GLUTATHIONE S-TRANSFERASE ACTIVITY FROM THE NORTHERN FRESH-WATER FISH UNDER MINERAL CONTAMINATION

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Molecular biomarkers attract ever-increasing interest as «early warning» tools for measurement of adverse effects of environment on organisms (de laTorre et al., 2005). Xenobiotic compounds in organisms undergo a series of biotransformation reactions catalyzed by phase I and II detoxification enzymes, the activation of which may point to pollution exposure. According to this detoxification enzymes are being extensively used as molecular biomarkers.

One of the potential biomarkers is glutathione-S-transferase (GST, EC 2.5.1.18) – key phase II detoxification enzyme. The phase II metabolism involves the conjugation of xenobiotics with endogenous substrate, thus facilitating their excretion (Van der Oost et al., 2003). GSTs catalyze binding and inactivation of wide range of both exogenous and endogenous electrophilic compounds and thought to play a significant role in the adaptation to the chemical stress. That is why it is important to study this biomarker in terms of its application and current limitations. But the data available are not consistent, with some reports indicating an increase in the enzyme activity under contamination in contrast with others that do not observe changes or even reported significant decreases (Tuvikene et al., 1999; Van der Oost et al., 2003). GST seemed to be sensitive to both pollution and natural factors, which can hinder the interpretation of the results.

Unlike the marine species, amount of detailed studies of fresh-water fish GST is not sufficient. Among northern ecosystem inhabitants only trout's (Almli et al., 2002; Uguz et al., 2003; Tuvikene et al., 1999; Lindström-Seppä, 1990) and salmon's GSTs have been studied intensively (Nóvoa-Valiñas et al., 2002; Greco et al., 2007; Arukwe A., Nordbø, 2008).

In our study whitefish *Coregonus lavaretus,* pike *Esox lucius* and roach *Rutilus rutilus* were chosen as a test objects. This species are common North-Europe inhabitants which are of commercial interest in the region. Catalytic activity of the GST was determined in fish captured in Karelian Republic lakes in the northwest of Russian Federation.

The contaminated lake Kostomukskoe is located in the source of upper effluent of the river Kem draining into the White Sea. The mining factory releases ore-dressing sewage in this lake leading to abnormally high mineralization (500 mg/l) with K^+, HCO_3^-, SO_4^{2-} and HSO_3^- ions prevalence (table 1). The presence of suspended particles in the water leads to high water feculence. Because the bottom of this lake mainly consists of alkaline rock, water pH level is relatively high (8,5). This could be a basis for natural barrier for heavy metals in this basin. Metal ions precipitate in alkaline water, thus their level is lower than Russian maximum allowable concentrations, except for molybdenum which is ten times higher (*Status of water objects..., 2007*).

The reference lake Kamennoe is situated in the source of the lower effluent of the river Kem on the territory of the Kostomuksha State Natural Reserve. This basin isn't subjected to any anthropogenic inputs and its water is characterized as pure (Table 1).

Mature fish were captured in June 2009 by netting. The animals were measured, weighed and sacrificed within 24 h after capture. After the dissection the tissues were frozen in liquid nitrogen and stored at –80 °C until they were assayed. For extract preparation the tissues were homogenized in 0,125 M PBS buffer (pH 6,5) by Potter homogenizer followed by centrifugation (100000g for 60 min at 4°C). The supernatant was used as an enzymatic solution.

Enzymatic assay was performed on fish livers, kidneys, gills and muscles. The activity was measured spectrophotometrically in cytosolic fractions according to Habig (Habig et al., 1974) using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates. The final reaction mixture contains 1 mM CDNB and 1 mM GSH.

Proteins were measured in supernatant spectrophotometrically at 205 nm as described by Noble and Bailey (2009), using BSA and reduced glutathione as a standard.

Differences between groups were tested by Manna-Witney's test at a 5% significance level, using the software Statistica 5.0 (Statsoft, Inc., 1995). Correlations between variables (enzymatic activity, age and weight) were examined based on Spearman's index.

