Marine Pollution Bulletin 138 (2019) 63–69

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Genomic damage in *Mugil curema* (Actinopterygii: Mugilidae) reveals the effects of intense urbanization on estuaries in northeastern Brazil

Anderson R.B. Lima[a,b,*], Rodrigo A. Torres[b], Uedson P. Jacobina[b], Marcelo A.A. Pinheiro[c], Mônica L. Adam[d]

[a] *Centro de Biociências, Programa de Pós-Graduação em Ciências Biológicas – PPGCB, Av. Prof. Moraes Rego, 1235, Universidade Federal de Pernambuco - UFPE, Cidade Universitária, Recife, Pernambuco, Brazil*

[b] *Centro de Biociências, Departamento de Zoologia, Laboratório de Genômica Evolutiva e Ambiental- LAGEA, Rua Prof. Nelson Chaves, s/n, Universidade Federal de Pernambuco - UFPE, Cidade Universitária, Recife, Pernambuco, Brazil*

[c] *Laboratório de Biologia de Crustáceos - Praça Infante D. Henrique, s/n, Universidade Estadual Paulista - UNESP, Campus Experimental do Litoral Paulista (CLP), São Vicente, São Paulo, Brazil*

[d] *Centro Acadêmico de Vitória – CAV, Rua do Alto do Reservatório, s/n, Universidade Federal de Pernambuco - UFPE, Bela Vista, Vitória de Santo Antão, Pernambuco, Brazil*

A R T I C L E I N F O

Keywords:
Comet assay
Micronucleus
Environmental risk
Estuary systems
Mullets
Water contamination

A B S T R A C T

The largest metropolitan centers in northeastern region of Brazil are all located near the coast, and industrial, tourist, and agro-industrial activities are the principal causes of water contamination due to discharges of untreated sewage. Adverse environmental conditions can often be detected by analyzing the genetic material of organisms exposed to pollutants, and furnish an overview of environmental quality. We evaluated possible damage to the DNA of one of the fish resources most widely consumed and commercialized by coastal communities in northeastern Brazil, *Mugil curema* ("tainha"). Erythrocytes from *M. curema* were analyzed by the presence of micronuclei and by comet assay (single cell gel electrophoresis, SCGE). Statistical comparisons to both tests revealed considerably greater genomic damage in polluted estuaries than in the control site ($p < 0.05$), suggesting strong genotoxic impacts on the specimens evaluated, principally among those taken near localities with dense demographic and industrial development.

1. Introduction

Intense industrial and urban development have resulted in strong and direct impacts on water resources throughout the world. The introduction of chemical compounds prejudicial to environmental health has impacted aquatic biodiversity, ecological equilibrium, and damage the fishing resources of subsistence communities (Amorim, 2003; Azevedo et al., 2013; Ghisi et al., 2014).

Globally, approximately 1.2 billion people live within 100 km of the coast and 100 m above sea level, resulting in human population densities three times greater than the mean global density (Small and Nicholls, 2003). That situation intensifies along low-elevation coastal areas (< 10 m above sea level), which represent only 2% of the global surface but are occupied by 13% of the world's urban population (McGranahan et al., 2007).This situation is due, in large part, to the commerce of agricultural and industrial goods, which inevitably culminates in the establishment of large, dense urban populations. Jiang et al. (2014) confirmed the occurrence of environmental contamination resulting from the discharge of domestic and industrial wastes into the coastal waters of southwestern Taiwan (where 8 million people reside). Similar situations have been reported in other countries in Asia, Europe, and the Americas (Hernández-Terrones et al., 2015; Spiteri et al., 2016; Wang et al., 2016), including Brazil, where the bulk of the population is concentrated near coastal or estuarine systems (IBGE, 2014). The high human population densities of those localities in Brazil result in high effluent discharges and reductions of adjacent water resource quality (Barletta and Costa, 2009; Souza et al., 2013).

Estuary and mangrove swamp systems are important biodiversity hotspots, as large numbers and wide diversities of organisms spend much (or all) of their lives in those environments, but the noxious ef-

* Corresponding author at: Centro de Biociências, Programa de Pós-Graduação em Ciências Biológicas – PPGCB, Av. Prof. Moraes Rego, 1235, Universidade Federal de Pernambuco - UFPE, Cidade Universitária, Recife, Pernambuco, Brazil.

E-mail addresses: anderson_rodrigues.184@hotmail.com (A.R.B. Lima), rodrigotorres@ufpe.br (R.A. Torres), pinheiro@clp.unesp.br (M.A.A. Pinheiro).

https://doi.org/10.1016/j.marpolbul.2018.07.037
Received 25 February 2018; Received in revised form 1 July 2018; Accepted 12 July 2018
0025-326X/ © 2018 Elsevier Ltd. All rights reserved.

fects of pollutants can affect the ecological equilibrium of those ecosystems, threaten the sustainable economic use of their resources, and compromise public health through the consumption of contaminated organisms (Abessa et al., 2008; Pereira et al., 2012). Those types of threats are recurrent in estuary and mangrove sites (Duarte et al., 2016, 2017), demanding the creation of diagnostic programs of environmental quality (Azevedo et al., 2013; Benincá et al., 2011; Pereira et al., 2012; Pinheiro et al., 2013).

Among the principal environmental contaminants of water resources are heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides – all of which are widely discharged into the environment to pollute rivers, lakes, and estuary systems – and becoming persistent contaminants due to the unique sedimentary characteristics of those sites (Bianchi et al., 2011; Maciel et al., 2015; Manzano et al., 2015; Pinheiro et al., 2013). Those contaminants have been confirmed to have genotoxic properties in numerous organisms and cause greater or lesser damage to their genetic material (De Flora et al., 1991; Pinheiro et al., 2013; Tsangaris et al., 2011) depending on their concentrations and the degrees of exposition (acute or chronic) of the affected species. As such, evaluations of the ecological health of estuary ecosystems are extremely important to the conservation of regional biodiversity, and bioindicators of genetic damage have been widely used to that end (Adam et al., 2010; Bolognesi and Hayashi, 2011; Catanhêde et al., 2014; Gusso-Choueri et al., 2015; Ohe et al., 2004).

