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Comparative genotoxicity as a baseline protocol for assessing environmental quality in Brazilian Marine Protected Areas: A case study of Grapsus grapsus (Brachyura: Grapsidae) from Trindade and Fernando de Noronha islands

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ABSTRACT

Marine Protected Areas (MPAs) aim to preserve the existing biological richness. However, anthropic actions can compromise the stability of the ecosystem even in areas of environmental protection. The present study aimed to evaluate the environmental quality of two Brazilian MPAs, the Natural Monument of the Islands of Trindade and Martim Vaz and of Mount Columbia and the Marine National Park of Fernando de Noronha, through the evaluation of genomic damage in hemocytes of the rocky crab *Grapsus grapsus*. The results indicated an environmental impact on the island of Fernando de Noronha, on the two evaluated beaches (Sueste and Sancho), demonstrated by high levels of micronucleated cells observed in the sampled animals. The likely explanation for this impact refers to the intense tourist activity on the island. Trindade Island was shown to be well preserved, with animals presenting basal levels of expression of genome damage possibly due to the minor human presence in the island and due to its distance from coastline (diluted pollutants). The results presented here highlight contrasting environmental circumstances in terms of effectiveness of conservation actions in Brazilian MPAs. In addition, the results also reaffirmed the accuracy of the environmental impact assessment protocol using the genome damage parameters to diagnose and monitoring the environmental quality.

1. Introduction

Environmental impacts are differentially established by human activities. Some impacts may be permanent, persisting in ecosystems and possibly resulting in biodiversity depletion or loss. On the other hand, some anthropic impacts are seasonal, when they expose organisms to different levels of environmental alterations and can affect their structure and population dynamics (Dias et al., 2009). The establishment of Conservation Units (CUs) aims to safeguard the representativeness of ecologically significant sections of different populations, habitats, and ecosystems, preserving the existing biological richness, in terms of mitigating the rapid loss of biodiversity and ecosystem services (Bernard et al., 2014; Oliveira-Roque et al., 2018; Mills et al., 2020). In addition, these areas guarantee the sustainable and rational use of natural resources to the populations, while allowing the development of sustainable economic activities (Silva, 2019). Brazil is home to one of the largest systems of protected areas in the world, with approximately 271 million ha (154 million ha of parks and other categories of protected lands) and over 117 million ha of indigenous lands, with threatened biomes being included in these areas (Oliveira-Roque et al., 2018). With regard to the marine environment, Brazil has 177 CUs named Marine Protected Areas (MPAs) comprising 26.4 % of the 3.5 million km^2 of the Exclusive Economic Zone. However, only 3.3 % of this area is made up of full protection CUs (SNUC-MMA, 2019).

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The Marine National Park of Fernando de Noronha was created with the aim of valuing natural environments and scenic beauty, protecting marine and terrestrial ecosystems, and preserving fauna, flora, and other natural resources. The park has a total area of 11,270 ha, which corresponds to 70 % of the island of Fernando de Noronha and the other islands of the Fernando de Noronha Archipelago (ICMBio, 2022a).

The urban area of the Fernando de Noronha Archipelago is located within the Environmental Protection Area (APA) of Fernando de Noronha - Rocas - São Pedro and São Paulo, which has a total area of 79,706 ha. The main objectives of the APA are to reconcile human occupation on Fernando de Noronha Island with the protection of the environment, to make tourism compatible with the conservation of natural resources and preserve the environmental quality and living conditions of people, and local fauna and flora (ICMBio, 2022a).

According to IBGE (2022), the population of Fernando de Noronha was estimated in 2021 at 3140 people. However, local tourist activity generates a floating population that contributes to the population density of the island. In 2019 this represented 106,130 people, while in 2020, due to the restrictions imposed by COVID-19, the island of Fernando de Noronha received only 33,836 visitors (G1 Pernambuco, 2022a). During 2021, a total of 114,108 tourists were registered, the largest flow of visitors that the island has ever received (G1 Pernambuco, 2022b). It is estimated that in the last decade, the increase in the total flow of tourists to Fernando de Noronha has been around 500 %. The growing numbers are worrying indicators of the threat to the conservation of local biodiversity (Zanirato and Tomazzoni, 2014).

With an area of approximately 40 million hectares, the Environmental Protection Area of the Island of Trindade and Martim Vaz, comprises the area of the Exclusive Economic Zone referring to the radius of two hundred nautical miles around the Islands of Trindade and Martim Vaz. The Natural Monument of the Islands of Trindade and Martim Vaz and Mount Columbia is made up of four areas that add up to approximately 7 million hectares (ICMBio, 2022b).

Although the islands of Fernando de Noronha and Trindade are conservation units, they have suffered alterations in their biodiversity. The current fauna and flora do not reflect the original biodiversity and ecological structure, demonstrating the fragility of island environments in terms of occupation, mainly endangering endemic species (Serafini et al., 2010; Magris et al., 2021).

Associated with the establishment of Protected Areas, biodiversity monitoring initiatives in tropical countries can improve understanding of the conditions and trends of biodiversity in the world (Oliveira-Roque et al., 2018), as well as global ecosystem dynamics that have been constantly and significantly altered by man (Vianna, 2020). The differentiation of natural processes from induced processes, as well as their consequences for the next generations and the ecosystem is crucial for the real effectiveness of biomonitoring (Vianna, 2020). In Brazil, Marine Protected Areas (MPAs) suffer from a lack of inspections, the absence of management plans and long-term monitoring programs, and a lack of financial and human resources, calling into question the effectiveness of these as protection areas (Motta et al., 2021).

