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Original Article

Cytotoxicity, genotoxicity, and impact on populations of the mangrove sentinel species, *Ucides cordatus* (Linnaeus, 1763) (Brachyura, Ocypodidae) after an environmental disaster at Cubatão, São Paulo, Brazil

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Abstract

In 2015, a serious environmental disaster occurred at ULTRACARGO - Aratu S/A Terminal (Cubatão, SP) causing a long-lasting, large-scale, fire that resulted in the release of various chemical pollutants, including those used to contain the fire. These pollutants affected adjacent regions and the innermost area of the Santos-São Vicente Estuarine System, requiring the assessment of environmental quality in two mangrove areas post-disaster (2016). This assessment considered biomarkers for the species including population density, structure, and cytogenotoxicity. The population structure and cytotoxicity of *Ucides cordatus* (Linnaeus, 1763) only changed slightly from pre-disaster (2013) to post-disaster (2016), as a consequence of the greater resilience and biological flexibility of this crab to environmental stress

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caused by pollutants. We recommend continuous monitoring be conducted using this species endemic to the mangroves of the study site, as this will make it possible to assess the magnitude of the chronic environmental impacts of the accident. In addition, it could guide environmental agencies in damage mitigation or in the quantification of possible future impacts.

Keywords
Biomarkers, crab, environmental disaster, estuary, *Ucides cordatus*.

Introduction

Mangroves are important coastal ecosystems that occur in the transition between marine and terrestrial environments (Schaeffer-Novelli, 1995). These regions are endowed with significant animal and plant diversity (Spalding *et al.*, 2010; Souza *et al.*, 2018), in addition to offering several ecosystem services (Twilley *et al.*, 1997; Worley, 2005; Lacerda *et al.*, 2013; Schaeffer-Novelli *et al.*, 2016) that stand out for their relevance.

In Brazil, mangroves in São Paulo State concentrate mainly in the central-south coast (24,051 ha), with 63.2 % occurring on the south coast and 36.8 % on the central coast (Cunha-Lignon *et al.*, 2011; Cunha-Lignon, 2014). Estuarine systems on the central coast are distributed near nine cities in the Baixada Santista Metropolitan Region (BSMR), which is home to 1.87 million people (IBGE, 2019). Therefore, they exist on the coastal stretch of São Paulo State considered one of the most contaminated sites in Brazil, due to its significant industrial, port, and sewage contamination (Pinheiro *et al.*, 2008; 2012).

These pollutants contaminate water and sediment and can accumulate and magnify in the food chain, harming human health (Duarte *et al.*, 2016; 2017). In this sense, studies that assess the impact on the estuarine biota are crucial for a better understanding of contamination processes. These processes can reduce biodiversity (Terlizzi *et al.*, 2005) and the abundance of organisms (Lotze *et al.*, 2006; Duarte *et al.*, 2016). This results in genetic (Nigro *et al.*, 2006) and physiological damage (Duarte *et al.*, 2019; 2020) that can culminate in the extinction of less tolerant species (Espinosa *et al.*, 2007).

Among benthic mangrove macroinvertebrates, brachyuran crabs stand out for their abundance and biomass (Wolff *et al.*, 2000). In this context, *Ucides cordatus* (Linnaeus, 1763) (Crustacea, Brachyura, Ocypodidae) is an extremely popular semiterrestrial crab, known in Brazil as 'caranguejo-uçá' (hereinafter referred to as 'uçá'-crab). It is an endemic species to the western Atlantic mangroves (Pinheiro *et al.*, 2016), where it digs galleries into the sediment, feeds on senescent leaves, and has economic importance for traditional communities (Pinheiro and Fiscarelli, 2001).

Benthic macroinvertebrates have been used in the assessment and monitoring of anthropogenic impacts (Goulart and Callisto, 2003), often revealing alterations in their population parameters (*e.g.*, abundance, density, demographic structure, and spatiotemporal distribution). Population density is a relevant parameter, particularly for species that have economic importance (Waiho *et al.*, 2015), but it is rarely explored in the literature (Alberts-Hubatsch *et al.*, 2016), and often disregarding the mobility and spatial distribution of species (Pinheiro and Almeida, 2015).

According to Macia *et al.* (2001), species density can be estimated by dividing the number of active specimens (or their galleries) by their area of occupation. In the case of *U. cordatus*, density estimates consider the number of active galleries in a mangrove area, comprising total open biogenic galleries (those associated with recent feces, tracks, and/or sediment movement) and closed galleries (Pinheiro and Almeida, 2015). Each gallery of the 'uçá'-crab is occupied by a single specimen (Pinheiro and Fiscarelli, 2001; Nordhaus *et al.*, 2009), which allows for more reliable estimates, as previously performed by Pinheiro *et al.* (2018).

From the previous use of geno- and cytotoxicity trials on target species, some biological markers have enabled the detection of stressors caused by extreme natural phenomena or by anthropogenic contamination by xenobiotics (Buss *et al.*, 2003).

In this sense, sentinel species have been used in environmental biomonitoring (Beltrame *et al.*, 2011; Berthet, 2013; Pereira *et al.*, 2014). This is the case for the *U. cordatus* crab, which can reveal an early reduction in the environmental quality of mangroves (Pinheiro *et al.*, 2012, 2013; Banci *et al.*, 2017; Duarte *et al.*, 2016). In addition, Duarte *et al.* (2017) confirmed the relationship of geno- and cytotoxicity biomarkers obtained from the hemolymph of this crustacean and reported reduction in the density of the 'uçá'-crab in contaminated mangroves.

