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Spatial-temporal genome damaging in the blue crab *Cardisoma guanhumi* as ecological indicators for monitoring tropical estuaries

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ABSTRACT

In this study, to better our understanding of the current state of conservation of *Cardisoma guanhumi* and its habitats, we evaluated the potential spatio-temporal genomic damage of this species across five estuaries in Brazil. The experiment was performed over two consecutive years, and the sampling was performed in the winter and summer seasons. Two genetic tests — micronucleus test and comet assay — were used to quantify the DNA damage. Unlike in the summers and in the winter of 2013, in the winter of 2012 a significant increase was noted in the frequency of micronucleated cells and genomic damage index. The occurrence of genomic damage coincided with the arrival of the harsh winter of 2012 as the water sourced from the coastal rivers significantly affected the estuarine species under study. Our results confirmed that this species was resilient to the atypical climatic conditions, which facilitated the generation of excessive waste.

1. Introduction

Of the coastal ecosystems, the estuarine systems are currently facing extreme threats, despite their economic significance importance (Barbier et al., 2011). These systems mostly form attractive and strategic locations that are preferred by humans for inhabitation, settlement, and for undertaking several types of anthropic activities. Owing to the ideal geographical features, estuaries are highly preferred locations for the establishment and development of large urban centers to facilitate industrial, marine, port, aquaculture, and agricultural activities (Elliott and Whitfield, 2011; Teichert et al., 2016). However, the estuarine systems are highly fragile biologically, with considerable biological diversity that has attracted their use for breeding purposes and as a tropic site for rearing several freshwater and marine species. The advancement in the world economic development and the population explosion has promoted the formations of alterations to these estuaries through loss of the environmental quality due to continuous contamination by several polluting sources, the change of their use purpose, or by complete suppression by land filling (Kennish, 2002).

The Brazilian coastal areas have always had heavy population densities, with unprecedented and unplanned growth explosion, particularly near the areas associated with the estuaries and their mangroves. For instance, the State of Pernambuco (Northeast Brazil) coastal area constitutes a 187 km extension that is distributed across 21 municipalities housing approximately 45% of the state's total population (Araújo et al., 2007). Such a high density has promoted the anthropic pressure on the estuaries (Kennish, 2002). Especially affected were the apicum areas, which are one of the main features of this ecosystem, as they have been systematically suppressed despite well-known biological significance. Notably, the apicum area is an important area of mangrove depreciation, with a specific flora and fauna population that are extremely sensitive to the variations in the anthropic pressures.

A huge amount of contaminated effluents flowing into the estuarine regions obviously increase the probability of the occurrence of any direct and/or indirect damages to the DNA molecules of living beings, resulting in mutagenic (alteration in the base sequence of the DNA

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molecule), aneugenic (abnormal chromosomal segregation), and clastogenic effects (alteration in the structure of the chromosomes) in the affected species (Frenzilli et al., 2009; Ohe et al., 2004). It may result, for example, in the reduction in the survival rate and recruitment, decreased reproductive capabilities, alteration in the mutation rates, migration, and the genetic constitution of the living population, thereby challenging their successful survival, conservation, and continuity (Adam et al., 2010; Thomas et al., 2014; Whitehead et al., 2003).

Recently, a growing interest in the use of genetic markers that can quantify the effects of exposure to contaminants has ensued the use of different sub-lethality procedures. One such genetic procedure, micronuclei testing, analyzes the extent of genomic damage in the coastal areas, especially in the estuaries. This method is preferred due to its relative speed, convenience in performance, low cost, and excellent discriminant capacity for the identification of genomic macrolesions (Duarte et al., 2017; Heddle, 1973; Pinheiro et al., 2013; Schmid, 1975; Siu et al., 2004). However, for this test, a complete cell cycle is required to be expressed, unlike in the comet assay. The latter presents with greater sensitivity for the detection of genomic microleads. The comet assay can identify damages in most eukaryotic cells at an individual level (Singh et al., 1988) as well as validate its use as an ecological indicator for the diagnosis and monitoring of coastal areas (Davanso et al., 2013; Rocha et al., 2015). We have observed the use of this kind of genetic markers in several vertebrate animals including mammals, amphibians, fishes (Arcaute et al., 2014; Frenzilli et al., 2009; Thomas et al., 2014), and invertebrates such as crustaceans (Nudi et al., 2010; Pinheiro et al., 2013).

Cardisoma guanhumi (Latreille, 1825) is a terrestrial blue crab with crustacean features, and it is widely distributed over the tropical region in the Western Atlantic from the United States to the southern Brazil. It has been mainly found in the sandy areas of the estuarine systems, either in the transition area between the mangroves and the more emergent areas (such as apicum), where arboreal rarefaction is known to occur (e.g., in the resting areas), or even in the Atlantic Forest forests. Burrows of this species have been identified up to 150 m away from rivers, although it requires brackish water for nurturing its eggs and larvae. The ovigerous females of this species lay about 300,000–700,000 eggs in these burrows during the breeding periods (Gifford, 1962; Hostetler et al., 1991). During adulthood, the capacity of *C. guanhumi* for geographical dispersion reduces such that it develops remarkable fidelity to its burrows throughout the life (Forsee and Albrecht, 2012). *C. guanhumi* is a crab with a low growth rate and, therefore, it takes time to reach its maximum size, being a testimony to the action of contaminants that may occur in the environment they occupy. The species feeds on plant material and has contact with the sediment and water inside the galleries, being in contact with three matrices that can become contaminated (water, sediment and food - vegetation). The wide geographic range of the species allows protocols to be established and its use as a bioindicator of contamination. This feature of *C. guanhumi* makes it a good indicator of its environmental quality. The environmental dispersal depends on the initial larval stages (zoeas) as the early larvae are transported for several hundred kilometers until recruitment finally occurs in the estuarine areas during the last larval stage (megalopa) (Abrunhosa et al., 2000; Costlow Jr. & Bookhout, 1968a, b).

