	4	9	-	0.13%
27.		***Perccottus glenii*** Dybowski, 1877	-	1	224	159	74	61	**3.51%**
28.		*Sander lucioperca* Linnaeus, 1758	-	-	9	2	-	-	0.07%
29.	SILURIDAE	*Ameiurus melas* Rafinesque, 1818	-	-	3	1	3	4	0.07%
30.		*Silurus glanis* Linnaeus, 1758	-	2	-	2	-	-	0.03%
		Species richness	**10**	**18**	**23**	**27**	**16**	**18**	**30**
		Number of individuals	**281**	**1309**	**3264**	**6691**	**1164**	**2091**	**14800**

Table 5. Within and between sites similarities (Jaccard index) of the fish fauna composition data. Diagonal (bold): within site similarities (mean±SD); above: p values of the Kruskal-Wallis tests (*=significant difference); below: similarity of two related sites (mean±SD). Impaired sites are underlined

	E1	*E2*	*E3*	*E4*	*L1*	*L2*
E1	**0.312 ± 0.12**	*0.058*	*<0.001**	*<0.001**	*0.001**	*0.026**
E2	0.249 ± 0.14	**0.405 ± 0.13**	*0.001**	*<0.001**	*0.113*	*0.852*
E3	0.194 ± 0.07	0.351 ± 0.10	**0.595 ± 0.13**	*0.709*	*0.027**	*0.004**
E4	0.196 ± 0.07	0.382 ± 0.13	0.614 ± 0.10	**0.539 ± 0.09**	*0.017**	*0.005**
L1	0.172 ± 0.08	0.310 ± 0.13	0.383 ± 0.10	0.376 ± 0.11	**0.503 ± 0.13**	*0.122*
L2	0.195 ± 0.16	0.359 ± 0.13	0.433 ± 0.11	0.445 ± 0.13	0.491 ± 0.13	**0.425 ± 0.14**

The lowest average similarity (0.199) was detected between the E1 and E4 sites, and the highest value (0.542) was found between the E4 and L2 sites.

Table 6. Within and between sites similarities (1 – Bray-Curtis distance) of the relative abundance data of fish. Diagonal (bold): within site similarities (mean±SD); above: p values of the Kruskal-Wallis tests (*=significant difference); below: similarity of two related sites (mean±SD). Impaired sites are underlined

	E1	**E2**	**E3**	**E4**	**L1**	**L2**
E1	**0.417 ± 0.19**	*0.08*	*<0.01**	*0.01**	*0.63*	*0.02**
E2	0.204 ± 0.14	**0.335 ± 0.20**	*0.02**	*0.01**	*0.29*	*<0.01**
E3	0.306 ± 0.10	0.341 ± 0.17	**0.552 ± 0.21**	*0.60*	*0.05**	*0.98*
E4	0.199 ± 0.15	0.338 ± 0.19	0.521 ± 0.16	**0.519 ± 0.20**	*0.04**	*0.60*
L1	0.224 ± 0.18	0.291 ± 0.18	0.451 ± 0.17	0.484 ± 0.23	**0.386 ± 0.21**	*0.02**
L2	0.245 ± 0.13	0.330 ± 0.20	0.537 ± 0.22	0.542 ± 0.17	0.496 ± 0.18	**0.549 ± 0.21**

The relative abundance data (Table 6) showes that the within-sites similarities ranged between 0.335 and 0.552. High values were found on the three, wastewater affected sampling sites. The values were significantly lower for the E1, E2 sites, but L1 did not show any significant differentiation. To visualize the relations of the three variable groups (hydrophysico-chemical, fish faunistic and relative abundance stabilities), the stability values of the six studied sites were presented in a bubble plot (Fig. 4). Where the stabilities of the hydrophysico-chemical data of sites represented on the X axis. The within sites stability values of fish relative abundance data showed on the Y axis. The size of circles correlates with the fauna composition stabilities positively

The Mantel test revealed significant relationship between the variances of faunistic and relative abundance datasets (R=0.86, p<0.01). Nevertheless no significant correlation was found between the temporal variability of fish assemblages and the spatial position of the sites along the longitudinal profile of the streams (for faunistic data: R_s=0.42, p=0.39; for relative abundance data: R_s=0.31, p=0.54).