Among the biomarkers that have been used, genotoxicity quantification by micronucleus assay (MN) employs the frequency of micronucleated hemocytes in the hemolymph. It consists of a simple, fast, and efficient method to assess the impact of contaminants on aquatic invertebrates (Pinheiro *et al.*, 2012; 2013). Similarly, the time of hemocyte cell membrane integrity, assessed by the neutral red retention time (NRRT), makes it possible to assess cell cytotoxicity. In this process, cell apoptosis occurs in less time in contaminated areas than in more pristine ones (Duarte *et al.*, 2019; 2020).

In the 1960s, Cubatão city (on BSMR), was considered one of the most polluted places in the world. The city housed several contaminants that affected its different coastal environments, including its flora, fauna, and even human beings (Damiani, 1985). It is a busy chemical and petrochemical industrial hub, where serious failures in filtering and precautionary systems already caused the almost total release of some contaminants, earning the designation as 'Vale da Morte' (Death Valley) (Ferreira, 1984; Alonso and Godinho, 1992; Paschoal and Silva, 1998; Araujo and Rosário, 2020).

Currently, these industries have more efficient accident prevention systems, but environmental disasters can still occur due to spills, explosions, or fires, bringing environmental damage that may be catastrophic. One of these most tragic disasters occurred for nine consecutive days (from April 2, 2015, to April 10, 2015). The accident consisted of a major fire due to operational failure and involved six fuel tanks (gasoline: 67 %; anhydrous alcohol: 33 %) of the Aratu Terminal – ULTRACARGO. So far, this was the second worst man-made disaster in terms of area impacted in the world (GT-CREA/SP, 2015) because various polluting chemicals flowed into the estuary adjacent to the Alemoa neighborhood, affecting the entire inner area of the Santos-São Vicente Estuarine System. About 8 billion liters of water from the estuary were mixed with 700,000 liters of foam concentrate (composed of fluorocarbon surfactants). This brought immediate death to nine tons of fish, represented by 142 species (MPF, 2018), 10.6 % of which were threatened with extinction (Carraschi *et al.*, 2012; MMA, 2014a; 2014b).

Silva *et al.* (2019) and Daniel *et al.* (2021) confirmed the foam concentrate toxicity, reporting its accumulation in various tissues of these animals (Taniyasu *et al.*, 2008; Oakes *et al.*, 2010; Awad *et al.*, 2011), as well as in people from the local community (Giesy and Kannan, 2001; Solla *et al.*, 2012). At the time, the company was held responsible under Law # 9,605/1998 (BRASIL, 1998), and was fined (CETESB, 2015) for mortality to estuarine fauna, damage to traditional fishing communities, and impacts on human health (MPF, 2018).

The dispersion and accumulation of pollutants in abiotic (water and sediment) and biotic compartments (vegetation and fauna) requires the assessment of their effects. Thus, using data obtained in a previous monitoring study developed with 'uçá'-crab, executed in 2013, it was possible to compare pre-disaster (2013) and post-disaster data (2016), and to evaluate possible changes in the population parameters of this species (population structure and density), as well as sublethal cytogenotoxic effects.

Material and Methods

Study site

Samples were taken in two mangrove areas in Cubatão municipality, São Paulo State, Brazil (Fig. 1), whose coordinates and distance/direction from the environmental disaster (ULTRACARGO - Aratu Terminal / AT) are: C1 (23°54'2.4"S 46°22'57"W), at 3.7 km NW; and C2 (23°55'8.0"S 46°23'4.8"W), at 2.8 km E. These areas showed a predominance of red mangrove, *Rhizophora mangle* Linnaeus, 1753 (Rhizophoraceae) (C1: 74 %; C2: 53 %), with similarity in canopy height (mean ± standard deviation, C1: 8.0 ± 2.1 m; C2: 9.4 ± 1.6 m), tidal flooding level (C1: 30.2 ±

4.9 cm; C2: 29.8 ± 10.1 cm), textural classification (C1: coarse silt; C2: medium silt), and local hydrodynamics (C1 and C2: moderate) (see Duarte *et al.*, 2016; 2017).

Population Structure, Density, and Extractive Potential

Study procedures were developed in two distinct time periods that bracket the environmental disaster in April 2015: pre-event (July 2013) and post-event (February 2016).

Ucides cordatus density estimation followed the method of Pinheiro and Almeida (2015) and considered the biological characteristics of this species (see Pinheiro and Fiscarelli, 2001; Wunderlich *et al.*, 2008). Four sampling quadrats (5 × 5 m each) were "randomly" arranged per mangrove area within a range of 10–50 m from the estuarine margin. In each quadrat, the total number of 'uçá'-crab galleries was counted (active open: with recent tracks, feces, and sediment accumulation close to the opening; closed: recently or not recently occluded).

According to Pinheiro and Fiscarelli (2001), each gallery excavated by *U. cordatus* is occupied by a single specimen. The authors calculated population density by dividing the total number of galleries by the area of each sampling square (25 m^2), expressed as the number of individuals per square meter (ind./m^2). Thus, population density was calculated for each mangrove area by the mean (± standard deviation) of the four sampling squares (replicates), both for the pre- (2013) and post-disaster (2016).