Rapid, precise, and low-cost diagnostic technologies have become increasingly popular, and one of the most efficient is the Comet assay (CA), which can detect breaks in DNA molecules (Singh et al., 1988) which, combined with Micronuclei (MN) tests, can be sufficiently sensitive to detect chromosome damage (Bolognesi and Hayashi, 2011). Those methods have already proven their efficiency with various groups of organisms, including fish (Adam et al., 2010; Barsiene et al., 2013; Ragugnetti et al., 2011; Sponchiado et al., 2011), crustaceans (Pinheiro et al., 2013), and mollusks (Rocha et al., 2014).

The family Mugilidae comprises 74 species of fish inhabiting coastal waters (Eschmeyer, 2017; Eschmeyer and Fong, 2017) that have relevant ecological and economic value (Durand et al., 2012). Of those 74 species, nine (12.2%) belong to the genus *Mugil*, and *M. liza*, *M. brevirostris*, and *M. curema* are widely distributed and found along the entire coast of Brazil (Barletta and Dantas, 2016). They are popularly known in Brazil as "tainhas" (mullets), and are considered optimal diagnostic sentinels of coastal environmental health (Hauser-Davis et al., 2016), especially of estuaries and mangrove swamps influenced by tides. A number of authors have reported *Mugil* spp. as an important bio-accumulator of heavy metals and other food chain pollutants (e.g., *M. liza* - Marcovecchio, 2004; *M. cephalus* - Dural et al., 2007; and *M. curema* - Carmo et al., 2013), especially in their gills and livers. It is important to note that those fish are consumed in many parts of the world, with artisanal fishing being one of the principal activities of small coastal communities, especially in northeastern Brazil (Araújo and Silva, 2013). *Mugil curema* (white mullet), for example, is one of the fishing resources most consumed in those communities in Brazil (Isaac et al., 2006), with catches estimated at > 18,000 tons/year (Pesca, 2011). Small (< 16 cm total length) juvenile mullet are also captured and used as bait for other fish (Ditty and Shaw, 1996; Ibáñez and Gutiérrez-Benítez, 2004).

In light of the importance of *M. curema* as a fishing resource, massive disturbances of coastal aquatic environments, and the potential for using Mugilidae as sentinels of environmental quality, the present study under took a diagnosis of the genomic damages suffered by *M. curema* as a measure of anthropogenic disturbance levels in estuary systems in northeastern Brazil.

Table 1
Estuaries along the coast of Pernambuco State, Brazil, and the numbers of specimens collected in each.

Estuary	N° of specimens collected
Goiana River	10
Jaguaribe River	14
Capibaribe River	10
Sirinhaém River	10
Formoso River	10

2. Materials and methods

2.1. Study area and sampling locations

Pernambuco State, Brazil, has a shoreline of 187 km, with 21 coastal municipalities that account for 44% of its population (Araujo et al., 2007). This high population density indicates the degree of disturbance experienced by mangrove swamps/estuaries in the region, as all of the wastes from human populations in the region are discharged into those environments.

Fifty-four specimens of *M. curema* from five river estuaries (Goiana, Jaguaribe, Capibaribe, Sirinhaém, and Formoso) in Pernambuco State, in northeastern Brazil, were sampled between December/2014 and February/2015 (Table 1 and Fig. 1). Those estuaries were selected based on their conservation statuses according to reports from the State Environment Agency (CPRH - PE) (Table 2). According to the CPRH, which undertakes systematic monitoring of water quality in the hydrographic basins of Pernambuco State, of those five estuaries, only that of the Formoso River demonstrated quality parameters within acceptable levels (as defined by Resolução CONAMA 357, de 17 de março de, 2005).

Specimens of *M. curema* were also obtained from the Una River estuary (the Juréia-Itatins Ecological Station), a protected area in São Paulo State, in southeastern Brazil, for comparative purposes, as Duarte et al. (2016 and 2017) described that region as having a pristine environment.

2.2. Slide preparation and analyses

2.2.1. Micronuclei tests (MN)

Smears of peripheral blood were prepared from the specimens collected. The slides were stained with Giemsa (7.5%) for approximately six minutes at room temperature, and the erythrocytes were then analyzed under an oil immersion microscope (100×). The criteria utilized to identify typical micronuclei were: their morphology (rounded); the size of the micronucleus (smaller than, or equal to, one third of the principal nucleus); and coloration (equal to the cellular nucleus). The total number of erythrocytes analyzed was standardized at 1000 cells/specimen. Two slides per animal were analyzed.

2.2.2. Comet assay (CA)

For the comet assay analyses, blood samples were removed from the caudal vein using heparinized hypodermic syringes. 10 μL blood samples from each specimen were mixed with 120 μL of low fusion point agarose (0.5%) and the suspensions then spread on slides previously coated with standard agarose (1.5%). The slides were subsequently immersed in a cold (4 °C) lysissolution (2.5 M NaCl; 100 mM EDTA; 1% Triton x-100; and 10% DMSO, pH 10) and maintained under refrigeration in the dark for one hour. After cell lysis, the slides were placed in an electrophoresis cube with TBE (TrisBoro EDTA 1×) buffer. The slides were left in that solution for 20 min before beginning the