The integration of biodiversity diagnostic and monitoring protocols that provide information on the state of biological diversity allows the availability of timely, comprehensive, and multi-scale information on biodiversity and ecosystem services. In this way, such integration allows the assessment of impacts and adequate decision-making for the conservation of Protected Areas (Oliveira-Roque et al., 2018). Therefore, monitoring systems based on different methods and specific bioindicators make it possible to carry out a diagnosis of the environmental quality of areas exposed to events that are harmful to the ecological balance, enabling identification of the cause-effect relationships between the aggressive agents and the environment (Ferreira and Olivati, 2017).

Due to the multiple ecological functions performed by crabs in terms of structure and function in the ecosystem, these species are considered good sentinels to assess the impacts of human activities on their environment (Ngo-Massou et al., 2018; Pragnya et al., 2021). *Grapsus grapsus* is a marine grapsid crab that inhabits rocky shores on oceanic islands, where it is endemic. This species had not yet been evaluated as a bioindicator of environmental quality, which translates as an unprecedented and original fact in the present study. Some species of brachyurous have already been evaluated in this regard, especially in the mangrove ecosystem (Pinheiro et al., 2017; Pinheiro et al., 2021). It is a species that is not under threat of extinction on the oceanic islands that are part of the Brazilian territory, where it is in the "Least Concern" category (LC - Least Concern) (Freire et al., 2024).

At this reasoning, to access the information on the genome of different species allows the monitoring of biological responses to different environments, as well as the identification, sustainable use, and conservation of exploitable biological resources. Genome toxicity (the effect of causing damage to the genome) occurs as a consequence of the action of a toxic agent (disturbing/genotoxic) that alters the structure and/or content of chromosomes (clastogenicity/aneugenicity) or the sequence of DNA base pairs (mutagenicity). Identification methods are based on the premise that a single molecule, interacting with DNA, can result in damage to the genetic material (Guérard et al., 2015). Therefore, even very low (or sublethal) concentrations of genotoxic agents in ecosystems are capable of affecting individuals, in all stages including reproduction, embryogenesis, development, growth and survival, contributing to carcinogenic processes and hereditary and teratogenic defects (Ali et al., 2014; Bolognesi and Cirillo, 2014; Silva et al., 2022; Yilmaz et al., 2014). In addition, exposure to toxicogenomic agents, including those of anthropic origin, can result in loss of the genetic diversity (genetic erosion) of a population faced to this type of stress. An eroded genetic variation compromises the resilience of species populations front to environmental demands over time, possibly leading to their extinction. Natural populations can be chronically exposed to pollutants for multiple generations, which can result in reduced genetic diversity, and consequently result in strong selection for tolerance or population decline (Nowak et al., 2009; Stefani et al., 2014).

To assess genome toxicity for testing environmental quality, the Micronucleus Test has been widely used (Falcão et al., 2020; Lima-Cardoso et al., 2018; Yáñes-Rivera et al., 2019), due to its speed, sensitivity, practicality, and low cost. The principle of the test is based on the fact that, during the cell cycle, especially in anaphase, the chromatids and acentric chromosome fragments (macrolesions) are not transported by the spindle fibers to opposite poles, while the fragments with centromeres are. After telophase, the undamaged chromosomes are included in the nucleus of each of the daughter cells. Elements that were not transported by the spindle can also be engulfed by the newly formed nuclei. However, some of these elements, normally very small, are not included in the newly formed nuclei, remaining in the cytoplasm in the form of micronuclei (Schmid, 1975). These genomic fragments are large-scale losses of genetic material that can compromise the cellular, tissue, and organic viability of individuals, leading from population depletion to their extinction. Therefore, the Micronucleus Test enables the sublethal detection of the effects of toxic substances on organisms, the assessment of the impact and effect of these substances on cells, tissues and organs, as well as inferences about possible metabolic disturbances. In summary, perturbed environments compromise the ecological stasis of biological communities, including humans.

Considering these assumptions, the current study aimed to contribute to the assessment of environmental quality in order to test the conservationist effectiveness of two contrasting MPAs (oceanic islands) in terms of anthropic effects, based on the assessment of genome toxicity through the analysis of micronucleated cells in *Grapsus grapsus*, as the sentinel species. In a more specific terms, this study tests the hypothesis that even areas designated for biodiversity conservation may have their environmental attributes compromised when subjected to different degrees of human occupation.

2. Materials and methods

2.1. Study areas and analyzed species

The Fernando de Noronha Archipelago is deeply representative in relation to natural ecosystems, with a high degree of complexity and intense metabolism in terrestrial and aquatic ecosystems, resulting in high productivity and biological diversity (Prazeres et al., 2012).

Trindade is an oceanic island occupied only by the Brazilian Navy and researchers during their scientific activities, which began on the island in early 1957, during the "International Geophysical Year" (Marinha do Brasil, 2022). However, in 2018, the following conservation units were created: Trindade Island Environmental Protection Area and Natural Monument of Trindade and Martim Vaz Islands and Mount Columbia (ICMBio, 2022b).

