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DNA damage in an estuarine fish inhabiting the vicinity of a major Brazilian port

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Abstract: The Itaqui Port Complex (northeastern Brazil) is one of the largest Brazilian port facilities, whose effluents and waste are dumped directly into the estuarine waters. Although environmental monitoring has been a concern around this site, there has been no toxicogenetics study on organisms living in this environment. Thus, we assessed the toxicogenetics potential of the estuarine waters surrounding Itaqui, using the native catfish *Sciades herzbergii* as a biomonitor. We found a significantly higher frequency of genetic damage and mutations in the animals collected near to Itaqui in both seasons compared to the reference site (distant from Itaqui with no port activities). We also quantified chemical elements in the surface water and sediments near the port and found that clorine, phosphorus, zinc, and boron were above the limits set by the Brazilian legislation. We suggest that such contaminants are involved in the origin of DNA damage. Moreover, we recommend including toxicogenetics assays in the environmental monitoring of pollutants, as well as in the definition of their allowable limits, as they could be used as law enforcement tools and help to predict large-scale contamination events associated with port activities.

Key words: Comet assay, estuary contamination, Itaqui Port, micronucleus test, *Sciades herzbergii*.

INTRODUCTION

Historically, coastal environments such as estuaries have been the main sites for human settlement. Such a preference imposes a large number of stressors on these areas, with chemical-derived xenobiotics posing the most important threat to the biota (Martins & Costa 2015). Xenobiotics are chemical element(s) or compound(s) found in an organism but that is not naturally produced or expected to be present, and can initiate a cascade of harmful consequences to cells, organs, the organism as a whole and even the population and entire communities (Amado et al. 2006, Grisolia et al. 2009).

One of the possible outcomes of xenobiotics is damaging the genetic material of organisms, in that many contaminants might have mutagenic and/or carcinogenic effects (Arslan et al. 2010), even at low concentration. Thus, toxicogenetics assays are highly effective methods for evaluating the impact of environmental contamination (Scalon et al. 2010, Martins & Costa 2015), especially considering that many substances released into natural environments (and their resulting metabolites) have not had their potential impacts been fully characterized yet (Van der Oost et al. 2003). For instance, genotoxic and mutagenic effects have been described in animals exposed to estuarine environments near ports (e.g., Barsiene 2002, Domingos et al. 2009, Barsiene et al. 2012).

The Itaqui Port Complex (IPC) is one of the largest ports on the Brazilian coast, located in the city of São Luís (state of Maranhão, northeastern Brazil), and surrounded by estuaries, mangroves and forests in the São Marcos Bay. The IPC can receive cargo with diversified products, including petroleum byproducts (gasoline, kerosene and gas), as well as a large amount of ores (e.g., iron, alumina, bauxite and manganese), aluminum, pig iron, fertilizers, malt, soy beans, caustic soda, coal, etc. (Acosta et al. 2011). Effluents and waste from the port, residences and industries are dumped directly into the estuarine waters and little is known regarding their effects on the local aquatic biota.

Previous studies have pointed out to stressful conditions in the vicinity of the IPC as fish exhibit more anatomical abnormalities and stress-derived physiological responses compared to fish from other distant areas (Carvalho-Neta et al. 2012, Carvalho-Neta & Abreu-Silva 2013, Sousa et al. 2013). Although environmental monitoring has been a concern in the IPC area, there has been no toxicogenetics study on organisms living in this environment. Therefore, the present study aimed to evaluate the genotoxic and mutagenic potential of estuarine waters surrounding the Itaqui Port Complex, using a native catfish (*Sciades herzbergii* Bloch 1794) as a biomonitor, to gain a better understanding of the impacts of port activities on the local aquatic biota.

MATERIALS AND METHODS

Sampling design

Fishes are most threatened by aquatic pollution and provide a suitable biomonitor due to their physiological similarity to mammals and their long-term exposure to the environment (Kime 1998). Moreover, they tend to accumulate metals and organic contaminants in their tissues,

reaching hazardous levels over time, even when their concentrations are below the limits set by environmental legislation. The bioaccumulation of chemicals in the biota is a prerequisite for adverse effects on ecosystems (Franke et al. 1994).

