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Article

Metallothionein and glutathione in *Lymnaea stagnalis* determine the specificity of responses to the effects of ionising radiation

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ABSTRACT **The aim of our study was to distinguish the stress-related molecular response of the pulmonate mollusc *Lymnaea stagnalis* from the Chernobyl area in comparison with the consequences of other harmful effects, including the short-term effects of radiation and heating. Specimens inhabiting ponds near the Chernobyl nuclear power plant, the cooling channel of the electric power station and the soil-reclamation channel (groups R, T and C, correspondingly), and specimens adapted to laboratory conditions (a control group (CL), a disposable group exposed to 2 mGy X-ray radiation over the body (RL), and a group exposed to 25 °C for 4 days (TL)) were compared. Despite high variability of responses, Principle Component Analysis distinctly separated the laboratory and feral groups into two sets. In the feral groups, low levels of the stress-related and metal-binding protein metallothionein (MT), protein carbonyls and lactate dehydrogenase in the digestive gland were indicated. The main separating criteria selected by classification and regression tree analysis were the protein carbonyls, cholinesterase and MT. Molluscs from group R were clearly distinguished by the lowest levels of MT, Mn-superoxide dismutase and lactate dehydrogenase, and the highest level of glutathione, demonstrating that the oppression of the gene-determined stress-related response and its partially metabolic compensation can be possible markers for chronic environmental effects of irradiation.**

Keywords: Mollusk / irradiation / metallothionein / oxidative stress

1. Introduction

Utilisation of molecular biomarkers is generally accepted to be the most adequate approach for early diagnosis of environmental impacts (Viarengo *et al.*, 2007). However, lack of detailed knowledge on their variability limits their use. Furthermore, genetic or adaptive differences between populations complicate the interpretation of studies on different sites. The particularly scant experience in the

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utilisation of the molecular approach to assess environmental effects is connected to the effect of ionising radiation. Existing experience in radiobiology is devoted more to the accumulation of radioactive particles in the organism than to the biomarkers of effects of environmental sources of radiation (Farcy *et al.*, 2007; Godoy *et al.*, 2008). The monitoring of radioactive contamination associated with the Chernobyl disaster with the use of molluscs is based solely on the measurement of radionuclides in their shells (Frantsevich *et al.*, 1996), whereas the assessment of their health status by biomarker approaches accepted in aquatic ecotoxicology is absent or limited to the characteristics of the genotoxicity of received doses (Ulsh *et al.*, 2003). Some attempts to elucidate the biological effects of radioactive discharges into the natural environment by study of other biomarkers, including stress-related proteins, were ineffective (Fetisov *et al.*, 1992; Farcy *et al.*, 2007; Guerlet *et al.*, 2007). This can probably be explained by the well-studied phenomenon termed radio-adaptation (Howell *et al.*, 2011). At the same time, it is known that low doses of γ-irradiation in laboratory conditions induced significant upregulation of the stress-related genes in aquatic animals (Cai *et al.*, 1999; Olsvik *et al.*, 2010). Study in the Chernobyl Nuclear Power station (ChNPS) area is of particular interest regarding the necessity to understand the remaining consequences of the Chernobyl accident.

Lymnaea stagnalis (Linné, 1758), a secondary-water lung pond mollusk, can be a suitable object for toxicological study. It belongs to dominant in freshwater zoobenthos species on the territory of moderate area of Eurasia. It is known to inhabit most polluted biotopes and accumulate pollution from the water and sediments (Golubev, 1995; Golubev *et al*., 2005; Croteau *et al.*, 2007). However, its molecular stress-related responses to inappropriate effects are little studied (Russo *et al.*, 2007; Gagnaire *et al.*, 2008). A key goal of this study was to distinguish stress-related molecular responses of *L. stagnalis* from the ChNPS area in comparison with the responses to other harmful effects; the continuous effect of elevated temperature in the native field surroundings, and short-term effects of irradiation and heating in laboratory conditions. The set of markers included characteristics of stress represented by lactate dehydrogenase activity and markers of oxidative stress (antioxidant enzymes, Mn- and Cu-Zn superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities, the concentration and redox state of glutathione (GSH), and the level of oxidative injury to proteins and lipids). The markers of exposure to certain kinds of pollution included the concentration of the metal-binding and stress-related proteins metallothioneins (MTs), and cholinesterase (ChE) activity as a marker of neurotoxicity (Viarengo *et al.*, 2007). Finally, to indicate the general and particular signs of responses, integrative statistical data processing was developed.