A total of 27 individuals of the gecarcinid crab species *Grapsus grapsus* (Fig. 1), which is endemic to oceanic islands in the South Atlantic, were collected at two Marine Conservation Units in Brazil. Ten specimens were sampled on Trindade Island (Andradas beach) adjacent to the Natural Monument of Trindade and Martim Vaz Islands and Mount Columbia and 17 more on Fernando de Noronha Island [Sueste beach (n = 10) and Sancho beach (n = 7)] in the National Marine Park of Fernando de Noronha (Fig. 2)

2.2. Sampling

Before collecting the hemolymph from the animals, an anticoagulant solution was prepared (0.49 M NaCl, 30 mM trisodium citrate, and 10 mM EDTA) according to Klobucar et al. (2012). For hemolymph collection, 1 mL syringes were prepared, with a 29 G needle, which were filled with 200 μL of anticoagulant solution. Each syringe was used to sample the hemolymph of a single specimen, which occurred by inserting the needle into the joint membrane of the third pair of pereiopods of the crabs.

Syringes (1 mL) were used, with 21 gauge needles (prevents stress to the hemocytes during puncture), inserted in the membrane of articulation between the propod and carpus of the first locomotor appendix (cheliped). All this information, as well as the preparation of anticoagulant solution, and its use in the syringe, prior to the puncture of the hemolymph, are already described in Pinheiro et al. (2013), Adam et al. (2023), and Duarte et al. (2016). After collecting the hemolymph, the animals were released in the same place where they were collected. This research was approved by SISBIO (Biodiversity Authorization and Information System) of ICMBio (Chico Mendes Institute for Biodiversity Conservation; license # 75834). The animals were not anesthetized before the extraction of hemolymph, since the method is non-invasive and because they are released alive soon after the collection of the samples, preserving animal welfare. All collection and handling methods, as well as the amount of specimens used as sentinels, always ensuring animal welfare, were authorized by SISBIO, via the competent agency (ICMBio).

2.3. Laboratory analysis

The Micronucleus Test was conducted according to the work by Siu et al. (2004), with the analysis of micronuclei (MN; showed in detail - Fig. 1) following the protocols of Heddle (1973) and Schmid (1975), with modifications. Cell evaluation was performed using hemolymph smears from the sampled animals. Once the smear had been produced, the slides were left at room temperature for drying, with subsequent fixation in absolute methanol for 5 min. The slides were then stained with Giemsa solution for 5 min. According to the criteria adopted by Moron et al. (2006), micronuclei were considered as nuclear fragments that have no connection with the main nucleus, that present the same color/intensity as the nucleus, and whose size does not exceed one third of the size of the main nucleus. In total, 1000 cells of each specimen of *Grapsus grapsus* were analyzed.

2.4. Data analysis

The data obtained for the frequency of micronucleated cells recorded per 1000 analyzed (MN), in each of the sampling sites, were arranged in electronic spreadsheets to obtain an analytical matrix, submitted to statistical packages available in *R* environment (R Core Team, 2021). Initially, the MN variable was submitted to a test of homogeneity of variances (*L*, Levene) and normality (*W*, Shapiro-Wilk). If normality was confirmed ($p > 0.05$), the data were submitted to a *t*-test or *ANOVA*. Otherwise, if the normality of the data was not confirmed ($p < 0.05$), the data were submitted to a non-parametric test (*KW*: ANOVA by Kruskal-Wallis ranks), where the medians would be compared "a posteriori", by Dunn's multiple comparisons test (Siegel and Castellan, 1988). Statistical analyses were set at 5 % significance, using *RStudio v. 1.4.1106*.

To test whether the observed differences in micronucleus frequency

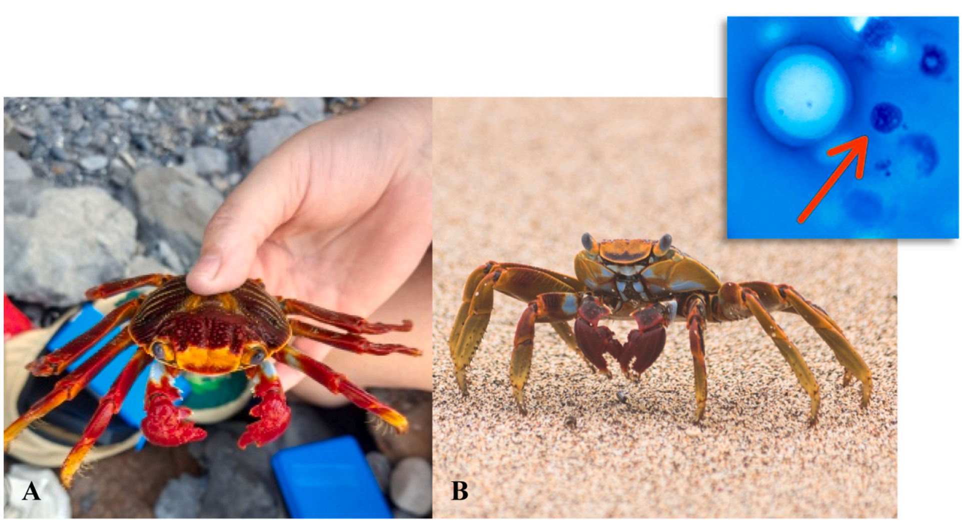

Fig. 1. Specimens of *Grapsus grapsus* collected in Sancho beach (A - Fernando de Noronha island) and in Andradas beach (B – Trindade island). In detail above an hemocyte (red arrow) from *G. grapsus* showing a large DNA mass (nucleous) and close to the right side a small DNA fragment (micronucleous). Photo credits must be addressed to Anderson R. B. de Lima (A; UFRuPE) and to Nikolas Krieger (B; UNESP IB/CLP - CRUSTA).