The sea catfish *Sciades herzbergii* (Ariidae) is an abundant estuarine-resident species along the coast of the state of Maranhão and commercially important to artisanal fisheries in the region (Ribeiro et al. 2011). We selected this fish as a biomonitor due to its wide distribution and tolerance to different environmental parameters as well as its benthic and sedentary habits (Carvalho-Neta & Abreu-Silva 2010, Carvalho-Neta et al. 2014). Moreover, this is the most used species for environmental biomonitoring in the region (e.g., Carvalho-Neta and Abreu-Silva 2010, 2013, Carvalho-Neta et al. 2012, 2014, Sousa et al. 2013, Marreira et al. 2017, Castro et al. 2018).

Specimens were caught at two sampling sites. Location 1 (L1) (02°31'45.21 "S, 44°5'11.87" W) was *Pau Deitado* in São José Bay, which is an estuarine environment located in the municipality of *Paço do Lumiar* with extensive and relatively preserved mangrove areas. It is distant from the IPC and urban centers/industrial areas, presenting less anthropogenic impacts. Therefore, we used it as a reference site for comparison to a potentially more disturbed environment near the IPC. The second location (L2) was *Irinema Pequeno* (02°34'50.3 "S, 44°21'42.4" W), which is a small mangrove stream in the vicinity of the IPC in São Marcos Bay, where two other ports are installed: Ponta da Madeira Port (owned by the mining company Vale) and ALUMAR Port (owned by BHP Billinton and ALCOA), both of which are involved with the transportation of ores (Figure 1).

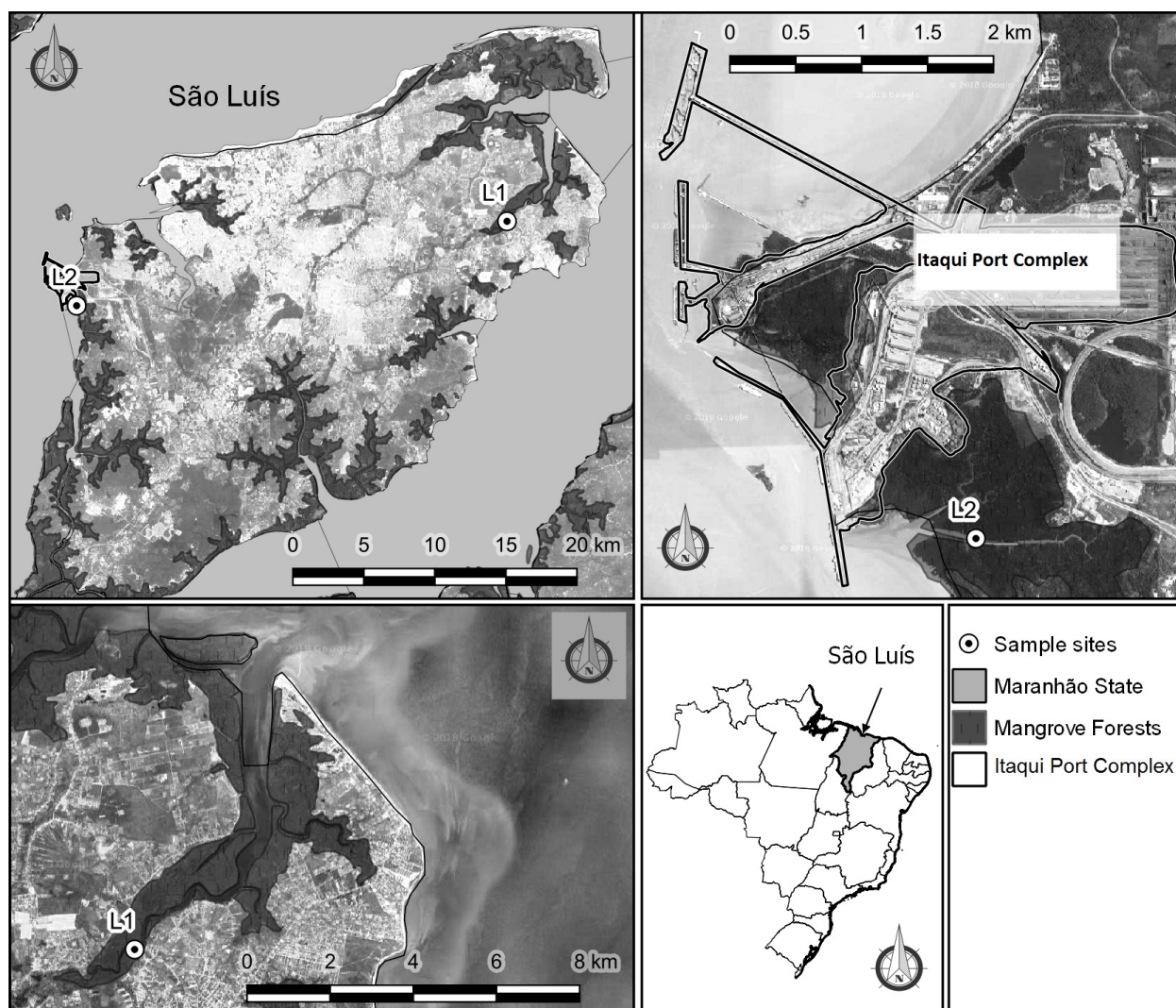Specimens were captured using nets measuring 100 m in length and 2.5 m in height

