

Society of Environmental Toxicology and Chemistry

**SETAC Europe 17th Annual Meeting
20-24 May 2007 Porto, Portugal**

Abstract Book

Multiple stressors for the environment and human health
present and future challenges and perspectives

SETAC Europe 2007 | Porto - PORTUGAL



www.setac.org

WE 398

Fish liver size and CAT activity: are they related to metals bioaccumulation?

Maria Salgado¹, Conceição Fernandes², António Fontainhas-Fernandes², Marta Ferreira², Amélia Cortez²

¹Instituto de Ciências Biomédicas de Abel, PORTO, Portugal

The hepato-somatic index reflects the status of liver, as energy store and metabolic centre, and is one of the most often associated with contaminant exposure (Dethloff and Schmitt, 2000). Several studies revealed that fish exposure to contaminants can also enhance intracellular formation of ROS, which are able to originate oxidative damage (Livingstone, 2003; Ferreira et al., 2005). ROS can be detoxified by an enzymatic defence system, that include catalase (CAT) (Halliwell and Gutteridge, 1989). Esmoriz/Paramos lagoon is located on the Northwest coast of Portugal in an area of industrial activity, where heavy metals are important anthropogenic inputs.

Total metal distribution of Cu and Zn in sediments revealed a relation Zn > Cu and the bioaccumulation factors (BAF) of these metals in mullet liver were 305% and 36% for Cu and Zn respectively (Fernandes et al., 2006).

In this study the uptake of metals by mullet was related with relative liver size (HSI) and CAT-liver activity. Fish captured in the lagoon showed higher HSI (1.76-4.38 %) compared with fish collected in the sea (0.36-2.21 %). Enzymatic activity changes were also observed for CAT between liver mullet from lagoon and from sea. Mullet in the lagoon showed higher CAT activity (39 mmol/min/mg prot.) than in the sea (22 mmol/min/mg prot.) and it was also positively related with Cu levels. These results suggest that a high CAT activity is needed to cope with Cu oxidative stress, and it may be linked to larger liver size. In conclusion metals chronic exposure in the lagoon seems to have conducted to adaptive metabolic fish responses.

References

- Dethloff G.M. and Schmitt C.J. (2000). Condition factor and organo-somatic indices. In: *Biomonitoring of Environmental Status and Trends (BEST) Program: selected methods for monitoring chemical contaminants and their effects in aquatic ecosystems*. Schmitt C.J. and Dethloff G.M. (Ed). U.S. Geological Survey, Biological Resources Division, Columbia, (MO): Information and Technology Report USGS/BRD-2000-0005.
- Fernandes, C., Fontainhas-Fernandes, A., Peixoto, F. & Salgado M.A. (2006). Bioaccumulation of heavy metals in Liza saliens from the Esmoriz-Paramos coastal lagoon, Portugal. *Ecotoxicology and Environmental Safety* (in press).
- Ferreira, M., Moradas-Ferreira, P. & Reis-Henriques, M.A. (2005). Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. *Aquatic Toxicology*, 71, 39-48.
- Halliwell, B. & Gutteridge, H.M.C. (1999). *Free radicals in biology and medicine*, Oxford: University Press.
- Livingstone, D.R. (2003). Oxidative stress in aquatic organisms in relation to pollution and aquaculture. *Revue Médicine Vétérinaire*, 154, 6, 427-430.

WE 399

Overall diagnosis of water: in vivo information with in vitro tools

Gregory Lemkine¹, gregory Lemkine², vincent Mataix¹, sophie Pallud-Mothre¹, anthony Sebillot²

¹WatchFrog, PARIS, France

²WatchFrog, PARIS, France

³EDF R&D, CHATOU, France

Biotechnology solutions are the only feasible way to accelerate environmental risk assessment whilst ensuring compatibility with managing a number of industrial plants. Electricité De France and the biotech company WatchFrog are working together to provide innovative tools to identify the impact of energy production installations on environmental health.

We have developed a tailor-made range of tests to monitor heavy metals bioavailability and detect endocrine disrupting chemicals.

The new generation of tests offered by WatchFrog is based on development of amphibian larvae that 'light up' (through emission of fluorescence) when a biological function is activated.

