Ecotoxicology and Environmental Safety 112 (2015) 153–160

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effect of different river flow rates on biomarker responses in common carp (*Cyprinus carpio*)

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ARTICLE INFO

Article history:
Received 10 January 2014
Received in revised form
13 October 2014
Accepted 16 October 2014
Available online 15 November 2014

Keywords:
Flow rate
Condition status
Oxidative stress
Biomarkers
Carp

ABSTRACT

The present study investigated effects of different river flow rates on basal activities of selected biomarkers and the occurrence of oxidative stress in the common carp (*Cyprinus carpio*). Juvenile carp were exposed to different river flow rates (5–120 cm/s) by caging for 3 weeks. After this period, one half of the fish were sacrificed and used for analysis. The other half received a single intraperitoneal injection of 3-methylcholanthrene (3-MC) and after 6 days were sacrificed and used for analysis. In order to investigate whether the physical activity of carp in the environment will influence the condition status of carp, following biomarkers were measured – activities of glutathione S-transferase (GST), catalase (CAT) and ethoxyresorufin-O-deethylase (EROD) and concentration of protein carbonyls (PC). The results showed that different flow rates significantly influenced biochemical biomarkers. The basal activity of GST did not change significantly after exposure to different river flow rates, whereas the activity of CAT increased with increasing river flow rates. The application of 3-MC caused significant increases in GST and CAT activities, but there were no difference between 3-MC control and 3-MC different flow rates. The occurrence of oxidative stress as a result of exposure to increased physical activity, i.e. increased river flow rates, was confirmed by measurement of PC levels – the level of PC increased with increasing river flow rates. Measurement of EROD basal activity showed that at lower river flow rates the EROD activity increased and at higher river flow rates decreased towards control levels demonstrating a close relationship between oxidative stress, PC levels and EROD activity. Obviously, biomarker responses in carp of different condition status can differ substantially. It can be concluded that flow rate may be an important factor in biomonitoring of rivers using biomarkers and since at different locations river water flow rate can vary significantly, the site selection is extremely important for proper design of river biomonitoring studies involving caging.

1. Introduction

Focusing only on chemical data (e.g. pollutant concentrations) in different environmental compartments is not sufficient to reliably assess potential risks, since different environmental factors influence the bioavailability of pollutants to organisms. Measurement of responses of organisms can serve as a functional measure of exposure to different stressors and can be used in environmental monitoring and assessment (van der Oost et al., 2003). Biomarkers at the molecular and biochemical level can provide insight into the mode of action of pollutants (Kammenga et al., 2000) and can be used as early warning signals of pollutant exposure. Consequently, biomarkers have been often measured in the different groups of aquatic organisms, and in fish species various biomarkers have been used as a tool for ecotoxicological assessments (Kirby et al., 2007). The inclusion of the biomarker approach in biomonitoring studies is common, however it is necessary to be aware of the potential confounding factors (Forbes et al., 2006). The confounding factors can be biological (e.g., age, sex, condition) and environmental (e.g., temperature, pH, oxygen) and can significantly influence biomarker responses.

Fish have proved to be good models for the evaluation of health status of aquatic ecosystems exposed to environmental pollution. Many fish species can be considered as top consumers in aquatic ecosystems (Dallinger et al., 1987) and it is likely that pollutants present in the aquatic environment will accumulate in fish and represent a potential risk for them and also to piscivorous birds and mammals (Adams et al., 1992). Monitoring of sentinel fish

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http://dx.doi.org/10.1016/j.ecoenv.2014.10.021
0147-6513/

species has been widely used in order to assess the degree of accumulation of pollutants and the effects on the health status of aquatic environments (De la Torre et al., 2000; Bervoets and Blust, 2003). In studies addressing the impact of environmental contaminants on fish biomarkers, several experimental designs can be applied – laboratory studies, field studies using wild-caught fish, and in situ studies using caged fish. In laboratory studies it is not possible to duplicate true field conditions so the results obtained in such studies may provide misleading conclusions (Hanson and Larsson, 2007; Martin-Diaz et al., 2008). Field studies using wild-caught fish require investigation of the same species at each site in order to be able to compare different sites. Since fish community structure depends on different abiotic and biotic factors present in the water body under investigation, it is practically impossible to find a species that is present at all sites of interest. Also, in wild-caught fish the high inter-variability in biomarker responses is often present due to mobility of fish and varied exposure histories (Hanson and Larsson, 2007). To minimize this problem, it is possible to conduct transplant experiments and active biomonitoring using cages has already been implemented using different organisms, including fish (Bervoets et al., 2009; De la Torre et al., 2007; Oikari, 2006; Reynders et al., 2008).