Due to the large body size of an adult (> 11-cm wide carapace) (Brasil/Ibama, 2011) as well as its pleasant taste, *C. guanhumi* is among the important resources relied on for the subsistence of the majority of the families living in the estuarine areas. Thus, this species is one of the most sought-after one. This fact along with the continuous contamination and suppression of its natural habitat has led to a significant reduction in the size and stock of this resource in the Brazilian coast (Amaral et al., 2015; Amaral and Jablonski, 2005). In 2004, this species was included among the Brazilian List of Aquatic Invertebrates as the "Overexploited or Threatened Fishes of Overexploitation". By 2014, they shifted to the category "Critically in Peril" (CR), which appeared in the Brazilian List of Fauna Species under the "Threat of Extinction" (MMA, 2004, 2014).

The larval (zoeas and megalopas) and the juvenile stages of the species are known to be sensitive to the discharges of contaminants from industrial complexes, ports, untreated domestic sewages, and, mainly, from carcasses (Galli et al., 2012). Even the adults of *C. guanhumi* may be sensitive to disturbances occurring due to terrestrial contamination from their food sources (Bright and Hogue, 1972) as well as from the degradation of estuarine systems as a result of diverse anthropic consequences. Accordingly, this species has a remarkable relevance in space-time monitoring considering its conservation and habitats, particularly in association with different pluviometric regimes to the "reference values" of genomic damages. The knowledge of these ecological indicators helps distinguish the influence of some environmental factors from that caused by exposure to genotoxic agents (Lacaze et al., 2011).

This study attempted to obtain the ecological indicatives of a genomic nature to diagnose and monitor the conservation status of *Cardisoma guanhumi* (Latreille, 1825) from across five tropical estuarine systems in the state of Pernambuco, Northeast Brazil. The investigation of the status of a species residence in its adaptive landscape, especially with reference to the estuaries, is important to comprehend the conservational status of the ecosystem in those areas. In addition, through this study, we could predict the magnitude of the ecological indicators depicting the genomic damage expected in an aqueous community of the coastal region using a representative species, for example, *C. guanhumi*, during a contiguous and oscillating climatic cycle resplendent with varying anthropic intervention regimes.

2. Methodology

2.1. Areas of study and sampling

Five tropical estuarine systems in the State of Pernambuco, Northeastern Brazil were selected as the study regions and categorized as three coastal regions for the study purpose, viz., 1) the North Coast, represented by the Goiana and Jaguaribe Rivers; 2) the Central Coast, with the Capibaribe River (the metropolitan area of Recife, the central landmark of the Pernambuco coast), and 3) the South Coast, which comprises the Sirinhaém and Formoso Rivers (Fig. 1). These areas were selected as they represent different variations of conservation, based on the information provided by the State Agency of Environment of Pernambuco (CPRH, 2010).

The northern coast of the state of Pernambuco is a preserved area with maintained biodiversity despite advancing industrial and domestic pollution, suppression/landfill of the mangrove swamps (Rio Goiana), predatory fishery exploitation (Jaguaribe River), threat of shrimp farming, and the expanding automobile industry. In contrast, due to the urban fabric of Recife (the capital of the State of Pernambuco), the estuarine and mangrove systems (Capibaribe River) of the central coast have been most affected by deforestation and other human activities such as land filling, real estate growth, and formation of public roads. Finally, in the southern coast of the state, the fauna and flora are satisfactorily conserved, which can be observed, for example, in the Sirinhaém and Formoso Rivers areas, although these areas are challenged by increasing predatory fishing exploitation (CPRH, 2010).

Ten specimens were sampled from the reference site located in the Una River at the Juréia-Itatins Ecological Station—a conservational unit located on the south-central coast of the State of São Paulo, Southeastern Brazil—as a part of a Mosaic of UCs (Lino and Albuquerque, 2007). Therefore, owing to this situation, the area is colonized by traditional communities of approximately 200 families comprising of 2000 people with a unique approach to environmental conservation and for remaining contaminant-free (Pinheiro et al., 2013). The animals collected at this reference site were considered as the control group given they live in a more pristine environmental