We perform direct analysis of water samples with dedicated robotized reading devices that permit real-time monitoring of biological effects of pollutants in industrial waste.

We have adapted the sensitivity of our tests in line with regulatory recommended concentrations of heavy metals.

Looking ahead, WatchFrog will provide follow-up and water analysis tools both for the water treatment and effluent management industries involving minimal handling on the part of users.

WE 400

A semi-quantitative study of the histopathological changes induced by waterborne copper in gills of Nile tilapia, *Oreochromis niloticus*

Sandra Monteiro¹, Eduardo Rocha², António Fontainhas-Fernandes³, Mário Sousa⁴

¹University of Trás-os-Montes and Alto Do, VILA REAL, Portugal

²ICBAS, Univ. Porto, CIMAR, PORTO, Portugal

³CETAV and UTAD, VILA REAL, Portugal

⁴Instit. Biomedical Sciences Abel Salazar, PORTO, Portugal

Gill histopathological changes are commonly known responses to environmental stressors. To test if their extension and severity can be used as indicators of the water toxicant levels, Nile tilapia were exposed to 40 and 400 microgram L⁻¹ of copper. The gill samples, collected after 3, 7, 14 and 21 days of exposure, were analysed by histopathology. The main lesions observed were edema, lifting, changes in filamentar epithelium thickness, lamellar fusion, vasodilation and aneurisms. Although less frequent, necrosis and lamellar epithelium proliferation were also observed. The extension and severity of each lesion were used to develop a severity gradation scale. Data revealed that edema and aneurisms may be used as acute biomarkers and that lifting and changes in filamentar epithelium thickness reflected the exposure time to 40 and 400microgram L⁻¹, respectively. Finally, lamellar fusion was an adequate biomarker of long exposure times. We conclude that gill histopathological changes, analysed globally, as cumulative severity degrees, provide a sensitive indicator of copper concentration and exposure time. This was corroborated by the correlation coefficients with plasma cortisol and gill copper deposition.

WE 401

Mullet chronic exposure to Cu and Zn: liver histopathological assessment and metabolic enzyme activities.

Maria Salgado¹, Conceição Fernandes², António Fontainhas-Fernandes², Eduardo Rocha³

¹Instituto de Ciências Biomédicas de Abel, PORTO, Portugal

²ICBAS-CIIMAR-C.I.Invest.Marinha e Amb., PORTO, Portugal

The presence of heavy metals in the aquatic environment has increased in different areas and several studies reported metal concentrations in wild fish species (Homung and Ramelow 1987; Romeo et al., 1999; Fernandes et al., 2006). Liver is the major organ of accumulation, biotransformation, and excretion of contaminants in fish (Triebkorn et al., 1997), therefore is usually the organ where the highest metal accumulation and greatest damage or impairment occur (Arellano et al., 1999).

Toxicological studies of acute exposure have shown that the concentration of pollutants can influence enzyme activities and often directly reflect cell damage in specific organs (Yang and Chen 2003). Histopathology is increasingly being used as indicator of environmental stress since it provides a definite biological end-point of historical exposure (Stentford et al., 2003). The type of liver injury is often dependent upon the length of exposure of various toxicants, such as metals (Yang and Chen, 2003; Au, 2004; Olojo et al., 2005).

The relation between hepatic lesions and metal concentrations (Cu and Zn) in liver tissue, as also the influence of these hepatic lesions on the plasma enzyme activities (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)) were pointed out in wild mullets caught at Esmoriz-Paramos coastal lagoon, Portugal. The prevalence of hepatic histological alterations was 9 % of non-neoplastic lesions (NNL), 34 % of foci of cellular alteration (FCAs), 31 % of foci of necrosis (FNs), 14 % of granulomas and 6 % of melanomacrophage centres (MMC). Cu-liver and Zn-liver were significantly higher in fish showing FCA than in fish without lesion, suggesting that the presence of these metals in liver may have an effect on the lesion development

Mullets from lagoon showed higher AST activity than mullets caught at sea. These results are in agreement with earlier reports on the effects of heavy metals on fish enzymes (Varanka et al., 2001; Zikic et al., 2001; Levesque et al., 2002). However, this increase may be an AST adaptive response rather than a consequence of

cell permeability and integrity alterations in liver caused by metals, since the activities of ALT and ALP observed in the two fish populations were not affected.