Crab population structure was evaluated by transforming the diameter of open galleries (GD), which were measured parallel to the sediment with a modified caliper (0.05 mm precision). Conversion between GD to crab size (CW, carapace width) was performed using the linear equation $CW = 13.21 + 0.9602 \cdot GD$, which was applied in previous monitoring (see Pinheiro and Almeida, 2015). Therefore, population structure was developed according to three parameters: 1) crab size (CW), comparing the means obtained in mangrove

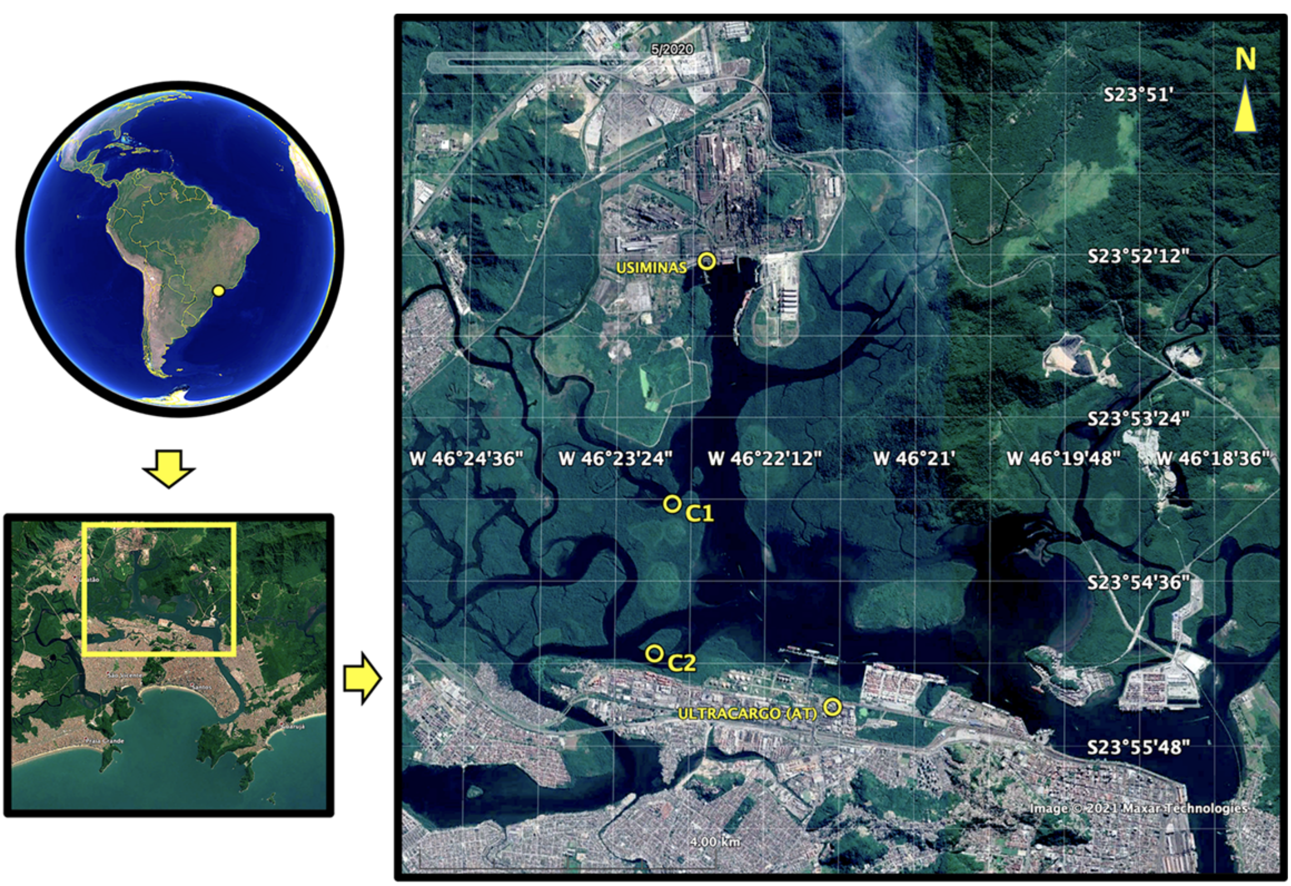

Figure 1. Inner region of the Santos-São Vicente Estuary, at Cubatão municipality, central coast of the State of São Paulo, Brazil, with two mangrove areas (C1 and C2), near to the environmental disaster which occurred at ULTRACARGO (AT). Source: modified from Google Earth® (Image © 2021 Maxar Technologies – Image from May, 24th 2020).

areas and the evaluated years; 2) extractive potential (see Wunderlich *et al.*, 2018), represented by immediate extractive potential (IEP, crabs with CW ≥ 60 mm) and future extractive potential (FEP, crabs with CW < 60 mm); and 3) skewness coefficient (*sk*), obtained by:

$$sk = \frac{n\Sigma(x_i - x)^3}{(n-1)(n-2)s^3} \quad (1)$$

Where: *sk*, skewness coefficient; *n*, number of crabs in each sample; x_i, value of the *i-nth* data; x, arithmetic mean; s, standard pattern. The generated curve will present a symmetric distribution (balance between juvenile and adult specimens) when the value is in the range $-0.5 \leq sk \leq 0.5$, being asymmetric when outside this range (positive: $sk > 0.5$; or negative: $sk < -0.5$). In addition, according to Wegner (2010), asymmetry can be moderate (positive, $0.5 < sk < 1$; or negative, $-1 < sk < -0.5$) or high (negative, $sk \leq -1$; or positive, $sk \geq 1$).

Sublethal Damage: Cytogenotoxicity Assessment

Ten adult male 'uçá'-crabs (CW ≥ 60 mm), at all stages of ecdysis, were manually collected in the mangrove areas (C1 and C2), in each of the years under analysis (2013 and 2016), following Pinheiro *et al.* (2013). Two hemolymph samples were obtained from each specimen, following the procedures of Pinheiro *et al.* (2013) for cyto- and genotoxicity assessments. The hemolymph of each specimen was removed with a hypodermic syringe (1 mL) equipped with a 21G needle. The procedure consisted of puncturing the articulating membrane of the locomotor appendages, preferably between the carpus and propodus of the major cheliped.

