Marine Pollution Bulletin 104 (2016) 229–239

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Assessing genotoxic effects in fish from a marine protected area influenced by former mining activities and other stressors

Paloma Kachel Gusso-Choueri [a,b,c,*], Rodrigo Brasil Choueri [d], Gustavo Souza Santos [a,e], Giuliana Seraphim de Araújo [c,f], Ana Carolina Feitosa Cruz [c,f], Tatiana Stremel [g], Sandro Xavier de Campos [g], Marta Margarete Cestari [a,e], Ciro Alberto Oliveira Ribeiro [a,b], Denis Moledo de Sousa Abessa [c]

[a] Graduate Program in Ecology and Conservation, Federal University of Paraná (UFPR), CP 19031, CEP 81531-990 Curitiba, PR, Brazil
[b] Cellular Toxicology Laboratory, Department of Cellular Biology, Federal University of Paraná (UFPR), CP19031, 81531-990 Curitiba, PR, Brazil
[c] Laboratory for the Study of Aquatic Pollution and Ecotoxicology (NEPEA), São Paulo State University, São Vicente Campus (UNESP Campus do Litoral Paulista), Praça Infante Dom Henrique, s/n, CP 11330-900 São Vicente, SP, Brazil
[d] Department of Marine Sciences, Federal University of São Paulo, Santos Campus (UNIFESP — Santos), Av. Almirante Saldanha da Gama, 89, CP 11030-490 Santos, SP, Brazil
[e] Genetics Department, Federal University of Paraná (UFPR), 81531-990 Curitiba, PR, Brazil
[f] Institute of Oceanography, University of São Paulo (IOUSP), Praça do Oceanográfico, 191, CEP 05508-120 São Paulo, SP, Brazil
[g] Research Group on Environmental and Sanitary Analytical Chemistry (QAAS), Ponta Grossa State University, Av. General Carlos Cavalcanti, 4748, CEP 84030-900 Ponta Grossa, Brazil

ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form 12 January 2016
Accepted 16 January 2016
Available online 25 January 2016

Keywords:
Estuary
Weight of evidence
Environmental quality assessment
Genotoxicity
Biomarkers
Multivariate approach

ABSTRACT

The goal of the current study was to evaluate different genotoxicity tools in order to assess a marine protected area (MPA) affected by former mining activities and urban settlements. A catfish (*Cathorops spixii*) was analyzed for genotoxic effects at the (i) molecular and at the (ii) chromosomal levels. Through factor analysis, genotoxicity was found to be linked to levels of metals bioaccumulated and PAH metabolites in the bile. Micronucleus and nuclear alteration were less vulnerable to the effects of confounding factors in mildly contaminated areas since they were more frequently associated with bioaccumulated metals than the DNA analysis. The different genotoxicity responses allowed for the identification of sources of pollution in the MPA. This approach was important for detecting environmental risks related to genotoxic contaminants in a mildly contaminated MPA.

1. Introduction

Metal contamination of aquatic ecosystems due to mining activities is of great environmental concern on a global scale and is one of the most serious threats to aquatic environments worldwide (Zhuang et al., 2014). This concern has arisen partly because mining activities are commonly performed but not controlled or monitored (Rybicka, 1996). Additionally, mine waste and tailings can have severe negative effects on the regions surrounding the mines (Fernández-Caliani et al., 2009). This effect often occurs because pollutants tend to be transported mainly via water (e.g. acid mine drainage) and air (atmospheric deposition, wind-blown particulate matter), and thus accumulate in various environmental compartments. Toxic metals from mining activities can therefore potentially impact the biota and human beings alike (e.g. Riba et al., 2005; Taylor et al., 2014; Camizuli et al., 2014; Molina-Villalba et al., 2015).

The Ribeira de Iguape River (RIR), located in southeastern Brazil, is an important mining region and represents an example of uncontrolled mining activity. Lead mines operated during the twentieth century, and their waste products were discharged into the river or on the river banks. High levels of metals such as Pb, Zn, Cu, Cr, and As have been recorded in river waters, bottom sediments, and suspended sediments in river headwaters (Eysink et al., 1998; Moraes et al., 2003; Guimarães and Sígolo, 2008). The RIR flows toward the Estuarine System of the Cananeia–Iguape–Peruíbe Environmental Protection Area (APA-CIP), recognized as a UNESCO World Natural Heritage Site. Metals in the APA-CIP estuarine sediments, which have been found at low levels in the past, increased substantially after the construction of an artificial connection between the river and the estuary (Mahiques et al., 2009). Recent studies have reported metals at moderate levels in the sediments from the estuary, quantities which have been attributed to the former mining activities as well as to contamination from urban settlements within the APA-CIP (Azevedo et al., 2011; Cruz et al., 2014; Gusso-Choueri et al., 2015).