References

- Arellano J.M., Storch V. and Sarasquete C. (1999). Histological changes and copper accumulation in liver and gills of the senegales sole, *Solea senegalensis*. *Ecotoxicology and Environmental Safety* 44: 62-72.
- Au D.W.T. (2004) The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. *Marine Pollution Bulletin* 48:817-834.
- Fernandes, C., Fontainhas-Fernandes, A., Peixoto, F. and Salgado M.A. (2006). Bioaccumulation of heavy metals in *Liza saliens* from the Esmoriz-Paramos lagoon, Portugal. *Ecotoxicology and Environmental Safety* (in press).
- Hornung H. and Ramelow G.J. (1987) Distribution of Cd, Cr, Cu and Zn in eastern Mediterranean fishes. *Marine Pollution Bulletin* 18:45-49.
- Levesque H.M., Moon T.W., Campbell P.G.C. and Hortela A. (2002) Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquatic Toxicology* 60:257-267.
- Olojo E.A.A., Olurin K.B., Mbaka G. and Oluwemimo A.D. (2005) Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to cadmium. *African Journal of Biotechnology* 4(1):117-122.
- Romeo M., Siau Y., Sidoumou Z. and Gnassia-Barelli M. (1999) Heavy metal distribution in different fish species from the Mauritania coast. *Science of The Total Environment* 232:169-175.
- Stentiford G.D., Longshaw M., Lyons B.P., Jones G., Green M. and Feist S.W. (2003) Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. *Marine Environmental Research* 55:137-159.
- Triebkorn R., Köhler H.R., Honnen W., Schramm M. and Adams S.M. (1997) Induction of heat shock proteins, changes in liver ultrastructure, and alterations in behaviour: are these biomarkers related and are they useful to reflect the state of pollution in the field? *Journal of Aquatic Ecosystem Stress and Recovery* 6: 1-10.
- Varanka Z., Rojik I., Varanka I., Nemcsók J. and Abraham M. (2001) Biochemical and morphological changes in carp (*Cyprinus carpio* L.) liver following exposure to copper sulfate and tannic acid. *Comparative Biochemistry and Physiology Part C* 128:467-478.
- Yang J.-L. and Chen H.-C. (2003) Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. *Zoological Studies* 42(3):455-461.
- Zikic R.V., Stajin A.S., Pavlovic S.Z., Ogrnjancic B.I. and Saicic (2001) Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminase activities in *Carassius auratus gibelio* Bloch. exposed to cadmium. *Physiological Research* 50:105-111.

WE 402

Assessment of ecotoxicological condition of Škadar Lake sediments using bacterial community diversity analysis and microbiological bioassays

Svetlana Perovic¹, Ales Lapanje², Andrej Perovic³, Lothar Erdinger³, Henner Hollert³, Rok Kostanjsek²

¹University of Montenegro, PODGORICA, Yugoslavia

²University of Ljubljana, LJUBLJANA, Slovenia

³University of Heidelberg, HEIDELBERG, Germany

This pilot study was performed to investigate the contribution of toxic effects caused by sediments on the Skadar lake aquatic ecosystem.

Specific toxic effects of sediments were measured using the microbial DHA test (*Arthrobacter globiformis*) and the Ames test (*Salmonella typhimurium* TA98 and TA100). Genetic diversity of the local bacterial community was analyzed as an indicator of the impact of sediments on the ecosystem. Bacterial DNA was isolated from sediment samples and fragments of bacterial 16S rRNA genes were amplified. Using Temporal Temperature Gel Electrophoresis (TTGE), fragments were finally separated and analyzed.

Analyses of bacterial community structure showed significant alterations and reduced genetic diversity of the bacterial community in the mouth Moraca river, the biggest tributary of Skadar Lake. Additionally, at the same sampling point both toxic and specific mutagenic effects were identified. This research was performed within the EULIMNOS project. It was supported by the German Rector's Conference (HRK), the Slovenian Ministry of science, sport and education and by the Montenegrin Ministry of science and education.

WE 403

Evoecotoxicology: Ontogenic features as a new datation method of the evolutionary process?