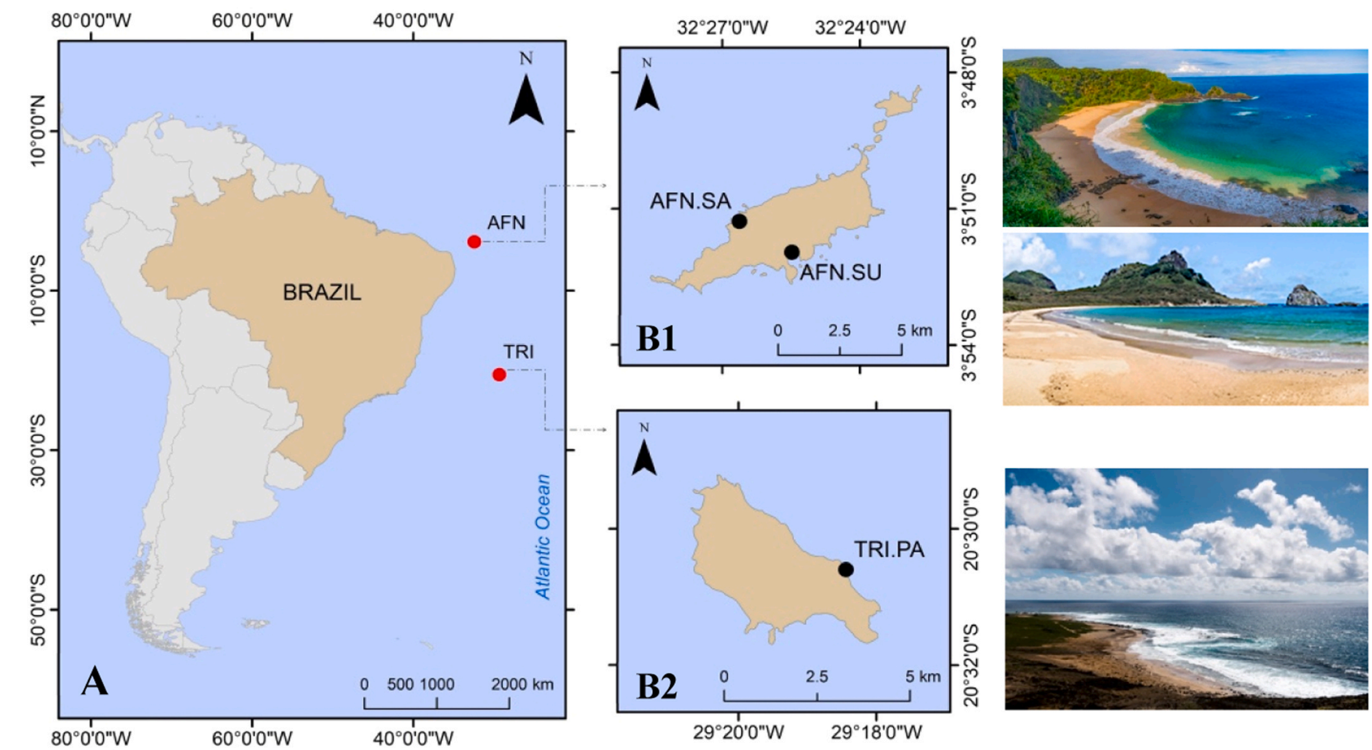

Fig. 2. Sampling sites of *Grapsus grapsus*. A) location of the two islands in the South Atlantic (AFN, Fernando de Noronha Archipelago; and TRI, Trindade Island); and B) location of the three sampling areas on Fernando de Noronha Archipelago (B1 - AFN.SA, Sancho beach; and AFN.SU, Sueste beach) and Trindade Island (B2 - TRI.PA, Andradas beach). From up to down, the first two photo beaches were captured from internet (https://blog.bonitour.com.br/praia-do-sancho-fernando-de-noronha/ and https://www.uol.com.br/nossa/noticias/redacao/2022/07/05/praia-do-sueste-em-noronha-e-liberada-para-turistas-apos-cinco-meses.htm, respectively). The credits to the photo in the bottom must be addressed to Nikolas Krieger (UNESP IB/CLP - CRUSTA).

between *Grapsus grapsus* populations from Trindade and Fernando de Noronha islands could be attributed to random processes (as other environmental variables), we applied the null model approach based on non-parametric bootstrapping. We specifically analyzed the proportion of micronucleated cells (prop_mn), calculated per individual as the number of micronucleated cells divided by the total number of hemocytes scored.

Prior to the simulations, the dataset was evaluated for adherence to the assumptions of parametric tests. Normality was assessed using the Shapiro-Wilk test, and homoscedasticity using Levene's test. Both island populations exhibited distributions consistent with normality (Trindade: $W = 0.850$, $p = 0.058$; Noronha: $W = 0.958$, $p = 0.763$) and equal variances ($F = 1.83$, $p = 0.193$), validating the dataset's structure. Nonetheless, we opted for a null model simulation to avoid relying on parametric assumptions and to provide a more robust inferential framework.