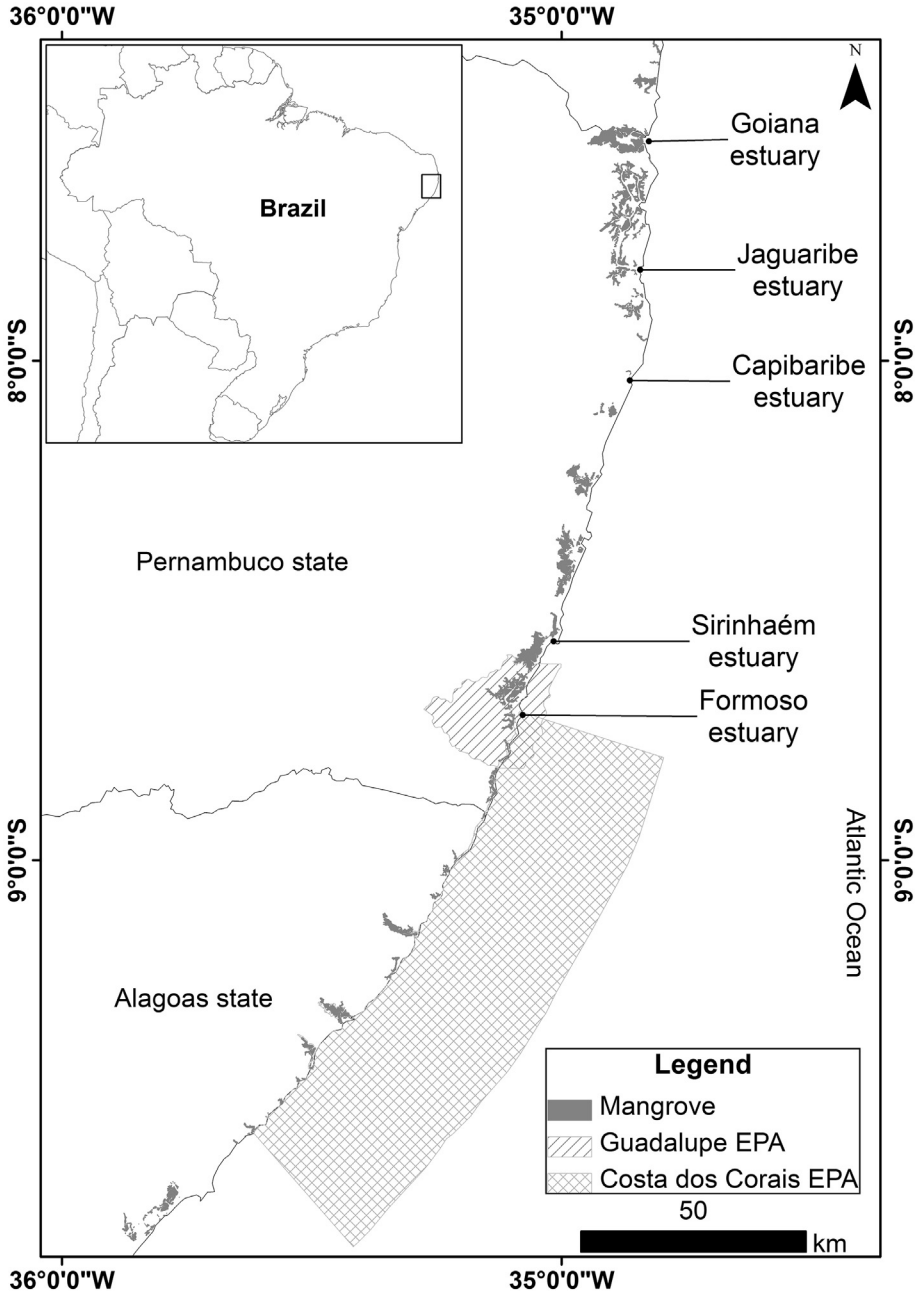

Fig. 1. The map showing the sampling sites of *C. guanhumi* for the five estuarine systems in the state of Pernambuco, Brazil during 2012–2013: North Coast (Goiana and Jaguaribe Rivers), Central Coast (Capibaribe River), and South Coast (Sirinhaém and Formoso Rivers).

situations. Therefore, given the evidence that this pristine environment interphere much less in the genome damage of the biota (Lima et al., 2019), we performed only one sampling at that location.

A total of 200 specimens of *C. guanhumi* were sampled for the analysis, including 10 for each estuarine system over two climatic seasons (the winter season lasting from June to September and the summer season lasting from December to March) between 2012 and 2013 (n = 50: winter of 2012; n = 50: summer of 2012; n = 50: winter of 2013; n = 50: summer of 2013). The experimental animals were captured by the local fishermen using traps with bits of citrus fruits acting as baits. The trapped animals were dispatched to the laboratory, followed by the collection of the hemolymphs within 24 h so as to minimize any possible alteration in the sample as a result of their removal from their natural habitat. After the collection of hemolymph, the animals were kept in the laboratory until they were returned to their collection sites. After the collection of hemolymph, the animals were kept in the laboratory until they were returned to their collection sites except those collected at the reference site (due to distance from the site) that were donated to a needy family as a food source.

2.2. Micronucleus test (MN)

The data on genomic damage was obtained by performing micronucleus test (MN) according to the methods described by Siu et al. (2004), Heddle (1973), and Schmid (1975), with slight modifications. Hemolymph was collected using a syringe (1 mL), with a 21G needle. The needle was inserted into the joint membrane of the pereopods with removal of 200 μL for the MN test. Two hemolymph smear slides were prepared per specimen; the slides were dried at the room temperature for 20 min, followed by fixing with absolute methanol and Giemsa (pure) staining. Each step of the procedure was performed for 5 min. The micronuclei were considered as nuclear fragments with no

correlation to the main nucleus, but with the same color and intensity as that of the nucleus, albeit being smaller (one-third the size of the nucleus) (Moron et al., 2006). A total of 1000 cells were analyzed for each animal under the optical microscope, and the micronucleated cells observed were counted to obtain the total MN‰.

2.3. Comet assay

The comet assay was performed as per the methodology of Singh et al. (1988), with slight modifications. An anticoagulant solution containing 0.49 M NaCl, 30 mM trisodium citrate, and 10 mM EDTA (Klobucar et al., 2012) was prepared and 200 μL of the anticoagulant solution and 400 μL of hemolymph, from each animal, were homogenized. This mixture was transferred to a 1.5 mL collection tube. About 70 μL of this solution was homogenized with 100 μL of 0.5% low-melting agarose at 37 °C. Approximately 170 μL of this solution (hemolymph + anticoagulant +0.5% low-melting point agarose) were spread onto two slides (previously covered with 1.5% agarose diluted in PBS ($KCl + KH_2PO_4 + NaCl + Na_2HPO_4$) and left to rest for 24 h), followed by covering with coverslips and incubating in a refrigerator for 15 min. After the agarose polymerization step, the coverslips were removed and the slides were immersed in the lysis solution (1 mL Triton X-100, 10 mL DMSO, and 89 mL lysis solution; pH 10, containing: 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 8 g NaOH in 890 mL of ultrapure water) for at least 24 h in the refrigerator. After the lysis, the slides were incubated in 300 mM NaOH +1 mM EDTA buffer (pH > 13) for 20 min to allow denaturation of the DNA molecules and then subjected to electrophoresis at 40 V and approximately 300 mA for 20 min. After the run, the slides were neutralized in a 0.4 M Tris solution for 15 min, followed by fixing in 96–100% ethanol for 5 min. The slides were then dried and stained with GelRed, and finally analyzed under a fluorescence microscope (400× magnification). A total of 100 cellular nucleoids were analyzed for each individual, with the scaling of cell damage established according to five classes: 0 (without damage) or I–IV (different extents of damage), according to the migration of the fragmented nuclear material in relation to the size of the remaining core. Thus, the percentage of Damaged Cells (sum of all damaged cells in relation to the total of cells analyzed) and the Genome Damage Index (ID) were determined. The ID was determined using the following formula:

ID = (number of cells of each class) x (class value)/number of individuals analyzed at each collection point.

In other words,

ID = (the number of cells with level of damage 0 × 0 + number of cells with damage level I x 1 + … + number of cells with damage level IV x 4)/number of individuals sampled (Collins, 2004).