Jorg Herkovits

Inst. de Ciencias Ambientales y Salud, BUENOS AIRES, Argentina

In spite of a large number of datation methods, for most of the evolutionary process, almost 4,000 million years, our understanding of both the environment and life forms is extremely limited and controversial. By means of a reciprocal illumination approach focusing on the rise of free Oxygen as the signature of a major change in the Earth environmental conditions around 2 billion years ago, versus the shift from anaerobic to aerobic metabolism during ontogenesis (e.g. at the stage in amphibians), living organisms at ontogenic stages could be considered as biomarkers of environmental changes during the evolutionary process as well as the complexity achieved by living organisms at that time. The shift in oxygen consumption in living forms moving towards aerobic metabolism both during the evolutionary process and at the present time during the ontogenesis of aerobic organisms, imply an increasing exposure to oxidative stress produced by ROS to aerobic metabolism as well as the adverse effects of other agents, e.g. metals and organic chemicals with oxidative stress as a main mechanism of toxicity. On the other hand other environmental agents like As, exerting adverse effects by affecting the respiratory metabolism, became highly toxic to aerobic organisms during the evolutionary process and now at the ontogenic stages in which the energy budget increasingly depend on aerobic metabolism. By focusing on the dependent susceptibility at early life stages to noxious agents exerting oxidative stress and/or disrupting aerobic metabolism, the correlation between the characteristics of the aerobic metabolism of the embryo and the magnitude of toxicity exerted by those agents at different embryonic stages became evident. Thus, from an Evoecotoxicological perspective the increasingly oxygen dependent living organisms, both during the evolution and ontogenesis, imply a shift towards a high energy metabolism associated to an increasing oxidative stress from metabolic origin and by a large number of environmental agents. This ongoing challenge, now is enhanced by the injection of huge amount of chemicals in the environment due to the anthropogenic activities.

WE 406

Pesticide poisoning of wildlife and domestic animals in Spain: A review of the Toxicology and Forensic Veterinary Service

Antonio Juan Garcia-Fernandez, Isabel Navas, Emma Martinez-Lopez, Pedro Maria-Mojica, Diego Romero, Pedro Jimenez, Alejandro Hernandez-Garcia, Pilar Gomez-Ramirez, Jose Peñalver, Jose Antonio Gonzalez

University of Murcia, MURCIA, Spain

Over a period of 15 years the Toxicology and Forensic Veterinary Service at the University of Murcia (Spain) investigated 510 suspected incidents of poisoning in wildlife, pets and livestock from different parts of Spain. In 2005, this Laboratory was accredited by SOS-Poison (Spanish National Programme 'Antidoto') as a reference laboratory for analyze suspected deliberate poisoning of animals. A total of 153 cases, mainly in wildlife and pets, confirmed like poisoning due to ingestion of pesticides have been used to realize this retrospective study. Eighty-five percent of them occurred from 1998 to 2006. The pattern of poisoning by groups of pesticides was similar to those described in previous studies: insecticides (n=94; 62%), rodenticides (n=39; 25%), molluscicides (n=9; 6%), herbicides (n=9; 6%) and fungicides (n=2; 1%). Carbamates and organophosphates were the commonest insecticides, 69 and 27%, respectively. Fifty of the 65 cases of carbamate poisonings were due to ingestion of aldicarb (n=36) and carbofuran (n=14). These substances were detected in cases of deliberate poisoning in mair wildlife and dogs from rural and hunting areas. The group of RODENTICIDES (n=39) was formed up anticoagulant rodenticides (n=24; 52%) and strychnine (n=15; 38%). Anticoagulant rodenticides more frequently detected were warfarin, brodifacoum and chlorophacinone, being detected mainly in cats and dogs. Strychnine was detected in both wildlife and pets, but in all the cases it was associated to rural areas. Metaldehyde, a MOLLUSCICIDE, was involved in both intentional and unintentional poisonings. Paraquat, a powerful HERBICIDE, was involved in the death of livestock and pets, but not in wildlife poisonings.

WE 407

The Environmental Impact of Anticoagulant Rodenticide Use on Wildlife in Scotland

Michael Taylor, Elizabeth Sharp, Kenneth Hunter, Laura Melton, Anna Giela

Scottish Agricultural Science Agency, EDINBURGH, United Kingdom