Figure 1. Sampling sites for *Sciades herzbergii* in Maranhão state, northeastern Brazil. L1 = reference site (*Pau Deitado*) distant from ports and urban centers/industries; L2 = *Irinema Pequeno* Stream at the vicinity of the Itaqui Port Complex (Map by Diego Campos).

(50 mm and 25 mm mesh sizes), and kept submerged in traps at the sampling sites until tissue collection to avoid stress due to capture (capture and transportation permit N.45384-1 from Brazilian Environmental Agency IBAMA/SISBIO). Twenty specimens were collected at each location in the rainy season (May and June, 2015). In the dry season (November and December, 2015), 20 specimens were caught at the reference site, but only 10 were caught at the IPC site. First, fish were anaesthetized and euthanized with clove oil (eugenol 100 mg L^{-1}), then blood samples were obtained by cardiac puncture (20 µl) using a heparinized syringe (Committee for ethics in animal experimentation of the Universidade Federal do Maranhão N° 23115.008075/2016-12). The blood samples were diluted in 1 mL of fetal bovine serum and stored on ice for ± two hours until the genotoxicity and mutagenesis assays.

Physical and chemical characterization of water and sediment from IPC

The water and sediment near the IPC are constantly monitored (Environmental Monitoring Program of the Itaqui Port, EMAP-FSADU-UFMA agreement). Physical and chemical variables (pH, salinity, temperature, dissolved oxygen and conductivity) were obtained from the two locations in both seasons (rainy and dry) using a multiparameter device (Hanna HI 9828) in the shallow surface water (20 cm in depth) at the same time fish were caught. Water chemistry data were available only for the IPC in June 2015 (rainy season) and November 2015 (dry season). The analyses were performed following the Standard Methods (APHA 2012) for the different matrices and chemical groups, and compared to CONAMA (Brazilian National Environmental Council) Resolution 357/05 (legislation applied to class 1 brackish water).

Comet assay

The comet assay was carried out following the method described by Singh et al. (1988). Ten μL of the blood solution (10 μL of blood plus 1 mL of fetal bovine serum) were mixed with 120 μL of low melting point agarose until homogenized. The mixture was dropped on a slide covered with regular melting point agarose (1.5%). The slides were covered with coverslips and left for 20 minutes at 4°C. The coverslip was removed and the slide was dipped in an ice-cold lysis solution (2.5 M of NaCl, 100 mM of EDTA, 10 mM of Tris, 10% dimethylsufoxide and 1% Triton X-100) and left at 4°C for 1 hour. The slides were then incubated for 25 minutes in an electrophoresis solution (200 mM of EDTA, 300 mM of NaOH, distilled H_2O, pH > 13) at 4°C. Electrophoresis was carried out for 25 minutes at 25 V and 300 mA. The slides were neutralized in 5 mL of neutralizing solution (0.4 M of Tris/HCL, pH 7.5) for 5 minutes (and repeated twice), and fixed with 100% ethanol for 5 minutes. Supercoiled loops of DNA linked to the nuclear matrix (nucleoids) were produced after these treatments. The slides were stained with 30 μl of ethidium bromide solution (30 μg/ml), covered with coverslips and immediately analyzed under a fluorescence microscope (BX51/BX52-Olympus; 516-560 ŋm filter; 590 ŋm filter barrier, 40 X objective).

We observed 100 nucleoids per animal and used the classification proposed by Speit & Hartmann (1995) according to the length of the nucleoid tail: Class 0 – no damage (< 5%); Class 1 – low level of damage (5-20%); Class 2 – medium level of damage (20-40%); Class 3 – high level of damage (40-94%); and Class 4 – total damage (≥ 95%). Counting the frequencies of each class (blind test) enabled calculating the damage score by multiplying the number of nucleoids in each class by the value of the respective class. The final score for each sample was obtained by dividing the sum of the values of each class by the number of nucleoids analyzed (Total score = (0 x n0) + (1 x n1) + (2 x n2) + (3 x n3) + (4 x n4) / number of nucleoids).

