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Enzymatic response of *Macrobrachium jelskii* (Miers, 1877) exposed to water from urban and rural rivers in Bahia, Brazil

Resposta enzimática de *Macrobrachium jelskii* (Miers, 1877) exposto à água de rios urbanos e rurais na Bahia, Brasil

Respuesta enzimática de *Macrobrachium jelskii* (Miers, 1877) expuesto al agua de ríos urbanos y rurales en Bahía, Brasil

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Abstract
Several biomarker enzymes such as catalase (CAT) and glutathione S-transferase (GST) can be used to measure oxidative stress in animals caused by exposure to xenobiotics. The objective of the present study was to characterize different points of the Capivari (CP1 and CP2), Paraguaçu (PG1 and PG2) and Subaé (SB1 and SB2) Rivers, state of Bahia, in relation to the presence of xenobiotics, using CAT and GST as bioindicators in *M. jelskii*. The water-sampling sites were considered urban or rural and in all of them signs of environmental degradation were observed. Therefore, acute exposure tests (96h) were performed with water samples collected during the dry and rainy seasons. Results showed that the activity of CAT and GST in prawns exposed to water from CP1 and CP2 were very similar, while those exposed to water from PG1, PG2, SB1 and SB2 formed distinct groups of data. Significant increase in the activity of at least one of the analyzed enzymes in each sampling site was observed, when compared to animals in the control group. This demonstrated a possible oxidative stress in *M. jelskii* caused by the presence of xenobiotics in the water (*e.g.*, domestic sewage, pesticides, oil, and heavy metals). Enzymatic activities were higher in animals from experiments carried out in the rainy season, except for the CAT activity of animals exposed to water from Subaé River. This study demonstrated the potential of *M. jelskii* as bioindicator and contributed to the knowledge of aspects of the antioxidant defense system of this species.
Keywords: Catalase; Glutathione S-transferase; Prawn; Ecotoxicology; Bioindicator.

Resumo
Várias enzimas biomarcadoras, como a catalase (CAT) e a glutationa S-transferase (GST), podem ser usadas para medir o estresse oxidativo em animais causado pela exposição a xenobióticos. O objetivo deste estudo foi caracterizar diferentes pontos dos rios Capivari (CP1 e CP2), Paraguaçu (PG1 e PG2) e Subaé (SB1 e SB2), localizados no estado da Bahia, em relação à presença de xenobióticos, utilizando CAT e GST como bioindicadores em *M. jelskii*. Os pontos de amostragem de água foram considerados urbanos ou rurais e em todos eles foram observados sinais de degradação ambiental. Para tanto, foram realizados testes de exposição aguda (96h), com amostras de água coletadas durante as estações seca e chuvosa. Os resultados mostraram que a atividade de CAT e GST em camarões expostos à água de CP1 e CP2 foram muito semelhantes, enquanto aqueles expostos à água de PG1, PG2, SB1 e SB2 formaram grupos de dados distintos. Observou-se aumento significativo da atividade de pelo menos uma das enzimas analisadas nos locais de amostragem, quando comparada aos animais do grupo controle. Isso demonstrou um possível estresse oxidativo em *M. jelskii*, causado

pela presença de xenobióticos na água (*e.g.*, esgoto doméstico, pesticidas, óleo e metais pesados). As atividades enzimáticas foram maiores nos animais dos experimentos realizados no período chuvoso, exceto para a atividade da CAT dos animais expostos à água do Rio Subaé. Este estudo demonstrou o potencial de *M. jelskii* como bioindicador e contribuiu para o conhecimento de aspectos do sistema de defesa antioxidante dessa espécie.
Palavras chave: Catalase; Glutadiona S-transferase; Camarão; Ecotoxicologia; Bioindicador.

Resumen
Varias enzimas biomarcadores, como la catalase (CAT) y la glutatión S-transferase (GST), pueden usarse para medir el estrés oxidativo en animales causado por la exposición a xenobióticos. El objetivo de este estudio fue caracterizar diferentes puntos de los ríos Capivari (CP1 y CP2), Paraguaçu (PG1 y PG2) y Subaé (SB1 y SB2), ubicados en el estado de Bahía, en relación a la presencia de xenobióticos, utilizando CAT y GST como bioindicadores en *M. jelskii*. Los puntos de muestreo de agua se consideraron urbanos o rurales y en todos se observaron signos de degradación ambiental. Para ello, se realizaron pruebas de exposición aguda (96h), con muestras de agua recolectadas durante la época seca y lluviosa. Los resultados mostraron que la actividad de CAT y GST en camarones expuestos al agua de CP1 y CP2 fueron muy similares, mientras que los expuestos al agua de PG1, PG2, SB1 y SB2 formaron distintos grupos de datos. Hubo un aumento significativo en la actividad de al menos una de las enzimas analizadas en los sitios de muestreo, en comparación con los animales del grupo de control. Esto demostró un posible estrés oxidativo en *M. jelskii*, causado por la presencia de xenobióticos en el agua (*e.g.*, aguas residuales domésticas, pesticidas, aceite y metales pesados). Las actividades enzimáticas fueron mayores en los animales de los experimentos realizados en época de lluvias, a excepción de la actividad CAT de los animales expuestos al agua del río Subaé. Este estudio demostró el potencial de *M. jelskii* como bioindicador y contribuyó al conocimiento de aspectos del sistema de defensa antioxidante de esta especie.
Palabras clave: Catalase; Glutatión S-transferase; Camarón; Ecotoxicología; Bioindicador.