2. Materials and methods

The experiments were carried out during August 2010. About 30 adult pulmonate molluscs *Lymnaea stagnalis* with shell height of 25 – 35 mm were caught in three aquatic bodies in the Republic of Belarus. Lake Perstok (51°30' N, 30°01' E) is located 14 km from the ChNPS (group R), in the river Pripyat land (group R). It is the most radiocontaminated water body in Belarus. In 2007–2008 average activities of ^{90}Sr and ^{137}Cs of *Lymnaea stagnalis* attained 18 938 and 625 Bq kg^{-1} body wet mass, respectively. The activities of principal dose-forming radionuclides in bottom sediments – ^{137}Cs, ^{90}Sr and ^{241}Am attained 2149, 1017 and 40 kBq m^{-2}, respectively (*Unpublished data*). Both other field sites are situated near the city of Belaazyorsk (52°27' N, 25°10' E) in an industrial area. Group T was sampled in the warm effluent channel of a heat electric power station in Belaazyorsk city (52°27'N, 25°10' E). In the sampling period water temperature there was about 30 °C. Group C originated from the sludgy soil-reclamation channel located near the warm effluent channel with a natural temperature regime (about 20 °C in the sampling period).

Individuals were transported to the laboratory in cages with native water and treated within a day after the sampling procedure. Snails from the pond in a clean forestry site located in the upstream portion of the river Seret (Ternopil region, Ukraine, 49°49' N, 25°23' E) were adapted to laboratory conditions for seven days. After that they were divided into three groups: the control group (CL), and two groups subjected to effects of radiation or elevated temperature (group RL and TL, correspondingly).

A single 2 mGy X-ray dose was administered to the snails from the group RL using a RUM-20 X-ray machine (Ternopil Oncology Centre, Ukraine), delivering 7.5 mGy per min. The exposure took 16 s in a waterless medium in plastic bags. The temperature of the irradiation chamber was about 20 °C. Whole molluscs were sealed in plastic bags and inserted into a plastic phantom with wall thickness of 0.4 g/cm^2, which is suitable for establishing electron equilibrium. Snails were studied for seven days. Group TL was exposed to 25 °C for four days before study in plastic holding containers with a Fluval M Aquarium Heater (EU). For the CL and RL groups, the temperature of the water was 18 ± 0.5 °C. Each group initially consisted of about 30 specimens. The mortality of snails was 40% in group RL and 75% in group TL. High mortality limited the periods of incubation of snails in the laboratory.

For each biochemical parameter eight digestive gland samples were prepared individually. For enzymatic measurements, tissue samples were homogenised (1/10 w/v) in 0.1 M pH 7.4 phosphate buffer containing 100 mM KCl and 1 mM

EDTA as well 0.1 mM phenylmethylsulphonyl fluoride for the inhibition of proteolysis. Homogenates were centrifuged at 6 000 g for 10 min and the resulting supernatant was used immediately for measurement. The protein concentration in the supernatant was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Each procedure of tissue analysis was carried out at a temperature around 4 °C.

Chemicals: 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), reduced glutathione (GSH), glutathione reductase, 2-vinylpyridine, 1-chloro-2,4-dinitrobenzene (CDNB) nitroblue tetrazolium (NBT), 2,4-dinitrophenylhydrazine (DNPH), serum albumin, phenazine methosulphate, phenylmethylsulphonyl fluoride, β-mercaptoethanol, NADH, NADPH, EDTA and acetylthiocholine iodide (ATCh) were purchased from Sigma. All other chemicals were of analytical grade.