Micronucleus test

We followed the methods of Schmid (1975) and Hooftman & Raat (1982) to determine the frequency of micronuclei in erythrocytes. A drop of blood was smeared on a slide, fixed with absolute methanol for 10 minutes and stained with Giemsa dye diluted in phosphate buffer (pH = 8.6) (1:10). The slides were rinsed in running water, dried at room temperature and observed under an optical microscope.

We counted the frequency of micronuclei in 1000 erythrocytes per animal considering only nucleated red cells with both intact membrane and cytoplasm. A micronucleus was defined as a particle that did not exceed 1/3 of the size of the nucleus, with distinguishable edges, as well as

the same color and refringence as the nucleus (Carrasco et al. 1990).

Statistical analyses

We tested data for normal distribution (Kolmogorov-Smirnov´s test) and homogeneity of variance (Levene's test). Parametric data were compared by the t-test for two independent samples. Non-parametric data were tested using the Mann-Whitney test (Wilcoxon Rank-Sum test). We considered the level for statistical significance to be 5% ($p < 0.05$). Both sampling sites and seasons were compared. All statistical tests were performed using the Prism version 6.0 (GraphPad software). All data generated or analyzed during this study are included in this published article and/or its supplementary information file.

RESULTS

Physical and chemical characterization of water and sediment from IPC

Surface waters at the reference site presented more variability in salinity, temperature, dissolved oxygen and conductivity when the seasons were compared (Table I). The water

chemistry analysis at the IPC revealed residual chlorine, total phosphorus, zinc and boron, in the rainy season, with values higher than those permitted by the Brazilian legislation. The concentration of manganese was just below the upper limit. In the dry period, the concentrations of all elements were below the cut-off values (Table II).

The sediment chemistry analysis at the IPC, in the rainy season, revealed quantifiable values of arsenic, lead, copper, chromium, nickel, zinc, benzo(a)anthracene, benzo(a)pyrene, chrysene, acenaphthene, acenaphthylene, anthracene, phenanthrene, fluoranthrene, fluorene, 2-methylnaphthalene, naphthalene, pyrene and total polycyclic aromatic hydrocarbons (PAH). In the dry period, arsenic, chromium, nickel and zinc were quantified. However, no elements or compounds in the sediments were above the levels permitted by legislation in both season (data not shown).

Comet assay

We found statistically significant differences between IPC and the reference site in the damage scores, although significantly marginal in the rainy season (Figure 2, Table III). Damage scores

Table I. Physical and chemical characterization of surface water at the reference site, distant from ports and urban centers/industries, and at the vicinity of the Itaqui Port Complex in Maranhão state, northeastern Brazil (dry and rainy seasons, 2015).

Season/Location	pH	Salinity (g/kg)	Temperature (ºC)	Dissolved Oxygen (mg/L)	Conductivity mS/cm
Rainy					
L1	7.47	8.44	24.97	6.24	14.54
L2	8.08	24.82	28.37	5.12	39.17
Dry					
L1	7.30	39.21	28.60	3.71	58.86
L2	7.70	37.36	29.31	4.41	56.40

L1: *Pau Deitado* or reference site distant from ports and urban centers/industries; L2: *Irinema Pequeno* Stream at the vicinity of the Itaqui Port Complex.

at the reference site did not differ significantly between seasons, whereas at the IPC they were significantly higher in the dry season (Figure 2, Table III).

Classes of DNA damage at the reference site were similar in both seasons, with zero being the most frequent class (77.3% and 85% for rainy and dry seasons, respectively), followed by the other classes in a descending trend (Figure 2, Table III). Scores for classes 3 and 4 at the reference site were lower than those calculated for IPC in both seasons. DNA damage was more pronounced in the dry season at the IPC, with a low frequency of class zero (9.6%) and higher frequencies of classes 2, 3 and 4 (46.3%, 5.9% and 5.7%, respectively) compared to the reference site (Table III).

Micronucleus test

For both the rainy and dry seasons, fish from the IPC had a significant higher mean frequency of micronuclei compared to those from the reference site ($p < 0.05$). Erythrocytes with micronuclei were significantly more frequent in the rainy season at both locations (Figure 3).