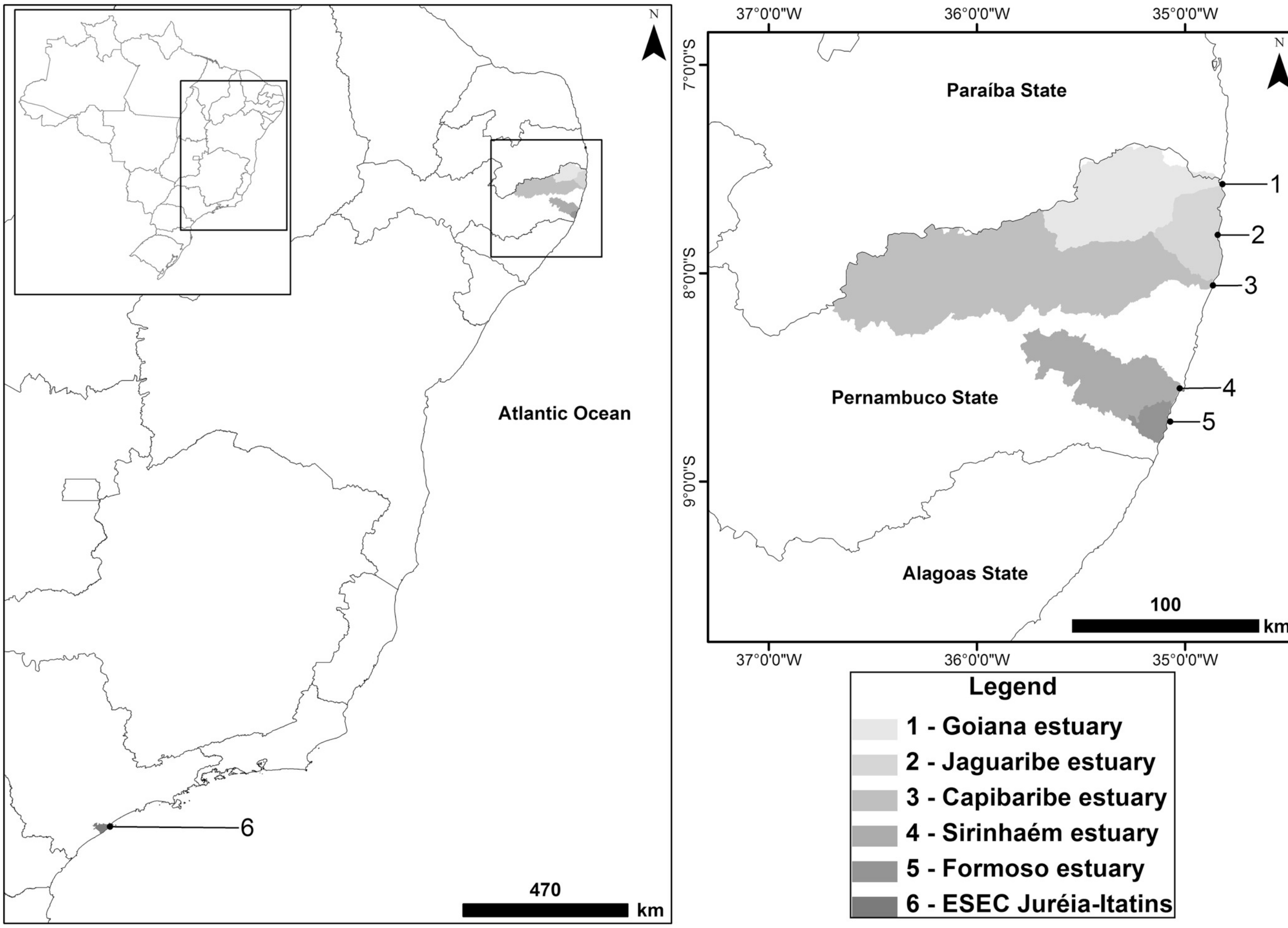

Fig. 1. Map of the Brazilian coast, indicating the locations of the estuaries evaluated and the control region.

Table 2
Descriptions of the estuary regions studied, according to the Pernambuco State Environmental Agency (CPRH).

Goiana River	Located in the extreme north of the state, formed by the Goiana, Megaó, Capibaribe Mirim, Tracunhaém, and Barra de Goiana rivers. The water pollution there is of industrial and domestic origin, and the site further suffers from deforestation of the mangrove swamp and landfill resulting from large carciniculture projects and represent serious threats to its preservation.
Jaguaribe River	The Jaguaribe River is 9 km long, and its margins harbor a great regional fauna and flora diversity. The construction of fish breeding tanks and predatory fishing represent the principal threats to that ecosystem.
Capibaribe River	The estuary of this river has historically lost extensive areas to urban expansion, with constant deforestation and landfill projects that have highly altered the mangrove swamp ecosystem.
Sirinhaém River	The estuary is quite exuberant, and occupies an area of approximately 3335 ha, from Porto de Galinhas to Serrambi. The rich diversity of fish and crustaceans species there represent an important source of income for many local populations.
Formoso River	The underwater reefs near the coastline act as natural barriers and guarantee low amplitude differences between the tides, favoring the local vegetation and fauna. As the estuary is large and well conserved, the diverse fauna there provides resources for many local communities.

Table 3
Comparison of the frequencies of micronucleated cells found in *Mugil curema* among estuaries evaluated by Tukey test (a posteriori).

	Juréia (1)	Goiana (2)	Jaguaribe (3)	Capibaribe (4)	Sirinhaém (5)	Formoso (6)
	M = 2.1 SD = 1.6	M = 5.6 SD = 2.5	M = 5.4 SD = 2.5	M = 8.2 SD = 2.0	M = 6.2 SD = 2.8	M = 4.6 SD = 2.8
(1)		0.0078**	0.0047**	0.0001***	0.0011**	0.1156
(2)	0.0078**		0.9999	0.1637	0.9933	0.9366
(3)	0.0047**	0.9999		0.0722	0.9705	0.9599
(4)	0.0001***	0.1637	0.0722		0.4331	0.0162*
(5)	0.0011**	0.9933	0.9705	0.4331		0.6707
(6)	0.1156	0.9366	0.9599	0.0162*	0.6707	

M - average frequency of micronucleated cells.
SD - Standard deviation.
Significant difference – level of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

electrophoretic runs, to allow for DNA denaturation. Electrophoresis was then initiated at 25 V and 300 mA, for 25 min. Upon terminating that procedure, the slides were fixed in absolute ethanol and stained with a Gel Red (1:100) solution. Two hundred nucleoids from each specimen were analyzed using an epifluorescence microscope; the visual classification system utilized was based on the migration of DNA fragments, ordered in classes that varied from 0 to 4 depending on the tail formed by the damaged DNA.

