Science of the Total Environment 586 (2017) 598–609

Contents lists available at ScienceDirect

Science of the Total Environment

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

A comparative approach using biomarkers in feral and caged Neotropical fish: Implications for biomonitoring freshwater ecosystems in agricultural areas

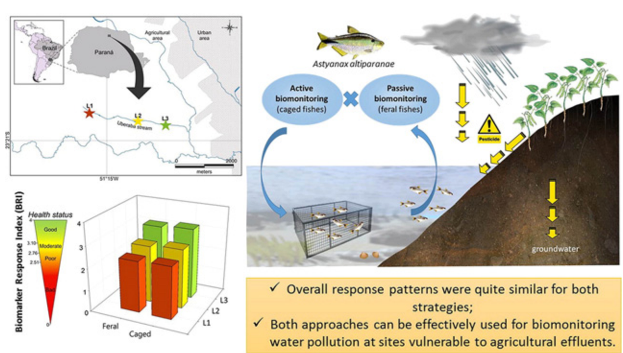
Carlos Eduardo Delfino Vieira [a], Patrícia Gomes Costa [b], Liziara Costa Cabrera [c], Ednei Gilberto Primel [c], Gilberto Fillmann [b], Adalto Bianchini [d], Claudia Bueno dos Reis Martinez [a,*]

[a] Departamento de Ciências Fisiológicas, Universidade Estadual de Londrina, Rod. Celso Garcia Cid, km 380, Londrina, Paraná 86057-970, Brazil
[b] Instituto de Oceanografia, Universidade Federal do Rio Grande, Rio Grande, Rio Grande do Sul 96203-900, Brazil
[c] Escola de Química e Alimentos, Universidade Federal do Rio Grande, Av Itália, km 8, s/n, Rio Grande, Rio Grande do Sul 96203-900, Brazil
[d] Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av Itália, km8, s/n, Rio Grande, Rio Grande do Sul 96203-900, Brazil

HIGHLIGHTS

- Biomarkers responses in feral and caged *Astyanax altiparanae* were determined.
- Fish were collected or confined (for 168 h) along a stream in an agricultural area.
- Most sensitive biomarkers were DNA breaks, lipid peroxidation and acetylcholinesterase activity.
- Both approaches were effective for discriminating contamination levels along the stream.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 13 January 2017
Accepted 3 February 2017
Available online 16 February 2017

Editor: D. Barcelo

Keywords:
Biotransformation
Oxidative stress
In situ tests
Environmental genotoxicity
Pesticides

ABSTRACT

The aim of this study was to investigate the responses of biomarkers in feral and caged fish and the capacity of these biomarkers to discriminate contamination levels along a stream located in an agricultural area in Southern Brazil. Specimens of the Neotropical fish, *Astyanax altiparanae*, were confined for 168 h in three lakes along the stream. Additionally, during the weeks of *in situ* exposure, wild specimens of this species were collected from the same sites. Biochemical biomarkers were analyzed, such as phase I biotransformation enzyme 7-ethoxyresorufin-*O*-deethylase (EROD) and phase II biotransformation enzyme glutathione *S*-transferase, and we also determined hepatic and branchial levels of non-protein thiols (NPSH), oxidative damage such as lipid peroxidation (LPO), and acetylcholinesterase (AChE) activity in muscle and brain. Genetic biomarkers such as DNA breaks (comet assay), frequency of micronuclei (MN) and erythrocytic nuclear abnormalities (ENA) were also examined. The results indicate that the most sensitive biomarkers for discriminating contamination levels are DNA breaks, LPO and AChE activity. Similar results were obtained for both caged and feral fish. The biomarkers that reflect the results of cumulative events, such as ENA, were more discriminative for chronically exposed specimens (feral fishes). Analyzing biomarkers using an integrated response index showed that both approaches (using feral and caged *A. altiparanae*) were effective for discriminating contamination levels along the stream, corroborating the results of chemical analyses for selected pesticides. Taken together, these results

* Corresponding author.
E-mail address: cbueno@uel.br (C. Bueno dos Reis Martinez).

http://dx.doi.org/10.1016/j.scitotenv.2017.02.026

highlight the importance of biomarker selection and show that both approaches (caged and feral fish) are satisfactory for evaluating water quality in streams impacted by agricultural activities.

1. Introduction

According to a recent review conducted by Albuquerque et al. (2016), Brazil is responsible for 20% of the total pesticides consumed in the world and is the world's largest consumer of pesticides. The process of agricultural expansion has resulted in domestic growth in pesticides of 194% over the last decade (IBAMA, 2013). In terms of Brazilian states, Paraná (Southern Brazil) is the third largest consumer of pesticides, with sales of approximately 55,000 tons of active ingredients in 2012 alone (IBAMA, 2013). The presence of pesticides in water bodies in agricultural areas of Paraná state was already detected (Vieira et al., 2016) at levels far in excess of those set by Brazilian environmental regulation (Resolution 357/2005 of the National Council for Environment - CONAMA).

The occurrence of pesticides in freshwater ecosystems has been well registered worldwide and is a major issue, causing concern at different geographic scales (Konstantinou et al., 2006). However, in Brazil, is difficult to evaluate the risks posed by pesticides to native aquatic fauna considering that data in peer-reviewed literature are still scarce (Albuquerque et al., 2016). Thus, environmental studies reporting the effects of these contaminants on aquatic biota are important, increasing the need for integrative approaches to the assessment of freshwater quality and the biological effects of pesticides on aquatic ecosystems (Vieira et al., 2016).