---

* Corresponding author at: Laboratory for the Study of Aquatic Pollution and Ecotoxicology (NEPEA), São Paulo State University, São Vicente Campus (UNESP Campus do Litoral Paulista), Praça Infante Dom Henrique, s/n, CP 11330-900 São Vicente, SP, Brazil.
E-mail address: pchoueri@ufpr.br (P.K. Gusso-Choueri).

http://dx.doi.org/10.1016/j.marpolbul.2016.01.025
0025-326X/© 2016 Elsevier Ltd. All rights reserved.

A multitude of ecotoxicological approaches and tools, from molecular testing to ecological response assessments, have been proposed over the last few decades for environmental assessment and monitoring (Van Straalen, 2003). Their application, however, has been tested and validated only at highly contaminated sites (e.g. Adams and Greeley, 2000; Galloway et al., 2004; Choueri et al., 2010; Torres et al., 2015). Mildly contaminated sites can also affect the health of the aquatic biota, since the organisms are subjected to long-term exposure (Nipper et al., 1998). This is the case of many marine protected areas (MPAs) influenced by urban or industrial settlements; these areas may be subjected to perhaps relatively moderate but nonetheless continuous inputs of contaminants produced on their outermost boundaries (Chou et al., 2004; Pozo et al., 2009; Perra et al., 2011; Araujo et al., 2013).

However, in mildly contaminated areas, cause-and-effect relationships between contamination and toxicity may not be as straightforward as they are at highly contaminated sites, especially in complex physical and chemical settings such as estuaries (Choueri et al., 2009). Tests on biological responses must be sensitive to low levels of contaminants, but still representative of an actual or potential risk to the individual organism or the population. Genotoxic responses may trigger a damaging chain of biological changes (e.g. reproduction disturbances, growth inhibition, carcinogenesis), some of which can be passed on to the next generations. These responses may also lead to a loss of genetic diversity (Mitchelmore and Chipman, 1998; Jha, 2004; Baršienė et al., 2013).

Different types of DNA damage may occur when organisms are exposed to environmental contamination. These types of damage include single- and double-strand breaks, inter-strand and intra-strand crosslinks, DNA adducts, and DNA protein crosslinks (Wood et al., 2001). Some metals and PAHs (especially high molecular weight PAHs) are known to cause genotoxicity. DNA damage caused by such contaminants can be characterized into three phases: (i) the formation of adducts with toxic molecules, followed by (ii) secondary DNA modifications, including single- and double-strand break, changes to DNA repair, base oxidation, and crosslinks (Fonseca et al., 2014), and an advanced stage (iii) in which cells present altered functions, cell proliferation, mutagenesis, and eventually carcinogenesis (Monserrat et al., 2007).

The quantification of DNA strand breaks (via electrophoresis or fluorescence measurement) is considered a sensitive indicator of genotoxicity at the molecular level (Olive, 1988; Gagne and Blasé, 1995; Silva et al., 2012; Maranho et al., 2012; Parolini et al., 2013). At the chromosomal level, the formation of micronuclei and nuclear abnormalities in fish erythrocytes has also been successfully used as indicators of genotoxicity caused by environmental contamination (e.g. Souza and Fontanetti, 2012; Hoshina et al., 2008). Several studies have reported increased frequencies of micronuclei and nucleus abnormalities in fish cells after exposure to different metals under both field and laboratory conditions (Al-Sabti and Metcalfe, 1995; Cavas et al., 2005; Cavaş, 2008; Isani et al., 2009; Yadav and Trivedi, 2009).

