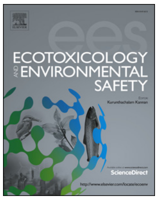
Ecotoxicology and Environmental Safety ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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



Upregulation of biotransformation genes in gills of oyster *Crassostrea brasiliana* exposed *in situ* to urban effluents, Florianópolis Bay, Southern Brazil

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form
24 March 2016
Accepted 4 April 2016

Keywords:
Sanitary sewage
Crassostrea brasiliana
Cytochrome P450
Glutathione *S*-transferase
Sulfotransferase
Biomarker

ABSTRACT

The release of untreated sanitary sewage, combined with unplanned urban growth, are major factors contributing to degradation of coastal ecosystems in developing countries, including Brazil. Sanitary sewage is a complex mixture of chemicals that can negatively affect aquatic organisms. The use of molecular biomarkers can help to understand and to monitor the biological effects elicited by contaminants. The aim of this study was to evaluate changes in transcript levels of genes related to xenobiotic biotransformation in the gills of oysters *Crassostrea brasiliana* transplanted and kept for 24 h at three areas potentially contaminated by sanitary sewage (Bücheller river, BUC; Biguaçu river, BIG; and Ratones island, RAT), one farming area (Sambaqui beach, SAM) and at one reference site (Forte beach, FOR) in the North Bay of Santa Catarina Island (Florianópolis, Brazil). Transcript levels of four *cytochrome P450* isoforms (*CYP2AU1*, *CYP3A-like*, *CYP356A1-like* and *CYP20A1-like*), three *glutathione S-transferase* (*GST alpha-like*, *GST pi-like* and *GST microsomal 3-like*) and one *sulfotransferase* gene (*SULT-like*) were evaluated by means of quantitative reverse transcription PCR (qRT-PCR). Chemical analysis of the sediment from each site were performed and revealed the presence of aliphatic and polycyclic aromatic hydrocarbons, linear alkylbenzenes and fecal sterols in the contaminated areas (BUC and BIG). Water quality analysis showed that these sites had the highest levels of fecal coliforms and other parameters evidencing the presence of urban sewage discharges. Among the results for gene transcription, *CYP2AU1* and *SULT-like* levels were upregulated by 20 and 50-fold, respectively, in the oysters kept for 24 h at the most contaminated site (BUC), suggesting a role of these genes in the detoxification of organic pollutants. These data reinforce that gills possibly have an important role in xenobiotic metabolism and highlight the use of *C. brasiliana* as a sentinel for monitoring environmental contamination in coastal regions.

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1. Introduction

Coastal ecosystems currently face increasing degradation all over the world. Considering that a great portion of global population lives in coastal areas and depends on its supplies and services for subsistence, the sustainable use of natural resources becomes a global challenge (Gesamp, 2001). Globally, untreated sanitary sewage discharges represent an important source of pollution to surface waters (Walker, 1996). This problem is especially evident in developing countries where more than 80% of untreated sewage is discharged directly into lakes, rivers and oceans (UN-Unicef, 2001), and the sanitary sewage outfall represents the main cause of contamination of estuarine and marine ecosystems (Abessa et al., 2005). In Brazil, it is estimated that only 55% of cities possess sanitation facilities, such as sewage collecting network and sewage treatment plants (IBGE, 2011). In Santa Catarina state (southern Brazil), where this study was carried out, this percentage drops to 35% (IBGE, 2011).

Sewage is a complex mixture of chemicals and can contain metals, hydrocarbons, pesticides, detergents, pharmaceuticals and personal care products (Gagné et al., 2007; Petrovic et al., 2003). In order to measure the potential impacts of these contaminants on aquatic organisms, the development of more sensitive tools in addition to analytical chemical methods is required (Cajaraville

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http://dx.doi.org/10.1016/j.ecoenv.2016.04.003
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et al., 2000). Biomarkers are defined as biological changes at molecular, cellular and/or physiological level that express toxic effects caused by the exposure to contaminants (Walker et al., 1996). Biomarkers represent early warning tools of long-term biological effects because they respond to environmental changes before deleterious impacts on population or community levels are detected (Cajaraville et al., 2000; de los Ríos et al., 2013).

Among the cellular responses of organisms to environmental contamination, the xenobiotic biotransformation comprises a number of cellular reactions that metabolize these chemicals into more soluble and easily extractable compounds (Oehlmann and Schulte-Oehlmann, 2003; Walker, 1996). Phase I reactions are catalyzed mainly by cytochrome P450 (CYPs) enzymes capable of adding a functional hydroxyl group to its substrates increasing the water solubility. Phase II comprises conjugation reactions catalyzed by transferase enzymes such as glutathione *S*-transferases (GSTs) and sulfotransferases (SULTs). These enzymes transfer soluble functional groups to their substrates increasing water solubility and hence enabling excretion (Oehlmann and Schulte-Oehlmann, 2003; Parant, 1998; Walker et al., 1996). Previous studies have established a positive relation between the presence of aquatic contaminants and the transcription of biotransformation genes in bivalve mollusks (Boutet et al., 2004; Cajaraville et al., 2000; Liu et al., 2015; Lüchmann et al., 2014, 2015; Milan et al., 2011; Zanette et al., 2010; Zhang et al., 2012b).