1. Introduction

In the past few decades, aquatic ecosystems have been significantly affected by various environmental factors and impacts caused by the expansion of human activity and so water pollution in the urban aquatic environment became a major concern (Chen et al., 2014; Mendoza et al., 2017; Restello et al, 2020). As a typical urban aquatic environment, the urban water channel has been regarded as the fore-end part of the natural water bodies and the primary pollutant carrier that receives wastewater and polluted surface runoff. The variation of anthropogenic associated pollutants makes it difficult for cities to maintain a good status of urban surface waters (Wang et al., 2020).

Often, quantifying the concentrations of xenobiotics present in water is costly and does not provide the real impact on the organisms that live in the environment (Schirmer et al., 2011). Thus, ecotoxicological tests with biomarkers have been used to assess the effects of toxic chemicals on different ecosystems (Adams & Greeley, 2000; Rosner et al., 2021). In addition, their responses can be used to estimate exposure to chemicals or resulting effects on aquatic organisms (Ramsdorf et al., 2012; Araujo et al., 2017).

Exposure of animals to xenobiotics can lead to the production of *Reactive Oxygen Species* (ROS), which in turn can induce changes in biochemical and cellular activities in animals, leading to the production or inhibition of substances that can be used as biomarkers (Van der Oost et al., 2003). Oxidative stress and production of ROS have, for example, been positively correlated with accumulation of heavy metals in aquatic organisms (Orebiyi et al., 2010; Tumminello & Fuller-Espie, 2013; Dedeke et al., 2016), exposed to hydrocarbons (Tim-Tim et al., 2009; Lushchak, 2011; Al-Fanharawi et al., 2018; Turja et al., 2020), pesticides (Lavarías et al., 2013; Lavarías & Garcia, 2015; Gaume et al. 2015; Lafontaine et al., 2017; Vieira et al., 2014), pharmaceutical products and surfactants (Canesi et al., 2010; Bolong et al., 2009; Saénz et al., 2010).

Several biomarker enzymes can be used to measure oxidative stress in animals. In this context, Catalase (CAT) is an antioxidant enzyme that acts against ROS by converting H_2O_2 into H_2O and O_2, and is mainly involved in the reduction of H_2O_2 produced from the metabolism of long-chain fatty acids into peroxisomes (Afiyanti & Chen, 2014; Liu et al., 2020). Similarly, enzyme glutathione S-transferase (GST) is a biochemical biomarker involved in cellular detoxification of electrophilic compounds and shows initial signs of stress caused by pollutants (Lushchak, 2011). GST combines with ROS and

interrupts the damage caused by these reactive species to the animal metabolism (Van Der Oost et al., 2003; Lushchak, 2011). Thus, biochemical markers are frequently used in toxicological studies to improve the understanding of the sublethal effects of pollutants on the health of organisms (Weis, 2014). Fish and prawn are considered potential bioindicators of the aquatic ecosystem for monitoring and evaluating water contamination (Hasan et al., 2020).

Macrobrachium jelskii (Miers, 1877) is a palemonid prawn with wide geographical distribution found in lotic or lentic environments with generalist feeding habits (Melo, 2003). Similarly, to other macroinvertebrates, the benthic habit of some decapod crustaceans enhances the likelihood of coming into contact with pollutants (Davanso et al., 2013; Key et al., 2006; Costa et al., 2008; Lavarías & Garcia, 2015), making them effective bioindicators.

Although several biological and ecological aspects of *M. jelskii* have been studied, data regarding toxicology is scarce, especially with respect to research exploring the potential biomarkers in this species. Thus, the objective of the present study was to identify the most contaminated sites in three urban rivers in the state of Bahia, using the response of two biochemical biomarkers of this prawn species. We focused on answering the following question: can the activity of CAT and GST enzymes of *Macrobrachium jelskii* be used as biomarkers of aquatic contamination? Therefore, we investigated (i) the activity of the CAT and GST enzymes of *M. jelskii* after 96 h of exposure to water from river sites; (ii) the relationship between the response of CAT and GST activity and the probable source of contamination in the rivers water; (iii) the potential of *M. jelskii* as bioindicator in ecotoxicological tests to determine water quality, and; (iv) the influence of seasonality on the activity of CAT and GST enzymes.