2.4. Pluviometric monitoring

The daily pluviometric record was obtained from the meteorological stations within < 60 km area for each estuarine systems and their respective mangrove systems between January 2012 and December 2013. These data were obtained from the website of the Pernambuco Water and Climate Agency (APAC) for seven monitoring stations of the studied estuaries, namely, Goiana River (Itapirema Station), Jaguaribe River (Igarassu Station, Barra do Catucá and Usina São José), Capibaribe River (stations of Alto da Brasileira and Várzea), and Sirinhaém and Formoso Rivers (Usina Cucaú Post).

The rainfall value for the years 2012 and 2013 were reached by averaging the monthly values of the seven monitoring stations to estimate the annual fluctuation during the study period. The monthly values were obtained by summing the daily data for each month so as to evaluate any possible refinements in the monthly rainfall value. Seasonal rainfall was also evaluated by grouping the rainfall volume for the winter and summer seasons by calculating the average for each climatic season using the data obtained from the seven monitoring stations.

2.5. Statistical analyses

The results of the micronucleus and comet assays were analyzed by using the GraphPad Prism 5.00 software (GraphPad Software Inc., San Diego, CA, USA). Data were presented as mean and standard error. The normality of the data was verified by the Shapiro–Wilk test, while the homoscedasticity of the variances was analyzed by the Bartlett's test. The data with normal distribution were evaluated using analysis of variance (ANOVA) followed by Tukey's test. For the data with non-normal distribution and non-homogeneous variance, they were analyzed by the Kruskal–Wallis test and then to the Dunn's multiple comparison test. The differences were considered to be statistically significant at *p values* 5, 1, and 0.1%.

3. Results

3.1. Micronucleus test (MN)

Independent of the climatic season or the year of sampling, the holistic analysis revealed no significant differences in the values of micronucleated cell frequencies (MN‰) for *C. guanhumi* among the estuaries ($p = 0.6752$). However, a significant variation of this parameter was noted as a function of the month, climatic season, and sample year ($p < 0.0001$). In general, the MN‰ during the winters was 4.9-times higher than that in the summer (1.747 ± 0.22 MN‰ and 0.354 ± 0.08 MN‰, respectively; $p < 0.0001$).

Table 1 show a notable expressive variation in the MN‰ during the winter of 2012, where a significant difference occurred as a function of the evaluated estuaries ($p < 0.0001$). The highest averages were recorded in the estuaries of the Goiana (4.10 ± 0.65 MN‰), Capibaribe (3.60 ± 0.60 MN‰), Sirinhaém (3.50 ± 0.82 MN‰), and Formoso (2.66 ± 0.55 MN‰) rivers (Fig. 2). No differences were identified when comparing the MN‰ obtained for the Jaguaribe (1.70 ± 0.42 MN‰) estuary and the control group (Estação Ecologica da Juréia) (0.20 ± 0.13 MN‰), which were similar to each other. However, no statistically significant difference was recorded between the MN‰ for

Table 1
Frequency of micronucleated cells (MN‰) recorded in *Cardisoma guanhumi* specimens collected from five estuarines areas of the State of Pernambuco (Goiana, Jaguaribe, Capibaribe, Sirinhaém and Formoso) and a control area in the State of São Paulo (Juréia Ecological Station -Itatins).

N = number of animals analyzed; Min = minimum value; Max = maximum value; X = arithmetic mean; ER = standard error of the mean.

Estuaries	Season	N	Min	Max	X ± EP
Goiana	Winter 2012	10	1	8	4100 ± 0.657
	Summer 2012	9	0	2	0.556 ± 0.242
	Winter 2013	8	0	1	0.125 ± 0.125
	Summer 2013	10	0	0	0.000 ± 0.000
Jaguaribe	Winter 2012	10	0	4	1700 ± 0.423
	Summer 2012	8	0	4	1125 ± 0.549
	Winter 2013	9	0	1	0.333 ± 0.167
	Summer 2013	10	0	0	0.000 ± 0.000
Capibaribe	Winter 2012	10	1	7	3600 ± 0.600
	Summer 2012	10	0	3	0.400 ± 0.306
	Winter 2013	10	0	1	0.100 ± 0.100
	Summer 2013	9	0	0	0.000 ± 0.000
Sirinhaém	Winter 2012	8	1	8	3500 ± 0.824
	Summer 2012	9	0	1	0.444 ± 0.176
	Winter 2013	8	0	0	0.000 ± 0.000
	Summer 2013	9	0	1	0.111 ± 0.111
Rio Formoso	Winter 2012	9	0	5	2667 ± 0.553
	Summer 2012	9	0	3	1000 ± 0.373
	Winter 2013	5	0	1	0.200 ± 0.200
	Summer 2013	10	0	1	0.100 ± 0.100
Jureia	October 2012	10	0	1	0.200 ± 0.133

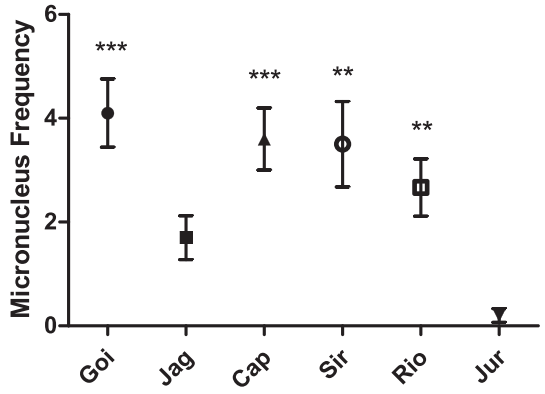

Fig. 2. Analysis of the frequency of micronucleated cells (mean ± SE) observed in the *Cardisoma guanhumi* specimens of the five estuaries (Goi: Goiana, Jag: Jaguaribe, Cap: Capibaribe, Sir: Sirinhaém, Rio: Rio Formoso) (Jur: Jureia) in the winter 2012 (**$p < 0.01$, ***$p < 0.001$ as compared to the Jureia control group).

the different estuaries in the other seasons analyzed (summer 2012, winter and summer 2013) ($p > 0.05$). The complete cycle analysis revealed a significant reduction in MN‰ from the data of winter 2012 in relation to that of the subsequent climatic seasons (Fig. 3).