Integrated and multidisciplinary studies combining biological and chemical evaluations represent a valuable approach for management and monitoring of heterogeneous aquatic environments (Bebianno et al., 2015). In this context, biomarkers can be valuable in providing information on the effects of contaminants that may impair the health of the organism (Moreira et al., 2004). As such, alongside chemical analysis, biomarkers could be incorporated into environmental monitoring programs as a fast-screening tool, prior to the implementation of preventive bioremediation strategies (Bebianno et al., 2015).

In situ tests using caged fish are a useful approach for assessing contaminant effects on the aquatic biota (Schlenk et al., 2008; Klobucar et al., 2010; Vieira et al., 2014, 2016). The active biomonitoring presents some advantages compared to passive approach, such as: the investigation of sites where feral specimens are difficult to capture or occur in unsatisfactory number; the exact knowledge of the exposure period, thus avoiding the possibility of organisms to acclimate to the new situation; and the standardization of organisms used in the tests (size, age, sex, reproductive stage of development), making it feasible the comparison of results from different sites (Wepener, 2013). According to Smolders et al. (2003), the parallel comparison between the wild and caged species may indicate to what extent the native organisms have adapted to conditions at the particular environment. Moreover, the approach using caged fishes can exclude adaptive factors reducing the influence of genetic and adaptive phenomena, which can impair the efficiency of biomonitoring to distinguish different levels of environmental contamination (Regoli and Principato, 1995).

Parallel feral and caged fish studies can allow comparison between the responses of both experimental approaches and provide information on susceptibility and/or possible adaptations of either group. Nevertheless, there are still few studies in which biomarker determinations have been undertaken in caged fish of any species, and there are even fewer studies in which comparisons with feral specimens of the same species have been made (Barra et al., 2001; de la Torre et al., 2002; Winter et al., 2004, 2005).

In Brazil, studies emphasizing the application of biomarkers in indigenous fish species as tools for assessing water quality are scarce (Wilhelm-Filho et al., 2001; Winkaler et al., 2001; Akaishi et al., 2004; Ramsdorf et al., 2009). Freshwater fish of the genus *Astyanax* have been used in environmental monitoring studies for determining biomarkers at sites affected by different levels of contamination (Winkaler et al., 2001; Silva and Martinez, 2007; Lemos et al., 2008; Trujillo-Jiménez et al., 2011; Vieira et al., 2014; Yamamoto et al., 2016). *Astyanax altiparanae* (Garutti and Britski, 2000) was selected for this study due to its reported biomarker sensitivity (Vieira et al., 2014; Bettim et al., 2016), its abundance at the studies sites and its availability as hatchery-reared specimens for a parallel caged-feral fish study.

Therefore, the goal of this study was to investigate multiple biomarker responses in *A. altiparanae* simultaneously caged at and collected from sites along a stream subject to pesticide contamination in order to understand how these organisms respond to acute and chronic exposure to environmental contaminants. In addition, the biomarker responses of feral and caged fish were integrated into a Biomarker Response Index (BRI) to grade the level of contamination along the stream and to identify the biomarkers that show the strongest responses to the environmental stressors present.

2. Material and methods

2.1. Study area

The sites investigated in this study are located along the Uberaba stream (Fig. 1), a small watercourse in the municipality of Londrina (Northern Paraná). This region shows intense rural activity, with a prevalence of non-perennial monocultures grown using heavy mechanization and pesticides, with the potential risk of contaminating the soil as well as ground and surface waters. Three sampling sites, characterized as artificial lakes, were delimited along the stream, from source to mouth, denoted lake 1 (L1), lake 2 (L2) and lake 3 (L3). In particular, L1, which is the source of the stream and surrounded by farmland, seems to be the location most susceptible to pesticide contamination (Fig. 1). During the study period, there was a transition from corn to soy monoculture, and different herbicides were applied and recorded, such as glyphosate and atrazine.

2.2. Test organisms and experimental design

Adults of *A. altiparanae* ($n = 120$, 5.6 ± 0.4 g body mass; 7.6 ± 0.1 cm total length, no sex differentiation) supplied by a local fish farming facility (Aqualina, Rancho Alegre, PR) were used in the *in situ* tests, carried out in May and June 2013. *In situ* tests were conducted for 168 h, at each site (L1, L2 and L3), along three subsequent weeks. At each site, sampling of caged fish was performed simultaneously with resident fish collection and sampling.

Before the *in situ* tests, one group of the 40 fish, for each experimental site, was acclimated under controlled laboratorial conditions (data are shown in Table 1) during one week in tanks with dechlorinated water and oxygenation and photoperiod of 12 h:12 h. During acclimation fish were fed every 2 days with commercial fish food (Guabi®, protein content 36%).

After acclimation, a group of fish ($n = 20$) was sampled in the laboratory (basal group) to determine biomarker baseline levels for this species. The results were used to calculate the Biomarker Response Index (BRI), according to Hagger et al. (2008, 2010). Another group of fish ($n = 20$ for each site) was transported (in transit for < 1 h) to the selected experimental site (L1, L2 or L3) in plastic bags containing water and