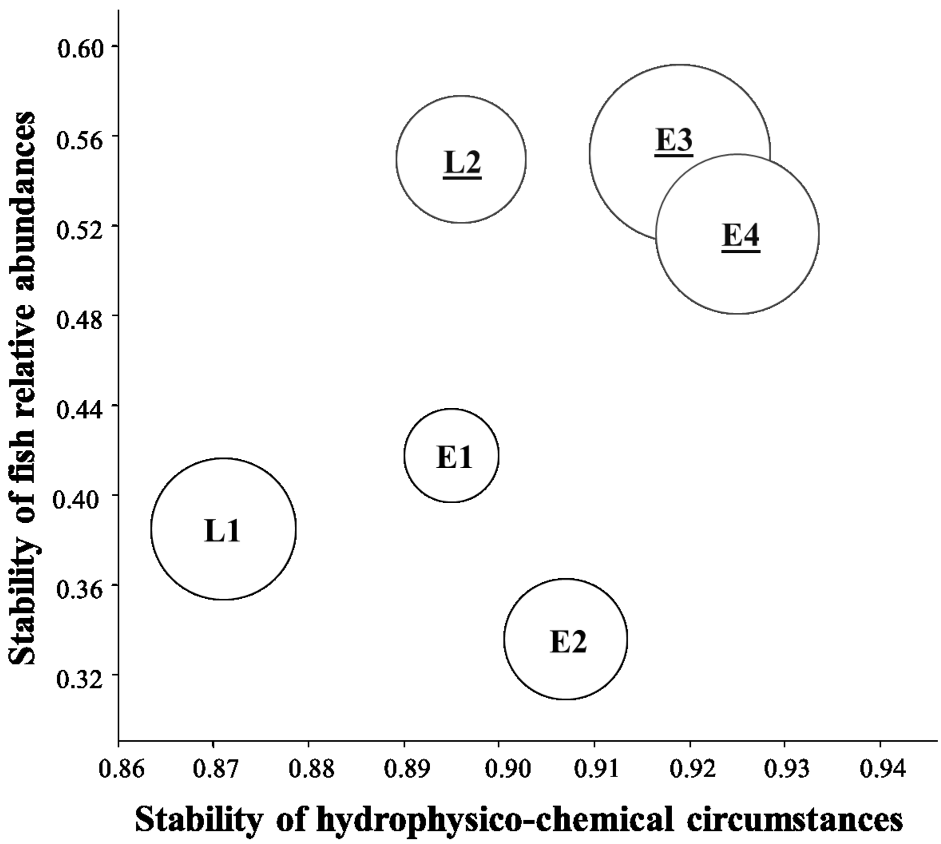

Fig. 4. Bubble plot of within site similarities (i.e.: 1 – Bray-Curtis distance) as “stabilities” of the hydrophysico-chemical (X axis) and fish relative abundance (Y axis) data. The size of circles correlates with the within site fauna composition similarities (i.e. Jaccard similarities) positively. Impaired sites are underlined. For numerical values see Tables 3., 5., and 6.

3.5. Discussion

Our results indicated that the municipal WWTP of Nyíregyháza removes high proportion of the organic matter from the sewage and did not exceed the emission limit in the studied period (Order in Council 2004). Notwithstanding, the discharge caused significant changes in most of the studied physico-chemical parameters on the affected channel sections. The nitrite and nitrate concentrations in the WWTP outflow increased ~39x and ~64x respectively. This load increased nitrite and nitrate concentrations at the E3 site 4x and 8x respectively, compared with the non-affected site (E2). Despite the high cleaning efficiency, the recipient channel system still receives a high nutrient load. Considering the amount of the outflowing sewage, the WWTP discharged ~5.7 tons/year nitrite and ~74.9 tons/year nitrate in the studied period. Moreover the total-phosphorus and total-nitrogen load of the recipient channel system was ~37 and ~153 tons/year respectively. Our results are in accordance with other publications (Brooks 2006; Spanhoff 2007; Canobbio 2009) which point out the strong effect of the wastewater inflows on the water quality of the recipient watercourses. Furthermore, the impact of the discharge is traceable more than 20 km downstream - (at the outermost sampling site – L2). This result is according with the findings of Marti et al., (2004) who showed that in streams the uptake length (measured as the index of the stream nutrient retention efficiency -Newbold et al., 1981) for dissolved inorganic nitrogen and phosphate forms can reach the 29 km and 14 km, respectively.