Data analyses were performed using two distinct parameters: (a) the damage index (DI), calculated as a result of multiplying the numbers of comets in each class by the denominator digit of that class (0, 1, 2, 3 or 4); and (b) the damage frequency (DF), calculated as the percentage of all of the comets in relation to the total number of nucleoids evaluated.

2.2.3. Statistical analyses

The data were tested for normality (Shapiro-Wilk) and homoscedasticity (Bartlett) that, if confirmed, would allow the means of the samples to be subjected to analysis of variance (ANOVA), followed by the application of the a posteriori Tukey test at a significance level of 5%.

3. Results

The mean numbers of MNs per estuary varied from 2.1 (Juréia-Itatins) to 8.2 (Capibaribe River). Erythrocyte analyses demonstrated significant differences in the quantities of MNs between the control site (Juréia-Itatins) and the most of the estuaries studied (Tukey, $p < 0.05$; Table 3). The Formoso River was the only estuary that demonstrated similar results to the control in terms of DNA macrolesions (as measured by micronuclei) (Tukey test, $p = 0.1156$; Table 3).

The mean numbers of DI per estuary varied from 14.6 (Juréia-Itatins) to 64.5 (Capibaribe River) (Table 4); and DF varied from 14.6 (Formoso River) to 108.3 (Capibaribe River) (Table 5).

The DI revealed statistically significant differences in the occurrence of comets in specimens of *M. curema* in most of the estuaries when compared to the control group ($p < 0.05$; Table 4). Statistically significant differences in the occurrence of comets (DI) among the specimens from each of the estuaries were confirmed by the Tukey test ($p < 0.05$; Table 4); only the Formoso River estuary demonstrated DI values similar to the control group ($p = 0.9611$; Table 4).

The results of the DF mirrored the patterns observed in the DI analyses, except in terms of the Jaguaribe site (which was not statistically different from the control site) ($p = 0.3779$; Table 5).

4. Discussion

Variations in the numbers of MNs and comets, as well as measures of their central tendencies per estuary in Pernambuco, suggested a critical situation for the conservation of *M. curema*, and reinforced the validity of using that species as a sentinel for evaluating estuary environments in light of its sensitivity to genomic damage. Our results supported the hypothesis of an elevated degree of disturbance in those estuaries as a result of anthropic activities as compared to the Juréia-Itatins Ecological Station (one of the few pristine estuary systems in São Paulo State) (Duarte et al., 2016, 2017). Studies of genomic damage in coastal species partially or totally isolated from anthropogenic impacts have shown in general less intense genetic damage than recorded in areas with dense human occupation (even past human occupation) (Catanhêde et al., 2014; Gusso-Choueri et al., 2015, 2016; Pinheiro et al., 2013). The significantly lower occurrence of macro- and microlesions in the low-impact Juréia-Itatins control area reflected that this area is largely free from anthropic disturbances and is only sparsely inhabited by traditional populations. The low levels of disturbance in Juréia-Itatins are likewise supported by studies of a marine invertebrates species (Duarte et al., 2016, 2017; Pinheiro et al., 2013). As such, our results reinforced the results of the water quality monitoring periodically undertaken by the environmental office of Pernambuco State (CPRH, 2010) indicating areas that are more or less well-preserved according to their different levels of genomic damage.

The MNs and comets data indicate a strong association between high levels of macro- and microlesions in the genome of *M. curema* and the proximity of estuaries to intensely urbanized regions, tourist activities, and sugarcane plantations. Tourism has been increasingly associated with anthropic disturbances in marine ecosystems along the northeastern coast of Brazil (Santos et al., 2015; Sarmento and Santos, 2011), with increasing releases of untreated sewage in those areas. Environmental disturbances originating from distinct sources of urban sewage have been well-documented in the southern Atlantic – increasing concern for the conservation of those areas (Berbel et al., 2015). Additionally, the use of herbicides and insecticides in rural environments (such as sugar cane plantations) can impact the ontogenetic development of vertebrate populations and their physiological activities (Procópio et al., 2014), resulting in expressive toxicity and genomic damage (Franco-Bernardes et al., 2014). The genomic damage observed in *M. curema* is similar to that reported for other species around the globe, such as the blue muscle *Mytilus edulis* along the southwestern coast of England (Dallas et al., 2013), and the fish species *Mugil cephalus* in coastal estuaries in Portugal (Carrola et al., 2014). Those sets of observations reinforce the severe impacts that *M. curema* suffers in the environments studied here, and provide evidence of the global deterioration of coastal ecosystems.

When the MNs data of the estuaries studied in Pernambuco was compared to that of the pristine estuary at Juréia-Itatins a statistically significant increase of 2.5 to 3.9 times ($p < 0.05$) more genomic (macrolesion) damage was observed. Those results perfectly reflect the urbanization patterns along the coast of Pernambuco in recent decades, and mirror the greatly increased establishment of industrial centers and tourist activities in coastal areas in many parts of the world that impact the physical and chemical characteristics of estuary waters (Berbel et al., 2015; Benincá et al., 2011; Galindo and Moreira, 2009; Sharif

Table 4
Comparison of genomic damage indices (DI) by comet assay observed in *Mugil curema* among estuaries evaluated by Tukey test (a posteriori).