2. Methodology

2.1 Bioindicators

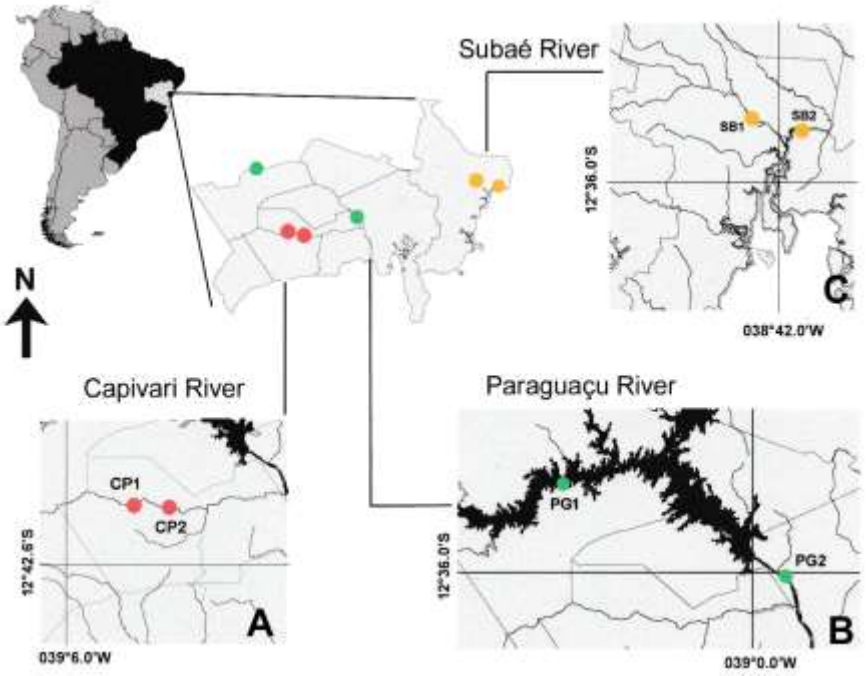
M. jelskii specimens were collected from an artificial pond at "Engenho São João" Farm (10°42'14.9"S; 039°02'58.9"W), in the countryside of the municipality of Cruz das Almas, state of Bahia. Individuals were sampled by sweeping a sieve (diameter of 50 cm, mesh of 0.5 mm) through the partially submerged vegetation along riverbanks. At the collecting site, specimens were stored in plastic containers with water from the pond and then transported to the laboratory.

In the laboratory, animals were submitted to depuration process for 10 days in order to observe health conditions, recover from stress caused by transport and adapt to new water conditions (Apha et al., 1998). For this, prawns were kept in glass containers (50 L) filled with water from the local supply system (previously dechlorinated) with constant aeration from air blowers. Water temperature was maintained at 24.0 ± 1.0°C, in room with air conditioning system and controlled photoperiod (12h / light: 12h / dark). During the depuration process, animals received fish feed *ad libitum*, feces were removed by siphoning and the water volume was replaced. Finally, feeding was suspended 24 hours before the beginning of the experiment.

2.2 Study area and collection of water samples

Water samples were collected from three rivers (Paraguaçu, Subaé and Capivari) in the "Recôncavo da Bahia" region, state of Bahia, Brazil (Figure 1) from February to June 2017. Water collection sites were chosen based on the characteristics of each area according to anthropic influence and history of pollution.

Figure 1. Map of South America, highlighting Brazil and the state of Bahia. Water sampling sites at: **A**- Capivari River (red circles); **B** - Paraguaçu River (green circles); and **C** - Subaé River (yellow circle). The black area in maps A and B indicate the Pedra do Cavalo Reservoir.



Source: Authors.

The spring of the Capivari River is in the municipality of Castro Alves and its mouth is in the municipality of São Félix, where it joins the Paraguaçu River downstream the Pedra do Cavalo Dam. The river runs through agricultural and pasture areas, and there are sources of discharge of domestic sewage along its course. Collection points were: CP1 - district of São José do Itaporã (12°38'43.00"S 039°7'15.40"W), located in the municipality of Muritiba, with intense rural activity; and CP2 - located within the city limits of Cruz das Almas (12°38'50.90"S 039°5'21.00"W). There is a community that lives in precarious conditions of sanitation and infrastructure between these two sampling sites. Therefore, part of the domestic sewage produced by this community is directly released into the Capivari River or kept in pits, which in turn can contaminate groundwater (Figure 2A,B).

The Paraguaçu River is one of the most important river systems in the state of Bahia, with approximately 600 km in length (Ingá, 2008). According to CRA (2011), one of the main uses of waters of the Paraguaçu River basin, in addition to supplying cities and industries, is for recreation and fishing (mainly in the estuarine zone). In its course, this river crosses several urbanized and agricultural areas and receives domestic and industrial sewage and pesticide residues. Sampling sites on the Paraguaçu River were: PG1 – at the Pedra do Cavalo Reservoir, municipality of Cabaceiras do Paraguaçu (12°30'49.6”S 039°11'14.1”W), where there is a ferryboat crossing, a small boat dock and the frequent use of jet skis for recreation; and PG2 - downstream of the Pedra do Cavalo Dam, municipality of Cachoeira (12°36'11.1”S 038°58'1.9”W), where there is a marked degree of urbanization and constant release of untreated domestic sewage (Figure 2C,D).

The spring of the Subaé River is in the municipality of Feira de Santana, extends for 55 km and its mouth is in the “Todos os Santos” Bay. This river has serious environmental impacts caused by the discharge of domestic and industrial effluents from agricultural and extractive activities. The sampling sites of the Subaé River were: SB1 - located on the urban limits of the municipality of Santo Amaro (12°32'17.10"S 038°43'38.92"W), near the deactivated lead factory Plumbum Mineração & Metalurgia Ltda; and SB2 - located downstream the downtown area of the municipality of Santo Amaro, where

untreated domestic and industrial effluents are released in the river water (Figure 2 E,F).

Figure 2. Photos from the water sampling sites at Capivari River (**A,B**), Paraguaçu River (**C,D**), and Subaé River (**E,F**).

Source: Authors.

Water collections were carried out in two seasonal periods: dry season (February / 2017) and rainy season (June / 2017). Each season was defined from rainfall data (calculated from a set of data of 30 years of observation) obtained from the "Clima Tempo" website (https://www.climatempo.com.br). Precipitation values below (dry) and above (rainy) 100 mm were considered. From each sampling site, 50 L of surface water were collected with the aid of previously decontaminated polystyrene gallons. Water samples were transported to the laboratory and transferred to glass containers (30 L) with aeration from air blowers. Each collection point was analyzed in duplicate, concomitantly.

