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The role of environmental transfer of macro-elements in the Brown trout *Salmo trutta*

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Abstract

In natural habitat of river Słupia researchers observe the fall condition among Brown trouts and their morbidity on ulcerative dermal necrosis. Trout's shape is strictly connected with elemental economy. In recent study we analyse the macroelements economy (Na, Mg, K, Ca, P) in trout's organism on different growth stages: young and adult individuals, inside trout's organs (muscles, gills, liver, fins), in periods of 2006-2007 and 2011-2012. We analyse the concentrations of chosen chemical elements directly in river Słupia and tributaries (small rivers: Glaźna, Kwacza, Skotawa, Kamienna) in demersal muds and river vegetation (2006-2007). We also analyse macroelements in trout's spawn and sperm (2012). In all kinds of samples we noted relatively homogeneous concentrations of macroelements. We obtain high correlations coefficients between elemental pool of trout's organizm (young or adult) and natural environment (r=0.74-0.99, p<0.05). Our results confirm that macroelemental balance in Brown trout's organism is exactly connected with natural habitat. We also demonstrated the importance of time which trout spends in environment in accumulation of macroelements (longer exposition on environmental factors - higher concentration of macroelements).

Key words: *Brown trout, macroelements, ulcerative dermal necrosis, condition, elemental economy*

1. Introduction

Brown trout *Salmo trutta* is one of indicatory species of qualitative state of northern Poland rivers [1,2,3,4,5]. The spawning of trouts takes place in basin of river Słupia, and their physiological condition is an index of qualitative state of northern Poland rivers. Life cycle of this fish became seriously violated by human activity, as a result of modifying the natural run of rivers, poaching and the uncontrolled fishings [2,3,4,5]. Destabilisation of Brown trout's life cycle influence on individuals' balance of chemical elements and the chemical balance of their environment. It also leads to destabilisation of macroelements economy. Additionally, ionizing radiation, sonication, or oxygenation of xenobiotics, institute a set of factors stimulating formation of free oxygen radicals (oxidative stress), which contribute to decay of cells on molecular level [6]. Trout is protected against reactive forms of oxygen by antioxidative enzymes like superoxide dismutase (SOD), catalase or glutathione enzymes which dispose the reactive forms of oxygen and non admitting to their rise [6,7]. However the phenomenon of breaking the defence line of organism on both levels antioxidative and immunological is more and more frequent. Extreme expression of fallen condition among trouts is their morbidity on illness called ulcerative dermal necrosis UDN [2,8].

1.1. Brown trout *Salmo trutta trutta*

Brown trout, is a fish from Salmonidae family, able to live both in fresh or sea water reservoirs (anadromic species) [9,10]. In Poland trout's spawning take place in river Słupia and it's tributaries [2,3]. Fry stays in river about two years then, young individuals (smolts) achieve 10-20 cm length and move to Baltic Sea. Adult trouts achieve over 1 m length and 30 kg weight [11]. Life cycle consists of spawning migrations since july till autumn as well as proper spawning from november to march. In case of Brown trout, similarly to many other migratory species appears the phenomenon of "homing". Individuals come back to spawning places thanks to olfactory memory [8,9,10]. Before reproduction period trout always alters it's environment from sea-water to fresh-water [10,12]. After spawning only part of individuals pretend to come back to sea. Many of them perish due to exhaustion [2,3]. Ichthyologists affirmed that *Salmo trutta trutta* initiates the species *Salmo trutta morpha fario* [13].

1.2. Ulcerative dermal necrosis

The increasing number of trouts infected by ulcerative dermal necrosis is alarming. Individuals with weak immunological system are particularly susceptible for infection [2,3]. Colonization of fish skin by becterium and fungus is the after-effect of weakness and decrease of mucus produced by epithelium [2,8]. Also exhaustion after migration favours potential infection [2,3]. Illness begins with skin injury. Progress occurs in presence of becteria like *Aeromonas sorbia*, *Aeromonas hydrophila complex*, *Streptococus mutant* [2,8]. Typical symptoms in ucerative dermal necrosis encloses local skin changes (spots altering into ulcers) colonized by *Saprolegnia parasitica* mould [2,8]. The last sickness stages happen when the mound covers the skin on fish head. Then the individuals die in slow current regions [2]. Males are more susceptible because their skin cells producing mucus undergo reduction during spawning. In this way the males lose their natural protection [2,8]. Human activity, especially the phenomenon of artificial spawning using ill individuals get the situation worse and contribute to spreading of disease, as well as quick propagation of pathogens with current of water [2,3,8].

1.3. Enzymic protective mechanisms

Occurrence of defensive antioxidative cellular systems is the characteristic fature of living organisms [6]. The main role of these mechanisms is to remove the reactive forms of oxygen RFT and toxic metabolites potentially harmful for living cells, as well as non admitting to their rise [2,3,6,7]. Pollution in trout's natural environment (for example by heavy metals) can intensify the process of formation of RFT, violating the ontioxidative system of organism protection [2,3,6,7]. Therefore, the efficiency of enzymic mechanisms and ontioxidative protection is decisive for healthfulness of organism. For millions of years atmospheric concentration of oxygen was stabilized on the level of 35% and just 500 million years ago finally achieved the present level 21% of atmosphere capacity. These facts were fundamental for uprising of cellular antioxidative defensive mechanisms against oxidative stress [7].

The results of investigations in Słupia River led by Kurhalyuk and collaborators (2009) comparing the healthy trouts with plagued - stricken ulcerative dermal necrosis individuals suggest, that in case of ill trouts the pathological effects of UDN apply to disorders of antioxidative barrier on catalase level [2]. In group of ill males quoted explorers noted the lowering activity of this enzyme by 75%. Smaller fall of activity was noted in females [2]. In case of decay of cellular membranes - in UDN males these processes occure more intensely, than in healthy individuals, and in females the explorers noted the opposite interaction - the fall of intensity of cellular membranes decay [2]. Explorers also noted the greater quantity of accumulated aldehydic and ketonic derivatives in cells of ill individuals [2]. These results confirm, that the redox equilibrium in conditions of oxidative stress is preserved in different way, dependent by sex [2,3]. *Salmo trutta* males are less resistant on negative changes caused by UDN [8]. Females seem to gain considerable resistance, particularly in time of spawning [2,3,8]. Additionally quoted explorers mention about important antioxidative role of superoxide dismutase SOD in stress conditions [2,3]. Authors also noted a positive correlation between activity of glutathione and intensity of lipid peroxidation, which shows a significance of this protein in antioxidative defense [3].