	Juréia (1)	Goiana (2)	Jaguaribe (3)	Capibaribe (4)	Sirinhaém (5)	Formoso (6)
	M = 14.6 SD = 10.9	M = 54.1 SD = 6.2	M = 29.1 SD = 13.8	M = 64.5 SD = 33.6	M = 42.6 SD = 11.3	M = 17.5 SD = 5.8
(1)		0.0001***	0.0004**	0.0001***	0.0001***	0.9611
(2)	0.0001***		0.0001***	0.0898	0.0415*	0.0001***
(3)	0.0004**	0.0001***		0.0001***	0.0046**	0.0196*
(4)	0.0001***	0.0898	0.0001***		0.0001***	0.0001***
(5)	0.0001***	0.0415*	0.0046**	0.0001***		0.0001***
(6)	0.9611	0.0001***	0.0196*	0.0001***	0.0001***	

M - average of the genomic damage indices.
SD - Standard deviation.
Significant difference – level of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

Table 5
Comparison of genomic damage frequencies (DF) by comet assay observed in *Mugil curema* among estuaries evaluated by Tukey test (a posteriori).

	Juréia (1)	Goiana (2)	Jaguaribe (3)	Capibaribe (4)	Sirinhaém (5)	Formoso (6)
	M = 15.3 SD = 8.3	M = 42.1 SD = 2.8	M = 20.6 SD = 6.8	M = 108.3 SD = 11,5	M = 30.2 SD = 5.5	M = 14.6 SD = 3.9
(1)		0.0001***	0.3779	0.0001***	0.0001***	0.9998
(2)	0.0001***		0.0001***	0.0001***	0.0059**	0.0001***
(3)	0.3779	0.0001***		0.0001***	0.0231*	0.3523
(4)	0.0001***	0.0001***	0.0001***		0.0001**	0.0001***
(5)	0.0001***	0.0059*	0.0231*	0.0001***		0.0002**
(6)	0.9998	0.0001***	0.3523	0.0001***	0.0002**	

M - average frequency of genomic damage.
SD - Standard deviation.
Significant difference– level of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

et al., 2014). Those same processes are modifying coastal areas in northeastern Brazil, particularly near large urban centers (with their alarming levels of pollution) (Maciel et al., 2015). Fortunately, many of those environmental changes are being more closely monitored in many parts of the world (Barsiene et al., 2013; Çavas and Ergene-Gozukara, 2005).

The comets data showed that pollution along the coast of Pernambuco State is affecting marine life at the molecular level ($p < 0.05$). The quantitative comets measurements from various estuaries there were very similar to data collected in other parts of the world (Lee and Steinert, 2003; Rank et al., 2005), altering the environmental characteristics of those regions, impacting the integrity of the genome of exposed species, threatening their survival, and consequently generating economic losses among traditional communities that depend on subsistence fishing (Araldi et al., 2015).

The results of the Tukey tests with MNs demonstrated significant differences ($p < 0.05$) between the Capibaribe, Goiana, Jaguaribe, and Sirinhaém river estuaries and the control region. The Formoso River estuary was the only site that generated data similar to the control group ($p > 0.05$), indicating it as the most conserved region among the five estuaries evaluated. This state of conservation, indicated by the evaluation of genomic damages observed here for the Formoso River estuary, is probably due to the fact that this estuary is in an area of environmental protection established by the Brazilian government. In other parts of the world, areas that comprise valuable water resources show problems related to human occupation similar to those of northeastern Brazil, with the same patterns of genomic damage (Arslan et al., 2015; Floehr et al., 2015). Although it is difficult to determine with certainty which contaminants are responsible to those damages, pesticides applied to sugarcane plantations are almost certainly one of the major causal agents of genotoxic damage (Arslan et al., 2015; Gutiérrez et al., 2015).

The Tukey analysis of comets revealed patterns similar to those of the MNs, suggesting that the DNA is being damaged at both macro and microlesion levels and the existence of significant impacts of pollution in those environments. The degrees of macro and microlesion damage to DNA (MNs and comets, respectively) considered together demonstrated a decreasing hierarchical ordering of the magnitudes of human impacts on coastal ecosystems: Capibaribe > Goiana > Sirinhaém > Jaguaribe > Formoso ≈ Juréia-Itatins (the control site).

The current panorama of global human coastal occupation will necessitate the constant monitoring of those natural resources to be able to protect their biodiversity richness (Carrola et al., 2014; Dallas et al., 2013; Hong et al., 2012). As such, the data presented here in can contribute to the proactive elaboration and implementation of conservation efforts to reduce (and hopefully revert) damage to coastal ecosystems in Brazil and the species they harbor, and contribute to the conservation of *M. curema* in the estuaries systems evaluated here. Our results could also be used to select priority areas for conservation measures, mainly in areas that do not yet show alarmingly high levels of degradation in terms of genomic damage – such as the Formoso River estuary – but which will continue to suffer progressive damage unless proactive measures are quickly adopted.

5. Conclusions

The results of the present study demonstrated that four of the five estuaries evaluated in Pernambuco State, in northeastern Brazil, show alarming levels of anthropogenic environmental impacts. Those four estuaries can be ordered in decreasing degrees of environmental degradation as: Capibaribe > Goiana > Sirinhaém > Jaguaribe. The Formoso River estuary (which is included in the Costa dos Corais Environmental Protection Area) was the only site that demonstrated environmental conditions similar to the control site.

The fish species *M. curema*, present in the four impacted estuaries, is threatened by high degrees of environmental pollution that provoke genotoxicity. It will therefore be necessary to initiate measures that can restore the environmental equilibrium of those ecosystems and help guarantee the survival of that species and other members of the estuarine fauna.

Artisanal fishing, often at the subsistence level, is threatened by negligence and by the lack of proactive environmental restoration efforts, and regular and detailed diagnoses of environmental degradation in coastal areas should be used to orient reparatory measures by environmental agencies.

Acknowledgements

The authors thank the Fundação de Amparo à CiênciaeTecnologia do Estado de Pernambuco (FACEPE) (Proc. n° APQ-0017-2.02/11) and the Instituto Nacional de CiênciaeTecnologia – INCT tropical environments/CNPq (Proc. n° 565054/2010-4) for their financial support throughout the present work; Caroline A. Souza (CRUSTA – UNESP IB/CLP), Isabela C. L. Costa, and Rafael Albuquerque (laboratory technician) for their help during the field expeditions and laboratory analyses. RAT and MAAP especially thank CNPq for the research fellowship grants awarded (Grants # 301208/2012-3 and 303286/2016-4 respectively).