Bivalve mollusks have been proposed as sentinels in environmental studies due to their sessile, filter-feeding habits, and wide geographical distribution. Bivalves are also early warning indicators of contamination because of their capability to bioaccumulate xenobiotics in their tissues, even when levels in the water are low (Oehlmann and Schulte-Oehlmann, 2003). Considering the deficiency in sewage treatment infrastructure and the socio-economic importance of bivalve aquaculture, this study aimed to evaluate changes in the transcription levels of *CYPs*, *GSTs* and *SULT* genes in the native mangrove oyster *Crassostrea brasiliana* (sin. *Crassostrea gasar*, Lazoski et al., 2011) exposed *in situ* to urban sanitary sewage discharges. The outcome from this study will contribute to a better identification of potential biomarkers of exposure possibly involved in the detoxification mechanisms in bivalve mollusks.

2. Materials and methods

2.1. Study area

The Northern Bay of Santa Catarina Island is located in Florianópolis, southern Brazil, and comprises a semi-enclosed water body. There are several agricultural activities, mollusk farms and urbanized area surrounding the bay. The *in situ* exposure was carried out in five areas (Fig. 1) displaying different levels of contamination by urban sanitary sewage. Bücheler river mouth (BUC) represents the most contaminated site due to discharges of untreated sewage (Souza et al., 2012; Flores-Nunes et al., 2015a). Biguaçu river (BIG) drains an agricultural basin and represents a potential source of pesticides. Ratones island (RAT) is located near an uninhabited island, placed in the center of the bay. Sambaqui beach (SAM) is a mollusk farming area near an urban area. Forte beach (FOR) also comprises a mariculture area but has a low urbanization density and therefore was chosen as the reference site.

2.2. Animals and experimental design

Mangrove oysters (*C. brasiliana*) were collected in the farming area of Marine Mollusks Laboratory (LMM, UFSC) at Sambaqui beach (SAM) and transplanted to BUC, BIG, RAT and FOR. After 24 h of exposure, oysters were collected at each site ($n=6$), gills were dissected and immediately placed in RNAlater™ (Qiagen) until molecular analysis.

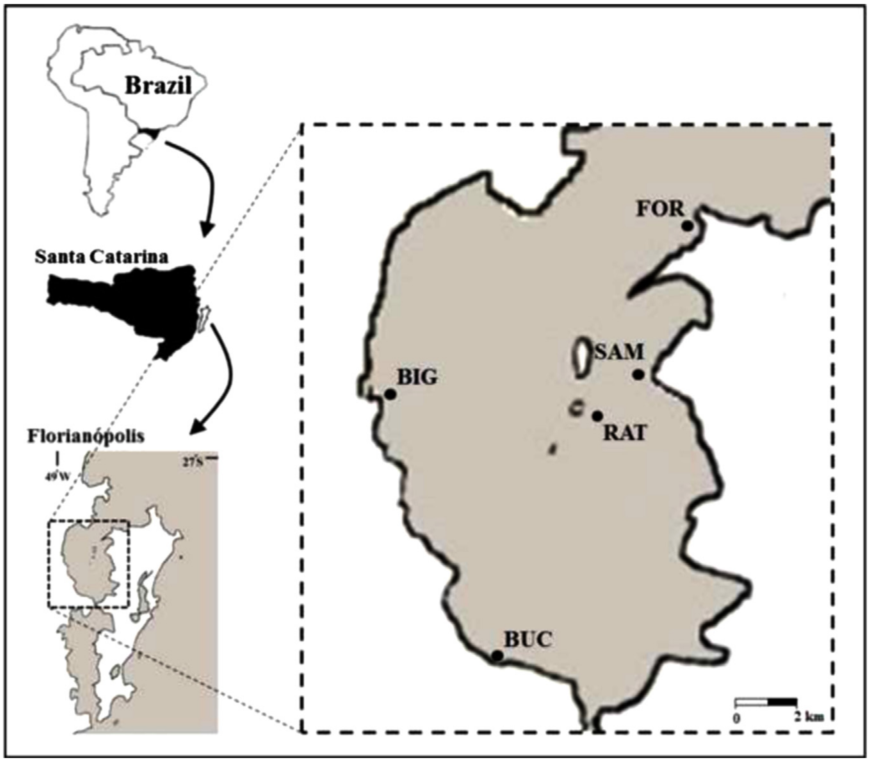

Fig. 1. Study areas in the Northern Bay of Santa Catarina Island (Florianópolis). 1 BUC: Bucheler river mouth, contaminated site; 2 BIG: Biguaçu river mouth; 3 RAT: Ratones island; 4 SAM: Sambaqui beach; FOR: Forte beach, reference site.

Please cite this article as: Pessatti, T.B., et al., Upregulation of biotransformation genes in gills of oyster *Crassostrea brasiliana* exposed *in situ* to urban effluents,.... Ecotoxicol. Environ. Saf. (2016), http://dx.doi.org/10.1016/j.ecoenv.2016.04.003

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