A COMPARATIVE TOXICOLOGICAL STUDY OF THE PIKE (*Esox lucius* L.) FROM TWO LOCALITIES IN THE RIVER DANUBE WITH DIFFERENT LEVELS OF POLLUTION

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Abstract

A comparative toxicological research of the pike (*Esox lucius* L.) from the River Danube was performed during a one-year cycle, at two localities with a different level and type of pollution. Comparative analyses of the enzyme activity (aspartate aminotransferase - AST, alanine aminotransferase - ALT, alkaline phosphatase - ALP), and concentrations of the total protein, urea and creatinine in the pike sera at these two localities, show that the occasional significant changes (Mann-Whitney U-test) in these parameters occur due to the sublethal pollution. Differences between the localities were also manifested as changes in the value of the hepatosomatical index (HSI). Under the influence of pollution, changes in the diet were present as well, determined by examining the stomach contents. These changes were accompanied by the significant changes in the slopes of the length-weight regressions ($\log W = a + b \times \log TL$). All changes were reversible.

Introduction

The pike (*Esox lucius* L.) represents a species with a wide circumpolar range in the Northern Hemisphere, having a commercial and ecological value both in North America and in Europe and Asia (Scott and Crossman, 1973). This species is a top predator in aquatic ecosystems. It generally represents a stationary species and is the predator of a sedentary type (Gonczi et al., 1985). All these figures, as well as the fact that many experiments were conducted on this species, both in laboratory conditions and in natural populations, make it very suitable for pollution monitoring in aquatic ecosystems (Johnson and Bergman, 1984; Balk, 1985).The aim of this research was to determine the importance of the biochemical parameters of the pike blood sera, hepatosomatical index, diet, and length-weight relationship as indicators of the population condition at two localities exposed to a different type and level of pollution.

Material and Methods

The pike was sampled monthly with an electrofishing appliance (230 V, 16.5 A) at two localities during one year. The upstream locality (km 1175) has a group of islets and channels that represent a typical pike habitat, while the downstream locality (km 1163) represents a true river course. There are no greater pollution spills at the upstream locality, except during the period of the low water level, when water from the channels and the flooded area carries a large amount of organic pollution. The downstream locality is constantly exposed to pollution from the largest city collector sewer and from the wastewater of a paper mill.

A total of 569 specimens of pike were sampled, of which 352 were from the upstream and 217 from the downstream locality. The analysed specimens were sexually mature (determined through hystological cross-sections of gonads). The total body length (TL, cm) and the total body weight (W, g) were measured in all specimens.

The sampling of blood and biochemical analyses (aspartate aminotransferase - AST, alanine aminotransferase - ALT, alkaline phosphatase - ALP, total protein, urea, creatinine), were done according to the procedure described in one of the previous papers by the author (Lenhardt, 1992).

The presence or absence of the food in the stomach was determined in all specimens.

The length-weight relationship was estimated for each month and for each locality using the linear regression of logarithmically transformed data for length and weight of the body:

$$\log W = \log a + b \times \log TL$$

The comparison of the values of biochemical parameters and the HSI was done with a test of rank sums (Mann-Whitney U-test). The significance of differences in percentage of the present dietary items was estimated with a Yates corrected χ^2 test. The comparison of the regression slopes was done with an analysis of variance.

Results

The comparative analysis of the biochemical parameters of the pike sera in specimens sampled at two localities indicates that there is a relative increase of certain parameters during the year (Table 1).

In February, at the upstream locality, water from the flooded area and the channels, loaded with a great amount of organic matter, entered into the main stream due to the low water level. During that time, a significantly higher activity of the transaminases (AST, ALT) and the increased concentration of urea were observed in specimens sampled at the upstream locality in comparison with the downstream locality (Mann-Whitney U-test). The analysis of the diet indicates that there are significant differences in the percentage of the full stomachs at two analysed localities during January ($\chi^2 = 9.02$, d.f.=1, $P<0.01$) and February ($\chi^2 = 4.72$, d.f.=1, $P<0.05$), with lower values detected at the upstream locality (Table 1). The comparative analyses show that there are significant differences between the regression slopes [$F_{(1,60)} = 7.655$, $P<0.01$] of the length-weight relationship in pike specimens sampled during February at two analysed localities, with lower values of the coefficient b at the upstream locality, which indicates poor dietary conditions at this locality.

During June, the activity of the transaminases was increased at the downstream locality, while the concentration of urea was increased both during June and July. The data show that there was a significantly less number of full stomachs (Table 1) during July ($\chi^2 = 9.31$, d.f.=1, $P<0.01$) at the downstream locality, as well as that the difference between the regression slopes of the length-weight relationship during June [$F_{(1,27)} = 6.900$, $P<0.05$], July [$F_{(1,53)} = 6.616$, $P<0.05$] and August [$F_{(1,52)} = 5.780$, $P<0.05$] was significant, with lower values of the coefficient b at the downstream locality.