References

Abessa, D.M.S., Carr, R.S., Sousa, E.C.P.M., Rachid, B.R.F., Zaroni, L.P., Pinto, Y.A., 2008. Integrative ecotoxicological assessment of a complex tropical estuarine system. Mar. Pollut. New Res. 1–36.

Adam, M.L., Torres, R.A., Sponchiado, G., Motta, T.S., Oliveira, C.M.R., Carvalho-Filho, M.A., Correia, M.T.S., 2010. Environmental degradation at a Public Park in southern Brazil as revealed through a genotoxicity test (MN) on peripheral blood cells from *Poecilia vivipara* (Teleostei). Water Air Soil Pollut. 211, 61–68.

Amorim, L.C.A., 2003. Os biomarcadores e sua aplicação na avaliação da exposição aos agentes químicos ambientais. Rev. Bras. Epidemiol. 6, 158–170.
Araldi, R.P., de Melo, T.C., Mendes, T.B., de Sa Junior, P.L., Nozima, B.H., Ito, E.T., 2015. Using the comet and micronucleus assays for genotoxicity studies: a review. Biomed Pharmacother 72, 74–82.
Araújo, A.R., Silva, F.D., 2013. Aspects of the fishery and biology of mullet, *Mugil curema* (Osteichthyes: Mugilidae), in the Vaza Barris River estuary, Sergipe State, Brazil. Arq. Cienc. Mar 46 (1), 29–38.
Araujo, M.C.B., Souza, S.T., Chagas, A.C.O., Barbosa, S.C.T., Costa, M.F., 2007. Análise da Ocupação Urbana das Praias de Pernambuco, Brasil. Revista da Gestão Costeira Integrada 97–104.
Arslan, O.C., Boyaciog, M., Parlak, H., Katalay, S., Karaaslan, M.A., 2015. Assessment of micronuclei induction in peripheral blood and gill cells of some fish species from Aliag˘a Bay Turkey. Mar. Pollut. Bull. 94, 48–54.
Azevedo, J.S., Braga, E.S., Silva de Assis, H.C., Ribeiro, C.A.O., 2013. Biochemical changes in the liver and gill of *Cathorops spixii* collected seasonally in two Brazilian estuaries under varying in fluences of anthropogenic activities. Ecotoxicol. Environ. Saf. 96, 220–230.
Barletta, M., Costa, M.F., 2009. Living and non-living resources exploitation in a tropical semi-arid estuary. J. Coast. Res. 56, 371–375.
Barletta, M., Dantas, D.V., 2016. Biogeography and distribution of Mugilidae in the Americas, 42–62. In: Crosetti, D., Blaber, S.J.M. (Eds.), Biology, Ecology and Culture of Grey Mullets (Mugilidae). CRC Press, Boca Raton (539p).
Barsiene, J., Rybakovas, A., Lang, T., Andreikenaite, L., Michailovas, A., 2013. Environmental genotoxicity and cytotoxicity levels in fish from the North Sea offshore region and Atlantic coastal waters. Mar. Pollut. Bull. 68 (1–2), 106–116.
Benincá, C., Ramsdorf, W., Vicari, T., Ribeiro, C.A.O., Almeida, M.I., Assis, H.C.S., 2011. Chronic genetic damages in *Geophagus brasiliensis* exposed to anthropic impact in Estuarine Lakes at Santa Catarina Coast–Southern of Brazil. Environ. Monit. Assess. 184, 2045.
Berbel, G.B.B., Favaro, D.I.T., Braga, E.S., 2015. Impact of harbour, industry and sewage on the phosphorus geochemistry of a subtropical estuary in Brazil. Mar. Pollut. Bull. 93 (1–2), 44–52.
Bianchi, J., Espindola, E.L., Marin-Morales, M.A., 2011. Genotoxicity and mutagenicity of water samples from the Monjolinho River (Brazil) after receiving untreated effluents. Ecotoxicol. Environ. Saf. 74 (4), 826–833.
Bolognesi, C., Hayashi, M., 2011. Micronucleus assay in aquatic animals. Mutagenesis 26 (1), 205–213.
Carmo, C.A., Abessa, D.M.S., Machado-Neto, J.G., 2013. Metals in muscles of mullet (*Mugil curema*) from a contaminated estuary: evidences of potential risks to public health. Nat. Res. 2, 81–94.
Carrola, J., Santos, N., Rocha, M.J., Fontainhas-Fernandes, A., Pardal, M.A., Monteiro, R.A., 2014. Frequency of micronuclei and of other nuclear abnormalities in erythrocytes of the grey mullet from the Mondego, Douro and Ave estuaries—Portugal. Environ. Sci. Pollut. Res. Int. 21 (9), 6057–6068.
Catanhêde, S.M., Medeiros, A.M., Ferreira, F.S., Ferreira, J.R.C., Alves, L.M.C., Cutrim, M.V.J., Santos, D.M.S., 2014. Uso de biomarcador histopatológico em brânquias de *Centropomus undecimalis* (Bloch, 1972) na avaliação da qualidade da água do Parque Ecológico Laguna da Jansen, São Luís – MA. Revista Arq. Bras. Med. Vet. Zootec. 66, 593–601.
Çavas, T., Ergene-Gozukara, S., 2005. Micronucleus test in fish cells: a bioassay for in situ monitoring of genotoxic pollution in the marine environment. Environ. Mol. Mutagen. 46, 64–70.
CPRH, 2010. Agência Estadual de Meio Ambiente/PE. http://www.cprh.pe.gov.br/perfis_ambientais/areas_estuarinas/39751%3B33012%3B1802%3B0%3B0.asp.
Dallas, L.J., Cheung, V.V., Fisher, A.S., Jha, A.N., 2013. Relative sensitivity of two marine bivalves for detection of genotoxic and cytotoxic effects: a field assessment in the Tamar Estuary, South West England. Environ. Monit. Assess. 185, 3397–3412.
De Flora, S., Bagnasco, M., Zanacchi, P., 1991. Genotoxic, carcinogenic, and teratogenic hazards in the marine environment, with special reference to the Mediterranean Sea. Mutat. Res. 258 (3), 285–320.
Ditty, J.G., Shaw, R.F., 1996. Spatial and temporal distribution of larval striped mullet (*Mugil cephalus*) and white mullet (*M. curema*, family: Mugilidae) in the northern Gulf of Mexico, with notes on mountain mullet, *Agonostomus monticola*. Bull. Mar. Sci. 59, 271–288.
Duarte, L.F.A., Souza, C.A., Nobre, C.R., Pereira, C.D., Pinheiro, M.A.A., 2016. Multi-level biological responses in Ucides cordatus (Linnaeus, 1763) (Brachyura, Ucididae), as indicators of conservation status in mangrove areas from the Western Atlantic. Ecotoxicol. Environ. Saf. 133, 176–187.
Duarte, L.F., Souza, C.A., Pereira, C.D.S., Pinheiro, M.A.A., 2017. Metal toxicity assessment by sentinel species of mangroves: in situ case study integrating chemical and biomarkers analyses. Ecotoxicol. Environ. Saf. 145, 367–376.
Dural, M., Göksu, M.Z.L., Özak, A.A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chem. 102, 415–421.
Durand, J.D., Shen, K.N., Chen, W.J., Jamandre, B.W., Blel, H., Diop, K., Nirchio, M., Garcia de León, F.J., Whitfield, A.K., Chang, C.W., Borsa, P., 2012. Systematic of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. Mol. Phylogenet. Evol. 64, 73–92.
Eschmeyer, W.N., 2017. Catalog of Fishes: Genera, Species, References. Disponível em. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (Acesso em: 04 set. 2017).