In this procedure, 10,000 simulations were conducted by randomly shuffling island labels among individuals, preserving the original group sizes. For each simulation, we calculated the difference in medians of *prop_mn* (Noronha - Trindade). The empirical *p*-value was defined as the proportion of simulations in which the simulated difference equaled or exceeded the observed one, following the methodology proposed by Gotelli and Graves (1996) and Manly and Alberto (2020).

This approach has been widely recommended in ecological and ecotoxicological studies, particularly when the number of populations is limited and the data are potentially skewed or influenced by outliers. It provides inferential strength even under suboptimal experimental designs, allowing for a more conservative and assumption-free assessment of non-random patterns.

3. Results

The frequency of micronucleated cells per 1000 analyzed (MN/1000) was recorded for *Grapsus grapsus*. The MN variable showed normal distribution and homoscedasticity, allowing the application of parametric tests to compare the averages obtained in different sampling sites.

The genotoxicity of *G. grapsus* was established for specimens collected in the two coastal islands Trindade (TRI, n = 10) and Fernando de Noronha Archipelago (AFN, n = 17). Table I shows that, on average, genotoxicity in AFN was 13.9 ± 3.1 MN/1000 (8–20 MN/1000), being 10.7 times higher than in TRI (1.3 ± 1.1 MN/1000, ranging from 0 to 3 MN/1000) (t = -12.27; P = $4.44 \cdot 10^{-12}$) (Table 1). The opposite occurred with the coefficient of variation (CV%), which in TRI (81.5 %) was 3.6 times higher than in AFN (22,5 %).

Table 1
Comparison of the means of micronucleated cells per 1000 analyzed (MN/1000) for the crab *Grapsus grapsus*, between two islands in the Atlantic Ocean, represented by Trindade Island (TRI) and Fernando de Noronha Archipelago (AFN). Where: n = number of samples; Min = minimum number; Max = maximum number; $\bar{x}$ = arithmetic mean; s = standard deviation of the mean; CV (%) = coefficient of variation of the mean in percentage; t = *t*-test; P = statistical significance. Means followed by distinct letters differed statistically from each other.

Oceanic Islands	Micronucleated cells per 1000 (MN/1000)					t	*P*
	n	Min	Max	$\bar{x} \pm s$	CV (%)		
TRI	10	0	3	1.3 ± 1.1 a	81.5	−12.27	$4.44 \cdot 10^{-12}$
AFN	17	8	30	13.9 ± 3.1 b	22.5		

Whereas in TRI the samplings were carried out in a single location (Andradas beach, n = 10), in AFN they were carried out in two locations (Sueste beach: FN.SU, n = 10; and Sancho bech: FN.SA, n = 7), the means were compared by ANOVA, in a multiple comparison between them. The means of genotoxicity, originating from the three sites analyzed (homogeneous groups) contrasted significantly with each other (F = 157.35; P = 0.0000) (Table 2, Fig. 3), with the following increasing hierarchy of genotoxicity: TRI (1.3 ± 1.1 MN/1000) < AFN.SA (11.3 ± 2.1 MN/1000) < AFN.SU (15.8 ± 2.3 MN/1000).

In terms of the null model analysis the observed median proportion of micronucleated cells was 0.003 for individuals from Trindade and 0.048 for those from Fernando de Noronha, resulting in a difference of 0.045 (Fig. 4). The null model simulations revealed that this difference was significantly higher than expected by chance (p = 0.0006; 10,000 simulations), indicating a statistically structured pattern that departs from randomness.

4. Discussion

The results of the different incidence of micronucleated cells presented by *G. grapsus*, collected on the two islands under study, suggest different degrees of conservation between the two environments, where Trindade Island presenting better conservation conditions than Fernando de Noronha Island.

The control of access and permanence of people on Trindade Island is exercised by the Brazilian Navy (Faria et al., 2012), and is restricted to a contingent of about 40 people (including military personnel and researchers), which may have contributed to the low genotoxic impact observed. The only access to the island is by official boats every two months, with no airstrips or seaports. This distinct characteristic of the island favors the low anthropic impact observed, demonstrated by the low frequency of micronucleated hemocytes in the animals collected on the island. The mean number of micronucleated hemocytes (1.3) observed in these animals probably corresponds to errors in the DNA repair systems inherent to the duplication process of the genetic material and cell cycle of these animals. Although probable direct anthropic effects were not observed in the cells of the animals, mainly due to the low population contingent on the island, the island is not exempt from the negative effects of anthropic pressures in an indirect way, such as overfishing, pollutants carried by sea currents, and stressors from climate change (Oliveira-Soares and Lucas, 2018), which, due to the lack of seasonal diagnosis and environmental monitoring, may compromise the real preservation of this remote Brazilian marine protection area.

The high frequencies of micronucleated cells observed in animals

Table 2
Comparison of the frequencies of micronucleated cells / 1000 analyzed (MN/1000) for the crab *Grapsus grapsus*, in three insular areas of the Atlantic Ocean, represented by Trindade Island (TRI) and Fernando de Noronha Archipelago (AFN.SA, Sancho beach; and AFN.SU, Sueste beach). Where: n = number of samples; Min= minimum number; Max= maximum number; $\bar{x}$ = arithmetic mean; s = standard deviation of the mean; CV (%)= coefficient of variation of the mean in percentage; F= ANOVA F test; P = statistical significance. Means followed by different letters differed statistically from each other.