In the rainy season, the frequency of micronuclei ranged from five to 19 per 1000 cells (mean = 13.0 ± 4.2) at the reference site and 13 to 65 per 1000 cells (mean: 29.1 ± 11.2) at the IPC. In the dry period, 5 to 15 micronuclei (mean = 9.7 ± 3.3) were counted at the reference site and 16 to 25 (mean = 20.1 ± 2.8) at the IPC.

Table II. Chemical analyses (concentration in mg/L) of water at the vicinity of the Itaqui Port Complex in Maranhão State, Northeastern Brazil (dry and rainy seasons, 2015), compared to the Brazilian environmental legislation (CONAMA-Brazilian National Environmental Council, Resolution 357/05, class 1 brackish water).

Chemicals	LQ[a]	Rainy	Dry	Upper limits CONAMA[c]
Copper (Cu)	0.005	<0.005	<0.005	–[d]
Dissolved copper	0.005	<0.005	<0.005	0.005
Iron (Fe)	0.01	1.92	0.935	–
Dissolved iron	0.01	0.0186	0.0926	0.30
Manganese (Mn)	0.01	0.0985	0.0156	0.10
Níckel (Ni)	0.01	<0.01	<0.01	0.025
Dissolved Aluminun (Al)	0.01	<0.01	<0.01	0.10
Arsenic (As)	0.01	<0.01	<0.01	0.01
Boron (B)	0.01	**2.82**	n.d[b]	0.50
Cadmiun (Cd)	0.005	<0.005	<0.005	0.005
Lead (Pb)	0.01	<0.01	<0.01	0.01
Residual Chlorine (Cl)	0.01	**0.47**	n.d	0.01
Chromium (Cr)	0.01	<0.01	<0.01	0.05
Total Phosphorus (P)	0.02	**0.18**	n.d	0.124
Mercury (Hg)	0.000075	<0.00007	<0.00007	0.0002
Zinc (Zn)	0.01	**0.519**	< 0.01	0.09

[a]LQ/Range: detection limit of the method (mg/L).
[b] not detected.
[c] Resolution 357/05 (class 1 brackish water) from National Council for Environment (Brasil 2005).
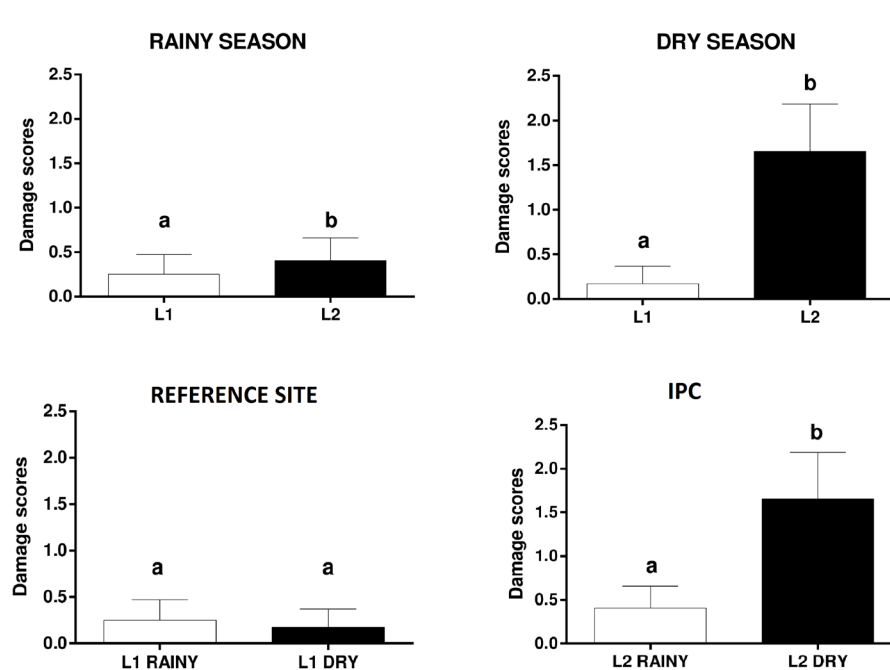[d] Not classified by Resolution 357/05.

Figure 2. Mean (with standard deviation) damage scores (comet assay) for *Sciades herzbergii* from Maranhão state, northeastern Brazil. Above: between-localities analysis by season. Below: between-seasons analysis by locality. L1: reference site distant from ports and urban centers/industries; L2 = *Irinema Pequeno* Stream at the vicinity of the Itaqui Port Complex. Different letters indicate statistically significant differences between means (Above left: t-test, p<0.05; above right: Mann-Whitney test, p<0.05. Below left: Mann-Whitney test, p<0.05; below right: t-test, p<0.05).