Eschmeyer, W.N., Fong, J.D., 2017. Catalog of Fishes: Species by Family/Subfamily. Disponível em. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp (Acesso em: 04 set. 2017).
Floehr, T., Scholz-Starke, B., Xiao, H., Koch, J., Wu, L., 2015. Yangtze Three Gorges Reservoir, China: a holistic assessment of organic pollution, mutagenic effects of sediments and genotoxic impacts on fish. J. Environ. Sci. 38, 63–82.
Franco-Bernardes, M.F., Maschio, L.R., Azevedo-Oliveira, M.T.V., Almeida, E.A., 2014. Biochemical and genotoxic effects of a commercial formulation of the herbicide tebuthiuron in Oreochromis niloticus of different sizes. Ecotoxicol. Environ. Contam. 9, 59–67.
Galindo, T.P., Moreira, L.M., 2009. Evaluation of genotoxicity using the micronucleus assay and nuclear abnormalities in the tropical sea fish *Bathygobius soporator* (Valenciennes, 1837) (Teleostei, Gobiidae). Genet. Mol. Biol. 32 (2), 394–398.
Ghisi, N.C., de Oliveira, E.C., Favaro, L.F., Silva de Assis, H.C., Prioli, A.J., 2014. In situ assessment of a neotropical fish to evaluate pollution in a river receiving agricultural and urban wastewater. Bull. Environ. Contam. Toxicol. 93 (6), 699–709.
Gusso-Choueri, P.K., Choueri, R.B., de Araújo, G.S., Cruz, A.C.F., Stremel, T., Campos, S., ... Ribeiro, C.A.O., 2015. Assessing pollution in marine protected areas: the role of a multi-biomarker and multi-organ approach. Environ. Sci. Pollut. Res. 22, 18047–18065.
Gusso-Choueri, P.K., Choueri, R.B., Santos, G.S., Araújo, G.S., Cruz, A.C.F., Stremel, T., Campos, S.X., Cestari, M.M., Ribeiro, C.A.O., Abessa, D.M.S., 2016. Assessing genotoxic effects in fish from a marine protected area influenced by former mining activities and other stressors. Mar. Pollut. Bull. 104, 229–239.
Gutiérrez, J.M., Villar, S., Plavan, A.A., 2015. Micronucleus test in fishes as indicators of environmental quality in subestuaries of the Río de la Plata (Uruguay). Mar. Pollut. Bull. 91, 518–523.
Hauser-Davis, R.A., Bordon, I.C.A.C., Oliveira, T.F., Ziolli, R.L., 2016. Metal bioaccumulation in edible target tissues of mullet (*Mugil liza*) from a tropical bay in Southeastern Brazil. J. Trace Elem. Med. Biol. 36, 38–43.
Hernández-Terrones, L.M., Null, K.A., Ortega-Camacho, D., Paytan, A., 2015. Water quality assessment in the Mexican Caribbean: impacts on the coastal ecosystem. Cont. Shelf Res. 102, 62–72.
Hong, Y.W., Yu, S., Yu, G.B., Liu, Y., Li, G.L., Wang, M., 2012. Impacts of urbanization on surface sediment quality: evidence from polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) contaminations in the Grand Canal of China. Environ. Sci. Pollut. Res. 19 (5), 1352–1363.
Ibáñez, A.L., Gutiérrez-Benítez, O., 2004. Climate variables and spawning migrations of the striped mullet and white mullet in the north-western area of the Gulf of Mexico. J. Fish Biol. 65, 822–831.
IBGE-Instituto Brasileiro de Geografia e Estatística, 2014. Estimativas de população por cidades. Avaliable from. http://cidades.ibge.gov.br/xtras/home.php.
Isaac, V.J., Martins, A.S., Haimovici, M., Andriguetto Filho, J.M., 2006. A pesca marinha e estuarina do Brasil no início do século XXI: recursos, tecnologias, aspectos socioeconômicos e institucionais. In: Editora Universitária UFPA, pp. 67–91.
Jiang, J.J., Lee, C.L., Fang, M.D., 2014. Emerging organic contaminants in coastal waters: anthropogenic impact, environmental release and ecological risk. Mar. Pollut. Bull. 85, 391–399.
Lee, R.F., Steinert, S., 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat. Res. 544, 43–64.
Maciel, D.C., Souza, J.R.B., Taniguchi, S., Bícego, M.C., Zanardi-Lamardo, E., 2015. Sources and distribution of polycyclic aromatic hydrocarbons in an urbanized tropical estuary and adjacent shelf, Northeast of Brazil. Mar. Pollut. Bull. 101, 429–433.
Manzano, B.C., Roberto, M.M., Hoshina, M.M., Menegario, A.A., Marin-Morales, M.A., 2015. Evaluation of the genotoxicity of waters impacted by domestic and industrial effluents of a highly industrialized region of Sao Paulo State, Brazil, by the comet assay in HTC cells. Environ. Sci. Pollut. Res. Int. 22 (2), 1399–1407.
Marcovecchio, J., 2004. The use of *Micropogonias furnieri* and *Mugil liza* as bioindicators of heavy metals pollution in La Plata river estuary, Argentina. Sci. Total Environ. 323, 219–226.
McGranahan, G., Balk, D., Anderson, B., 2007. The rising tide: assessing the risks of climate change and human settlements in low elevation coastal zones. Environ. Urban. 19, 17–37.
Ohe, T., Watanabe, T., Wakabayashi, K., 2004. Mutagens in surface waters: a review. Mutat. Res. 567 (2–3), 109–149.
Pereira, C.D., Martín-Díaz, M.L., Catharino, M.G., Cesar, A., Choueri, R.B., Taniguchi, S., 2012. Chronic contamination assessment integrating biomarkers responses in transplanted mussels-a seasonal monitoring. Environ. Toxicol. 27, 257–267.
Pesca, B.D., 2011. In: M. D. P. E. Aquicultura (Ed.), Boletim Estatístico da Pesca e Aquicultura.
Pinheiro, M.A., Duarte, L.F., Toledo, T.R., Adam, M.L., Torres, R.A., 2013. Habitat monitoring and genotoxicity in Ucides cordatus (Crustacea: Ucididae), as tools to manage a mangrove reserve in southeastern Brazil. Environ. Monit. Assess. 185 (10), 8273–8285.
Procópio, S.O., Fernandes, M.F., Teles, D.A., Sena-Filho, J.G., Cargnelutti-Filho, A., Resende, M.A., Vargas, L., 2014. Toxicity of herbicides used in the sugarcane crop to diazotrophic bacterium *Herbaspirillum seropedicae*. Semina Cienc. Agrar. 35 (5), 2383–2398.
Ragugnetti, M., Adam, M.L., Guimarães, A.T.B., Sponchiado, G., Vasconcelos, E.C., Oliveira, C.M.R., 2011. Ibuprofen genotoxicity in aquatic environment: an experimental model using *Oreochromis niloticus*. Water Air Soil Pollut. 218, 361–364.
Rank, J., Jensen, K., Jespersen, P.H., 2005. Monitoring DNA damage in indigenous blue mussels (*Mytilus edulis*) sampled from coastal sites in Denmark. Mutat. Res. 585, 33–42.
Resolução CONAMA 357, de 17 de março de, 2005. Conselho Nacional de Meio Ambiente.
Rocha, T.L., Gomes, T., Cardoso, C., Letendre, J., Pinheiro, J.P., Sousa, V.S., 2014. Immunocytotoxicity, cytogenotoxicity and genotoxicity of cadmium-based quantum