The methods applied in this study are described in detail in (Falfushynska *et al.*, 2010; Gnatyshyna *et al.*, 2011). Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridovich (1971). In order to assess Mn-SOD activity, the supernatant was preincubated for 60 min at 0 °C in the presence of 5 mM KCN, which produced total inhibition of Cu-Zn SOD. Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decomposition of 10 mM H_2O_2 at 240 nm according to Aebi (1974). Glutathione-S-transferase (GST, EC 2.5.1.18) activity was measured using CDNB as the substrate (Habig *et al.*, 1974). Total glutathione concentration was quantified by the glutathione reductase recycling assay (Anderson, 1985). To estimate the oxidised glutathione (GSSG) level, the protein-free sample was treated with 2-vinylpyridine prior to the assay run (Griffith, 1980). The glutathione redox index (RI GSH) as the ratio of concentrations ([Total glutathione]–[GSSG])/[Total glutathione] was calculated. Lipid peroxidation was determined by the production of TBA-reactive substances (TBARS) (Ohkawa *et al.*, 1979). Protein carbonyl (PC) content, as an index of protein oxidation, was measured in the resulting supernatants by the reaction with DNPH (Reznick *et al.*, 1994). The activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was determined from the pyruvate-dependent NADH oxidation (Bergmeyer *et al.*, 1974). Cholinesterase (ChE, EC 3.1.1.7) activity as the biochemical marker of neurotoxicity was determined as the ATCh-cleaving ChE activity according to the colorimetric method (Ellman *et al.*, 1961). Metallothioneins (MTs) were determined from thiol measurement with DTNB after ethanol/chloroform extraction (Viarengo *et al.*, 1997). Results were expressed as means ± standard deviation (SD) of eight individuals for each biochemical endpoint. Since data were not normally distributed (Lilliefors test), non-parametric tests (Kruskall-Wallis ANOVA and Mann-Whitney *U*-test) were performed (significant at $p < 0.05$).

Data were subjected to principal component analysis (PCA) to evaluate the biomarkers' relation both in feral and experimental groups. A classification tree was built using the classification and regression tree (CART) algorithm. All statistical calculations were performed with Statistica v7.0 and Excel for Windows 2000.

3. Results and discussion

The results (Fig. 1) showed that the field groups were distinctly distinguished from the laboratory groups by low MT, LDH (particularly in group R) and PC levels. In group R, the lowest MT and highest GSH levels combined with rather high RI GSH and GST activity within the field groups. Additionally, group R demonstrated the lowest Mn-SOD and LDH activities. Group C could be qualified as the most injured group due to having the lowest levels of RI GSH and Cu-Zn SOD, and the highest level of TBARS. Groups T and TL were characterised by the highest antioxidant activity according to high CAT levels and low TBARS and PC levels in the correspondent set of groups. However, activity of the system of GSH in these snails was low.

According to PCA (Fig. 2), 69.9% of the data belonged to Factors 1 and 2. PCA confirmed the differences between the field and laboratory groups. One cluster (with the value of p1 less than 0) combined markers that described snails from the laboratory. Another set (with the value of p1 more than 0) included groups from field sites. Within each cluster, the groups C and CL, T and TL, and R and RL had similar locations. R and RL groups were situated in the middle of the clusters. The similarity of the responses of MT, PC, LDH, RI GSH and GST, in opposition to the responses of GSH and TBARS (Factor 1), was also confirmed by PCA (Fig. 2B). The responses of ChE and CAT were different from other markers since they belonged to Factor 2.

To elucidate the main partitioning markers, we used the CART algorithm (Fig. 3). When all of the biological parameters in the six groups were compared (Fig. 3A), the PC level in the digestive gland persisted as a splitting variable through two prunings of the tree, that resulted in a 6-node tree. MT and LDH levels in the digestive gland were represented at the nodes as partitioning criteria for the field groups, whilst ChE activity distinguished the laboratory groups RL and TL. None of the terminal nodes contained misclassified snails. The resulting confusion matrix showed an overall classification accuracy of 93%. The best classification was predicted for group CL, followed by groups TL and RL. Comparison of the laboratory groups separately demonstrated that the main partitioning criteria were MT and RI GSH levels (Fig. 3B).