2.3 Acute Exposure Test

After 24 h of filling containers with water samples, 32 *M. jelskii* specimens were transferred to each container. As a standardization criterion, only adult prawns were used in experiments, with cephalothorax length above 6.7 mm (Rocha &

Barbosa, 2017). In the control group (CTR), prawns were exposed to previously dechlorinated laboratory water. In total, 540 *M. jelskii* specimens were used in experiments, with 253 animals in the dry season and 286 in the rainy season.

The test was of static type, with controlled photoperiod (12 Light / 12 Dark) and duration of 96 h. Animals were not fed during experiments, while physicochemical parameters of water were daily monitored with a multiparameter meter (Hanna HI 9828). Mortality rate was assessed throughout the exposure period, with dead individuals counted and discarded.

2.4 Biochemical Biomarkers

After 96 h of exposure to water samples, prawns were individually removed from containers and analgesized in water containing ice (1:1) for one minute. Specimens were sexed according to presence (male) or absence (female) of the *appendix masculina* on the second pair of pleopods. Total body length (TL = from the tip of the rostrum to the end of the telson) and carapace length (CL - from the post-orbital margin to the posterior limit of the carapace) were measured to the nearest 0.01 mm with digital caliper. Finally, to obtain wet weight (W), precision scale (range: 0.01 g) was used.

The hepatopancreas of the animals were removed with the aid of surgical material, weighed and homogenized in a proportion of 1:10 (m/v) in potassium phosphate buffer (Tris-HCl, 50 mM, 0,15 M KCl, pH 6,8). Due to the small size and weight of the hepatopancreas of *M. jelskii*, it was necessary to compose the sample from a pool. Thus, the hepatopancreatic tissue of three adult individuals was combined in order to compose each sample pool, with volume of at least 0.04 g required for analysis.

The homogenized hepatopancreatic tissue was centrifuged for 25 minutes (4 °C) at 12.300 g. After this procedure, the supernatant was separated and frozen at -80 ºC for later determination of the activity of CAT and GST enzymes. CAT activity (µmol H_2O_2 min^{-1} $mg.pt^{-1}$) was determined based on the degradation of exogenous H_2O_2 by CAT, generating H_2O and O_2 as by-products (Aebi, 1984). The readings were performed on the UV/Vis spectrophotometer (Reaction kinetics software) in quartz cells and wavelength of 240 nm. GST activity (nmol CDNB min^{-1} $mg.pt^{-1}$) was determined catalyzing the substrate conjugation reaction 1-26 chlorine-2,4-dinitrobenzene (CDNB) with reduced glutathione, in acrylic cells and wavelength of 340 nm (Keen, Habig & Jakoby, 1976). The total protein concentration in the hepatopancreas was quantified using commercial kit (Interkit®).

2.5 Statistical analysis

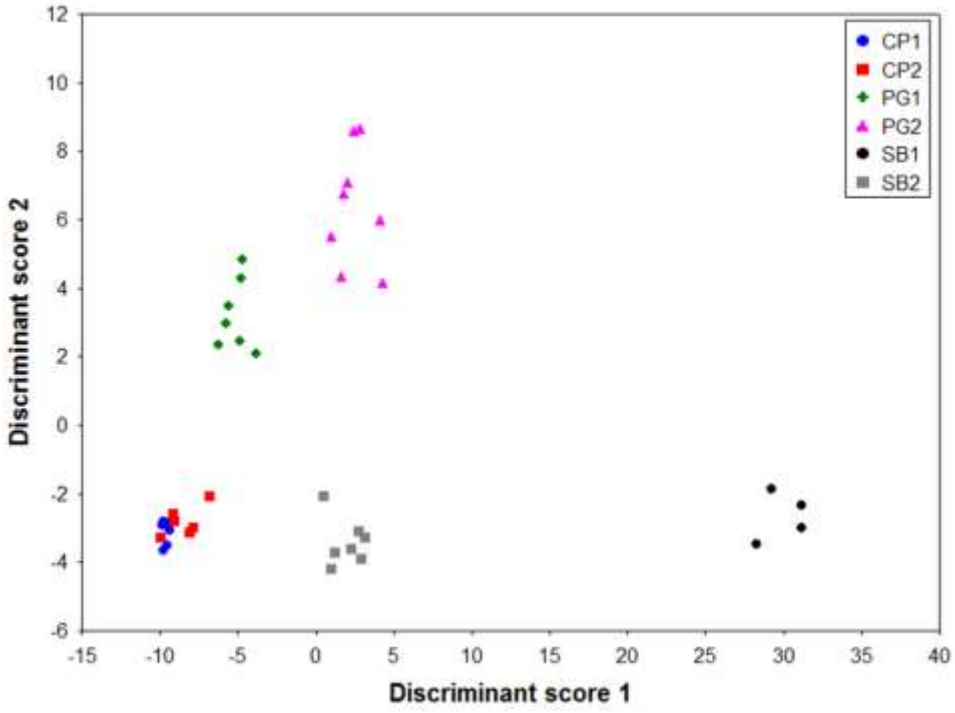
In our work, we used the experimental method of a quantitative nature (Pereira et al., 2018), with the analysis of the mean and standard deviation values of the analyzed biomarkers. Discriminant Analysis in a descriptive context (Williams, 1984) was used to evidence the most similar sampling points according to responses of biochemical biomarkers of prawns. For that, four variables were used to group sampling points: CAT activity in the dry and rainy season and GST activity in the dry and rainy season.