The increased values of biochemical parameters during May at the upstream locality were probably caused by the time interval between sampling at the downstream and the upstream locality (15 days). During this time the water temperature increased from 16°C to 20°C, which probably influenced the increase of the concentration of creatinine, which is correlated with water temperature (Lenhardt, 1992), while the increased values of ALT can be explained with the correlation of this parameter with the photoperiod (Lenhardt, 1997).

Urea had significantly higher values at the downstream locality four times during the year, in the period of low water level, while in November the activity of transaminases and the concentration of creatinine were increased too, which points to a higher frequency of the sublethal pollution at this locality.

The increased values of creatinine in males, in March and April, at the downstream locality, were probably related to spawning activities, because the individuals with groups of unabsorbed spermatozoa (cross-sections of gonads) were found until mid June at the downstream locality (Lenhardt, 1997).

Occasionally there was a relative increase of the total proteins in males during March and June at the downstream locality, whilst at the upstream locality this increase occurred both in males and females during September.

The relative increase of the HSI during certain periods of the year was probably related to the reproductive cycle.

Table 1. Comparative analysis of investigated parameters at upstream (UL) and downstream locality (DL), F-female, M-male, * $P<0.05$, ** $P<0.01$.

month			Dec	Jan	Feb	Mar	Apr	May
AST (Ul^{-1})	F	UL	310.4	411.0	286.6	583.8	314.9	481.6*
		DL	301.6	374.0	221.3	441.5	424.0	275.4
AST (Ul^{-1})	M	UL	357.4	386.1	302.6*	445.0	345.9	421.4
		DL	290.8	395.8	264.8	451.1	388.8	385.2
ALT (Ul^{-1})	F	UL	10.6	11.2	7.4	5.7	5.3	11.0*
		DL	10.6	10.7	4.4	5.9	5.6	6.6
ALT (Ul^{-1})	M	UL	7.0	8.8	6.4*	5.8	5.2	10.5
		DL	7.4	8.6	3.6	5.5	4.7	7.5
ALP (Ul^{-1})	F	UL	50.9	54.2	44.2	91.8	56.8	59.4
		DL	47.1	67.1	58.6	59.5	47.1	53.6
ALP (Ul^{-1})	M	UL	45.9	39.5	39.5	55.2	46.4	63.4
		DL	45.2	42.7	38.8	48.9	54.8	57.7
total protein (gl^{-1})	F	UL	36.0	30.7	31.0	28.2	34.8	33.3
		DL	41.2	34.6	31.1	37.6	33.7	36.4
total protein (gl^{-1})	M	UL	39.0	36.3	37.4	27.7	36.2	34.8
		DL	36.9	36.0	40.0	33.5*	41.7	33.8
urea (mmoll^{-1})	F	UL	0.63	0.57	0.62	0.83	1.16	1.18
		DL	1.18	0.94*	0.58	1.00	0.77	1.22
urea (mmoll^{-1})	M	UL	0.85	0.68	0.68*	1.06	1.17	1.07
		DL	1.07	0.91*	0.52	0.93	1.15	0.98
creatinine (µmoll^{-1})	F	UL	0.28	0.28	0.24	0.26	0.49	0.72*
		DL	0.26	0.28	0.32	0.36	0.44	0.57
creatinine (µmoll^{-1})	M	UL	0.48	0.46	0.42	0.39	0.54	0.83*
		DL	0.49	0.46	0.48	0.52*	0.66*	0.55
HSI	F	UL	3.61	4.16*	2.66	1.57	1.32	1.28
		DL	3.26	3.22	3.38	1.86	1.50	1.53
HSI	M	UL	2.20	1.87	2.06	1.40	1.38	1.34
		DL	1.81	1.87	2.26	1.48	1.26	1.44
regression slope - b		UL	3.22	3.08	3.07	2.91	3.10	3.37
		DL	3.15	3.16	3.23**	3.19	3.10	3.38
full stomachs (%)		UL	52.0	10.0	7.4	37.5	28.6	26.7
		DL	60.0	58.3**	33.3*	57.1	44.4	44.4

Table 1. (continued)