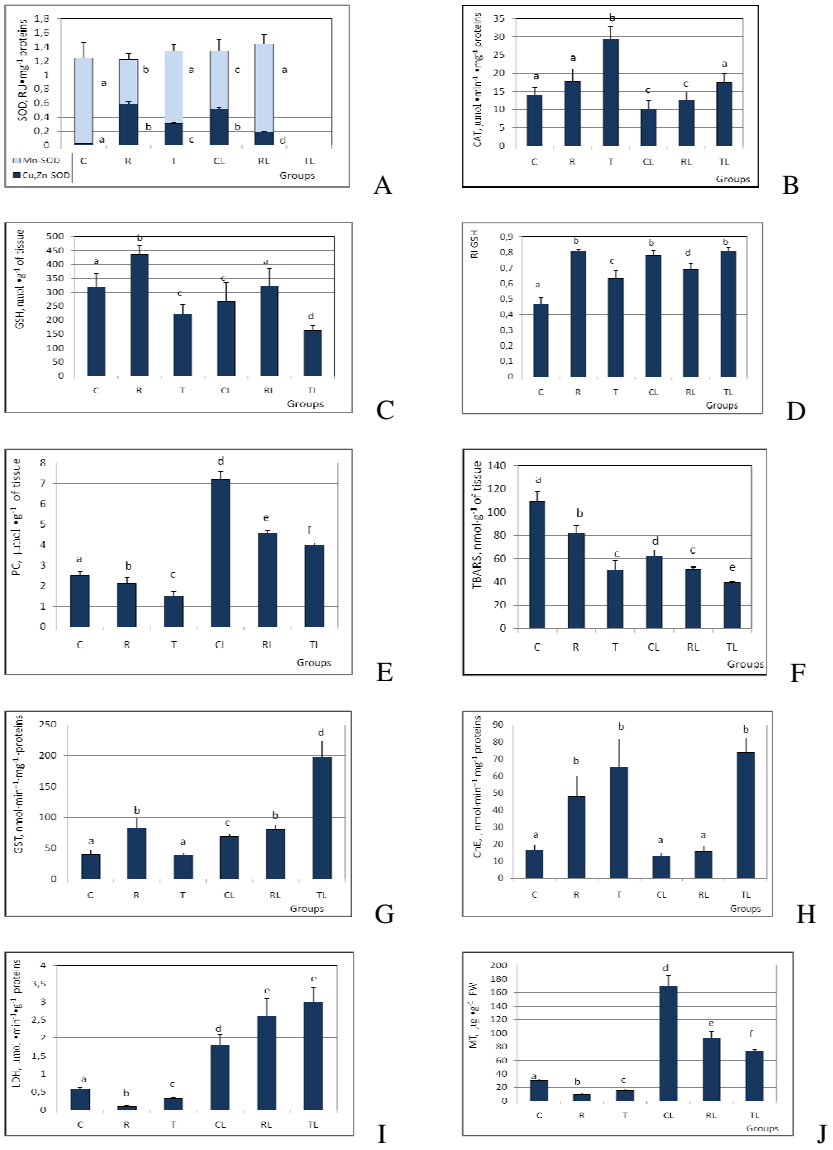

Figure 1 – ***Biomarkers of stress and toxicity in the digestive gland of Lymnaea stagnalis. Data for A, SOD; B, CAT; C, reduced GSH; D, RI GSH; E, PC; F, TBARS; G, GST; H, ChE; I, LDH; J, MT are present as means ± SD (N = 8). The same letters correspond to values of biomarkers that are not significantly different, always p > 0.05.***

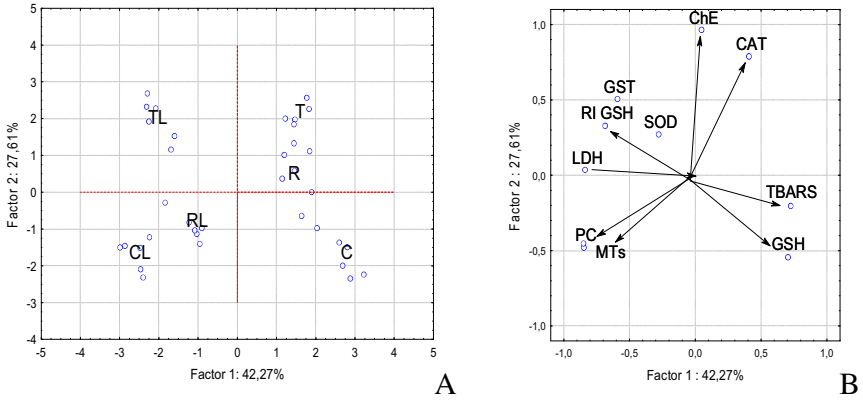

Figure 2 – ***Centroid grouping (A) and principal component (B) analysis of the Lymnaea stagnalis parameter dataset in the digestive gland from feral (C, R, T) and laboratory (CL, RL, TL) groups.***