Oceanic Islands / Sampling Site	Micronucleated cells per 1000 (MN/1000)					F	P
	n	Min	Max	$\bar{x} \pm s$	CV (%)		
TRI	10	0	3	1.3 ± 1.1 a	81.5	157.35	0.0000
AFN.SA	7	8	14	11.3 ± 2.1 b	18.2		
AFN.SU	10	12	20	15.8 ± 2.3 c	14.6		
TOTAL	27	0	20	9.3 ± 6.7	72.5		

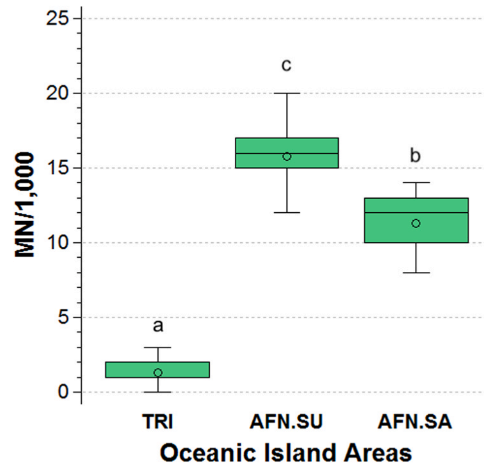

Fig. 3. Comparison on the frequencies of micronucleated cells (MN) in *G. grapsus* collected on the islands of Trindade (TRI) and Fernando de Noronha [AFN; Sueste (AFN.SU) and Sancho (AFN.SA) beaches]. The letters (a), (b), and (c) indicate different statistical significances among the data obtained from different beaches.

collected on the two beaches of the Fernando de Noronha Archipelago, around 10.7 times greater than the average observed on Trindade Island, calls attention to the conservationist efficiency of this MPA. Due to its exuberant beauty and paradisiacal environments, the Fernando de Noronha archipelago is a highly desired and visited tourist destination, by both Brazilians and foreigners. However, the tourist infrastructure of Fernando de Noronha cannot support the number of tourists allowed on the island. Thus, tourism has been considered one of the main impact factors of anthropic pressures on this ecosystem (Feitosa and Gómez, 2013; Cristiano et al., 2020; Grillo and Mello, 2021), as evidenced by the high genotoxic index in the hemocytes of the crabs in this study.

In addition to tourism, the resident population on the island, around 2630 people according to the last census (IBGE, 2022), also contributes to the anthropic impacts on the island. Considering both the resident and tourist population contingents, the effects related to the environmental impact on the island are worrying, mainly due to the demand for basic sanitary conditions. According to Cristiano et al. (2020) the main problems found on the island refer to deficiencies in the treatment of domestic sewage and the separation of solid waste. Included in this panorama are litter on the beach, the irregular disposal of effluents from the waste stabilization pools, that reach the beaches, effluents from the water desalination process, and the inefficient domestic sewage system on the island, these factors together presenting a great genotoxic potential (Pannetier et al., 2019; Rosner et al., 2023; Siqueira et al., 2021). It should be noted that only 65.71 % of residents have a sewage system, while 31 % have aseptic tanks and 3.29 % discard their waste in open ditches. The overflow of sewage containment pools to the beaches often occurs in the rainy season and in the high tourist season (Cristiano et al., 2020). Feitosa and Gómez (2013) add the burning of fossil fuels as a negative source of impact on this ecosystem.

The genotoxic potential of domestic sewage is already well known by the scientific community. An interesting result was presented by White and Rasmussen (1998) who demonstrated the genotoxic potential of household waste, highlighting the mutagens present in human feces, such as polycyclic aromatic hydrocarbons, aromatic amines and, mainly, fecapentenes, as they are a potent direct-acting mutagen (Gupta et al., 1983). According to the same researchers, the by-products of fossil fuel combustion also contribute to the genotoxic effects and enter the environmental impact system through surface runoff from roads. The genotoxic effects of fossil fuel by-products are already well established in the scientific literature (Rocha et al., 2015; Johann et al., 2020).