dots in the marine mussel Mytilus galloprovincialis. Mar. Environ. Res. 101, 29–37.
Santos, G.S., Burgos, D.C., Lira, S.M.A., Schwamborn, R., 2015. The impact of trampling on reef macrobenthos in northeastern Brazil: how effective are current conservation strategies? Environ. Manag. 56, 847–858.
Sarmento, V.C., Santos, P.J.P., 2011. Trampling on coral reefs: tourism effects on harpacticoid copepods. Coral Reefs 31, 135–146.
Sharif, A., Monperrus, M., Tessier, E., Bouchet, S., Pinaly, H., Rodriguez-Gonzalez, P., 2014. Fate of mercury species in the coastal plume of the Adour River estuary (Bay of Biscay, SW France). Sci. Total Environ. 496, 701–713.
Singh, N.P., Mccoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.
Small, C., Nicholls, R.J., 2003. A global analysis of human settlement in coastal zones. J. Coast. Res. 584–599.
Souza, I.C., Duarte, I.D., Pimentel, N.Q., Rocha, L.D., Morozesk, M., Bonomo, M.M., 2013. Matching metal pollution with bioavailability, bioaccumulation and biomarkers response in fish (Centropomus parallelus) resident in neotropical estuaries. Environ. Pollut. 180, 136–144.
Spiteri, C., Roddier-Quefelec, C., Giraud, J., Hema, T., 2016. Assessing the progress in depolluting the Mediterranean Sea. Mar. Pollut. Bull. 102, 295–308.
Sponchiado, G., Reynaldo, E.M.F.L., Andrade, A.C.B., Vasconcelos, E.C., Adam, M.L., Oliveira, C.M.R., 2011. Genotoxic effects in erythrocytes of Oreochromis niloticus exposed to nanograms-per-liter concentration of 17β-estradiol (E2): an assessment using micronucleus test and comet assay. Water Air Soil Pollut. 218, 353–360.
Tsangaris, C., Vergolyas, M., Fountoulaki, E., Nizheradze, K., 2011. Oxidative stress and genotoxicity biomarker responses in grey mullet (*Mugil cephalus*) from a polluted environment in Saronikos Gulf, Greece. Arch. Environ. Contam. Toxicol. 61 (3), 482–490.
Wang, P., Lu, Y., Wang, T., Meng, J., Li, Q., Zhu, Z., Sun, Y., Wang, R., Giesy, J.P., 2016. Shifts in 724 production of perfluoroalkyl acids affect emissions and concentrations in the environment of 725 the Xiaoqing River Basin, China. J. Hazard. Mater. 307, 55–63.