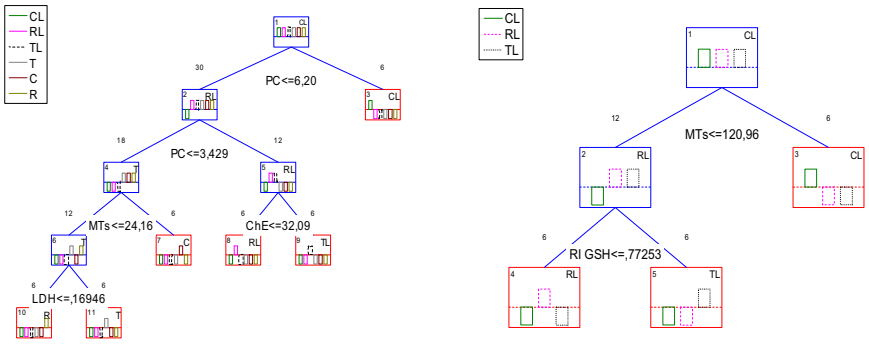

Figure 3 – ***Classification tree models. Terminal nodes identify the groups and the number of molluscs represents all studied specimens (A) and groups only exposed in the laboratory (B).***

The pulmonate mollusc *L. stagnalis* is a haemoglobin-free species (Bugge *et al.*, 1999), which probably makes this snail very sensitive to oxidative stress. The SOD-catalysed reaction can be the source of endogenous oxygen in these circumstances. As was shown, in general SOD activity remained stable in all studied groups. Moreover, the low level of LDH activity confirmed the sufficient level of metabolism in the field groups of snails. However, the lower level of Mn-SOD may be a result of the particular vulnerability of mitochondrial-dependent processes in the snails from the Chernobyl area. The sensitivity of mitochondria to adverse effects was also described in the snail *L. stagnalis* (Masola *et al.*, 2008) and bivalve molluscs (Ivanina *et al.*, 2008).

Among the studied biomarkers, low-molecular-weight intracellular thiols, MTs and GSH, that perform crucial biological functions involved in the general stress response, particularly scavenging of reactive oxygen species, and storage and transport of metal ions (Cai *et al.*, 1999; Viarengo *et al.*, 2007) were selected as important indices for the distinguishing of groups (Fig. 3). Upregulation of these stress-related thiols is considered to be one of the mechanisms involved in the adaptive response to low-dose radiation exposure (Cai *et al.*, 1999; Olsvik *et al.*, 2010). On the other hand, the decreased level of GSH under the influence of low-level radiation (below 20 cSv), accompanied by increases in the level of lipid peroxidation products in human plasma was revealed long after the accident (4-7 years) in the liquidators and children of exposed mothers from the ChNPS area (Ivanenko *et al.*, 2008).

The presence of MT in the cells may provide protective effects from radiation-induced genotoxicity and cytotoxicity (Cai *et al.*, 1999). However, the main feature of the molluscs from the Chernobyl area was the depletion of MT: it was probably a result of the exhaustion of the stress-induced gene regulation. GSH-dependent functions could partly compensate for this lack of MTs in the studied snails. The opposite location of MTs and GSH by PCA confirms this assumption.

Despite the unique experience of the radioactive pollution related to the Nuclear Disaster in the ChNPS, the data concerning the responses of aquatic animals in the area are scant. Variation in blood cell DNA in *Carassius carassius* from ponds near the ChNPS determined about ten years after this disaster demonstrated that the abnormalities were not correlated with known contaminant distributions (Lingenfelser *et al.*, 1997). Genetic studies, by electrophoresis, of seven *Dreissena polymorpha* populations in the water basins near the ChNPS also revealed that the differences in populations were apparently governed by conditions at the breeding site and not by thermal or radioactive contamination (Fetisov *et al.*, 1992). So, peculiarities found in the MT concentration deserve attention and further study.

ChE in the digestive gland revealed differences between two sets of groups, with low levels in C, CL and RL groups and high levels in R, T and RT groups. It is difficult to explain this pooling of groups with such different kinds of exposure. The level of ChE activity obtained in our study for C, CL and RL groups was similar to those reported in the literature for several molluscs including gastropods (Gagnaire *et al.*, 2008). Therefore, it can be classified as a normal level and the level of ChE activity in the groups R, T and TR can be classified as an elevated one. ChE inhibition is widely used as a specific and sensitive biomarker of neurotoxicity in different animals, mainly vertebrate, but including molluscs and certainly snails (Gagnaire *et al.*, 2008). This inhibition is particularly caused in