The panorama described above applies to Fernando de Noronha,

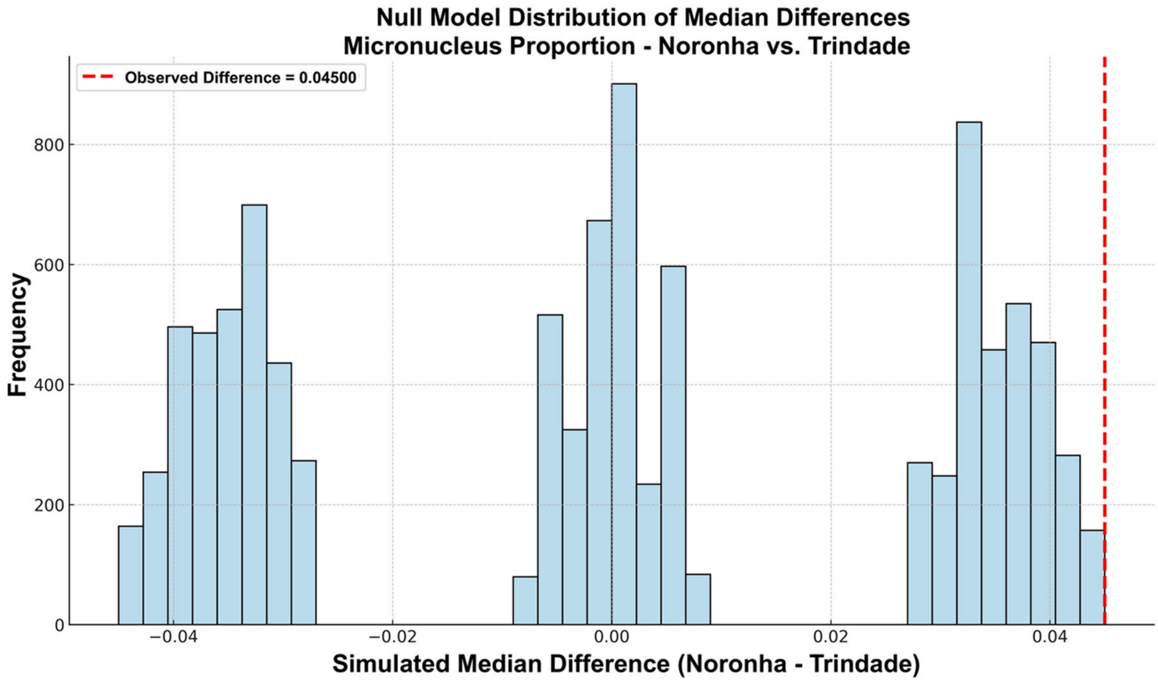

Fig. 4. Null model distribution of 10,000 simulated differences in the median proportion of micronucleated cells between *Grapsus grapsus* individuals from Noronha and Trindade islands. In each simulation, island labels were randomly reassigned while preserving the original group sizes, and the difference in medians was computed. The red dashed line represents the observed difference (0.045), which lies far outside the distribution generated under the null hypothesis, indicating a statistically significant and non-random pattern ($p = 0.001$). The discretized appearance of the histogram results from the limited number of unique values and sample size per group ($n = 10$), which causes multiple simulations to converge on the same median differences.

bearing in mind that the deficiency in the domestic sewage system, and the intense flow of cars on the island, which also contribute to the increase in sounds and noise, may have contributed to the increased expression of genomic damage in crab erythrocytes. With regard to the increase in noise, Adam et al. (2011) demonstrated, in an animal model, the genotoxic effect resulting from the stress generated by the increase in noise. In general, it has been demonstrated that crabs can react to sounds changing their behavior (Hughes et al., 2014) as a result of a physiological cascade episode. Also, it is known that crabs are very affected by noise from ships showing alterations in the oxygen consumed, indicating a higher metabolic rate and potentially greater stress (Wale et al., 2013). On Fernando de Noronha, in addition to the noise generated in the terrestrial environment, the noise caused in the aquatic environment due to the intense flow of vessels may also have contributed to the expression of genomic damage in *G. grapsus*, since these animals spend the longest period of their life in the aquatic environment.

The results of the frequency of micronucleated cells in *G. grapsus* referring to the two beaches of Fernando de Noronha were different from each other, as well as in relation to Trindade Island. In Fernando de Noronha Archipelago, it was found that the genotoxicity for Sueste beach was 39.8 % higher than for Sancho beach. Considering that Trindade Island is a pristine environment with basal genotoxicity, it is possible to estimates that the anthropic impact on the Fernando de Noronha Archipelago would be 9–12 times higher on Sancho and Sueste beaches, respectively, given the differences observed in the frequency of micronucleated cells.

The genotoxic differences found between the two beaches (Sueste and Sancho), may have occurred, mainly, due to the divergent distinct characteristics of each one. Sueste beach, located in Mar de Fora/Parque Nacional Marinho (PARNAMAR; windward) is characterized by a beach protected by islands and is home to the only insular mangrove in the South Atlantic, which, during the rainy season establishes a connection with the sea. Access control to the beach is carried out by the Chico Mendes Institute for Biodiversity Preservation (ICMBio), through an Information Center and Control Point (PIC) (Cristiano et al., 2020). Despite the entrance control, this beach is frequently visited due to the attraction provided by the great biodiversity present, mainly sharks, that are of great interest to tourists who seek the adventure of observing and swimming among these animals. Contributing to the negative environmental effects of the tourist flow, suggested by the higher incidence of micronucleated cells observed in the animals collected there, the proximity of the car park very close to the beach line results in increased pressure from environmental disturbing agents, such as noise and the effects of fossil fuels.

Sancho beach is located within PARNAMAR, but in Mar de Dentro (leeward), and access to the beach is also controlled by ICMBio. However, access to the beach is more difficult due to the accentuated relief, being carried out through stairs located on the slope of the rocks of the Quixaba Formation (Manso et al., 2011). The control of the number of people accessing the waterfront is also stricter. The beach is far from parking areas and the control point, which minimizes the impact expected from this proximity. Another access to the beach is via boats that travel to the beach and allow tourists to swim there. The difficulty and greater control of access to this beach and the distance from the parking areas may have been a contributing factor to the lower genotoxic effect observed in the analyzed animals. However, the higher levels of micronucleated cells observed in animals collected on this beach compared to those from Trindade, demonstrate a high genotoxic impact. These results suggest that even with lower values of genomic damage than the animals from the Sueste beach, the animals collected from Sancho beach provide a warning of the impact of the anthropic disturbances to which they are exposed.