3.2. Comet assay

The genome damage by the Comet Assay revealed an increase in the specimens during the winter season in 2012, specifically in the estuaries of Goiana, Jaguaribe, and Capibaribe rivers (Table 2). These estuaries were different from those of the southern coast of the State of Pernambuco (Sirinhaém and Formoso) ($p < 0.0001$). The latter shared similarity to the control estuary (Fig. 4). Moreover, no significant increase was noted for the five estuaries during the summer of 2012 and the winter and summer of 2013 ($p > 0.05$). In addition, a holistic analysis of the time-course of studies indicated a significant decline in the level of genomic damage observed during the winter season in 2012 throughout the other climatic seasons ($p < 0.05$) (Fig. 5).

3.3. Pluviometric monitoring

The mean of the total rainfall volume recorded for the seven monitoring stations in the estuaries did not differ for the years 2012 (109.1 ± 28.35 mm) and 2013 (158.9 ± 34.34 mm) (Fig. 6A). However, the rainfall average was higher in the month of July 2013 than in July 2012 (356.0 ± 29.12 mm and 265.0 ± 17.02 mm, respectively; $p = 0.007$). Moreover, the sum of the two-month daily rainfall values (July 12 and July 13) indicated an event with higher rainfall volume in the early July 2012 (632.5 mm), which was twice as high as the rain of any day of July 2013.

4. Discussion

The pluviometric analysis was recorded in the municipalities of Pernambuco, where the estuaries occurred and evidenced a pattern with a higher incidence of rain during the winter months, which is similar to that in other states of the Brazilian Northeast. Specifically, the Pernambuco coast presents with a gradual increase of precipitation from the month of February (50–100 mm), to reach the maximum levels of rainfall in June (> 600 mm), with subsequent decrease in the following months. The rainy season that affects the coast of Pernambuco in the month of June is related to the position and intensity of the high pressure of the South Atlantic and the temperature of the South Atlantic (MMA, 2004b).

The diagnostic analysis and monitoring of the conservation status of *C. guanhumi* by the two genetic methods across the five estuaries of Northeast Brazil hinted at a significant increase in the genomic damage during the winter of 2012. The significant values of genomic damage (MN‰) for the specimens in the Pernambuco estuaries suggested that the local populations of *C. guanhumi* were exposed to contaminants at an expressive level. Rapid growth and development of the human population has resulted in the release of potentially genotoxic and carcinogenic contaminants in water resources or other coastal environments, including mangroves and estuaries (Frinhani and Carvalho, 2010; Jha, 2008). The estuaries of the Goiana rivers (Goiana city, on the north coast of Pernambuco) and Capibaribe (Recife city, on the central coast and capital of Pernambuco) have faced harsh pollution as a result of the extensive industrial, domestic, and hospital sources, and the landfill of mangrove areas for the implantation of shrimp farming projects is also a major setback (Dantas, 2008; Araújo and Oliveira, 2013). On the other hand, the estuaries of the rivers Formoso and Sirinhaém are considered as the two Areas of Environmental Protection (State APA of Guadalupe and APA Federal Coast of the Corals), although subject to a wide historical cultivation of sugarcane that occupies almost 100 km away that connect these municipalities to the city of Recife. Therefore, the high frequency values of the micronucleated cells (MN‰), observed in the winter of 2012, were associated to the probable discharge of different contaminants released from the surrounding of estuaries studied.

Several studies have associated the increase in MN‰ among the marine organisms with continued exposure to contaminants for > 96 h (Erbe et al., 2011; Nudi et al., 2010). However, when considering the other climatic stations, the MN values did not differ in relation to that of the control group. Such evidence thus suggests that the differences in the rainfall levels over the years have influenced the establishment/dispersion of contaminants in the coastal environments, especially in the estuaries, considering the gradual decrease in the genomic damage during the low rainfall period.

Several studies conducted in the tropical coastal environments in Brazil or elsewhere suggest that estuarine environments under intense perturbation fix contaminant compounds of several classes and many of them have a proven association with genomic damage (Carreira et al., 2015; Davanso et al., 2013; Oliveira et al., 2014; Sukhdhane et al., 2015). Therefore, the analyses of the chemical composition of the sediment around the crab burrows could reinforce our hypothesis of direct association between the frequency of genome damage and the occurrence of a strong chemical disturbance in the studied estuaries.

The increase in the frequency and ID, as observed by the comet assay in animals from the Goiana and Capibaribe rivers, corroborated the results obtained by the micronucleus test in the winter of 2012. The different types of lesions found included micro and macrolesions, which are consistent with a great perturbation in the estuarine dynamics, since the occurrence of different classes of damage in the genome suggests the existence of different classes of contaminants in the environment. Our results thus coincide with the increase in the process of industrialization in the region that recently housed a complex with numerous industries in the automotive sector.

The comet assay also revealed a pronounced increase in the DNA damage (microlesions) in the animals from the Jaguaribe River, not detected by the micronucleus test (macrolesions). Despite the low incidence of micronucleated cells in Jaguaribe, a high incidence of cells in advanced degree of deterioration (apoptotic cells - not counted) was observed. Thus, the presence of microlesions, combined with the absence of the macrolesions, as well as the incidence of cellular deterioration in individuals of *C. guanhumi* in the Jaguaribe River may be result of the induction of cell death. Similar results were also obtained by Arcaute et al. (2014), who reported that some of the cells could not complete the cell division cycle to evidence the formation of micronucleated cells.