month			Jun	Jul	Aug	Sep	Oct	Nov
AST (Ul^{-1})	F	UL	252.0	313.5	307.8	321.5	301.3	306.7
		DL	386.0	436.7	323.6	325.3	331.0	442.4*
AST (Ul^{-1})	M	UL	274.1	415.0	308.8	313.8	342.8	335.0
		DL	439.0*	365.5	328.8	304.0	308.8	451.3*
ALT (Ul^{-1})	F	UL	6.1	5.9	8.4	5.0	5.1	7.4
		DL	9.6	7.2	7.8	5.6	5.6	8.8
ALT (Ul^{-1})	M	UL	7.8	5.9	8.0	4.9	5.2	7.4
		DL	11.4*	6.6	7.9	5.1	5.6	8.3*
ALP (Ul^{-1})	F	UL	50.3	57.4	62.9	87.5	58.4	49.4
		DL	66.2	53.8	57.6	62.5	46.3	63.7
ALP (Ul^{-1})	M	UL	60.9	52.3	64.0	54.0	57.1	56.0
		DL	64.9	58.3	61.3	60.4	66.2	69.6
total protein (gl^{-1})	F	UL	31.6	36.6	36.1	40.1*	34.5	32.9
		DL	36.0	38.0	34.2	29.6	33.8	36.8
total protein (gl^{-1})	M	UL	34.3	37.8	38.1	37.5*	33.8	38.7
		DL	39.6*	40.5	36.8	32.8	35.2	39.5
urea (mmoll^{-1})	F	UL	1.12	0.99	1.28	1.46	1.34	1.03
		DL	1.20	1.48*	1.40	1.58	1.35	1.40*
urea (mmoll^{-1})	M	UL	1.11	1.12	1.45	1.52	1.38	0.99
		DL	1.50*	1.84*	1.44	1.59	1.38	1.43*
creatinine (μmoll^{-1})	F	UL	0.84	1.13	1.34	0.56	0.37	0.31
		DL	0.66	1.19	0.92	0.62	0.31	0.32
creatinine (μmoll^{-1})	M	UL	0.94	1.24	1.20	0.84	0.47	0.42
		DL	0.82	1.17	1.16	0.74	0.54	0.52*
HSI	F	UL	1.28	1.72	1.71	1.49*	2.02	2.61
		DL	1.24	1.35	1.28	1.06	1.94	2.27
HSI	M	UL	1.48	1.61	1.57*	1.45*	1.40	1.84*
		DL	1.46	1.54	1.27	1.04	1.16	1.34
regression slope - b		UL	3.06*	3.11*	3.11*	3.08	3.27	3.16
		DL	2.66	2.90	2.97	3.06	3.21	3.11
full stomachs (%)		UL	54.5	76.9**	25.0	25.7	32.4	51.9
		DL	33.3	31.6	20.0	36.4	16.7	37.9

Discussion

The presented results point to the importance of biochemical parameters as "early warning indicators" of water pollution (Hodson, 1986), but also to the complexity of such an approach to pollution monitoring. Field-sampled fish generally show greater variability and wider range of values of blood parameters in comparison with the laboratory-reared fish (Edsall, 1999). Therefore, to use these parameters for diagnostic purposes, it would be necessary to acquire an estimate of their standard values (Lockhart and Metner, 1984; Folmar, 1993), as well as their correlation to exogenous and endogenous factors (Larsson, 1985). The relative differences in activities of transaminases (AST, ALT) obtained in this research can be explained as a sublethal effect of toxicants, as presented in some earlier papers (Asztalos et al., 1988, 1990). The changes obtained in the concentration of urea could be caused by a gill dysfunction (Lockhart and Metner, 1984), since the urea is excreted mainly through the gills (Smith, 1929). The differences in the concentration of creatinine in certain periods of the year could be related to differences in spawning activities at the two analysed localities, and the differences observed in November could be the consequence of a kidney dysfunction (Lockhart and Metner, 1984). The differences in the concentration of proteins are hard to explain due to their different functions in the fish blood (Sandnes et al., 1988).

The values of the HSI, the diet, the length-weight relationship and the condition factor are all being used as indicators of the population condition in various papers. While the differences in the values of the HSI in this paper can be attributed to differences in the reproductive cycle as well, the differences in diets and length-weight relationships are probably caused by the influence of toxicants. However, Koss et al. (1986) cite that the field sampled pike did not show any changes in the HSI values and length-weight relationship under the influence of xenobionts. The research results obtained for *Thymalus arcticus* indicate that the diet disturbances and growth reduction occur under the influence of toxicants (McLaey et al., 1987), while Hoque et al. (1998) cite that the HSI represents a more sensitive indicator of pollution than the Fulton's condition factor.

All parameters analysed in this paper indicate that the sublethal pollution led to changes both in biochemical parameters and in the diet and length-weight relationship. Unfortunately in this case, this was not confirmed by a chemical analysis of water, although it was visible at the upstream locality, while at the downstream locality, which was constantly loaded with wastewater from the city collector and the paper mill, it was probably caused by water level and water temperature. This research showed that the analysed parameters are sensitive to water pollution, and also that their changes due to the pollution were reversible, i.e. that they returned to the normal ranges after the cessation of the sublethal pollution. Similar observations of the reversibility in the enzyme activity (AST, ALT, ALP) in the sera and of gill lesions under the influence of sublethal concentrations of copper sulphate were observed in experiments conducted on the carp as well (Karan et al., 1998).

This research showed that the relative increase of the analysed parameters can point to a deterioration of environmental conditions. Also, such an approach is rather complex, having in mind the relation of significant changes in analysed parameters to changes in exogenous (photoperiod, water temperature) and endogenous (diet, reproductive cycle) factors during the annual cycle. The presented data also point to the necessity of a comparative monitoring of biochemical blood parameters and pathological changes in the tissue (gills, kidney, liver), in order to use these biochemical parameters as indicators of organ dysfunction.

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