Table 1. Water chemical constituents in studied lakes

	Ions concentration in the Kostomukskoe lake, mg/l *	Ions concentration in Kamennoe, mg/l *
Total mineralization	500	17,4
K^+	146	0,45
Na^+	15	0,7
Ca^{2+}	30	1,5
Mg^{2+}	10	1,2
Cl^-	7	1,7
HCO_3^-	145	1–9,7
NO_3^-	9	
NH_4^+	0,05	0,08
NO_2^-	0,03	
N organic	3	0,4–0,7
SO_4^{2-}	172	4,5–6
N total	13	0,3
P total	0,02	0,04
Suspended particles	1,34	0,3–1,8
Fe total	0,3	0,8–1

* (Status of water objects..., 2007).

GST activity was found to positively correlate with age in pike's gills (r_s= –0.62), whitefish kidneys (r_s= –0.63) and roach's kidneys (–0.87) and liver (r_s = –0.87). Concerning to this, this species samples should be chosen very carefully considering its natural variability.

GST activity was found to be sex dependent only in pike's kidneys. It was elevated in female in comparison with males in pikes captured in the Kostomukskoe lake.

The induction of the GST activity was found in pike's gills and kidneys and in whitefish's livers from contaminated lake in comparison with fish from reference site (table 2). GST activity was also altered in whitefish kidneys, there it was significantly higher in the reference than in the contaminated lake.

The altered GST activity in fish from the mining factory lake compared with the unpolluted lake from the same region indicates the bio-protection system response to the mining factory influents. This response in the contaminated site indicates general metabolic stress in water inhabitant under the excessive mineralization. Adverse conditions may modify metabolic processes in tissues, leading to elevated production endogenous reactive GST substrates, for example products of lipids peroxidation.

The absent of roach tissues response indicate its weak adaptive potential to mineral contamination, which can lead to further elimination this species from the contaminated lake. Abnormally low percent of males in population and growth delay of roach from this site can support this assumption.

Table 2. Glutathione S-transferase activity in tissues of fish collected from the lake situated near the mining factory (Kostomukskoe lake) and reference lake (Kamennoe lake)

	pike *Esox lucius*		whitefish *Coregonus lavaretus*		roach *Rutilus rutilus*	
	Kostomukskoe	Kamennoe	Kostomukskoe	Kamennoe	Kostomukskoe	Kamennoe
Liver	699.87	704.62	123.23	75.98*	98.07	71.88
Muscels	7.37	6.35	10.37	7.78	8.22	8.50
Kidneys	31.96	6.58*	16.22	45.71*	3.79	13.55
Gills	819.92	465.95*	9.41	8.94	16.03	29.71

* – groups are significantly different compared to reference lake ($p \leq 0,05$.)
Values are presented as median.

Surprisingly high level of the GST activity was found in pike's liver and gills in comparison with whitefish and roach livers and gills activity, which was on the average 9 and 30 times lover. As obligatory predators pikes seems to have protecting mechanisms against toxicants accumulation during food chain. Elevated biotransformation enzymes level is possibly an adaptation resulting from evolution of this species.

It could be concluded that glutathione S-transferase from pike's kidneys and gills and whitefish's kidneys and liver may be applied as an indicator of fish exposure to non-specific contamination. The GST activity response was observed at polluted lake compared with the control, indicating adverse effect of elevated mineralization on fish detoxification system. Because whitefish, pike and roach are common in northern European lakes this data may be of importance for ecological monitoring in this region.

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MYOSIN EXPRESSION LEVEL IN WHITE MUSCLE AS A MARKER OF FISH GROWTH

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One of the main questions in biotechnology of aquaculture is the search of convenient markers of fish growth. Methods for growth rate maximizing have been tested for many years in aquaculture. Examples, of such methods are recombinant growth hormone injection, use of transgenic fish, variation in type and regimes of nutrition, etc. That is why it is important to assess accurately the effect of use a new techniques. However, there are numerous ways of assessing growth, from traditional and direct measurements such as length, weight and condition factor, to the newest techniques that measure gene expression. Measuring biological macromolecules has been hypothesized by many investigators to be a more sensitive indicator of overall fish condition than traditional morphometric variables. It is considered that muscle-specific genes (Myogenic regulatory factors, myostatin, myosin) are the most reliable markers for assessment of fish growth. The myosin hard chain (MyHC) gene expression has been tested as possible indicator of muscle growth during the last decade since Overturf and Hardy (2001) demonstrated the