1.4. Aims of invastigation

1. Analysis of correlation between macroelemental pool (Na, K, Ca, P, Mg) in trout's organism and natural environment.

2. Estimation of the expansion of macroelements concentrations (Na, K, Ca, P, Mg) in trout's organism and in natural environment. On this basis inference about differentiation or homogeneity of chemical elements on different growth stages (from fertilized spawn to adult individuals).

3. Examining the relationship between the pool of chemical elements in demersal muds and river vegetation in trout's natural habitat.

4. Comparison of trout's elemental composition in growth stages to check, how the time which trout spends in natural environment (time of exposition on environmental factors) determines the macroelemental concentration in organism.

5. Comparison of elemental composition in young or adult trouts and the concentrations of chemical elements in vegetation and muds as well as examining the correlation between pools of elements. On this basis inference, how natural environment determines trout's elemental economy.

6. Opinion about current condition of Polish trouts from river Słupia and the influence of biogeochemical factors in natural environment, with special regard of ulcerative dermal necrosis.

2. Investigation area

Field investigations were led in 2006-2007 and 2011-2012, in basin of river Słupia (the longest river in North Poland) and it's tributaries. The terrain was convenient to led the analyses, because of close neighbourhood of Baltic Sea and presence of numerous populations of Brown trout. Słupia and tributaries are also known from high intensity of ulcerative dermal necrosis occurrence. Material to investigations was taken from 80 young (2012) and 25 adult (2011) Brown trouts. Additionally we analysed samples of river muds and vegetation from tributaries of Słupia: river Glaźna (15 km length), Kwacza (21 km), Kamienna (30 km) and Skotawa (45 km). Concentrations of macroelements in trout's spawn and sperm (2012) were also analyzed.

2.1. Materials and methods

The material to investigations was gained in result of fishings (with direct electricity aggregate). From each tributary (Glaźna, Kwacza, Skotawa, Kamienna) 20 young trouts were taken to analysis, as well as 25 adult individuals directly from Słupia. The fishing took place in co-operation with Lanscape Park "The Valley of Słupia” and the Board of Polish Angling Circle in Słupsk. The fragments of tissues: liver, muscles, gills and fins were the basic investigative material. The procedures of material preparation encloses homogenization and mineralisation of respective samples. Finally we got 180 samples to analyse (Tab. 1).

Table 1

Informations about kinds of material and amount of samples

Kind of material	Amount of samples
Fins (2011)	25
Gills (2011)	25
Muscles (2011)	25
Liver (2011)	25
Smolts Kamienna (2012)	20
Smolts Kwacza (2012)	20
Smolts Skotawa (2012)	20
Smolts Glaźna (2012)	20
Together	**180**

The preparation of samples started with drying in temperature 60°C to solid mass, in thermal analysis chamber type Memmert, as well as their homogenization by crumbling in porcelain mortar. In mineralisation we used maximum 200 mg of dry mass by one sample. We conducted the mineralisation with underpressure method. In this procedure we used MWS -2 type Berghoff apparatus. Samples were placed in special containers, then every sample was flooded with 5 ml of ultraclean nitrogenous acid (65%). Single mineralisation contained 10 samples and consisted with following stages: 1 hour in temperature 100°C, then 1 hour in temperature 150°C, finally in temperature 200°C, also by hour. When mineralisation was finished, from single sample we got about 0,2 ml of solution. When it cool, we diluted the samples with distiled water (up to 25 ml). The samples were mixed and poured to leakproof poliethyl containers (Bem and collaborators 1986).

Using the method of ICP MS (mass spectrometry with inductively coupled plasma stimulation) the concentrations of Na, Mg, K, Ca and P were marked in analysed samples. In this procedure was used the apparatus type ICP - MS Agilent 7500CE, consisting of nebulizer (micro - mist) as well as the mist chamber (double - pass), cooled termoelectrical by Peltier` effect. Apparatus was also equipped in reaction chamber (ORS), quartz burner, ion lens (CE), as well as sammler and skimmer nickel cones. Turbomolecular pump and oil entrance pump were used as vacuum system of apparatus. As a distributor was used kwadrupol with hyperbolical rods producing electric field. The electron duplicator (pulsating and analog modes) was used as the crystal set with nine stages dynamic range. External patterns (^{45}Sc, ^{89}Y as well as ^{159}Tb) minimalized the effects of work, and helped to stabilise the apparatus. Argon high cleanness 5.0. was used as transfering gas. Hydrogen 6.0 and helium 6.0 were used as reaction gases to eliminate the interference effect.

2.2. Statistical analysis

The concentrations of macroelements previously marked in quantitative analysis: Na, K, Ca, P, Mg were analysed statistically. Data were divided into four representative groups called accordingly: adult individulas (quantitative analysis from 2011 and 2007), young individuals (2012 and 2007), trout's spawn and sperm (2012), water vegetation and river muds (2007 and 2006). To examine the expansion of concentrations of chemical elements we conducted Shapiro - Wolf test. On this basis we affirmed non - parametric expansion. Therefore the model of Spearman's correlation of ranks was used in further part of our work, on the stage of correlational analysis. We used STATISTICA v.10 as well as Microsoft Excel programmes.

3. Results

3.1. Concentrations of macroelements in adult trouts

The quantitative analysis of macroelements Na, K, Ca, P, Mg ($mg*L^{-1}$) in organs of adult trouts (in muscles, gills, liver and fins) permitted to establish, which macroelements were mostly accumulated in particular organs. It was also possible to estimate the degree of differentiation of these concentrations (Tab. 2). Comparing the average concentrations of macroelements with their standard deviations, we can infer about relatively homogenous expansions of macroelemental concentrations: in majority of cases arithmetical averages values were higher than SD, for example magnesium in gills (average 19.775, SD 6.131), calcium in muscles (average 104.1557, SD 73.24909); Fig. 1. These results show that macroelements achieve the homogenous values of concentrations. The majority of quantitatively measured elements were strongly accumulated in trout's gills (K was the exception); Fig. 2.