In order to reduce the variability and to more closely resemble true field exposure conditions, in the present study in situ caged fish exposure was applied. Environmental conditions present at different sites of interest can affect organisms and consequently cause differences in the biomarker responses which are not derived exclusively from exposure to pollutants. Both organism condition and an understanding of how changes in environmental conditions may change organism response to pollutant exposure are essential for successful biomonitoring. It is known that each species prefers certain environmental conditions, and changes in these conditions can possibly affect the responses of organisms. Regarding the river flow rate, it is also known that some fish species prefer slow moving waters whereas other prefer fast moving waters. Although the influence of river flow rates on fish biomarker responses has not been investigated so far, it can be assumed that higher rates will lead to greater physical activity and consequently lead to stress in organisms. Namely, in the laboratory study it has been shown that oxidative stress induced by intensive exercise and $CdCl_2$ affects 7-ethoxyresorufin O-deethylase (EROD) inducibility, concentration of thiobarbituric acid reactive substances (TBARS) and protein carbonyls (PC) in common carp (Stepić et al., 2012). So in order to investigate whether the different river flow rates, i.e. the increased physical activity of carp under field conditions, will influence the condition status and the inducibility of EROD, the present study was undertaken. Fish were exposed in situ to different river flow rates and besides EROD activity, widely used biomarker of environmental pollution in aquatic environments, following biomarkers were also measured – concentration of PC and activities on enzymes catalase (CAT) and glutathione S-transferase (GST).

Cytochrome P450 (CYP) is one of the most intensively studied biomarkers, in both laboratory and field conditions (Stegeman, 2000). The CYP450 enzymes belong to the large family of hepatic mixed-function oxidase enzymes of phase I xenobiotic biotransformation that are involved in reactions related to the biotransformation of many endogenous and exogenous substances, and this enzyme system has been detected in all organisms examined, from bacteria to mammals. Among them, one of the best studied parameters used as a biomarker of exposure of aquatic organisms to pollutants in the environment is the induction of CYP450 1A (Cheevaporn and Beamish, 2007; Flammarion et al., 1998; Flammarion et al., 2002; Mayon et al., 2006). Induction of CYP450 1A occurs after ligand-activation of the aryl hydrocarbon receptor (AhR). This receptor is typically, but not exclusively, activated by planar, polycyclic and aromatic compounds including polyaromatic hydrocarbons (PAHs), dioxins, and polychlorinated biphenyls (PCBs) (Denison et al., 2002). However, there is an increasing evidence that activation of AhR is also triggered by other compounds, including some pharmaceuticals and personal care products and pesticides (Fernández-Cruz et al., 2011; Casado et al., 2006). The induction of EROD activity is a commonly used biomarker for exposure to CYP450 1A inducers and the advantages of using EROD activity as a biomarker are the specificity for CYP 1A in fish, high sensitivity, feasibility and simplicity of its measurement (Arinç and Sen, 1994; Arniç et al., 2000; Bucheli and Fent, 1995).

Oxidative stress occurs when the generation of reactive oxygen species (ROS) in a system exceeds the system's ability to neutralize and eliminate them. In fish, antioxidant defenses are sensitive to exposure to environmental pollutants and can therefore be used as indicators of aquatic environmental health (Sturve et al., 2008). When antioxidant defenses are impaired or overcomed, oxidative stress can damage all types of biological molecules and the most common perturbation resulting from oxidative stress is protein carbonyl formation (Shacter, 2000a). Oxidative modifications of proteins can lead to diverse functional consequences, e.g. they can inhibit a wide array of enzyme activities (Stadtman, 1990) and can also lead to loss of function (Stadtman and Oliver, 1991). Enzyme CAT plays an important role in maintaining reactive oxygen homeostasis and also regulating pathophysiology of the organisms (Deisseroth and Dounce, 1970). This enzyme catalyzes the conversion of hydrogen peroxide to oxygen and water and is regarded as one of the most important specialized antioxidant enzymes for the detoxification of oxidative stress in the cellular antioxidant mechanism (Gravato et al., 2005; Michiels et al., 1994). Enzyme GST belongs to a phase II family of detoxifying enzymes and neutralizes a broad range of xenobiotics and endogenous metabolic by-products via enzymatic glutathione conjugation, glutathione-dependent peroxidase activity or isomerisation reactions (Hayes et al., 2005). This enzyme catalyzes the conjugation of reduced glutathione (GSH), an efficient scavenger against reactive oxygen species, with a group of compounds having electrophilic centers (Hayes et al., 2005).

The aim of the present study was to investigate the changes in basal activities of selected biomarkers and the occurrence of oxidative stress in regard to different river flow rates and to assess the impact of condition status on the inducibility of EROD activity in common carp (*Cyprinus carpio*). Common carp, fish species commonly used in monitoring of freshwater systems, is usually found in still or slowly flowing waters at low altitudes and are capable of tolerating a range of environmental conditions. However, although carp is considered as tolerant species, there are no studies investigating the effects of different river flow rates on its physiology. To our knowledge, this is the first study investigating the response of biomarkers to current variation using caged carp, i.e. investigating the effects of river flow rate on fish biomarkers under field conditions.

2. Materials and methods

2.1. Animals

Juvenile common carp (*C. carpio*, L.) (mean body weight 28.39 ± 5.98 g) were obtained from the fish-farm "Grudnjak" (Virovitica-Podravina County, Croatia). The fish were acclimatized for 1 week before experiments. They were kept unfed in tanks with aerated, filtered, dechlorinated tap water (hardness 380.3 mg/L as $CaCO_3$, pH 7.1 ± 0.2). The temperature of the water was maintained at 15 ± 1 °C and light in the room followed a 12: 12 h photoperiod.