DISCUSSION

In this study, we demonstrate that fish collected near the Itaqui Port Complex have more DNA damage and mutations, suggesting the presence of xenobiotics capable of inducing genetic harm and mutagenesis in this area. When comparing the levels of genomic damage and mutations between seasons at each locality, only mutagenesis was higher in the dry period.

DNA damage caused by xenobiotics can be repaired by the DNA repair system of the cell, impeding the damage from becoming mutations. However, when the rate of harm is continuous, the repair system may be overwhelmed even with low dosages of compounds, making the fixation of mutations more common than would be expected by the background mutation rate. This suggests that waters near the IPC have higher levels of genotoxicity and mutagenicity due to the chronic effects of xenobiotics, which have been associated with the production of reactive oxygen species (ROS) in aquatic organisms (Livingstone 2001). ROS interact with DNA, causing genomic damage and, eventually, mutations. When this affects a large number of individuals, the viability and maintenance of wild populations are jeopardized.

Sciades herzbergii was proved to be a sensitive biomonitor for environmental ecotoxicology studies, with applicability in monitoring water resources. Carvalho-Neta & Abreu-Silva (2010) report the higher activity of catalase (an enzyme involved in cell defense against oxidative damage induced by ROS) and glutathione S-transferase [GST (an enzyme involved in detoxification of xenobiotics)] as well as a decreased gonadosomatic index [GSI (evidence of endocrine disruption in the reproduction regulatory framework)] in females of this species sampled from sites in São Marcos Bay near another port (ALUMAR), when compared to a distant location. The same authors report increased GST activity and a decreased GSI for *S. herzbergii* juveniles and mature females associated with contaminants above the limit

Table III. Nucleoid class frequencies and DNA damage scores (comet assay) on erythrocytes from *Sciades herzbergii* (Maranhão state, northeastern Brazil) at the reference site, distant from ports and urban centers/industries, and at the vicinity of the Itaqui Port Complex (IPC) (dry and rainy seasons, 2015; n = 20 per site/season, except IPC dry season where n= 10). Different letters indicate statistically significant differences between means (rainy season: t-test, p < 0.05; dry season: Mann-Whitney-test, p < 0.05).

Season/sampling sites	Classes (%)					Scores
	0	**1**	**2**	**3**	**4**	**Mean ± SD**
Rainy						
L1	77.3	20.7	1.5	0.3	0.2	0.25 ± 0.22 [a]
L2	62.0	35.7	2.1	0.2	0.0	0.40 ± 0.25 [b]
						p = 0.0497
Dry						
L1	85.0	13.4	1.2	0.3	0.1	0.17 ± 0.19 [a]
L2	9.6	32.5	46.3	5.9	5.7	1.65 ± 0.53 [b*]
						p < 0.0001

L1: *Pau Deitado* or reference site distant from ports and urban centers/industries; L2: *Irinema Pequeno* Stream at the vicinity of the Itaqui Port Complex.
SD: Standard deviation.
* indicates difference between seasons (t-test, p<0.05).

set by the Brazilian legislation (Al, Cd, Pb, Cr, Fe, Hg, benzene, total phenols, tributyltin and polychlorinated biphenyl) both in the water and sediment in São Marcos Bay near the IPC (Carvalho-Neta & Abreu-Silva 2013).

In another study, specimens of *S. herzbergii* collected along a pollution gradient, including the IPC surroundings, also exhibited increased GST activity and gill lesions at most contaminated sites (Carvalho-Neta et al. 2012). Several histological abnormalities have also been found in *S. herzbergii* collected near the IPC (Sousa et al. 2013). Therefore, multiple studies on the same species and using different biomarkers to evaluate environmentally-driven damage have come to the same conclusion: fishes inhabiting the vicinity of the IPC exhibit more abnormalities compared to samples from areas with low levels of disturbance. The present findings lend support to this notion by demonstrating damage on the DNA level.

The chemical analysis of the surface water from the IPC in the rainy season indicates the presence of potentially toxic elements, which, even at low concentrations (by the standards of Brazilian law), may be causing harm to *S. herzbergii*, as well as, possibly, to the remaining biota. For instance, the mangrove oyster (*Crassostrea rhizophorae*) (C. Rocha-Junior, unpublished data) exhibited a high rate of DNA damage associated with oxidative stress when exposed to water samples from the IPC compared to water samples from a distant and little disturbed site.