Another differentiating factor between the beaches of Sueste and Sancho, which may have contributed to the differential results of genotoxic impact observed in animals on the two beaches, is the amount of litter transported by the sea. According to Ivar do Sul et al., (2009) the rotation of the northern South Atlantic current is the main factor promoting the transport of floating marine debris, directly influencing the Fernando de Noronha Archipelago, where the beaches of Mar de Fora (windward), such as Sueste beach, are more impacted by the contribution of floating marine litter than those of Mar de Dentro (leeward), where Sancho beach is located. According to Grillo and Mello (2021) windward beaches are more affected by plastic debris from bottle caps and hospital waste while leeward beaches are more affected by disposable plastics and cigarette butts. In addition, there is a vast amount of data demonstrating the genotoxic effect of debris on marine biota (Gomiero et al., 2018; Brandts et al., 2018; Molino et al., 2019; Cole et al., 2020; Giovani et al., 2022). Thus, the frequencies of micronucleated hemocytes of *G. grapsus* obtained in this study were able to discriminate the different impacts of anthropic disturbances on the two islands (Trindade and Fernando de Noronha), as well as differentiate the effects of these disturbances on beaches with natural characteristics and distinct anthropic influences. The anthropogenic disturbance observed in Fernando de Noronha was also proven by Araújo et al. (2023). This study observed high frequencies of micronucleated cells and nuclear morphological alterations in species of sharks (*Negaprion brevirostris* - Lemon Shark, *Galeocerdo cuvier* - Tiger Shark and *Ginglymostoma cirratum* - Nurse Shark). These evidences were related to the presence of heavy metals and surfactants in the water of the archipelago. The presence of surfactants, as reported, is directly related to the human presence, given that such chemical compounds are components of cleaning products, of personal hygiene and of pesticides. Also, spinner dolphins (*Stenella longirostris*) from Fernando de Noronha showed some preoccupant levels of DTT and HCH (organochlorines), and HMS (Homosalate) pollutants in blubber samples. These data were associated to the increasing degree of island human occupation and tourism (Combi et al., 2022)

Decreto no 92.755 (1986) and 9312 of March 2018 (Executive Power - Brazilian Government), which established the Federal Territory of Fernando de Noronha, Atol das Rocas, Penedos de São Pedro and São Paulo, and the Archipelago of Trindade and Martins Vaz, as Environmental Protection Areas, were premised on the fact that protected areas would represent one of the best approaches for resource management and for the conservation and/or restoration of biological communities. With the creation of such areas, it was expected that the level of protection would become the primary factor, with a direct positive influence on marine biodiversity. However, the results presented in the current study call attention to the effectiveness of this protection system. The high frequencies of micronucleated hemocytes in the animals collected in Fernando de Noronha are opposed to the objectives of a MPA, where the level of anthropic disturbance is evident, mainly due to the tourist flow on the island and the lack of management of these effects.

The statistical analysis revealed a clear and consistent pattern: individuals from Fernando de Noronha exhibited significantly higher proportions of micronucleated cells compared to those from Trindade. The difference was robustly supported by the null model approach, which ruled out stochasticity as a plausible explanation ($p < 0.001$).

Although both island populations passed tests of normality and homoscedasticity, the use of a bootstrap-based median comparison provided an additional layer of inferential strength by minimizing assumptions. The significantly higher genotoxic response in crabs from Noronha — an island with high human density and touristic activity — suggests a narrow association between anthropogenic pressure and DNA damage in insular environments. These results align with previous findings (Combi et al., 2022; Lima e Silva et al., 2022; Araújo et al., 2023, 2024) that link human presence to increased genotoxicity in sentinel organisms, reinforcing the relevance of *Grapsus grapsus* as a bioindicator species in oceanic islands.

In contrast, Trindade Island, primarily due to its remote location and absence of tourist interference, has successfully maintained its status within the criteria and objectives of an MPA. The findings of this study highlight the pristine condition of Trindade Island, evidenced by genotoxicity indices near baseline levels of micronucleus expression observed in the collected specimens. This original and unpublished information serves as a valuable reference for future research on oceanic islands globally. Furthermore, it provides a robust foundation for monitoring practices and the enhancement of Brazil's conservation strategies, aiming for more effective management of MPAs and the preservation of marine biodiversity. These results underscore the critical need for implementing sustainable measures to ensure adherence to the criteria established for the designation and maintenance of MPAs.

5. Conclusions

Genome toxicity varied significantly among the MPAs studied, revealing distinct levels of environmental impact and hierarchical conditions of ecosystem health. Specimens from the beaches of Fernando de Noronha Island exhibited the highest levels of genomic damage, with statistically significant differences between the two beaches, highlighting ongoing and discontinuous environmental impacts at this MPA. In contrast, *Grapsus gra*psus from Trindade Island exhibited lowest levels of genomic damage, underscoring its pristine environmental condition. These findings demonstrate hierarchical differences in environmental health among the studied MPAs and affirm *Grapsus grapsus* as a reliable sentinel species for assessing ecosystem health in insular environments especially at those with different human densities.

CRediT authorship contribution statement

Anderson R B Lima: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Harry Boos:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marcelo A A Pinheiro:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Adam Mônica L:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Rodrigo A Torres:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of Competing Interest

The authors signed below declare that there are no competing interests regarding employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registration, and grants or other funding related to the present research.

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Data availability

Data will be made available on request.

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