non-target organisms by insecticide contamination, as its inhibition occurred at low concentration without mortality. Besides pesticides, irradiation is also known to produce significant ChE depression, for example in the blood of rats, on the tenth day at a dosage level of 75 r (Williams *et al.*, 1961). However, contradictory results are reported for molluscs, particularly in field studies, in which fluctuations in dissolved oxygen or temperature, or any combined effects, can modulate the typical response (Gagnaire *et al.*, 2008). In the study of Corsi *et al.* (2007), invasive species of *Anadonta*, *Anodonta woodiana*, well adapted to new surroundings, showed significantly greater ChE activity than the endemic species (*Anodonta sp*.). This effect, the increased ChE activities, observed in oysters upon the second spray events of organophosphate (Bolton-Warberg *et al.*, 2007), was explained as a ''hormetic'' response (Stebbing, 1982), where a toxic substance acts as a stimulant in small doses. ChE activity was significantly elevated in the subcellular fractions of the cerebellum of adult rats as a late radiation effect (Adlard *et al.*, 1972). Elevated levels of ChE activity were observed in rhabdomyosarcoma tumours and in the small intestine in correlation with doses of neutron radiation (2.0, 3.8 and 7.0 Gy) (Szeinfeld *et al.*, 1993). In our case, we could speculate that a similar effect on ChE in the field groups R and T and group TL exposed in the laboratory can be provoked for different reasons and needs additional study for explanation. In the case of groups R and RL, the difference can be explained by the late and recent effect of radiation.

Some contrasting regularity was found between field and laboratory groups. An increase in anaerobic glycolysis is a well-known sign of toxic effect in molluscs (Anestis *et al.*, 2007). In our study, despite the favourable regime of respiration, laboratory groups had comparatively high LDH activity that attests to the shift to anaerobiosis. They also showed high levels of PC, MTs and RI GSH. That could be explained by slow removal of damaged proteins and maintenance of the reset state in the conditions of low metabolic activity. On the other hand, low levels of PC in the field groups may be a result of high turnover of proteins (Ivanina *et al.*, 2008). The enhancement of pulmonary respiration in *L. stagnalis* in these groups has been found to be associated with a rise in levels of GSH and TBARS in the tissue (Sidorov *et al.*, 2008). The relation between GSH and TBARS levels was confirmed in our study by PCA.

Obviously, as is shown above, snails with different life histories and under different exposures demonstrated high variability of molecular responses. Only the elucidation of the interrelation of separate characteristics and, based on this issue, classification of molecular responses, remains a very current technology in environmental risk assessment. In accordance with our aim to distinguish between the state of molecular markers in snails originating from the ChNPS area (group R) and snails subjected to other long- or short-term effects, we can conclude that the

particularities that we observed in the group R could correspond to an overwhelming of the stress-related systems, based probably on the inhibition of the expression of correspondent proteins. On the other hand, elevated temperature provoked the activation of these systems, as is evident from our data and other results on molluscs (Farcy *et al.*, 2007) and in fish (Lushchak *et al.*, 2006).

To conclude, the particular effect of prolonged radioactive pollution was the exceeding of the resilience of the gene-determined adaptive stress response connected to depletion of MT and its partially metabolic compensation by GSH in the digestive gland of the mollusc. This makes MT and GSH evaluation a possible marker for chronic environmental effects of irradiation.

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REFERENCES

Adlard B.P.F., Dobbing J. (1972) Permanent changes in the activity and subcellular distribution of acetylcholinesterase and lactate dehydrogenase in adult rat cerebellum after X-irradiation in infancy, *Exp. Neurol.* **35(3)**, 547550.

Aebi H. (1974) Catalase. In: Bergmeyer HU (Ed.) *Methods of Enzymatic Analysis*. Academic Press, London, 671–684.

Anderson M.E. (1985) Determination of glutathione and glutathione disulfide in biological samples, *Methods Enzymol.* **113**, 548-555.

Anestis A., Lazou A., Portner H.O., Michaelidis B. (2007) Behavioral, metabolic, and molecular stress responses of marine bivalve Mytilus galloprovincialis during long-term acclimation at increasing ambient temperature, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**, 911-921.

Beauchamp C., Fridovich I. (1971) Superoxide dismutase: improved assay and an assay applicable to acrylamide gels, *Anal. Biochem.* **44**, 276-287.