To identify significant differences in the responses of biomarkers (CAT and GST) among sampling points, three-way ANOVA was used (Sokal & Rohlf, 1995). Two three-factor ANOVA were performed, one using the CAT enzyme activity as response variable and the other using the GST enzyme activity as response variable. The three categorical variables were: i) three different rivers (Capivari, Paraguaçu and Subaé); ii) two different seasons (dry and rainy); and iii) three types of points (control points, points upstream the river (point 1) and points downstream the river (point 2). Differences among rivers, season, and points were considered significant at probability value < 0.05.

3. Results

Discriminant analysis showed that the CAT and GST activity in prawns exposed to water samples collected in both sites of the Capivari River were very similar, composing a single group of data (Figure 3). On the other hand, data sets of the enzymatic activity of prawns exposed to water from the Paraguaçu and Subaé rivers composed distinct groups of data for each sampling site (Figure 3). In this context, a slight difference in the enzymatic activity of animals exposed to water from both sites at Paraguaçu River was observed. Conversely, it was observed that the results of biomarkers evaluated in prawns exposed to water from SB1 were quite different, when compared to result of exposure to SB2 and even to the other points sampled in the other two rivers (Figure 3).

Figure 3. Enzymatic activity variation across all sample points of Capivari (CP), Paraguaçu (PG), and Subaé rivers (SB), using the two main discriminant factors of the Discriminant Analysis.



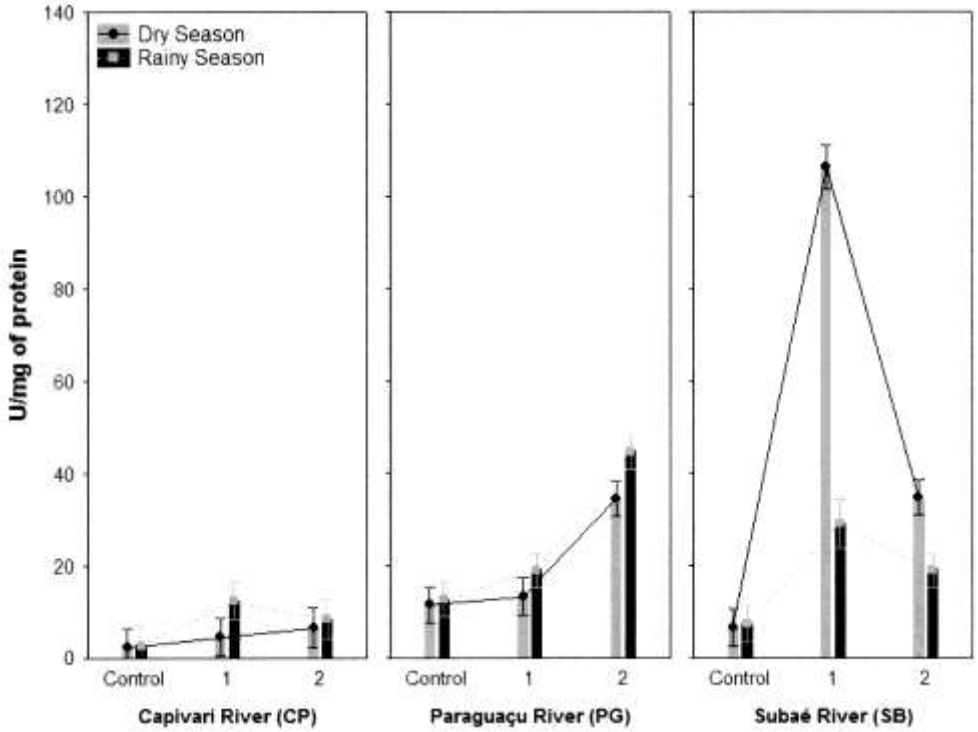
Source: Authors.

Catalase activity in prawns was significantly different among points, seasons, and rivers ($F = 61.19$, $p = 0$; specific differences can be viewed comparing interval confidences in Figure 4). In the Capivari River, results showed significant difference when CP1 (12.53 ± 0.3 U.mg^{-1} protein) with CTR (2.8 ± 0.05 U.mg^{-1} protein) were compared during the rainy season. Furthermore, in CP1, the CAT activity was significantly higher in the rainy season, when compared with the dry season (4.54 ± 0.1 U.mg^{-1} protein). On the other hand, in CP2, the CAT activity did not vary between dry and rainy seasons.

In the Paraguaçu River, the CAT activity was significantly higher in PG2, when compared to CTR and PG1. This result was observed in both seasons. In the Paraguaçu River, the highest values of CAT activity were observed in animals exposed to water from PG2, in the dry season (33.78 ± 0.4 U.mg^{-1} protein) and in the rainy season (45.41 ± 1.4 U.mg^{-1} protein), when compared to CTR and PG1. Comparing seasonality, it was observed that the CAT activity was higher in PG2, in the rainy season.

Finally, in the Subaé River, the CAT activity of SB1 and SB2 was significantly higher than that of CTR, both in the dry and rainy seasons. Comparing both sampling sites, the activity of this enzyme was significantly higher in SB1 in both seasons (106.20 ± 1.1 U.mg^{-1} protein in the dry season and 29.07 ± 1.1 U.mg^{-1} protein in the rainy season). Furthermore, higher CAT activity was observed in animals exposed to water samples from SB1 and SB2 collected during the dry season.