Table 2

Concentarations of Na, Mg, K, Ca and P (mg*L^{-1}) in adult trout's *Salmo trutta* muscles, gills, liver and fins

Macroelement	Organ	n	Arithmetical average	Min.	Max.	SD
Na	Muscles	157	65.5325	8.18	286	81.40948
Mg	Muscles	157	12.6115	4.85	21.6	3.12794
K	Muscles	157	126.7129	23.5	211.8	34.88872
Ca	Muscles	157	104.1557	5.81	403	73.24909
P	Muscles	69	91.00435	27.5	357	48.64808
Na	Gills	115	87.7153	14.7	211.2	27.7161
Mg	Gills	115	19.775	7.24	46.52	6.131
K	Gills	115	71.5751	21.2	163	19.3945
Ca	Gills	115	510.9817	116	1083	161.4105
P	Gills	25	306.772	89.3	464	109.1463
Na	Liver	243	57.5017	3.041	256	62.923
Mg	Liver	243	8.2338	0.919	19.72	4.41845
K	Liver	243	107.2014	6.471	285.6	68.90944
Ca	Liver	243	8.424	1.5152	81.77	6.43351
P	Liver	69	123.2362	12.9	220	56.38174
Na	Fins	25	27.7692	6.82	70.3	18.7257
Mg	Fins	25	8.35	2.7	19.6	4.0964
K	Fins	25	28.18	11.3	55.7	12.1898
Ca	Fins	25	383.1	88.5	1201	291.1162
P	Fins	25	208.104	49	636	161.8661

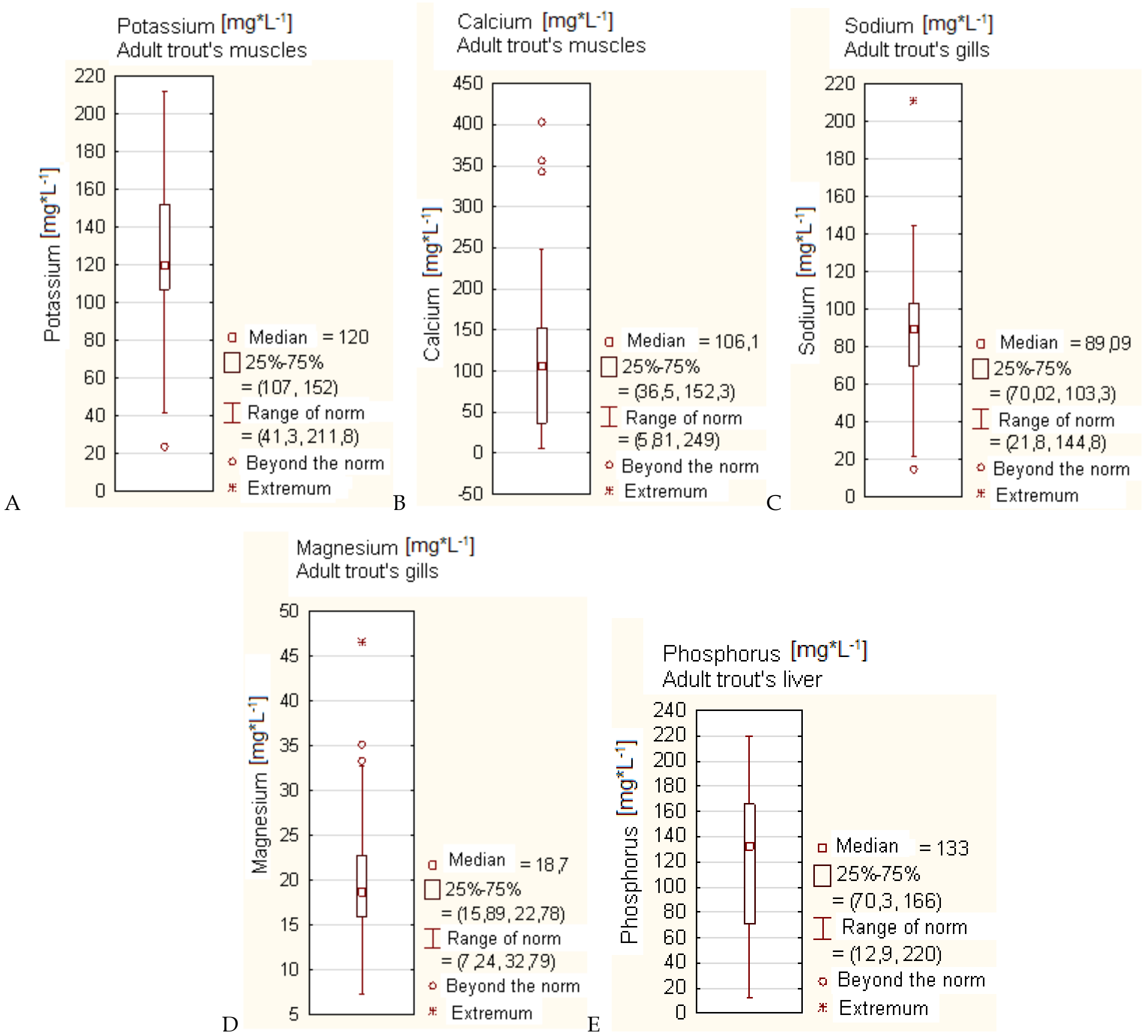
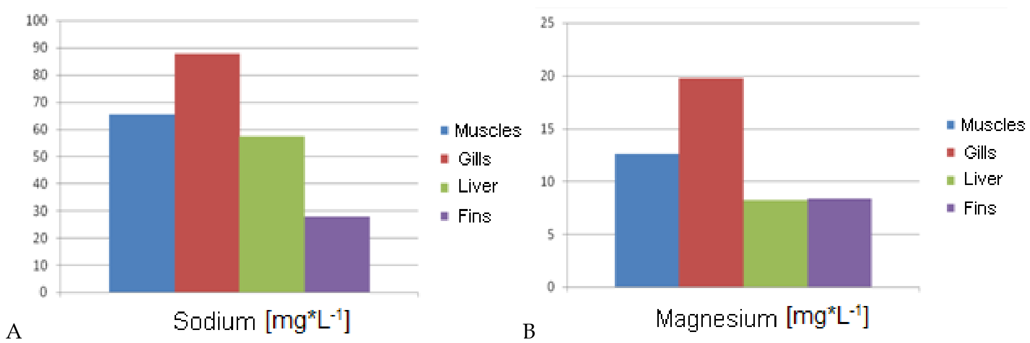
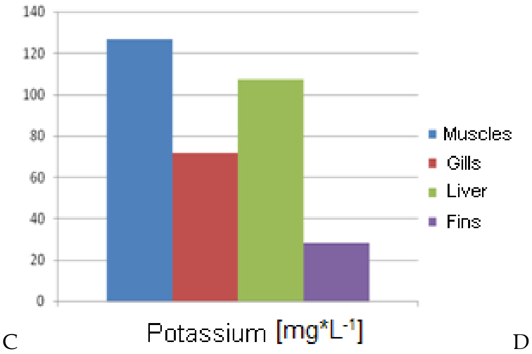


Fig. 1 (A-E). Expansion of K, Ca, Na, P and Mg (mg*L-1) in adult trout's organs (muscles, gills, liver). Relatively homogenous expansion of concentrations (single extremes for Na and Mg).