Genotoxicity was evaluated by the micronucleus assay (MN), a successful protocol for *U. cordatus* (see Pinheiro *et al.*, 2013; and Duarte *et al.*, 2016; 2017; 2019; 2020). For this purpose, two hemolymph smear slides per specimen were prepared and viewed under a common optical microscope (400 ×) to record hyalinocyte hemocytes with micronuclei, as well as to determine their frequency in 1,000 evaluated cells. All slides were stained for 15 minutes using Giemsa solution (2 %) ($Na_2HPO_4 + KH_2PO_4$, pH 6.8), washed with deionized water and air-dried. Following Countryman and Heddle (1976), only micronucleated cells having the following characteristics were counted: 1) micronucleus with color similar to that of the cell nucleus; 2) micronucleus size < 1/3 cell nucleus size; and 3) lack of connection between the micronucleus and the cell nucleus. Cells with more than three micronuclei were not considered in the count.

Cytotoxicity was evaluated by lysosomal neutral red retention time (NRRT) according to the protocol established by Duarte *et al.* (2016) and adapted for *U. cordatus*. For this purpose, microscope slides, previously treated with a diluted solution of poly-L-lysine (1:10), were used to adhere live hemocytes after their treatment with neutral red solution, saline solution, and anticoagulant solution (2.05 g of glucose, 0.8 g of sodium citrate, 0.42 g of sodium chloride, and 100 mL of distilled water). In this treatment, the hemolymph of each specimen (0.5 mL) was diluted with a syringe (1 mL) with the same volume of anticoagulant solution. This content was transferred, without use of the needle, to microtubes (2 mL) that, after being slightly homogenized, were kept at rest (15 – 20 min). Subsequently, 40 µL of each microtube were transferred with a micropipette to the slides, which were kept in a humid dark chamber (15 min) for greater adherence of hemocytes to the surface. Then, each slide received 40 µL of the neutral red solution and was left again to rest (15 min), after which they received a cover slip. In the first hour, the slides were examined (every 15 min) under an optical microscope (400 ×), and then examined for a second hour (every 30 min) when necessary. Retention time analyses focused on ten hemocytes per slide (Matozzo and Marin, 2010), with annotation of those undergoing apoptosis at each inspection time. To determine cell stress, the size, cell shape, and color of lysosomes were analyzed, which help to indicate the impact of contaminants (Collier *et al.*, 2013).

Statistical Analysis

Statistical analyses were performed in RStudio (version 1.2.1335 – R Core Team, 2019), following the statistical indications of Sokal and Rohlf (2003). Empirical data on quantitative parameters (crab density and size; micronucleated cells; and cell membrane integrity time) were previously submitted to a variance homogeneity test (*L*, Levene) and a normality test (*W*, Shapiro-Wilk).

Confirmation of the normality and homoscedasticity of these variables allowed the means to be compared by ANOVA, with *a posteriori* Tukey test. In cases of no confirmation, the means are compared by Kruskal-Wallis, with *a posteriori* Nemenyi test (Zar, 1999; Pohlert, 2014). Thus, data on each parameter were compared between the mangrove areas (C1: 3.7 km; and C2: 2.8 km) and either side of the environmental disaster (2013: pre-disaster; 2016: post-disaster).

The extractive potential of the 'uçá'-crab was established by the IEP and FEP percentages for each mangrove area and sampling year, being compared by chi-square test (χ^2), based on its assumptions, at 5 % significance level.

After conversion of gallery diameter (GD) to carapace width (CW), crab size data for each mangrove area and sampling year were submitted to skewness coefficient analysis (Meyer *et al.*, 2021).

The dataset obtained was submitted to a multifactorial analysis, with the means of each response variable disposed in a principal component analysis (PCA), using similarity distances and cluster analysis (Euclidian metric and Ward method) according to Kaufman and Rousseeuw (1990). These variables were mainly calculated using the FactoMineR package (Le *et al.*, 2008; Husson *et al.*, 2018), making it possible to classify mangrove areas and disaster years in groups (Q mode), by similarity.

Results

Population Structure, Density and Extractive Potential

None of the variables had a normal distribution or homogeneous variance (according to the Shapiro-Wilk and Levene tests, respectively; $p < 0.05$). Therefore, they were submitted to Kruskal-Wallis (KW) analysis, with pairwise contrasts by a post-hoc Nemenyi test.

The size of *U. cordatus* crabs ranged from 15.4 to 95.0 mm CW (67.0 ± 14.9 mm CW). Crabs from the C1 area (52.1 ± 16.3 mm *CW*) were smaller than those from the C2 area (56.6 ± 15.9 mm CW) (KW = 15.14, $p = 9.96 \times 10^{-5}$). The interaction between area and year was also contrasting, with the following size hierarchy: C1/2016 < C1/2013 < (C2/2013 = C2/2016) (KW = 19.32, $p = 2.35 \times 10^{-4}$). Furthermore, the mean size of crabs was 9.3 % smaller in the post-disaster (54.9 ± 16.3 mm CW*)* than in the pre-disaster (60.5 ± 14.4 mm CW) (KW = 7.42, $p = 0.0065$) (Fig. 2A).

Population density, in turn, ranged from 0.6 to 2.8 ind./m^2 (1.4 ± 0.7 ind./m^2), with no significant differences between the study areas (C1: 1.5 ± 0.9 ind m^2; C2: 1.4 ± 0.3 ind./m^2) (KW = 1.51, $p = 0.219$) or regarding the disaster (pre-disaster: 1.3 ± 0.6 ind./m^2; post-disaster: 1.7 ± 0.7 ind./m^2; KW = 3.22, $p = 0.359$) (Fig. 2B).