Figure 4. Mean catalase activity values in the hepatopancreas of *Macrobrachium jelskii* exposed for 96h to water samples from Capivari, Paraguaçu and Subaé rivers in the dry and rainy seasons. Bars represent confidence intervals at 0.95 level.



Source: Authors.

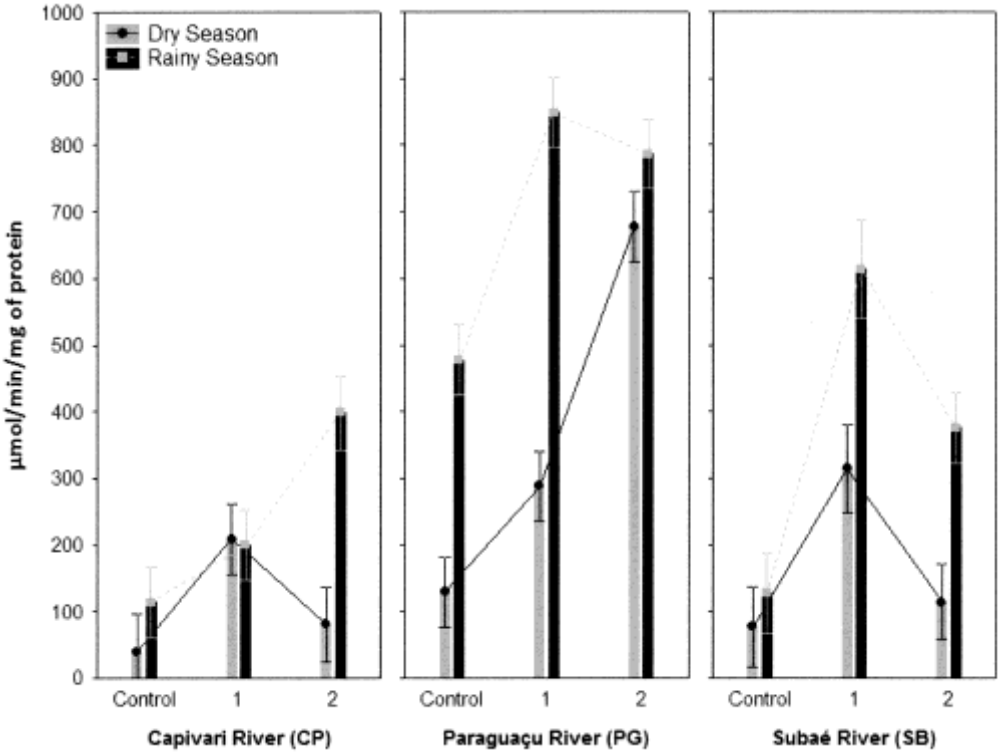
In benthic macroinvertebrates, the catalase activity differs broadly from taxa to taxa, ranging from 3.0 to 100.0 U (Berra et al., 2004). However, as far as we know, specific data about this enzyme in *M. jelskii* are not available in the literature. The present study showed that the catalase activity in *M. jelskii* ranged from 2.4 to 106.2 U, which is similar to that reported by Berra et al. (2004).

The GST activity in prawns was significantly different among points, seasons, and rivers (F = 28.90, p = 0; specific differences can be viewed comparing interval confidences in Figure 5). In the Capivari River, CP1 had significantly higher activity when compared with CTR and CP2. However, in the rainy season, the highest GST activity was recorded in animals exposed to water from CP2 (194.2 ± 0.1 µmol.min^{-1}mg^{-1} protein). Comparing dry and rainy seasons at the same sampling site, it was observed that the GST activity was significantly higher in CP2, in the rainy season (Figure 5). In experiments with water samples from the Paraguaçu River, the GST activity was significantly higher in prawns exposed to water from both sampling sites, when compared to CTR, in both seasons. In the dry season, the highest GST activity was observed in PG2 (676.7 ± 0.1 µmol.min^{-1}mg^{-1} protein), while in the rainy season, the highest GST activity was observed in specimens exposed to water from PG1 (849.3 ± 24.1 µmol.min^{-1}mg^{-1} protein).

Comparing the dry and rainy seasons, the GST activity was higher in PG1 and PG2 during the rainy season; the same pattern was observed for animals in the CTR group (Figure 5). In the Subaé River, the GST activity was significantly higher in

SB1 and SB2 when compared to CTR, in both seasons. In addition, the activity of this enzyme was significantly higher in animals exposed to water from SB1, in both seasons (dry season: 314.4 ± 23.6 μmol.min^{-1}mg^{-1} protein and rainy season: 613.2 ± 73.6 μmol.min^{-1}mg^{-1} protein). Considering seasonality, higher enzymatic activity was observed during the rainy season in both sampling sites (Figure 5).

Figure 5. Mean glutathione S-transferase activity values in the hepatopancreas of *Macrobrachium jelskii* exposed for 96h to water samples from Capivari, Paraguaçu and Subaé rivers in the dry and rainy seasons. Bars represent confidence intervals at 0.95 level.



Source: Authors.

4. Discussion

Biomarkers are defined as alterations at cellular, physiological or biochemical level in response to a stress condition. In this context, biomarkers of oxidative stress detect alterations resulting from increased exposure to oxidant agents or reduction in antioxidant defenses (Biazus *et al*., 2015). In benthic macroinvertebrates the analysis of oxidative stress parameters is less widespread, but there are studies with promising results in this field (see Berra et al., 2004; Prat et al., 2013; Biazus 2015 for review).