Bergmeyer H.U., Bernt E. (1974) Lactate dehydrogenase U.V. assay with pyruvate and NADH, In: Bergemeyer HU (Eds.) *Method of Enzymatic Analysis*, Academic Press, New York. II, 574–579.

Bolton-Warberg M., Coen L.D., Weinstein J.E. (2007) Acute Toxicity and Acetylcholinesterase Inhibition in Grass Shrimp (*Palaemonetes pugio*) and Oysters (*Crassostrea virginica*) Exposed to the Organophosphate Dichlorvos: Laboratory and Field Studies, *Arch. Environ. Contam. Toxicol.* **52**, 207-216.

Bugge J., Weber R.E. (1999) Oxygen binding and its allosteric control in hemoglobin of the pulmonate snail, Biomphalaria glabrata, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **276**, 1-19.

Cai L., Satoh M., Tohyama Ch., Cherian M.G. (1999) Metallothionein in radiation exposure: its induction and protective role, *Toxicology* **132**, 85-98.

Corsi I., Pastore A.M., Lodde A., Palmerini E., Castagnolo L., Focardi S. (2007) Potential role of cholinesterases in the invasive capacity of the freshwater bivalve, *Anodonta woodiana* (Bivalvia: Unionacea): a comparative study with the indigenous species of the genus, *Anodonta* sp., *Comp. Biochem. Physiol.* **145 C**, 413-419.

Croteau M.N., Luoma S.N. (2007) Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand and biodynamics using enriched stable isotopes, *Environ. Sci. Technol.* **41**, 3140-3145.

Ellman G.L., Courtney K.D., Andres V.J., Featherstone R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* **7**, 88-95.

Falfushynska H.I., Gnatyshyna L.L., Farkas A., Vehovszky A., Gyori J., Stoliar O.B. (2010) Vulnerability of biomarkers in the indigenous mollusk *Anodonta cygnea* to spontaneous pollution in a transition country, *Chemosphere* **81**, 1342-1351.

Farcy E., Voiseux C., Lebel J.-M., Fievet B. (2007) Seasonal changes in mRNA encoding for cell stress markers in the oyster *Crassostrea gigas* exposed to radioactive discharges in their natural environment, *Sci. Total Environ.* **374**, 328-341.

Fetisov A.N., Rubanovich A.V., Slipchenko T.S., Shevchenko V.A. (1992) The structure of *Dreissena polymorpha* populations from basins adjacent to the Chernobyl atomic power station, *Sci. Total Environ.* **112**, 115-124.

Frantsevich L., Korniushin A., Pankov I., Ermakov A., Zakharchuk T. (1996) Application of molluscs for radioecological monitoring of the Chernobyl outburst, *Environ. Pollut.* **94**, 91-100.

Gagnaire B., Geffard O., Xuereb B., Margoum Ch., Garric J. (2008) Cholinesterase activities as potential biomarkers: characterization in two freshwater snails, *Potamopyrgus antipodarum* (Mollusca, Hydrobiidae, Smith 1889) and *Valvata piscinalis* (Mollusca, Valvatidae, Müller 1774), *Chemosphere* **71(3)**, 553-560.

Gnatyshyna L.L., Falfushynska H.I., Golubev A.P., Dallinger R., Stoliar O.B. (2011) The roles of metallothioneins in adaptation of pulmonate mollusk *Lymnaea stagnalis* under condition of environmental pollution, *Hydrobiol. J.* **47(5)**, 56-66.

Godoy J.M., de Oliveira M.S., de Almeida C.E.B., de Carvalho Z.L., da Silva E.R., da Costa Fernandes F., Pitanga F.L., Danelon O.M. (2008) 210Po concentration in *Perna perna* mussels: looking for radiation effects, *J. Environ. Radioactivity* **99**, 631-640.

Golubev A.P. (1995) General thermoresistance and radioresistance of the populations of Lymnaea stagnalis (Gastropoda, Pulmonata) from the reservoirs with different forms of anthropogenic loading, Doklady Akademii Nauk **342(2)**, 280-283.

Golubev A., Afonin V., Maksimova S., Androsov V. (2005) The current state of pond snail *Lymnaea stagnalis* populations from water reservoirs of the Chernobyl nuclear accinent zone, *Radioprotection* **240**, 511-517.

Griffith O.W. (1980) Determination of Glutathione and Glutathione Disulfide Using Glutathione Reductase and 2-Vinylpyridine, *Anal. Biochem.* **106**, 207-212.