The number of adults (CW ≥ 60 mm) exceeded that of juvenile specimens in the two mangrove areas by about 3 to 10 times (C1 and C2, respectively), a pattern that was maintained both in the pre- and post-disaster periods (Tab. 1). Moreover, the future extractive potential (FEP) increased by about three times in the post-disaster (see C2 and overall total), implying a consequent reduction in the respective percentages of IEP. The limitations of the χ^2 test made it impossible to compare the percentages of juveniles and adults for the C1 area. The percentage reduction of adults was similar in the mangrove areas (22 and 17 %, respectively). Meanwhile, the recruitment of juveniles was 62 % higher in the area closest to the disaster (C2) than in C1, corresponding to percentages of 71 and 27 %, respectively.

In the pre-disaster, the population structure of *U. cordatus* showed a moderate negative asymmetry (*sk* = –0.79), differing between areas (C1: symmetry = –0.05; and C2: moderate negative asymmetry = –0.84). The structure started to have a symmetric distribution in the post-disaster (*sk* = –0.15), with the following values in the mangrove areas (C1: –0.13; and C2: –0.43). Overall, the level of negative asymmetry decreased by 5.3 times in the post-disaster, with a reduction in adults and an increase in juvenile specimens in the two mangrove areas.

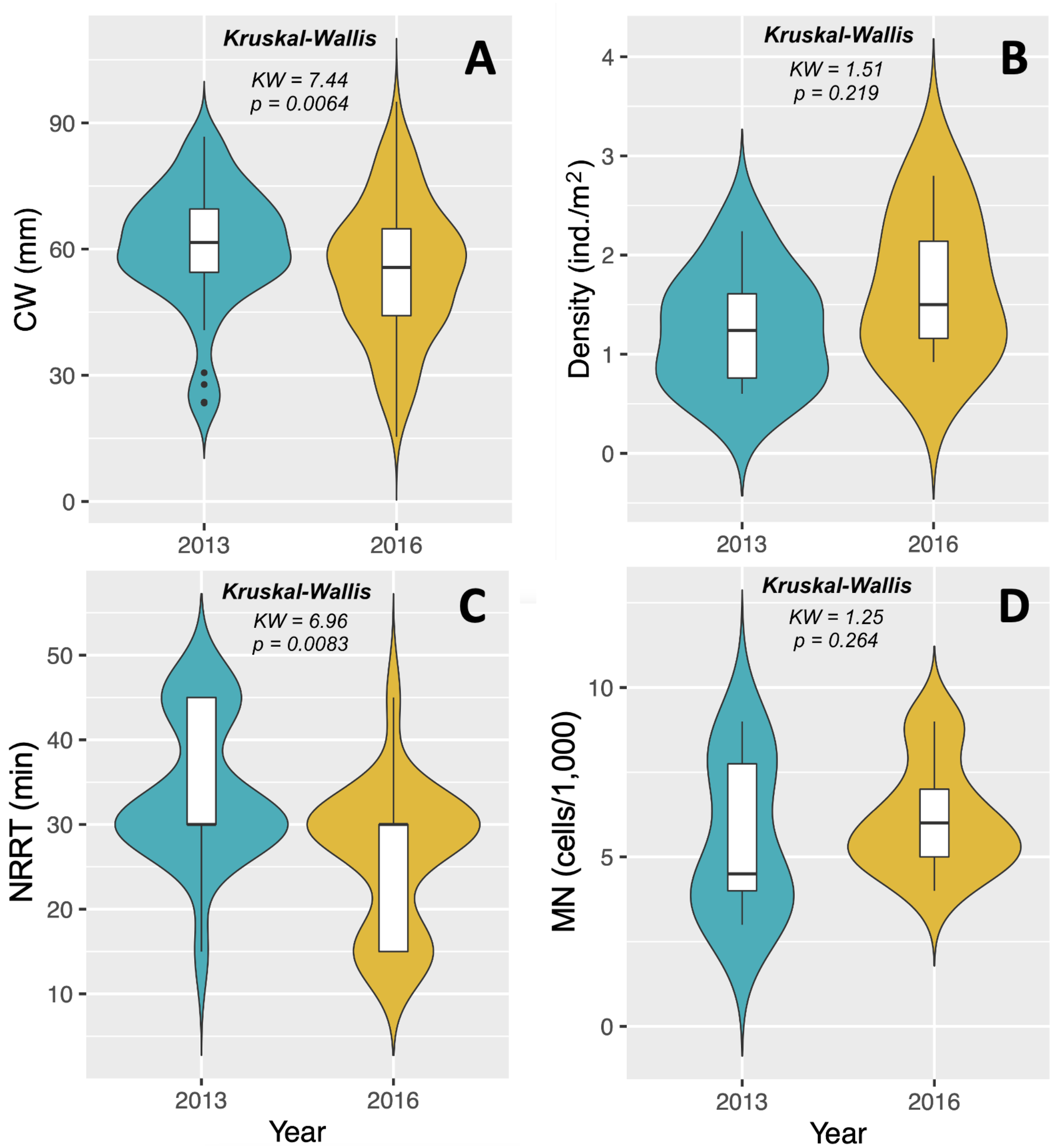

Figure 2. Population parameters and cytogenetic markers registered in the mangrove crab (*Ucides cordatus*), from Cubatão municipality (Brazil), based on two temporal periods bracketing an environmental disaster (2013: pre-disaster; and 2016: post-disaster). (**A**) crab size (CW, carapace width in millimeters); (**B**) population density (ind./m 2); (**C**) cytotoxicity biomarker (NRRT, neutral red retention time, in minutes); and (**D**) genotoxicity biomarker (MN, micronucleus assay, as micronucleated cells/1,000). Where: violin plot, represents kernel probability density; box plot, represented by median (horizontal line), box (IQR, interquartile range) and whiskers (1.5 times plus IQR).