However, abiotic conditions may have contributed synergistically to the effects of the contaminants. Higher salinity levels similar to marine waters in the dry and rainy seasons may have imposed hyperosmotic stress on *S. herzbergii*, which has preference for estuarine waters. Increased salinity has been related to oxidative stress in fish (e.g., Martínez-Álvarez et al. 2002, Hossain et al. 2016), which could
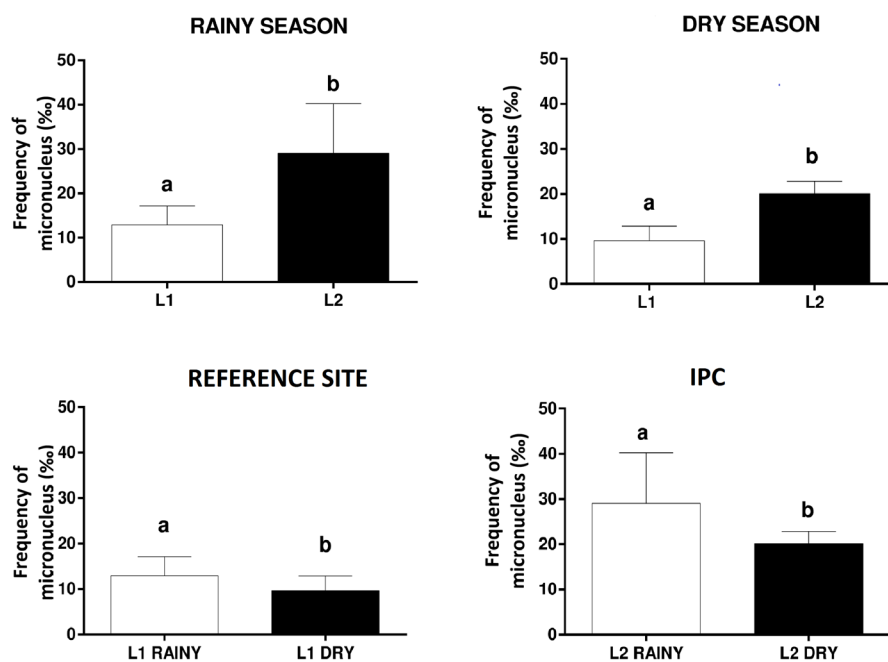
Figure 3. Mean (with standard deviation) frequencies (‰) of erythrocytes containing micronuclei in *Sciades herzbergii*. Above: between-localities analysis by season. Below: between-seasons analysis by locality. L1: reference site distant from ports and urban centers/industries; L2 = *Irinema Pequeno* Stream at the vicinity of the Itaqui Port Complex. Different letters indicate statistically significant differences between means (Above left: Mann-Whitney test, p<0.05; Above right: t-test, p<0.05. Below left: t-test, p<0.05; below right: Mann-Whitney test, p<0.05).

also explain the increase in genetic damage. Increased salinity requires a rapid physiological modulation in estuarine fishes, which are hypo-osmotic to water, with greater water intake required to counterbalance the loss imposed by the osmotic gradient. With the increase in the water intake rate, fishes also swallow a larger amount of particulate matter, which may contain pollutants, such as heavy metals, potentially enhancing the genotoxic and/or mutagenic effects when absorbed by the digestive tract.

Other reason to state that the IPC area is unsuitable for *S. herzbergii* is that our sample size was lower in the dry season at this site (n = 10) than that obtained in the rainy season (n = 20), suggesting that this environment did not have adequate conditions for the species at the time of sampling, despite the considerable sampling effort. In the dry season, salinity and rainfall are altered, causing changes in the bioavailability of pollutants, which may increase the rate of DNA damage, overloading the DNA

repair system and increasing the probability of the fixation of mutations.

A higher frequency of micronuclei was detected in the rainy season at both locations. At the time of the sampling, salinity was less than 30% (brackish water) and some contaminant levels were detected above the limit stipulated by Brazilian law. Thus, the greater exposure to contaminants in the rainy season is likely associated with the increase in mutations among the fish collected in this season.