In addition, non-significant values were obtained to the comet test for the animals from the south-coast. The differences in the results of these tests can be attributed to their specific characteristics. For

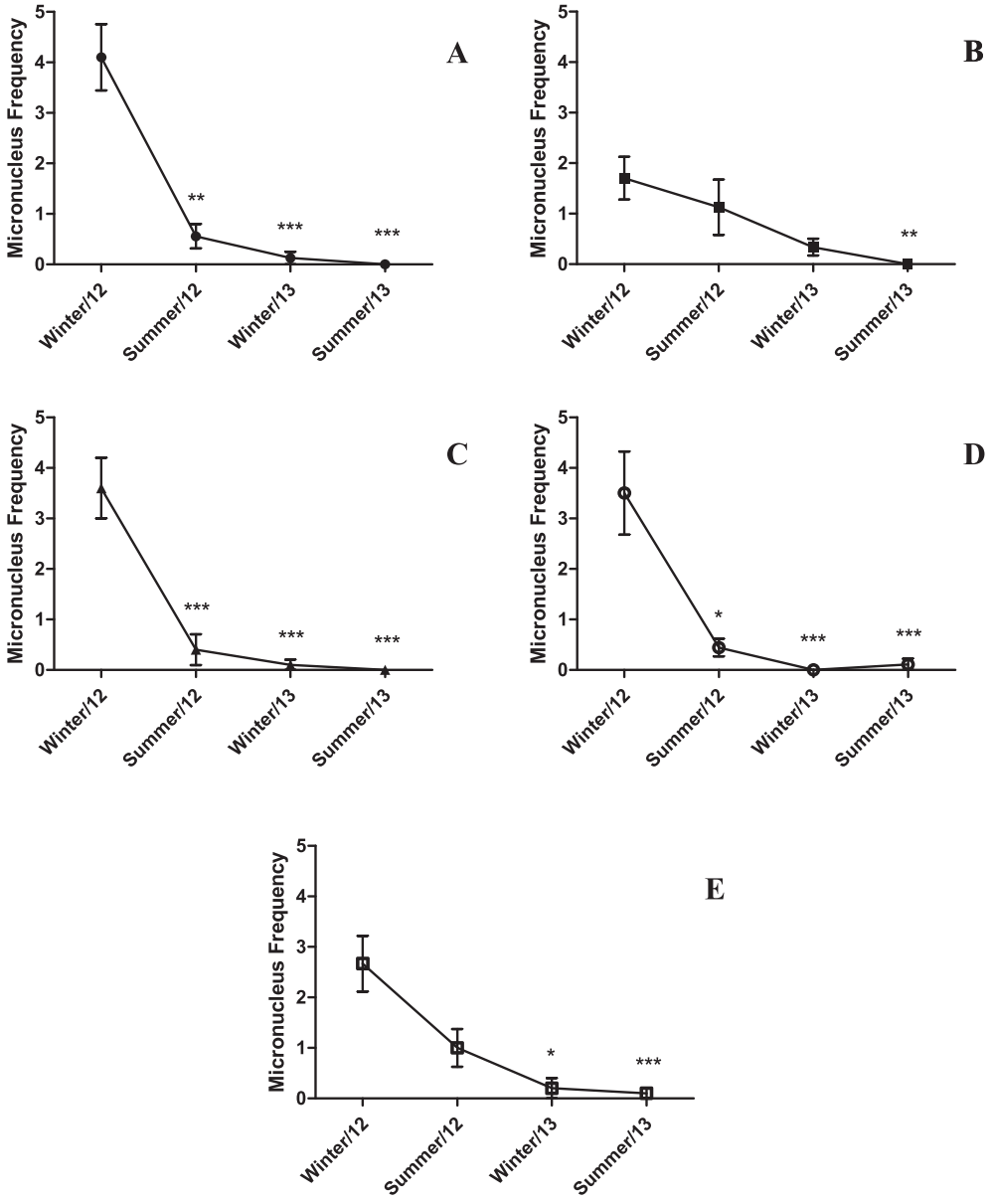

Fig. 3. Temporal analysis of the frequency of micronucleated cells (mean ± SE) observed in the *Cardisoma guanhumi* specimens from the five estuaries (A, Goiana, B, Jaguaribe, C, Capibaribe, D, Sirinhaém and E, Rio Formoso) during the winter and summer seasons of 2012 and 2013. The values of significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) confronted each season with the winter of 2012.

example, in the comet assay, greater sensitivity was detected in the detection of genomic lesions (Cotelle & Ferard, 1999; Jha, 2008). In addition, some past researchers have reported that the increase in genomic damage by the comet assay is mostly detectable in the first few days of the exposure, which then reverts from the 10th day (Sponchiado et al., 2011). This particular phenomenon could probably be due to the ability of DNA repair that can occur from minutes to hours (Collins and Horvathova, 2001; Schmezer et al., 2001). Moreover, comet assay for lobster cells also indicated an increased ability to stabilize genomic damage against prolonged exposure to contaminants (Klobucar et al., 2012). With no definitive explanations for these results, we can conclude that the lack of evidence of genomic damage detectable by the comet assay for the estuaries of the south coast can be attributed to the longer time lapse between the episodes of intense rainfall in the early July and the collection of the experimental specimens. In addition, the difference in the methodologies due to the difference in the response mechanism to certain contaminants with varying genotoxic effects can be held responsible.

Moreover, we examined whether the variations in the ecosystem negatively influences the integrity of a genetic material. Previously, the densification of the human population in the coastal areas was reported to affect the integrity of their ecosystems significantly (Costa et al., 2017; Lin et al., 2016). The results of the population density in the coastal zone of the state of Pernambuco revealed a variation of 50–100 inhabitants/km^2 to > 500 inhabitants/km^2. However, the demographic density of the South and North coasts in Pernambuco was found to be 181.3 inhabitants/km^2 and 446.4 inhabitants/km^2, which indicate a clear discrepancy between the North and South coasts. Therefore, the lower concentrations of microlesions in the tested specimens of the southern coast reflect the higher conservation status of the estuaries in this region despite severe climatic changes in that region.

The progressive decrease in the genomic damage subsequent to the winter of 2012 reinforces the possibility of a point event. Considering the natural increase in the rainfall index during the winters in the estuaries from Pernambuco State, any direct association of increased genomic damage with greater precipitation season (winter) should

Table 2
Analysis of the damaged cells (%) and the genomic damage index (ID) recorded for the *Cardisoma guanhumi* specimens captured from five estuaries in the State of Pernambuco (Goiana, Jaguaribe, Capibaribe, Sirinhaém, and Formoso) and a control area in the State of São Paulo (Juréia-Itatins Ecological Station).