Sublethal Damage: Cytogenotoxicity

As an indicator of cytotoxicity, neutral red retention time (NRRT) in *U. cordatus* ranged from 15 to 45 minutes (30 ± 9 min), not differing between the study areas (C1: 30.8 ± 9.1 min and C2: 29.3 ± 9.1 min; KW = 7.24, $p = 0.064$). However, the response time decreased by 22.2 % in the post-disaster (26.3 ± 8.3 min) in relation to the pre-disaster (33.8 ± 8.4 min) (KW = 6.96, $p = 0.008$) (Fig. 2C). In turn, genotoxicity ranged from 3 to 9 MN‰ (5.9 ± 1.8 MN‰), not differing significantly between mangrove areas (C1: 5.6 ± 1.8 MN‰; and C2: 6.2 ± 1.9 MN‰; KW = 1.25, $p = 0.264$) or regarding the disaster (pre-disaster: 5.5 ± 2.3 MN‰; and post-disaster: 6.1 ± 1.6 MN‰; KW = 2.86, $p = 0.41$) (Fig. 2D).

The first two dimensions of the PCA explained 87.7 % of cluster variance in a significant way ($p < 0.01$).

Table 1. Extractive Potential (%) recorded for the mangrove crab (*Ucides cordatus*) in two mangrove areas (C1 and C2), municipality of Cubatão (Brazil), regarding the environmental disaster (2013: pre-disaster; and 2016: post-disaster), that occurred in 2015 at ULTRACARGO (AT). Where: FEP, future extractive potential (juvenile crabs: carapace width < 60mm); and IEP, immediate extractive potential (adult crabs: carapace width ≥ 60mm); χ^2, chi-square test.

Year (disaster situation)	Area	n	FEP (%)	IEP (%)	χ^2
2013 (pre-disaster)	C1	16	31.3	68.8	- [na]
	C2	48	8.3	91.7	33.33 *
	Total	**64**	**14.1**	**85.9**	**33.06 ***
2016 (post-disaster)	C1	77	42.9	57.1	1.57 [ns]
	C2	84	28.6	71.4	15.43 *
	Total	**161**	**35.4**	**64.6**	**13.72 ***

* $p < 0.05$; [ns] $p > 0.05$; [na] not applied (n < 20).

The first dimension (Dim1) was the main discriminant between the pre- and post-disaster situation (2013 and 2016, respectively) involving the mangrove areas (C1 and C2), with the following clustering variables (correlation coefficient): *IEP* (0.71), *FEP* (–0.70), *CW* (–0.18), *NRRT* (0.91), *MN* (–0.68), and *crab* (–0.83) (Fig. 3).

The *PCA* data generated two distinct clusters depending on the environmental disaster (2013: pre-disaster; and 2016: post-disaster), each represented by the two mangrove areas (C1 and C2) affected. The post-disaster cluster (2016: C1 and C2) was more heterogeneous than the pre-disaster cluster (2013: C1 and C2), highlighted by the antagonism between the variables MN *vs.* NRRT and IEP *vs.* FEP.

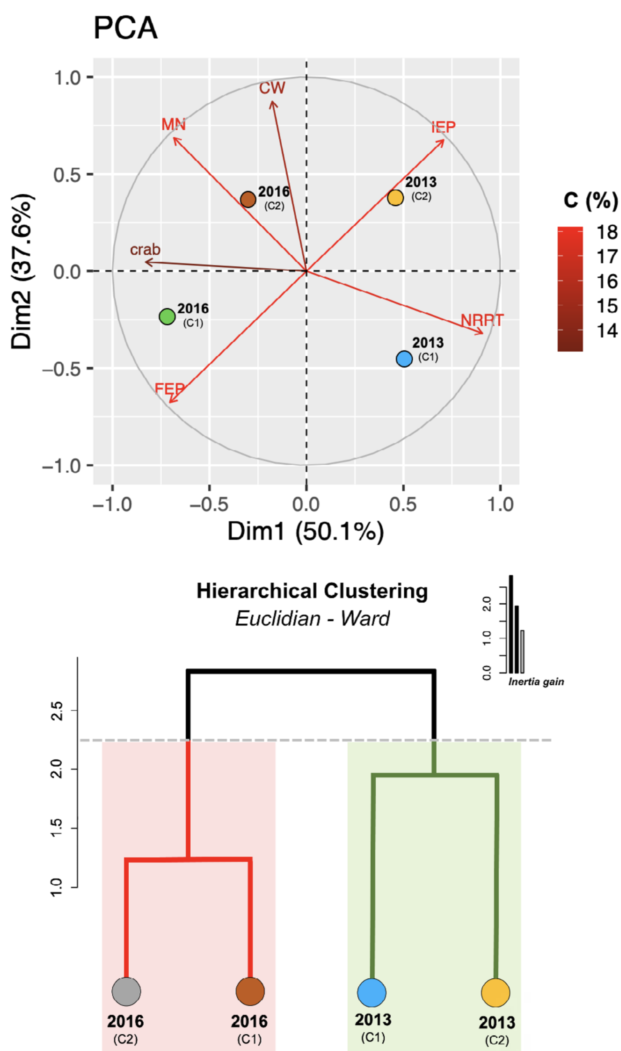

Figure 3. Principal Component Analysis (PCA) using a biplot function, with the percentual contribution (C) of each variable; and Hierarchical Clustering of similarity expressed by a dendrogram using the Euclidian metric and Ward method. A total of six variables based on 'uçá'-crab (*Ucides cordatus*) were used, in two mangrove areas (C1 and C2) at Cubatão municipality, bracketing an environmental disaster (2013: pre-disaster; and 2016: post-disaster). Where: CW, carapace width (mm); crab, population density (ind./m^2); FEP, future extractive potential; IEP, immediate extractive potential; MN, frequency of micronucleated cells (micronucleated hyalinocyte cells/1,000); and NRRT, neutral red retention time (min).