Genotoxic and mutagenic effects have been described in animals exposed to estuarine environments near discharges of agricultural, domestic and industrial effluents, as well as waste from port activities (e.g., Barsiene 2002, Domingos et al. 2009, Barsiene et al. 2012). For instance, bivalve and gastropod mollusks inhabiting sites around a port area in Lithuania (Baltic Sea) in 1995 and 1996 exhibited high levels of genotoxicity (Barsiene 2002). After dredging, which resulted in the removal of the contaminated sediments, these animals

exhibited a significant decrease in cytogenetic damage in the period from 1997 to 1999, suggesting that pollutants in the sediment were involved in the DNA damage (Barsiene 2002). In the same area, a study with mussels revealed a significant increase in the frequencies of micronuclei, nuclear buds and fragmented-apoptotic cells after an accidental oil spill in comparison to the frequencies before the spill (Barsiene et al. 2012). A multi-biomarker approach (comet assay, micronucleus test, cholinesterase activity and histopathological findings) indicated the negative impacts on fishes from estuaries near another major Brazilian port (Paranaguá Port in the state of Paraná) (Domingos et al. 2009).

Many of the contaminants found in estuaries around the world are metals resulting from industrial and agricultural waste, domestic sewage and other human activities. Heavy metals (e.g., lead, nickel, mercury, cadmium, chromium, etc.) pose, by far, the most serious threat to the health of aquatic biota. These metals are stable and persistent in the environment and most are not involved in any cell metabolism pathway, implying that they may have toxic effects on organisms (Barbosa et al. 2010). Heavy metals have been widely implicated in mutagenesis in aquatic environments (e.g., Cavas et al. 2005, Pinheiro et al. 2013, Yazici & Sisman 2014, Akhtar et al. 2016). Even those metals essential to cell functioning (e.g., manganese, copper, zinc, iron, magnesium, cobalt, boron, molybdenum, etc.) can be toxic at high concentrations (Tchounwou et al. 2012).

In the present study, the sediments near the IPC had different organic and inorganic compounds in both seasons, although these compounds did not exceed the maximum limits established by Brazilian law. However, the combined effects of these pollutants, even at low concentrations, may shed some light on the origin of the genetic damage revealed

herein. For instance, zinc interacts with essential oligoelements, such as iron and copper as well as non-essential elements (Bagdonas & Vozyliene 2006). The rainbow trout exhibited higher frequencies of micronuclei when exposed to a copper + zinc mixture than when exposed to each element alone (Bagdonas & Vozyliene 2006). Both copper and zinc are aneugenic agents, inducing aneuploidy (abnormal migration of chromosomes during cell division) and, consequently, micronuclei (Bagdonas & Vozyliene 2006).

In the rainy season, zinc (0.519 mg/L) exceeded about five times the permitted limit (0.09 mg/L) for brackish water (CONAMA 357-Brasil, 05). This is also quite above the highest concentration tested by Bagdonas & Vozyliene (2006) (0.25 mg/L). The high levels of zinc and other contaminants in São Marcos Bay suggest considerable availability of the element at this site in the rainy season (J.K.C. Sousa, unpublished data). Thus, zinc may be implicated as having a higher mutagenic effect on fishes sampled near the IPC. Other contaminants found in the rainy season (Cu, Pb and Cd) are also reported in São Marcos Bay (J.K.C. Sousa, unpublished data), although at low concentrations in the water and sediment that do not exceed the upper limits established by law.

Water and sediment analyses are important to the evaluation of environmental contamination by heavy metals in estuarine systems, but do not provide real data on the availability of the elements to aquatic organisms (Guimarães & Sígolo 2008). To accomplish such a task, it is necessary to correlate chemical pollutants with bioindicators such as the biota inhabiting these ecosystems. Even at small concentrations, some contaminants and their combined effects can cause damage to genetic material and lead to mutations (e.g., Bagdonas & Vozyliene 2006). Moreover, mutagenic

and carcinogenic compounds often occur in aquatic environments as mixtures, while the guidelines stipulated by legislation are issued for individual compounds (Martins et al. 2015). Therefore, we emphasize the need for Brazilian legislation to stipulate the use of genotoxic and mutagenic assays to determine the allowable limits of contaminants in aquatic ecosystems, since such tests are capable of revealing threats that cannot be detected through chemical or physical analysis alone.

Once in the aquatic environment, hazardous compounds can travel through several levels of the food web, eventually making their way to human populations (Al-Sabti & Metcalfe 1995). Therefore, it is essential to expand aquatic monitoring activities near ports to assess their threat to coastal ecosystems, given the genetic risks that contaminants pose to the local biota and human populations. The inclusion of genotoxic and mutagenic tests in the environmental monitoring of pollutants, as well as in the definition of their allowable limits is highly recommended. Such methods could serve as law enforcement tools and could help predict large-scale contamination events associated with port activities.

Acknowledgments

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