Estuaries	Season	N	Damaged Cells (%)	ID
Goiana	Winter 2012	10	66.8	116.9
	Summer 2012	9	20.7	36.5
	Winter 2013	10	15.8	27.8
	Summer 2013	10	5.1	9.8
Jaguaribe	Winter 2012	10	72.0	121.8
	Summer 2012	10	2.0	2.8
	Winter 2013	10	19.0	25.6
	Summer 2013	10	8.8	15.2
Capibaribe	Winter 2012	10	49.8	99.6
	Summer 2012	9	8.7	14.5
	Winter 2013	10	4.0	4.9
	Summer 2013	9	4.9	6.0
Sirinhaém	Winter 2012	6	18.7	21.0
	Summer 2012	10	21.8	55.8
	Winter 2013	10	9.0	12.4
	Summer 2013	9	7.1	10.0
Rio Formoso	Winter 2012	10	13.0	16.4
	Summer 2012	8	24.4	33.4
	Winter 2013	10	23.2	42.7
	Summer 2013	10	15.8	28.0
Juréia	October 2012	12	5.0	7.5

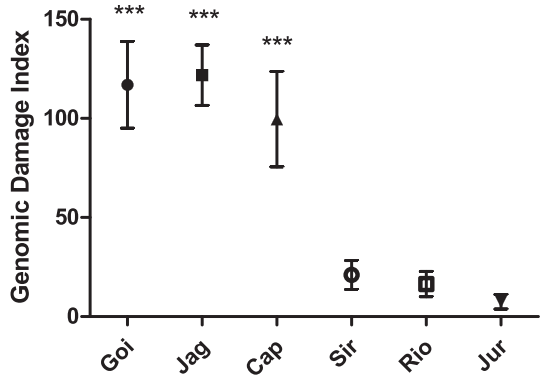

Fig. 4. Analysis of the genome damage index by the comet assay (mean ± SE) for specimens collected from across five estuaries (Goi: Goiana, Jag: Jaguaribe, Cap: Capibaribe, Sir: Sirinhaém, Rio: Rio Formoso and Jur: Jureia) in the winter season of 2012 (***$p < 0.001$ as compared to the Jureia control group).

present similar results to the winter of 2013, which did not occur. Winter et al. (2004) and Lacaze et al. (2011) reported no differences in the indices of the genomic damage in the exposed animals during both the winter and summer seasons, which supports the lack of any obligatory relationship between genomic damage and a particular climatic condition. However, there is a slight possibility of occurrence of such a phenomenon in areas that have a characteristic rainfall pattern similar to that in the State of Pernambuco, that is, with the winters coinciding with the heaviest rainfall. Thus, with the lack of records about occurrence of such large pluviometric events after the winters in the Brazilian northeast region and considering that these events occur sporadically, susceptibility relation of the damage can be perceived with the climatic seasons.

The monthly analysis of the rainfall pattern verified that the intensity of the winter 2013 was greater that of the winter 2012. However, a daily analysis of the precipitation pattern in the month of July in both the years revealed that, despite the comparatively smaller occurrence of rainfall in the winter 2012, it was concentrated in just a single day, and not uniformly distributed over several days as in 2013. In the July of 2012, a large volume of rainfall occurred, causing flooding and overflowing of the rivers across several cities in the State of Pernambuco, which in turn accelerated the washing off of the soils from the adjacent farmlands. This event led to the addition of a large volume of untreated waters in the rivers and rain gutters, which flooded the estuaries with a huge amount of diverse contaminant types. Thus, a positive correlation between high rainfall precipitation and increased incidence of genomic damage in the tropical estuarine systems is in concordance with some previous findings (Polard et al., 2011; Werner et al., 2002; Whitehead et al., 2004) related to the relation of increase in the amount of pesticides and a greater incidence of genomic damage after the excessive rainfall. This observation further supports that this is the most appropriate model for the diagnosis and monitoring of endemic species of tropical estuaries as they are subject to disturbance regimes even on an extremely short time scale.

The findings of the present study thereby suggest that the genotoxicity indexes for *C. guanhumi* are associated more to the rain volume concentrated than to the precipitation volume that is uniformly precipitated. We can thus argue in the favor of a global predictive scenario relative to the harmful (and eventually fatal) effects of genomic damage due to climatic conditions on the species' populations, biotic communities, and their ecosystems involved in the episodes of intense water volume, which reinforces similar scenarios reported earlier (Galloway, 2006).

In addition, the decrease in the IDs also revealed an interesting ecological peculiarity of the *C. guanhumi*, which could recover from the disturbances in winter 2012 in a very short period of time, followed by the successful recovery in winter 2013. This species possesses great population resilience potential. Moreover, recent studies have revealed that the genomic damage in a fish may reverse shortly after the discontinuation of the specimen's exposure to xenobiotics (Groff et al., 2010; Hasue et al., 2013; Mohanty et al., 2011). The combination of these evidences reinforces the hypothesis that a point event carried genotoxic compounds into the estuaries during winter 2012.

The decrease in the genomic damage among the analyzed animals during the experimental period coincides with the decrease in the daily rainfall indices and therefore a decrease in the pollutant contribution in the estuaries throughout the experimental period. Thus, *C. guanhumi* can be considered to be a good bioindicator species with probable application in the diagnosis and monitoring of estuarine and tropical mangrove systems owing to its sensitivity to environmental disturbances even on a short time scale and its resilience power. The high sensitivity and resilience to xenobiotics of the blue crab (*C. guanhumi*) makes it a potential sentinel of the environmental quality of the Western Atlantic tropical estuarine systems, similar to that for the crab *Ucides cordatus* as reported in past studies (Nudi et al., 2010; Pinheiro et al., 2013).

Diagnosis and environmental monitoring are relevant aspects to gain knowledge regarding the conservation status of a species and for planning concrete conservational actions to regain prior status. It is already well known that genomic damage is related to the decreased state of fitness of animals. Included among the impacts commonly related to the damage occurring from mutagenic or clastogenic effects is the increase in the frequency of gametic loss due to cell death, altered growth in individuals, decreased reproductive success, and physiological abnormalities (Anderson and Wild, 1994; Anderson & Wild, 1994; Matson et al., 2006).