Discussion

The Juréia-Itatins Ecological Station (EEJI) is a Conservation Unit in São Paulo State that has extremely pristine coastal ecosystems, especially mangroves (Pinheiro *et al.*, 1013). According to Duarte *et al.* (2016), EEJI mangroves had a higher mean density of the 'uçá'-crab (1.9 ± 1.0 ind./m^2) than another five mangrove areas evaluated in the center-south coast of São Paulo State (represented by Cananéia, Iguape, Cubatão, São Vicente, and Bertioga). These authors confirmed that population density was associated with other biomarkers (MN, NRRT, and solid residues), allowing an indication of nonimpact damage category for EEJI (density > 1.7 ind./m^2). The 'uçá'-crab density in EEJI was 60 % higher than that of Cubatão (1.0 ± 0.7 ind./m^2), a region that suffered high impact damage at that time.

In the present study, the population density of *U. cordatus* did not differ depending on the mangrove area or the environmental disaster, being well represented by the overall mean (1.4 ± 0.7 ind./m^2). The result is very similar to that of Duarte *et al.* (2016), highlighting it as a variable little affected by chemicals from the environmental disaster.

Even in mangroves affected by diffuse contamination, as is the case in the inner area of the Santos-São Vicente Estuarine System belonging to Cubatão city, the density of *U. cordatus* varies according to its spatial distribution; as reported by some authors (see Góes *et al.*, 2010; Pinheiro and Almeida, 2015; Pinheiro *et al.*, 2018). According to them, *U. cordatus* density in mangroves is lower in the fringe zone (closer to the margin, with less topography and greater flooding), gradually rising towards innermost areas (basin zone and transition zone). This increase is more pronounced in the transition zone (known as "apicum zone"), as it is less immersed and little affected by flooding.

When in equilibrium, the population structure of *U. cordatus* tends to have a similar proportion between juvenile and adult specimens. This fact is supported by the size at maturity, which is reached with about half of the maximum average size (CW_{max}) according to Dalabona *et al.* (2005) (CW_{max} = 55.7 %, based on CW_{males} = 52.7 % and $CW_{females}$ = 58.7 %) and Pinheiro and Fiscarelli (2001) (CW_{max} = 58.3 %, based on CW_{males} = 61.5 % and $CW_{females}$ = 55.1 %). Pinheiro (2020) mentions that size distribution for *U. cordatus* may vary depending on tidal flooding level. The author reports a tendency toward negative asymmetry (predominance of adults) in areas with greater tidal flooding (fringe zone) and a tendency toward positive asymmetry (predominance of juvenile specimens) in less immersed areas with little flooding (transition zone). However, a joint analysis involving population structure (asymmetry coefficient) and extractive potential (FEP and IEP) showed that population asymmetry decreased in the post-disaster due to an increase in the number of juvenile specimens in relation to adults, with the distribution becoming symmetric.

The potential effect of contaminants from the environmental disaster may explain the population changes of the present study for the mangroves of Cubatão. In this area, the incidence of adult specimens decreased by 24.8 % (IEP: CW ≥ 60 mm), while the incidence of juvenile specimens increased by 39.8 % (FEP: CW < 60 mm), which brought balance and symmetry to population distributions. One of the effects of the post-disaster was the mortality and leaf fall of the mangrove arboreal vegetation, especially in areas close to the event (C2). This reduced the availability of leaves on the mangrove sediment, which are the main food item for adults of the species (Christofoletti *et al.*, 2013).

Considering the overall occurrence of vegetation damage in the entire inner area of the Santos-São Vicente Estuarine System, the reduction of adults may have occurred due to migration in search of food. Moreover, it may also be due to mortality from starvation, since leaf litter is the main food source of adult *U. cordatus* (81 % of its total food, according to Nordhaus *et al.*, 2006). On the other hand, the significant increase in the number of juveniles in the mangrove areas under study, especially those close to the disaster (C2), may be seasonal. This is because the recruitment of the 'uçá'-crab occurs mainly in the transition zone ("apicuns") of mangroves (Schmidt *et al.*, 2010; Pinheiro, 2020), which is less emerged and was less affected by flooding by contaminated estuarine waters.

Furthermore, feeding in the tiny juvenile stages of *U. cordatus* depends directly on the amount of microphytobenthos, meiobenthos, and sediment organic matter (Diele, 2000). Juveniles of this species usually dig their galleries in sediment previously bioturbated by adult specimens (Kassuga and Masunari, 2015). Codependence between the ontogenetic phases of *U. cordatus* is due to the availability of space (ecological niche) necessary for the excavation of galleries in the sediment. Hence, density is inversely proportional to the crab size, being also modulated by the amount of food available to adults from mangrove trees.

The results show that *U. cordatus* is extremely resilient to stress caused by environmental changes, showing adaptability and biological tolerance. Duarte *et al.* (2019; 2020), for example, confirmed this special ability in experiments of exposure to Cd and Pb. The authors showed that crabs residing in a pristine location (mangroves of the Juréia-Itatins Ecological Station) suffer more sublethal damage in the presence of these contaminants, accumulating greater concentrations of these metals in the gills (primary contact tissue — see Pinheiro *et al.*, 2012), both in their total and biologically active (toxic) form. On the other hand, in animals residing in a polluted place (mangroves of Cubatão), these metals accumulated more in the hepatopancreas (main detoxifying tissue — see Pinheiro *et al.*, 2012), but in low toxicity forms, which may denote a pathway of tolerance to these metals. The results corroborate the hypothesis of biological adaptation acquired over time by crabs from the polluted area.