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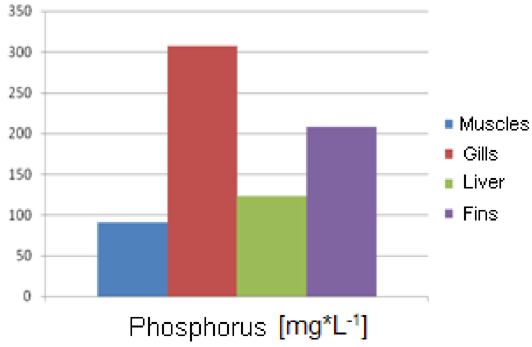

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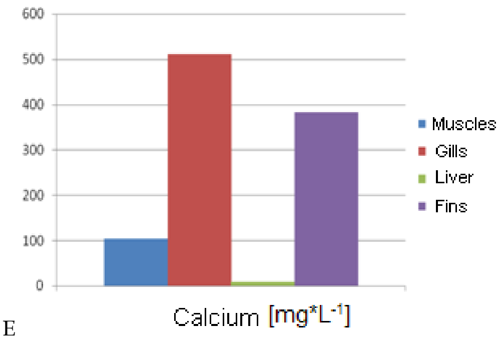


E

Fig. 2 (A-E). Concentrations of Na, K, Mg, P, Ca (mg*L-1) in adult trout's muscles, gills, liver and fins. Ca and P achieved the highest concentrations. Gills are the organ of the highest bioaccumulation of macroelements.

3.2. Concentrations of macroelements in young trouts

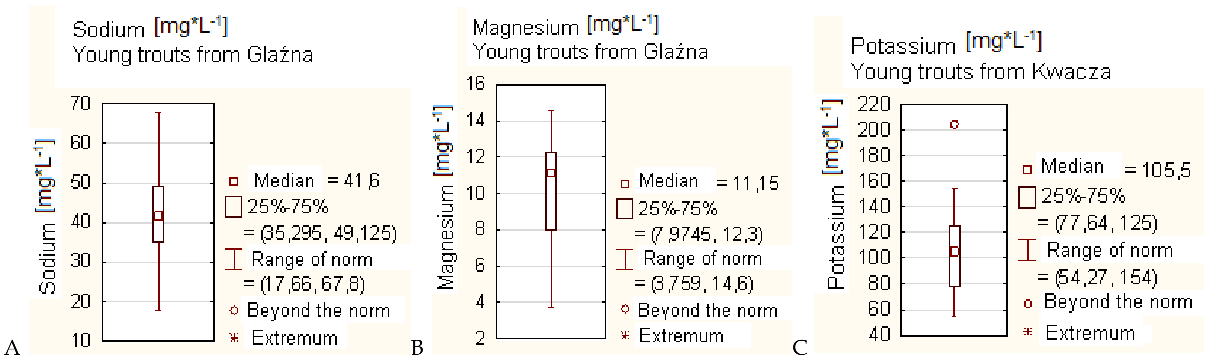
The similar analysis of Na, K, Ca, P and Mg concentrations (mg*L-1) was conducted for four populations of young trouts from tributaries of Słupia (Glaźna, Kwacza, Skotawa, Kamienna). Analysis of averages and standard deviations confirmed relatively homogenous expansion of macroelements in populations (Tab. 3). We did not observe any extreme values of concentrations among macroelements (Fig. 3). Young trouts from particular tributaries of Słupia were loaded with macroelements quite evenly (Fig. 4).

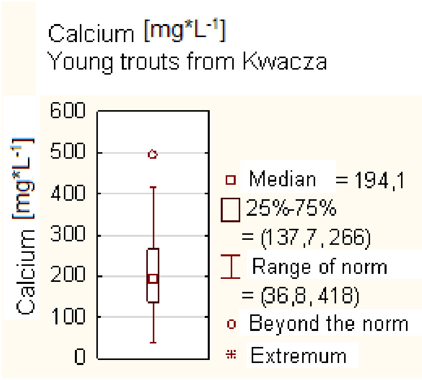
Table 3

Concentrations (mg*L-1) of Na, Mg, K, Ca and P in young trout's organisms (populations from four tributaries of river Słupia: Glaźna, Kwacza, Skotawa, Kamienna)

Macroelement	River	n	Arithmetical average	Min.	Max.	SD
Na	Glaźna	52	42.6006	17.66	67.8	11.0833
Mg	Glaźna	52	10.1056	3.759	14.6	2.7374
K	Glaźna	52	91.7513	31.27	160	30.8047

Ca	Glaźna	52	246.1808	53.5	612	121.8484
P	Glaźna	30	251.5	113	487	103.6202
Na	Kwacza	50	42.4604	14.7	71	12.1822
Mg	Kwacza	50	10.9046	4.586	16.3	3.3005
K	Kwacza	50	103.9852	54.27	205	31.4172
Ca	Kwacza	50	212.836	36.8	497	103.0503
P	Kwacza	32	205.5844	70.7	365	76.92908
Na	Skotawa	56	40.8475	16.7	58.9	7.8649
Mg	Skotawa	56	10.7067	6.357	16.1	2.5061
K	Skotawa	56	98.8555	52.7	169	30.8266
Ca	Skotawa	56	245.4643	70.1	522	104.2242
P	Skotawa	34	223.7118	87.2	397	75.32282
Na	Kamienna	57	47.5511	13.2	101	24.62274
Mg	Kamienna	57	10.8684	3.451	17.7	4.50718
K	Kamienna	57	96.6030	28.09	173	40.35886
Ca	Kamienna	57	208.8158	22.6	475	96.40993
P	Kamienna	32	191.3188	88.3	392	62.09344