Guerlet E., Ledy K., Meyer A., Giambérini L. (2007) Towards a validation of a cellular biomarker suite in native and transplanted zebra mussels: a 2-year integrative field study of seasonal and pollution-induced variations, *Aquat. Toxicol.* **81(4)**, 377-388.

Habig W.H., Pabst M.J., Jakoby W.B. (1974) Glutathione S-Transferases: The first enzymatic step in mercapturic acid formation, *J. Biol. Chem.* **249**, 7130-7139.

Howell E.K., Gaschak S.P., Griffith K.D.W., Rodgers B.E. (2011) The effects of environmental low-dose irradiation on tolerance to chemotherapeutic agents, *Environ. Toxicol. Chem.* **30**, 640-649.

Ivanenko G., Goloshchapov A., Fatkullina L., Burlakova E. (2008) Relationship Between Plasma Glutathione Levels and Lipid Antioxidants of People Exposed to Low Doses of Radi, *Radioprotection* **43(5)**, 54.

Ivanina A.V., Sokolova I.M., Sukhotin A.A. (2008) Oxidative stress and expression of chaperones in aging molluscs, *Comp. Biochem. Physiol.* **150 B**, 53-61.

Lingenfelser S.K., Dallas C.E., Jagoe C.H., Chesser R.K., Smith M.H., Lomaki M.D. (1997) Variation in blood cell DNA in *Carassius carassius* from ponds near Chernobyl, Ukraine, *Ecotoxicology* **6**, 187-203.

Lushchak V.I., Bagnyukova T.V. (2006) Temperature increase results in oxidative stress in goldfish tissues. 2. Antioxidant and associated enzymes, *Comp. Bioch. Physiol.* **143 C**, 36-41.

Masola B., Chibi M., Kandare E., Naik Y.S., Zaranyika M.F. (2008) Potential marker enzymes and metal-metal interactions in *Helisoma duryi* and *Lymnaea natalensis* exposed to cadmium *Ecotoxicol. Environ. Saf.* **70**, 79-87.

Ohkawa H., Onishi N., Yagi K. (1979) Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction, *Anal. Biochem.* **95**, 351-358.

Olsvik P.A., Heier L.S., Rosseland B.O., Teien H.C., Salbu B. (2010) Effects of combined gamma-irradiation and metal (Al+Cd) exposures in Atlantic salmon (*Salmo salar* L.), *J. Environ. Radioactivity* **101**, 230-236.

Reznick A.Z., Packer L. (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay, *Methods Enzymol.* **233**, 357-363.

Russo J., Lefeuvre-Orfila L., Lagadic L. (2007) Hemocyte-specific responses to the peroxidizing herbicide fomesafen in the pond snail *Lymnaea stagnalis* (Gastropoda, Pulmonata), *Environ. Pollut.* **146**, 420-427.

Sidorov V., Maslova G.T. (2008) Antioxidant defense in the central nervous system of *Lymnaea stagnalis* at different environmental temperatures, *News Biomed. Sci.* **1-2**, 74-78.

Stebbing A.R.D. (1982) Hormesis- the stimulation of growth by low levels of inhibitors, *Sci. Total Environ.* **22**, 213-234.

Szeinfeld D, de Villiers N. (1993) Cholinesterase response in the rhabdomyosarcoma tumor and small intestine of the BALB/c mice and the radioprotective actions of exogenous ATP after lethal dose of neutron radiation, *Strahlenther Onkol*. **169(5)**, 311-316.

Ulsh B., Hinton T.G., Congdon J.D., Dugan L.C., Whicker F.W., Bedford J.S. (2003) Environmental biodosimetry: A biological relevant tool for ecological risk assessment and biomonitoring, *J. Environ. Radioactivity* **66**, 121-139.

Viarengo A., Lowe D., Bolognesi C., Fabbri E., Koehler A. (2007) The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comp. Biochem. Physiol.* **146 C**, 281-300.

Viarengo A., Ponzano E., Dondero F., Fabbri R. (1997) A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic Molluscs, *Mar. Environ. Res.* **44**, 69-84.

Williams M.W., Baker R.D., Covill R.W. (1961) Effect of Gamma Irradiation and AET on Rat Blood Cholinesterase, *Exp. Biol. Med.* **106(3)**, 603-605.