Contaminant-tolerant populations though, have lower biological performance due to the cost that biological tolerance imposes. The intense use of defense mechanisms and damage repair increases metabolic expenditure and, consequently, physiological stress, which may affect the population in the long term (Amiard-Triquet *et al.*, 2013; Ortega *et al.*, 2016; 2017).

The mangrove fringe zone is the most impacted by contaminants from the environmental disaster, due to the greatest time (and frequency) of high tide flooding. It should be kept in mind that both mangrove areas under study have similar flooding levels. Despite this, C2 suffered a more intense negative effect due both to the proximity to Ultracargo - AT (disaster area) and to the lower depth along the margin, resulting in a smaller volume of water, but greater concentration and residence of the pollutants released. The lower heterogeneity of the population structure of C2 (variation coefficient, 19.9 %) in relation to C1 (24.8 %) confirms this aspect, since the negative effect was less intense in the latter area. The explanation for this has to do with the existence of a deep navigation channel close to C1 for the access of large ships to USIMINAS ('Usinas Siderúrgicas de Minas Gerais SA' — see Fig. 1). The area thus holds a greater volume of water and, consequently, a lower concentration of xenobiotics from the accident. Gimiliani *et al.* (2016) estimates the residence time of contaminants in the innermost waters of the Santos-São Vicente Estuary to be 8 days. This period may allow acute (or even chronic) effects, with consequences in the short or long term, depending on the specific characteristics and structure of each mangrove. This negative effect also affected the community of crab collectors who live directly from the sale of this resource (see Machado *et al.*, 2018), with a 17 to 22 % reduction in immediate extractive potential (IEP).

The genotoxicity analysis (MN assay) indicates more obvious and chronic changes due to pollutants. In contrast, the cytotoxicity analysis (NRRT assay) indicates acute physiological effects that rapidly change after reducing or suppressing the intensity of the stressor (Pinheiro *et al.*, 2017). The genotoxicity of *U. cordatus* was high in the two mangrove areas (> 5 MN‰), characterizing a high anthropogenic impact (see the environmental quality scale proposed by Duarte *et al.*, 2016). This parameter increased by 9.8 % in the post-disaster, although without statistical confirmation.

On the other hand, regarding cytotoxicity, environmental quality in the mangrove areas of Cubatão decreased by 22.2 % in the post-disaster. This fact was confirmed by the statistics, characterizing the area with high anthropogenic impact (NRRT < 60 min, according to Duarte *et al.*, 2016). This significant increase in the time of physiological integrity of hemocytes (cytotoxicity), as well as the change in the population structure of *U. cordatus*, confirms the use of this species as an important biological model, as well as its status as a sentinel species. As already mentioned, the 'uçá'-crab is extremely resilient due to its adaptation and tolerance to metallic contaminants. The species shows great plasticity in the treatment of environmental stress by xenobiotics, even in serious cases such as the disaster that occurred in the Aratu Terminal - ULTRACARGO (Cubatão, SP).

Even in the absence of confirmation of a significant genotoxic effect in the post-disaster (possibly due to the known history of contamination in Cubatão), cytotoxic data confirmed the damage. In this context, such damage can be considered biologically reversible to the 'original' levels, which we call pre-disaster, reinforcing that the environmental deterioration in the mangroves of Cubatão is systemic, taking place even before the fire disaster.

According to Pinheiro *et al.* (2017), the classic scheme suggested by Adams *et al.* (2001) was corroborated with this finding, where organic stress due to environmental impact can determine sublethal damage at different levels of biological organization and at different times (in hours, days, weeks, months, years or decades). Thus, biomarkers (MN and NRRT) can be used to detect effects at lower temporal levels, while population parameters (density and structure) are only evidenced after years, especially when considering that the average lifetime of *U. cordatus* is 10 years (Pinheiro *et al.*, 2005). Due to the relevance of this topic, continuous monitoring of mangroves should take place using *U. cordatus* as a sentinel species for recording relevant population parameters (*e.g.*, density and structure), as recommended by Pinheiro and Almeida (2015). The low cost of the process and the involvement of traditional artisanal fishing communities are highlights of such a procedure.

The size and scope of the environmental impact caused by the disaster at ULTRACARGO in 2015 includes likely reproductive damage to the population under study. Therefore, a conspicuous population imbalance is expected after the generation time of the species, estimated between 7.5 and 8.7 years, for males and females, respectively (Pinheiro *et al.*, 2016). Moreover, the easier applicability of some biomarkers is supported by the high speed and efficiency, low cost, and easy training of applicators, as is the case with genotoxicity markers (*e.g.*, MN assay).

Environmental disasters have acute and chronic effects, with extreme consequences for local fishing communities, especially traditional ones. In this sense, aiming at the prevention and mitigation of damage, Gillam and Charles (2018) mentioned as essential that the state and federal government employ efforts in the region. These efforts should favor: (1) the application of policies that ensure that the environment is protected from polluting activities of chemical and petrochemical industries; (2) the application of adequate financial compensation in environmental disaster situations; (3) effective support for artisanal fishermen, minimizing their subsistence losses and thus preventing them from changing their professional occupation; and (4) the adoption of appropriate conservation measures, supporting community participation, with the aim to restore coastal habitats, their marine resources, and the ecosystem services they provide.

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