The antioxidant defense system activates repair mechanisms, which include enzymes that regulate the reactive intermediates produced in the cells (Paital, 2018). The superoxide dismutase enzyme catalyzes the dismutation of the superoxide radical (O_2^-) into O_2 and H_2O_2 whereas, the catalase (CAT) catalyzes the conversion of H_2O_2 (toxic to cells) into H_2O and O_2 (Benavides et al., 2016). Thus, variations in the activity of CAT may be related to the presence of stressing substances in the water. Similarly, the GST enzyme is also important in the defense against oxidative stress, since it protects proteins, lipids and DNA from oxidation (Benavides et al., 2016). According to Zanette et al. (2011) and Turja et al. (2020),

GST is a phase-II enzyme that conjugates electrophilic compounds with reduced glutathione (GSH), acting in the detoxification of organic contaminants (*e.g.,* PAHs).

In our study, the highest values of CAT and GST activity were observed in animals exposed to water from sampling points with the greatest anthropic influence, mainly by domestic sewage (CP2, PG2, SB1 and SB2). Domestic sewage in Brazilian urban areas presents high concentration of pharmaceutical products, personal hygiene and surfactants, which can act as endocrine disruptor chemicals, which can cause increase in the CAT and GST activity in mussels (Zanette et al., 2008; Canesi et al., 2010; Bolong et al., 2009; Saénz et al., 2010). Thus, it could be inferred that the activity of CAT and GST enzymes of *M. jelskii* individuals exposed to water from CP2, PG2, SB1 and SB2 was influenced by the presence of xenobiotics from domestic sewage contamination. Another fact that supports this hypothesis is that the activity of both enzymes was also higher than the control group in animals exposed to water from these sampling points.

Pesticides can also cause oxidative stress in aquatic organisms (Lushchak, 2011; Vieira et al., 2014), including other prawn species. For instance, exposure to sublethal "fenitrothion" concentrations (insecticide used to control agricultural pests) increased the activity of CAT and GST enzymes in *Macrobrachium borellii* (Nobili, 1896) and *Palaemonetes argentinus* (Nobili, 1901) (Lavarías et al., 2013; Lavarías & Garcia, 2015). These results suggest that these enzymes would be strongly involved in the detoxification of fenitrothion products during their metabolization (Lavarías et al., 2013). Gaume et al. (2014) showed that the organochlorine pesticide Chlordecone induced expression of enzymes involved in biotransformation and detoxification processes (*e.g.,* cytochrome, P450 and GST) in *Macrobrachium rosenbergii* (De Man, 1879). Finally, Lafontaine et al. (2017) demonstrated the accumulation of the same pesticide in the hepatopancreas of *M. rosenbergii* and the action of detoxifying enzymes in this organ. In this context, it is important to note that the three rivers under study show intense agricultural activity along their courses (authors personal observation), which can cause contamination by pesticides (even if this is not the main source of water contamination), and it may have also interfered in the response of CAT and GST enzymes.

It has already been determined that antioxidant enzymes can also be good biomarkers of exposure to metals (Van der Oost, 2003; Luchchak, 2011). According to Varol & Sen (2011) one of the main anthropogenic sources of heavy metal contamination are mining and smelting activities, in which sediments of sites downstream a copper mine plant in Turkey showed significant Cd, Co, Cu, Pb and Zn concentrations, indicating metallic discharges. In addition, metals such as Cu, Pb and Zn are components of household waste and more than 50% of these metals may come from urban sewage (Souza et al., 2016).

In our study, CAT and GST showed high activity in experiments using water from SB1. Given the history of contamination of this site (da Silva et al., 2017; Silva Júnior, 2020), the activity of such enzymes could also be related to exposure to heavy metals. In the Subaé River, in addition to inactivated lead smelter, paper and food industries, several sources of untreated sewage discharge can contribute to metal contamination (Hatje & Barros, 2012). Hatje et al. (2009) concluded that the Subaé River, particularly in its low course (where the city of Santo Amaro is located), has high level of contamination, based on the concentration of trace metals (Co, Cu, Pb, Zn). In addition, the Subaé River bottom sediment is also highly rich in Cd, Pb and Zn (Hatje et al., 2006; Hatje et al., 2009). Thus, we can infer that the defense system mediated by both enzymes in *M. jelskii* responded positively to reduce the damage caused by ROS.

Metals, such as Cd, Ni, Cr, Pb and Hg are toxic in aquatic organisms mainly due to their oxidative potential bioaccumulation and slow biodegradation (Vlahogianni et al., 2007; Duarte et al., 2017; Carvalho Neta, 2019). Previous studies have shown that both enzymes used in our study usually respond significantly to heavy metal contamination in a variety of animals, including crustaceans. In the Saronikos Gulf (Greece), the activity of CAT enzyme in the mussel *Mytilus galloprovincialis* Lamarck, 1819 increased 2–3 times in a site contaminated with heavy metals compared to control site

(Vlahogianni et al., 2007). In his review, Lushchak (2011) mentioned several studies in which the antioxidant enzymes (*e.g.*, CAT and GST) of various fish species responded to contamination by metals (*e.g.,* Cu, Hg). Harayashiki et al., (2018) measured the CAT and GST activities in *Penaeus monidon* Fabricius, 1798 juveniles fed on food contaminated with mercuric chloride and observed that it caused oxidative stress. Similarly, Capparelli et al. (2020) observed that the GST activity was higher in the hepatopancreas of the fiddler crab *Uca rapax* (Smith, 1870) (now *Minuca rapax*) exposed to copper when compared to control group. In our study, we also observed higher activity of GST in animals exposed to water from the SB1. However, in our case, CAT seems to be a biomarker more sensitive to the presence of metals, since the highest peak of activity of this enzyme was precisely in animals exposed to water from SB1.