D 

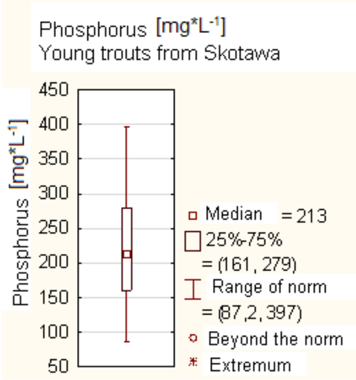
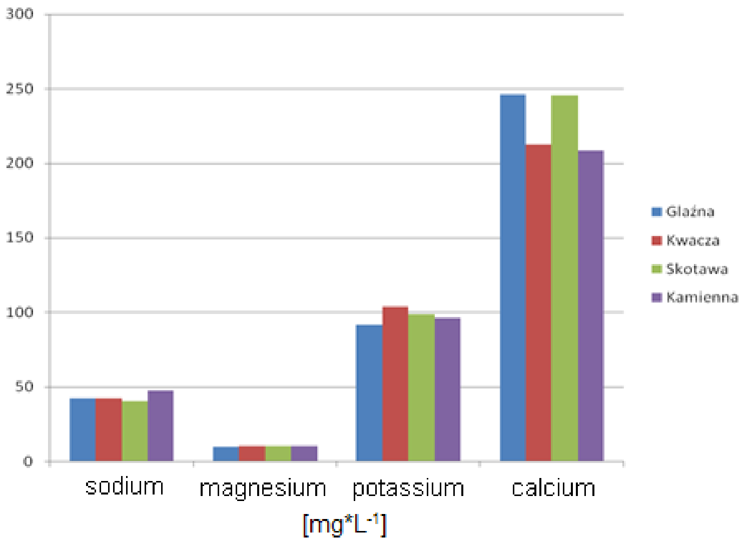
E 

Fig. 3. Expansion of concentrations of Na, K, Ca, Mg and P (mg*L-1) in young trouts from tributaries of river Słupia (Glaźna, Kwacza, Skotawa). Relatively homogenous expansion of concentrations in populations (lack of extremes).



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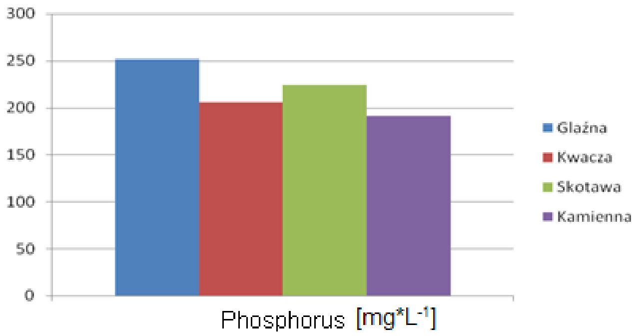

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Fig. 4. Similar levels of concentrations (mg*L-1) of Na, K, Mg, Ca and P in young trouts populations from tributaries of river Słupia (Glaźna, Kwacza, Skotawa, Kamienna).

3.3. Concentrations of macroelements in trout's spawn and sperm

Comparing the average concentrations of Na, K, Ca, P and Mg (mg*L^{-1}) in trout's spawn and sperm, we noted that they differ in these two kinds of material. Concentrations of sodium, potassium and phosphorus in sperm achieved higher values than in spawn (Na: 19.385 mg*L^{-1} in spawn, 46.5333 mg*L^{-1} in sperm; K: 44.135 mg*L^{-1} in spawn and 69.0889 mg*L^{-1} in sperm; P: 86.935 mg*L^{-1} in spawn and 416.5556 mg*L^{-1} in sperm). We also noted extremely high concentration of phosphorus in trout's sperm. In case of Mg and Ca we observed the opposite dependence - higher concentration in spawn than in sperm (Mg 19.31 mg*L^{-1} in spawn, 7.3256 mg*L^{-1} in sperm; Ca: 51.215 mg*L^{-1} in spawn, 13.7222 mg*L^{-1} in sperm). Details in table 4. Comparison of averages and SD affirmed relatively homogenous expansion of elements in analysed samples (arithmetical average higher than SD).

Tab. 4.

Concentrations of Na, Mg, K, Ca and P (mg*L^{-1}) in trout's spawn and sperm

Macroelement	Material	n	Arithmetical average	Min.	Max.	SD
Na	Spawn	20	19.385	12.1	27.2	5.70164
Mg	Spawn	20	19.31	15.2	25.4	2.90297
K	Spawn	20	44.135	33.4	54.8	6.12633
Ca	Spawn	20	51.215	34.4	84.5	16.29256
P	Spawn	20	86.935	78.9	93.6	3.94559
Na	Sperm	9	46.5333	36.1	57.8	7.35034
Mg	Sperm	9	7.3256	7.04	8.2	0.36511
K	Sperm	9	69.0889	54.8	76.6	8.08771
Ca	Sperm	9	13.7222	11.9	16	1.34516
P	Sperm	9	416.5556	388	444	22.61698

3.4. Increasing trend

We noted a trend of increasing concentrations of macroelements in successive developmental stages of trout. For example calcium in spawn achieved 51.215 mg*L^{-1}, in young individuals from river Kwacza 212.836 mg*L^{-1}, and in adult trout's gills 510.9817 mg*L^{-1}. Similarly the phosphorus in spawn achieved 86.935 mg*L^{-1}, in young trouts from river Kamienna 191.3188 mg*L-1, and in adult individual's gills 306.772 mg*L-1 (Fig. 5). This trend applied also to other macroelements. This observation confirms, that the time trout spends in natural environment favours the accumulation of macroelements in organism (longer exposition on environmental factors cause higher accumulation of macroelements in body).

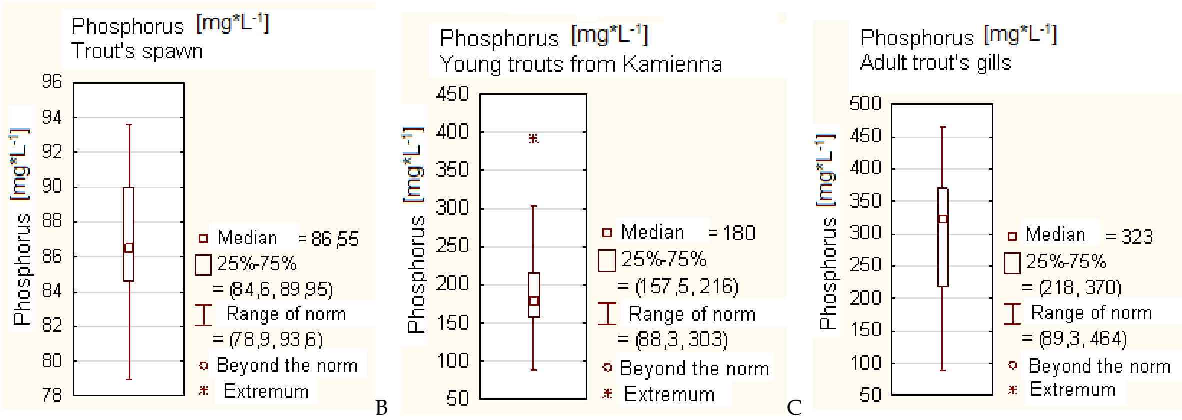


Fig. 5. Increment of phosphorus accumulation (mg*L-1) in successive developmental stages of trout. Concentrations of macroelements increase with time which trouts spend in natural environment.