In addition, several studies have suggested a relationship between DNA lesions and variation in the genetic structure of a population. The various aggressions to the structure and sequence of nucleic acids may cause a decrease in the genetic variability of the populations, with selection of the genotypes more resistant to the contaminants and disappearance of the less tolerant ones (Bickham et al., 2000; Thomas et al., 2014; Whitehead et al., 2003; Wolf et al., 2004). After the winter of 2012, *C. guanhumi* showed a good recovery rate from the genomic damage, although the genotoxic analysis could not evaluate the possible damages suffered by this species alone. Thus, it is recommended that complementary studies be performed to evaluate the

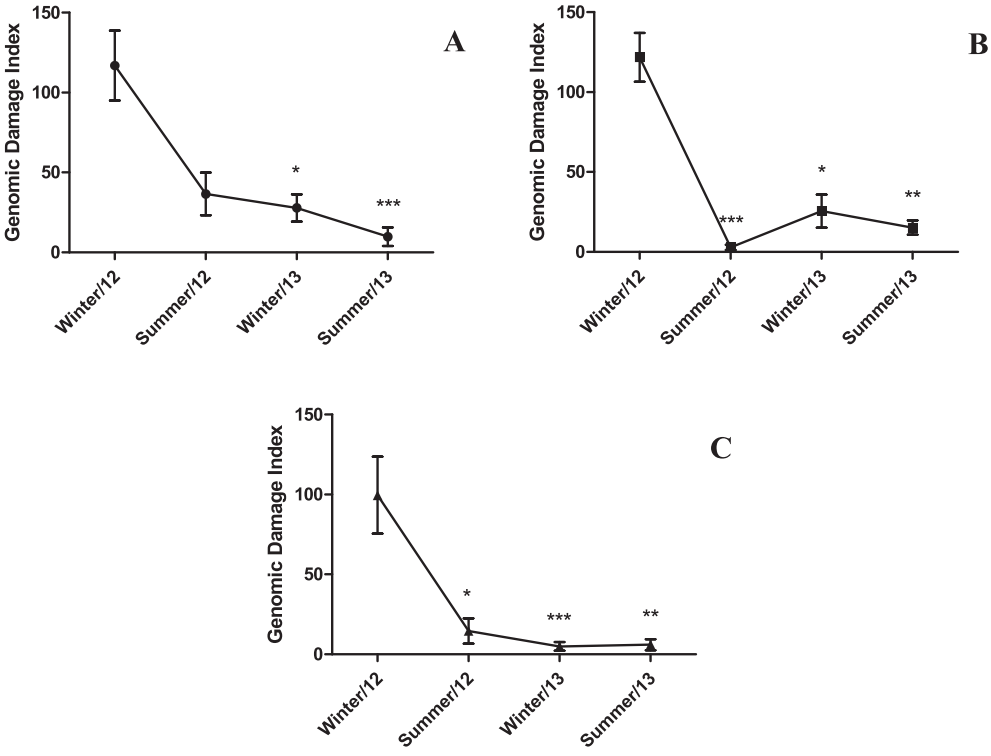

Fig. 5. Temporal analysis of the genome damage index observed by the comet assay (mean ± SE) in the *Cardisoma guanhumi* specimens from the three estuaries (A, Goiana; B, Jaguaribe; and C, Capibaribe), during the winter and summer seasons of 2012 and 2013. The values of significance ($p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) confronted each season with the winter of 2012.

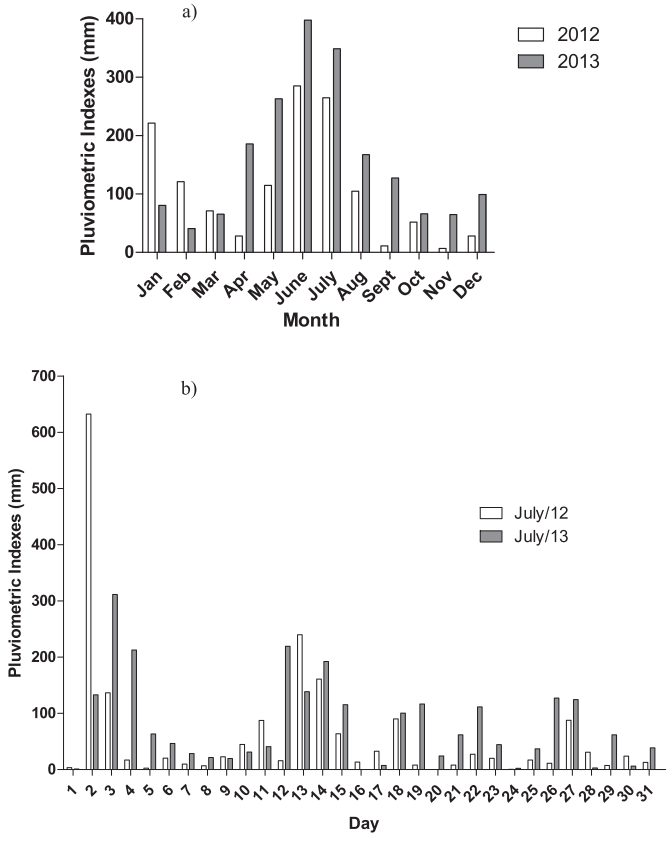

Fig. 6. The monthly averages of the rainfall indices over the years 2012 and 2013 (a) and the sum of daily rainfall indices in July 2012–2013 (b) were recorded across seven rainfall monitoring stations using the data available on the website of Agência Pernambucana de Águas e Climas (APAC) (http://www.apac.pe.gov.br/), namely: Goiana ('Itapirema'); Igarassu ('Igarrasu', 'Barra do Catucá' and 'Usina São José'); Recife ('Alto da Brasileira' and 'Várzea'); and Rio Formoso ('Usuca Cucaú').

conservational status of *C. guanhumi* specifically.

5. Conclusion

Genomic damage cannot be related directly to certain potential chemical compounds without screening for the main sources and types of pollutants. These studies are essential for the monitoring of genomic damage in the estuaries during the periods after the incidence of intense rainfall, as in the present study. The implementation of such monitoring steps would achieve the results and their application by the competent agencies to trace conservation measures to minimize the potential damage occurring after these events in the ecosystems as threatened and as relevant as estuaries and mangroves.

Declaration of competing interest

The authors declare that there is not any financial or personal conflict of interest in the present research.

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