On the other hand, GST activity was also high in animals exposed to water from PG1. In this sampling site, we observed the presence of a ferry boat and other vehicles powered by diesel oil. Crude oil and its by-products, which could be the main source of contamination at PG1, may affect aquatic organisms in many ways and oxidative stress is one of the key elements of their toxicity (Lushchak, 2011). The main pollutants from petrochemical activities include polycyclic aromatic hydrocarbons (PAHs), alkylphenols, diesel fuel, and hydrocarbons (Martínez-Gómez et al., 2006; Nudi et al., 2010; Lushchak, 2011; Zanette et al., 2011; Turja et al., 2020). For instance, PAHs are a group of organic pollutants of great impact to environment and human health (Celino et al., 2010; Sedeño-Díaz & López-López, 2013; Turja et al., 2020). In freshwater environment, contamination by PAHs is particularly serious because the solubility of these chemicals increases in low salinities (Ramachandran et al., 2006). Furthermore, many authors have already demonstrated that environmental pollution by oil and its by-products changes the patterns of GST activity (among other enzymes) in vertebrates and invertebrates (Tim-Tim, 2009; Zanette et al., 2011; Wu et al., 2011; Moreira & Guilhermino, 2005).

Considering the seasonal factor, there are two possibilities for increased concentration of contaminants in aquatic environments. First, during periods of greater precipitation, there is greater supply of potentially toxic substances due to leaching processes and the carrying of contaminants (Hatje & Barros, 2012; Souza et al., 2016). Second, during dry periods, there is greater water evaporation and less flow, with consequent increase in the concentration of contaminants in water, especially of heavy metals (Obasohan & Eguavoen, 2008; Ahmad et al., 2010; Mohiuddin et al., 2012; Shanbehzadeh et al., 2014; Mohammad Ali et al., 2016). Our results demonstrated that both possibilities seem to occur in the three rivers, since increase in the CAT and/or GST activity was observed in both seasons. However, at the rainy season, the GST activity was significantly higher at all sampling sites on the Paraguaçu (PG1 and PG2) and Subaé (SB1 and SB2) rivers. Thus, the greater GST activity in *M. jelskii* exposed to water collected from the rainy season may be related to the greater supply of potentially toxic substances as a result of leaching processes and the carrying of contaminants (Hatje & Barros, 2012). Finally, it is noteworthy that the rainy season can also promote dilution of xenobiotics (Mohiuddin et al., 2012), which induces less oxidative stress (*i.e.,* less enzymatic activity) during this season, as reported by Sedeño-Díaz & López-López (2013) for the fish *Chirostoma jordani* Woolman, 1894 from Yurira Lake, Mexico. However, this does not seem to be the case in the present study, since, during the rainy season, we observed an increase in the activity of at least one of the analyzed enzymes in each sampling site.

Specifically in the case of the Capivari River, less prominent enzymatic responses were observed. Possibly, it occurs because this river is of lesser magnitude and/or because it crosses less urbanized areas, with predominance of agricultural or pastures activities (authors personal observation; Figure 2). Thus, it is possible that the rainfall regime had less influence on the activity of biochemical biomarkers of *M. jelskii* exposed to water from this river.

The results of the discriminant analysis revealed a strong relationship between CP1 and CP2, which indicates similar characteristics, regarding the presence of chemical substances (*e.g.*, xenobiotics) in the water from the Capivari River. In fact, field observations (during water collection) found the presence of agricultural and pasture activities, waste and evidence of

contamination by domestic sewage in both points. PG1 and PG2 showed different enzymatic responses, which can be explained by field observations, where these two points have different contaminant sources. Consequently, PG1 was probably contaminated by oil products, while PG2 had signs of untreated domestic effluent discharge, and these different contaminants resulted in greater distance between PG1 and PG2. A similar situation occurred in SB1 and SB2. The isolation of the points that represent the enzymatic activity in animals exposed to water from SB1 proves the existence of a different type of contamination at this site (Figure 3).

5. Final Considerations

The present study demonstrated that the activity of CAT and GST enzymes can be used as biomarkers in *M. jelskii* and this species can be used as a bioindicator in ecotoxicological tests to determine water quality. Our results also contributed to the knowledge of aspects regarding the antioxidant defense system of this species. Furthermore, this study was the first ecotoxicological evaluation using freshwater prawn biomarkers to characterize urban and rural rivers in relation to aquatic contamination in the state of Bahia. However, further studies should be carried out, including the evaluation of other biochemical exposure biomarkers in conjunction with the determination of toxic substances present in water.

In general, the enzymatic activity was significantly higher in animals exposed to water samples collected from the three rivers. Such enzymatic activities were higher in animals of experiments carried out in the rainy season, in comparison to those from the dry season. These responses may be an indicative of activation of the antioxidant defense mechanism of *M. jelskii*. Further studies, using a greater number of biochemical biomarkers, are necessary to correlate the response of biochemical biomarkers with the probable source of contamination of the studied rivers.

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