3.5. Concentrations of macroelements in trout's natural environment

Concentrations of Na, K, Ca and Mg (mg*L-1) in demersal muds and river vegetation in particular tributaries of Słupia were relatively even (Tab. 5, Fig. 6). We noted a trend of higher accumulation of macroelements in vegetation, than in river muds. The tendency, with few exceptions, appeared in case of all tributaries of Słupia. This observation was compatible with our expectations, because plants accumulate chemical elements and keep up their concentrations on stable level (similarly to trout's organism). Oppositely, the elemental composition of river muds changing more dynamically and depend on many external factors (Fig. 7).

Table 5.

Concentrations of Na, Mg, K, Ca (mg*L-1) in demersal muds (results from two measuring places in each tributary of river Słupia) and river vegetation (results from two measuring places in each tributary of river Słupia)

Macroelement	River	Material	Arithmetical average	Min.	Max.	SD
Na	Glaźna	muds	2.388	2.09	2.686	0.421436
Mg	Glaźna	muds	2.253	1.841	2.665	0.582656

K	Glaźna	muds	3.197	2.635	3.759	0.794788
Ca	Glaźna	muds	12.19	10.08	14.3	2.983991
Na	Glaźna	vegetation	15.839	4.148	27.53	16.5336
Mg	Glaźna	vegetation	11.3885	7.367	15.41	5.6873
K	Glaźna	vegetation	85.8865	6.373	165.4	112.4491
Ca	Glaźna	vegetation	116.305	98.81	133.8	24.7417
Na	Kwacza	muds	8.0655	5.651	10.48	3.41462
Mg	Kwacza	muds	10.615	2.54	18.69	11.41977
K	Kwacza	muds	72.0255	3.151	140.9	97.40325
Ca	Kwacza	muds	57.67	22.95	92.39	49.10149
Na	Kwacza	vegetation	21.675	20.08	23.27	2.25567
Mg	Kwacza	vegetation	11.99	10.01	13.97	2.80014
K	Kwacza	vegetation	54	41.31	66.69	17.94637
Ca	Kwacza	vegetation	118.01	72.02	164	65.03968
Na	Skotawa	muds	3.0635	2.91	3.217	0.217082
Mg	Skotawa	muds	3.501	2.755	4.247	1.055003
K	Skotawa	muds	1.822	1.645	1.999	0.250316
Ca	Skotawa	muds	21.005	16.55	25.46	6.300321
Na	Skotawa	vegetation	29.31	17.92	40.7	16.10789
Mg	Skotawa	vegetation	17.125	13.17	21.08	5.59321
K	Skotawa	vegetation	150.3	107.6	193	60.38692
Ca	Skotawa	vegetation	106.935	70.17	143.7	51.99356
Na	Kamienna	muds	1.831	1.248	2.414	0.824487
Mg	Kamienna	muds	4.273	3.881	4.665	0.554372
K	Kamienna	muds	2.6975	2.476	2.919	0.313248
Ca	Kamienna	muds	22.75	20.17	25.33	3.648671
Na	Kamienna	vegetation	17.4825	6.465	28.5	15.5811

Mg	Kamienna	vegetation	16.94	12.81	21.07	5.8407
K	Kamienna	vegetation	76.4	27.6	125.2	69.01362
Ca	Kamienna	vegetation	92.405	84.21	100.6	11.58948

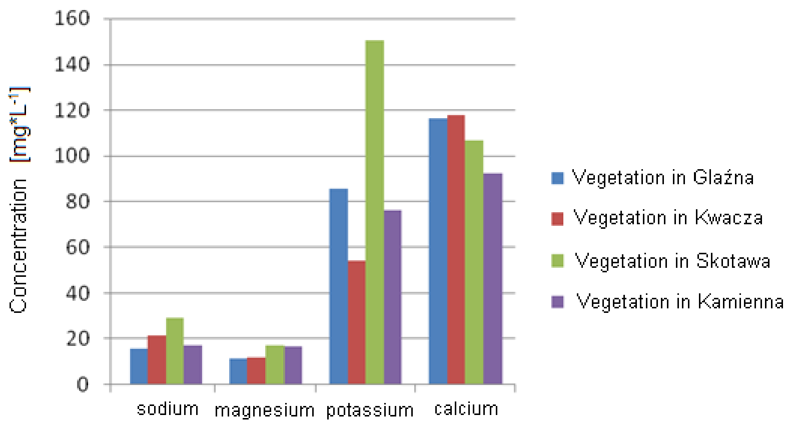
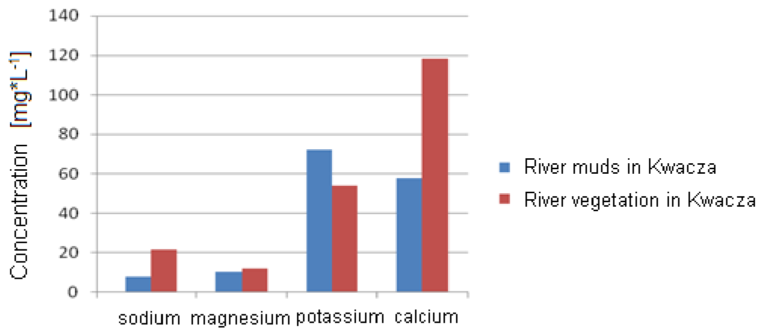
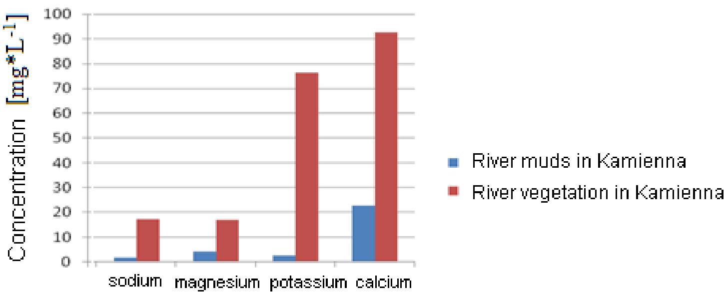


Fig 6. Concentrations of Na, Mg, K and Ca (mg*L^{-1}) in vegetation from river Słupia tributaries. Apart from few exceptions (increased K level in Skotawa) macroelemental loadings are relatively even.