The Marine Strategy Framework Directive (2008/56/CE) has recently proposed the use of genotoxicity endpoints as a tool to help characterize the biological status of marine water bodies. In addition, different genotoxicity assays have been used in environmental monitoring programs (e.g. ICES Working Group on Biological Effects of Contaminants and the Mediterranean Pollution Programme) (Davies and Vethaak, 2012). Although this approach has been widely used for environmental quality assessment and monitoring, no attempts have been made to assess the suitability of these tools for monitoring mildly contaminated sites or MPAs. These studies on mildly contaminated sites are important because genotoxic responses can be affected by many factors, including natural environmental conditions, diet, and hormonal status (Mitchelmore and Chipman, 1998). These confounding factors may be exacerbated from mildly contaminated environments.

The current study aimed to evaluate different genotoxicity tools used on fish in order to assess an MPA affected by former land-based mining activities and current urban settlements. We hypothesized that the different genotoxicity endpoints would be positively associated with contaminant body burden. We also hypothesized that different tissues would respond differently to the contamination.

Cathorops spixii was tested for genotoxic effects through (i) the quantification of DNA damage in different tissues by means of two different methodologies (the comet assay with blood tissue, and the alkaline precipitation assay with kidney, liver, and gill tissues), and (ii) cytogenotoxicity assessment, which was performed using the micronucleus test (MN) and tests on nuclear alterations (NA) in erythrocytes. The bioindicator specie (*C. spixii*) was chosen because it is a species potentially exposed to contaminated sediments in the estuary, since it has demersal habits, preys mainly upon zoobenthos (especially crustaceans and polychaetes) (Fishbase, 2014) and spends its whole life cycle in muddy-bottom estuaries (Azevedo et al., 1999). Additionally, *C. spixii* is considered as an important artisanal fishing resource in tropical and sub-tropical South American Atlantic coasts (Melo and Teixeira, 1992; Álvarez-León and Rey-Carrasco, 2003).

The relationship between environmental contamination and genotoxicity was assessed by integrating genotoxicity data with metal body burdens (liver and muscle) and levels of PAH metabolites in bile by using a multivariate approach. The results of this study can subsidize the definition of suitable genotoxic tools to assess and monitor MPAs.

2. Materials and methods

2.1. Study area

The Cananéia–Iguape–Peruíbe Environmental Protection Area, or the APA-CIP (24°40′S and 25°05′S) (Fig. 1) is an estuarine–lagoon ecosystem recognized by UNESCO as part of the Biosphere Reserve of the Atlantic Rainforest. It is an area of priority to be included on the list of Brazilian wetlands of international importance within the scope of the Ramsar Convention (Brazil, 2012). The region is on the list of UNESCO World Heritage Sites.

The main freshwater contributor to the estuarine lagoon is the Ribeira de Iguape River (RIR), which meets the lagoon through a water channel known as Valo Grande. This lagoon deviates approximately 70% of the river toward the lagoon waters. For many years, mine tailing and metallurgical slags such as blast furnace were directly dumped into the RIR. After the establishment and enforcement of Brazilian environmental laws in the early 1990s, the mining industry halted its release of waste into the river, but began disposing of it on the river banks instead. The waste and riverbanks were exposed to weathering and subjected to lixiviation (Guimarães and Sígolo, 2008; Abessa et al., 2014).

High levels of some metals (Pb, Zn, Cu, Cr) and As were recorded in the river waters, as well as on riverbeds and in suspended sediments (Eysink et al., 1998; Corsi and Landim, 2003; Moraes et al., 2003; Guimarães and Sígolo, 2008). Meanwhile, in the estuarine lagoon, metals were found at only moderate levels in the sediments (Mahiques et al., 2009) as defined by international Sediment Quality Guidelines (Long et al., 1995; Environment Canada and Ministère du Développement durable, de l'Environnement et des Parcs du Québec, 2007).

In addition to former mining activities, other sources of contaminants to the APA-CIP include the three cities placed within the limits of the protection area (Iguape, Ilha Comprida, and Cananéia), with a total estimated population of approximately 51,900 inhabitants (IBGE, 2014), and which lack adequate sanitation infrastructure (Morais and Abessa, 2014).

2.2. Fish collection and sample preparation

The sampling sites were set with the aim of encompassing the main potential contaminant sources along the APA-CIP (Fig. 1). Thus, site P1 is the closest to the mouth of the RIR, and site P4 is the closest to the city of Cananéia. Fifteen specimens of *C. spixii* were collected at each sampling