The WWTP effluent doubled the average flow rate of the recipient channel. This high flow rate combined with the relatively constant composition of the inflowing sewage makes the regime and the physico-chemical characteristic of the recipient channels more stable as well. The highest difference in the stability of the hydrophysical and chemical parameters was found between L1 and E4 (Table 3, Fig. 4). This probably is the result of water quality in the L1 section once it the water arrives from the eastern part of the catchment area not being affected by the sewage. The lowest stability index value and the highest standard deviations of the L1 section water parameters maybe caused by the hectic regime of the upper sections of the LMC. Higher stability index value of the L2 section point to the stabilizing role of the inflowing EC water. The permanent load and balanced regime makes the water quality parameters more stable on the L2 sites, than on the L1.

The fish community of the studied area proved to be species-rich, which may be attributable to the vicinity of the River Tisza, the second largest tributary of the Danube, which contains approximately 50 fish species (Harka and Sallai 2004). The studied assemblages were dominated by common and tolerant species which are widely distributed in the waters of Hungarian Great Plain Ecoregion. The fish assemblage structure (e.g. the dominant species) showed only a slight change in relation to the longitudinal profile of the river-system, and were not significantly affected by the wastewater discharge.

The largest fish stocks and the highest value of species richness were found at the affected channel sections. However, Shannon diversity values of fish communities did not differ significantly between the impaired and the unaffected sites. Our results support the criticisms made by Lenat (1983), Metcalfe (1989) and Cao (1996) who argue that diversity indices are not always appropriate for assessing the effects of point source effluents. The within-site assemblage similarities were found to be higher in the vicinity of the WWTP outflow for both species composition and relative abundance data (Figure 4). In these cases no significant correlations were found with the longitudinal location of the sites. Our results can be explained by the multiple effects of the wastewater load. The discharge creates stable environmental conditions and the flow regime in particular is more stable thus favouring the persistence of stable fish assemblages (Paller, 2002).

In addition, a previous study Deák (2006) made on the macrozoobenthos of these channel sections showed that the species richness decreased on the impaired sites as few taxa (e.g.: Chironomidae, *Asellus aquaticus*, Oligochaeta, Simulidae) can tolerate the wastewater input. At the same time the biomass of the impoverished community did not differ significantly. Furthermore, the permanent and high organic load, via the increased bacterial biomass (Wittner and Takács 2005) ensures sufficient food source for a larger and more diverse fish community (Northington and Hershey 2005; Tsai et al., 1991). Our results are in concordance with the Perturbation Theory (Odum et al., 1979), since more different assemblages appeared at the disturbed sites. Also, these results point out that the different animal groups (e. g. macrozoobenthos and fish), because of their different tolerance limits and motility may show highly different reactions to altered environmental conditions.

Moreover, the observed processes caused by the sewage afflux in these semi-natural habitats are so similar to those observed in lakes for fishery production, where, additional nutrient load by manuring and/or foraging is provided to enchance productivity (Hall et al. 1970, Wasilewska 1978, Baluyut, 1989). On the other hand the dimension of the nutrient load of the studied channels can easily exceed the tolerance level of the fish communities. For example the maximum BOD_5 concentrations at E3 and E4 sites were around the tolerance limit of freshwater fish (~10 mg•l^{-1}) established by Gafny et al. (2000). Based on our results it appears that the fish community in the recipient channels is able to tolerate the current discharge regime. However, any increase in the load (e.g. elevated quantity or concentrations of the discharge) may cause the collapse of the fish communities. Consequently, the likelihood of massive die offs occurs is remarkably high. In agreement with Gücker et al (2006) we suggest that the routing of the treated wastewater through lotic networks the adequate load, and dilution rates should always be considered. Beside of this, the insertion a controlled stream mesocosm (Craggs et al., 1996, Kutty et al., 2009) or a reed-bed system as a tertiary treatment process can reduce the effect of the WWTP load on these semi-natural low flow channel systems.

5. Conclusions

1. Although the municipal WWTP can be characterised by appropriate cleaning efficiency, has qualitatively and quantitatively altered the discharge regime with significantly more stable environmental conditions in the recipient channels than would have occured naturally.

2. The largest and most diverse fish communities were found in the vicinity of the WWTP outflow.

3. The permanent discharge altered not only the stock sizes and species richness, but also caused significant decrease in the variability in fish assemblage structure (a characteristic attribute of fish assemblages inhabiting lowland streams) in these perturbed, semi-natural habitats.

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