A



B

Fig. 7. Concentrations of Na, Mg, K and Ca (mg*L^{-1}) in river vegetation and demersal muds of Kwacza (A) and Kamienna (B). Macroelements achieve higher concentrations in vegetation than in muds (potassium in Kwacza is a single exception). This trend applies to all analyzed tributaries of river Słupia. Vegetation accumulates chemical elements and keep up their stable levels. On the contrary - elemental composition of river muds changes more dynamically.

3.6. Correlations between concentrations of macroelements in trout's organism and natural environment

Correlations between concentrations of Na, K, Ca and Mg in young trouts from four tributaries of Słupia (Glaźna, Kwacza, Skotawa, Kamienna), and the same elements in their natural environment ($p < 0.05$) were relatively high ($r = 0.74-0.99$); Tab. 6. We also analysed correlations between widened elemental pool: Mn, Fe, Co, Cu, Zn, Cd, Pb, Na, Mg, K and Ca in young trouts, and the same elements in their natural environments ($p < 0.05$). Again we got high correlation coefficients ($r = 0.77-0.92$); Tab. 7. Finally we examined correlations between Mn, Fe, Co, Cu, Zn, Cd, Pb, Na, Mg, K, Ca in organs of adult trouts, and the same elements in vegetation and river muds of Słupia tributaries ($p < 0.05$), $r = 0.7-0.96$; Tab. 8.

Table 6

Correlation coefficients between concentrations of Na, Mg, K, Ca (mg*L^{-1}) in young trouts from four tributaries of river Słupia and the same elements in river vegetation and demersal muds; $p<0.05$. Statistically significant results bolded.

	Muds in Glaźna	Vegetation in Glaźna	Muds in Kwacza	Vegetation in Kwacza	Muds in Skotawa	Vegetation in Skotawa	Muds in Kamienna	Vegetation in Kamienna
Young trouts in Glaźna	**0.970306**	0.912638	-	-	-	-	-	-
Young trouts in Kwacza	-	-	0.745586	**0.996780**	-	-	-	-
Young trouts in Skotawa	-	-	-	-	0.906414	0.800000	-	-
Young trouts in Kamienna	-	-	-	-	-	-	0.884613	0.906354

Table 7

Correlation coefficients (Spearman's model) between Mn, Fe, Co, Cu, Zn, Cd, Pb, Na, Mg, K and Ca (mg*L^{-1}) in young trouts from four tributaries of river Słupia and the same elements in river vegetation and muds; $p<0.05$. Statistically significant results bolded.

	Muds in Glaźna	Vegetation in Glaźna	Muds in Kwacza	Vegetation in Kwacza	Muds in Skotawa	Vegetation in Skotawa	Muds in Kamienna	Vegetation in Kamienna
Young trouts in Glaźna	**0.845455**	**0.918182**	-	-	-	-	-	-
Young trouts in Kwacza	-	-	**0.909091**	**0.927273**	-	-	-	-
Young trouts in Skotawa	-	-	-	-	**0.845455**	**0.918182**	-	-
Young trouts in Kamienna	-	-	-	-	-	-	**0.772727**	**0.863636**

Table 8

Correlation coefficients (Spearman's model) between Mn, Fe, Co, Cu, Zn, Cd, Pb, Na, Mg, K and Ca ($mg*L^{-1}$) in adult trout's organs and the same elements in river vegetation and muds; $p<0.05$. Statistically significant results bolded.

	Muds in Glaźna	Vegetation in Glaźna	Muds in Kwacza	Vegetation in Kwacza	Muds in Skotawa	Vegetation in Skotawa	Muds in Kamienna	Vegetation in Kamienna
Gills	**0.872727**	**0.936364**	**0.890909**	**0.927273**	**0.900000**	**0.909091**	**0.809091**	**0.854545**
Muscles	**0.890909**	**0.945455**	**0.954545**	**0.954545**	**0.854545**	**0.963636**	**0.809091**	**0.890909**
Liver	**0.836364**	**0.863636**	**0.890909**	**0.890909**	**0.772727**	**0.909091**	**0.709091**	**0.800000**
Fins	**0.900000**	**0.954545**	**0.945455**	**0.963636**	**0.890909**	**0.954545**	**0.827273**	**0.900000**

4. Discussion

Results achieved in this work confirm that the elemental balance of Brown trouts is connected with elemental economy of their natural environment. Chemical elements in river vegetation were closer correlated with elemental pool of trout organism, than river muds and trout's organism. This observation (with few exceptions) applied both to young and adult individuals. The statistical significance of correlation coefficients supports the hypothesis about exact relationship between trout's condition, and the quality of their natural environment.

4.1. River Słupia chracteristics

Słupia is the river of Wschodniopomorskie and Zachodniopomorskie Lake Districts as well as Koszalińskie Sea-coast. It beginnins on peatbogs of Kashubian Lake District in neighbourhood of Sierakowska Huta (commune Sierakowice). It passes to Baltic Sea near Ustka [14]. River Słupia creates a profitable environment for Salmonidae fish. However the naighbourhood of large industrial works have an influence on water quality [2,3,4,5]. Moczulska and collaborators (2006) recognized the anthropogenical pollution of bottom run of Słupia as the most serious threat. The examples of municipal sewage treatment plant located in Słupsk (transfer 20000 m^3 of sewages a day) and sewage treatment plant in Ustka (3990 m^3 a day) confirm this diagnosis [4]. Other important factor influencing on fish's life cycle is man's interference in natural river run. Hydrotechnical buildings (water power stations) along river bank, impede fish in performing their spawning migrations [4,5]. Also man's interference in reproduction of fish is the source of anxiety. Ill or weak individuals are often used in procedure of "artificial spawning" [8]. All these factors influence on trout's elemental balance. The multitude of threats created the need of periodical monitoring of qualitative state of Słupia waters. Elemental pool of fresh waters changes more dynamically, than in extensive sea water reservoirs. Therefore the estimation of chemical composition of Słupia is difficult [15]. Results presented by Jarosiewicz and callaborators (2008) show a seasonal changeability in concentration of dissolved oxygen, total nitrogen, and phosphorus. This observations confirm a periodical hesitation of water quality in Słupia [5]. For all these quality parameters quoted explorers noted higher values in winter months [5]. Besides authors mention that the decisive influence on concentration of nitrogen and phosphorus in water has the soil fertilization, from which in result of erosion and rinsing, elements penetrate to water [4,5].

There are various activities undertaking to improve the condition of fish living in river Słupia. Reproduction of spawning grounds, reconstruction of previously regulated sections of rivers, introducing the periods of fish's protection, applying the modern systems of sewages cleaning in industrial works, propagating of natural reproduction to obtain the optimal shape of fry, afforestation of shore lines (willows) in aim to create a natural buffer line for pollution, the realization of project "Nature 2000" foundations to assure a legally protected natural areas [2,3,4,5].

4.2. Macroelements and trout's condition

It's widely known that macroelements are essential for proper growth and development of living organisms. The subgroup of macroelements called biogenes: oxygen, carbon, nitrogen and hydrogen appears to be most important [15,16]. Oxygen – the indispensable breathing gas – except for it's life-giving function – can be potentially harmful for living cells [2,3,6,7]. This feature follows high reactivity of oxygen. Similarly to carbon and nitrogen – oxygen constitutes multiple linkages and stable electron configurations with other chemical elements [16]. Because of that propriety reactive forms of oxygen are so dangerous for cells [2,3,6,7]. Their negative effects enclose: oxygenation of haemoglobin and glutathione, degradation of collagen, inactivation of enzymes and transport proteins, breakages of DNA, damages of chromosomes and many more [6].

The utility of other biogens is undeniably. Carbon – present in organic substances – essential for nutrition and synthesis of complex nutritious substances. Nitrogen – the component of amino acids, proteins and nucleic acids. Hydrogen – fundamental for synthesis of carbohydrates and lipids [16]. Beyond the biogens group: sodium, potassium, magnesium, calcium, phosphorus are also significant [16]. Sodium and potassium enable to keep proper osmotic balance and nervous conductivity. Magnesium is an anti-inflammatory factor, equally important in enzymes activation and muscles contraction. Calcium assures the endurance of bone structures, as well as the excitability of nerves and muscles. Finally phosphorus is a component of ATP – mediates in transport and storage of energy [15,16]. However in all cases – the concentration of each chemical element in organism determines if it is harmless or harmfull [15,16].

In recent study we identified anomalies of Brown trout's condition base on occurrance of extreme concentration values of macroelements (Na, K, Ca, Mg, P) in their organisms. As we observed – in spite of relatively homogenous expansions of macroelements – there appeared extremely high values of concentration in each developmental stage of trout. For adult individuals we observed several such situations in case of sodium in trout's muscles and liver when SD achieved higher values than arithmetical average (sodium SD: 81.40948, average: 65.5325 in muscles; SD: 62.923, average: 57.5017 in liver). Besides we observed single extremes for magnesium (in gills), phosphorus (in muscles and fins), potassium (in gills) and calcium (in fins). However in these cases SD was still lower than arithmetical average, because the extremes were not numerous. In young trouts we practically did not note any extremes. Only one appeared in case of phosphorus in population of smolts from river Kamienna. Also in trout's spawn and sperm extremes hardly ever appeared. Although we still noted single extreme in case of magnesium in trout's sperm.

The values of extremes that we observed were mostly twice higher than on the average (Fig. 1 C, D and Fig. 5 B). Rarely extremes were even higher. For example extreme value of phosphorus in adult trout's muscles achieved 350 $mg*L^{-1}$ while the range of norm reached to about 150 $mg*L^{-1}$. In adult trout's gills we noted extremely high concentration of potassium: 160 $mg*L^{-1}$ while the normal values achieved about 117 $mg*L^{-1}$. Similarly magnesium in gills: extremely 47 $mg*L^{-1}$, range of norm up to 32 $mg*L^{-1}$. Calcium in fins: extremely 1200 $mg*L^{-1}$, the range of norm reached 550 $mg*L^{-1}$. Even if extremes were not numerous, we can deduce about the reasons of destabilisation of trout's macroelemental economy. Probably this destabilisation is an aftereffect of destructive impact of reactive forms of oxygen [6,7]. Furthermore the negative influence of human activity like water pollution, cutout of trees, modification of river runs or artificial spawning could play the part [2,3,4,5,8]. Heavy metals appear to be especially dangerous, mostly for organs of high energy and matter conversion like liver, muscles or fish gills [16]. What is more, pollution as well as sonication or ionic radiation act potentially stimulative for RFT uprising [6]. Undeniably destabilisation of chemical elements economy also leads to diseases and ulcerative dermal necrosis could be the aftereffect of such destabilisation [2,3,8].

5. Summary

1. Economy of macroelements in organisms of Brown trouts remains in exact relationship with elemental pool of their natural environment.

2. In all developmental stages of trouts, macroelements (Na, Mg, K, Ca, P) achieved high levels of cencentration. The analysis of elements in adult trout's organs (muscles, gills, liver, fins) confirmed, that the majority of macroelements achieves the highest concentration in gills.

3. Population of young trouts (smolts) from Słupia tributaries showed homogenous concentrations of macroelements. Among populations of four tributaries (Glaźna, Kwacza, Skotawa, Kamienna) macroelemental loadings were relatively comparable.

4. Concentrations of macroelements in trout's sperm and spawn were differentiated. Concentration of phosphorus was the most significant difference between these two kinds of material (400 $mg*L^{-1}$ in trout's sperm).

5. In demersal muds of river Słupia tributaries we noted relatively homogenous concentrations of macroelements (however river Kwacza was an exception with slightly higher concentrations in comparison to other tributaries). We also observed that macroelements achieved higher concentrations in river vegetation, than in demersal muds (elemental accumulation in vegetation). In demersal muds and in river vegetation concentrations of macroelements tend to be lower, than concentrations of the same elements in adult trout's organisms. Therefore natural environment determines trout's elemental economy.

6. We noticed a trend of increasing concentrations of macroelements in successive developmental stages of trout (from fertilized spawn to adult form). This observation confirms the essential role of time spending in natural environment (longer exposition on environmental factors cause greater accumulation of elements in organism).

7. Correlation coefficients between elements in demersal muds and in young trout's organisms in majority were lower, comparing to correlations between vegetation and young trouts (0.84 for muds and 0.91 for vegetation; $p < 0.05$). Probably the decisive factor was an accumulation of elements in vegetation, similarly to accumulation in trout's organism and oppositely to more changing elemental composition of muds. Statistical significance of correlation coefficients confirms the exact relationship between macroelemental pool of natural environment and trout's organism. Therefore we can speculate that macroelemental economy in early stages of trout's growth influances on their later development, if there will not appear a radical changes in environmental canditions.

8. Economy of macroelements in trout's organism is determined by biogeochemical transformations of their environment. We did not affirm a significant improvement in life cycle conditions of Brown trout, which is connected with spreading of ulcerative dermal necrosis. Destabilisation of macroelemental concentrations in trout's organism can be the after-effect of this illness.

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