Contrasting tourism regimes due to the COVID-19 lockdown reveal varied genomic toxicity in a tropical beach in the Southern Atlantic

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Abstract Tourist occupancy in coastal environments threatens the stability of various coastal ecosystems and is thus a cause for concern for the environmental sector. As such, it is important to perform environmental monitoring in a way that analyses and quantifies the environmental impact of coastal ecosystems. Porto de Galinhas beach (Pernambuco – Brazil) has one of the highest visitation rates in Brazil and suffered from restrictions to human mobility due to the COVID-19 pandemic. These restrictions allowed for the evaluation of the impact of tourism on Porto de Galinhas beach and the effects that the lack of tourist occupancy had during the lockdown period of 2020. Blood samples from the species *Abudefduf saxatilis* were collected monthly over a period of 1 year and during the lockdown quarter, in order to perform micronucleus (MN) and nuclear morphological alteration (NMA) tests, and data were analyzed at a seasonal level (dry/rainy period) using a comet assay. For the control group, *A. saxatilis* samples were collected in an environmentally protected area on Tamandaré beach (68 km from Porto de Galinhas). The MN and NMA tests showed a greater frequency of genomic damage when there was greater tourist flow. In relation to rain seasonality, the comet assay showed a greater incidence of genomic damage during the dry period, where there was a higher rate of tourist migration, compared to the rainy period. The lockdown period presented a lower incidence of genotoxic damage compared to the period without restrictions on human mobility and the control. The results show that tourism has been causing a significant environmental impact on Porto de Galinhas beach. The data collected during the lockdown period demonstrated how the absence of human movement results in changes that are favorable to environmental recuperation, as illustrated by the lower frequency of genomic damage.

Keywords Environmental monitoring · Beaches · Genotoxicity · Tourism impact · COVID-19 pandemic

Introduction

The rapid expansion of the tourism industry, resulting from the growing rate of people visiting new places,
has highlighted the social and economic importance of this activity (Barbosa, 2020; Canteiro et al., 2018). In 2019, the tourism sector increased by 4%, with approximately 1.5 billion observed tourist visitations worldwide (UNWTO, 2020). Tourism is also one of the most important economic activities in coastal regions, especially in tropical countries (Gößling et al., 2018). Due to the benefits that coastal regions provide to human populations, such as the availability of a wide variety of ecosystem services, these environments undergo rapid development which is mainly stimulated by tourism and residential expansion. The most common ecosystem in tropical coastal regions are beaches, where interactions between species and humans can determine the structure of animal and vegetation communities (Araújo et al., 2018; Cowburn et al., 2018).

Natural and anthropogenic threats can influence the dynamics of these ecosystems, and the extent of these impacts can be measured by the severity of anthropogenic pressure and ecosystem vulnerability (Soto et al., 2021;Suciu et al., 2017). The intensive use of coastal environments can result in a reduction of available ecosystem services and consequently, an increase in poverty and inequality (Canteiro et al., 2018). The degradation of the environment (terrestrial and marine), associated with the loss of biodiversity, is classified as one of the five main risks to the global economy (Díaz et al., 2019;Coll, 2020). Therefore, understanding and quantifying these effects has become imperative for implementing strategies with the aim of promoting sustainable development and maintaining the balance of interactions between humans and wildlife (Coll, 2020).

It can be challenging to establish sample locations for comparisons (controls) in order to quantify the impacts of tourism on beaches, due to the unlikely existence of similar undisturbed environments (Soto et al., 2021). Although tourist beaches with annual visitation experience reduced anthropogenic pressure during the off season, they continue to be affected by environmental disturbances, albeit at lower intensities (Reyes-Martínez et al., 2015; Soto et al., 2021).

The lockdowns resulting from the WHO COVID-19 pandemic decree in March of 2020, led to the confinement of thousands of people worldwide. Tourism activities during the COVID-19 pandemic were strongly affected globally, experiencing a global reduction of approximately 73% and an estimated economic loss of almost 2 trillion dollars in 2020 compared with 2019 pre-pandemic year (UNWTO, 2021). As such, coastal tourism was one of the most affected sectors (Ormaza-González et al., 2021). Social distancing and lockdowns imposed by many countries considerably reduced the level of anthropic pressure on environments. Therefore, this situation provided a rare opportunity to investigate the extent of the ecological damage caused by human intervention (Edward et al., 2021). As such, the COVID-19 pandemic facilitated experimental comparisons between an environment that experiences high tourist impact with minimally disturbed environment, since beach visitation restrictions established a unique situations, thus allowing for the evaluation of the effects of the temporary absence of tourists in this environment (Coll, 2020; Manenti et al., 2020; Ormaza-González et al., 2021; Rume & Islam, 2020; Arora et al., 2020; Zielinski & Botero, 2020; Soto et al., 2021; Edward et al., 2021).

As Brazil is a tropical country with more than 8500 km of coast and favorable climate, especially in the North-eastern region, it has great tourist potential (Araújo et al., 2018). However, during the social restriction period, this economic sector suffered an intense reduction of more than 80% in hotel occupancy rate (Barbosa, 2020). One such example of the impact of the COVID-19 pandemic on tourism is Porto de Galinhas beach (Ipojuca, Pernambuco, Brazil), which, due to its naturally attractive flora and fauna and good hotel and culinary infrastructure, is classified as one of the most attractive touristic Brazilian beaches (Araújo et al., 2018; Barbosa et al., 2015). The decree no 667/2020 which banned visitation to all coastal beaches, rivers, and mangroves of the municipality of Ipojuca (PE), had a severe impact on tourism in Porto de Galinhas, as the most famous beach tourist attraction, boat trips to the coral pools, was also suspended (Prefeitura de Ipojuca, 2020).

What effects can tourism and disorganized occupancy have on beaches and the species that inhabit these areas? The evaluation of several physical, chemical, and biological parameters of bioindicator species can contribute to answering this question.

All organism characteristics depend on a complex genetic arrangement that is selected throughout evolutionary time. Thus, species’ genomes preserve all the biological information that allows for their persistence despite environmental disturbances, thus guaranteeing the possibility of ecosystem
sustainability and preservation (Cai & Des Marais, 2021; Grant et al., 2017; Wytock et al., 2020). However, environmental disturbances can alter the structure and/or content of species’ genetic material (Falcão et al., 2020; Kumar et al., 2017) and are known as genotoxic agents. Even at very low concentrations (considered sublethal), these agents are capable of damaging individual genomes. Such genomic damage can alter reproductive, embryogenetic, developmental, growth, and survival mechanisms, as well as favoring carcinogenic processes and hereditary and teratogenic defects (Gallão et al., 2019; Gutiérrez et al., 2019). Additionally, genomic damage can cause a loss of genetic diversity (genetic erosion), which can result in the inability of populations to persist when faced with disruptive agents (Garcia-Ulloa et al., 2020). Thus, identifying and quantifying the effects of environmental disturbances is fundamentally important for recognizing the limits of species resilience and for improving the management of ecosystem use by human-mediated activities. The quantification of genomic damage has been one of the most effective approaches in the management of aquatic environmental quality. The most commonly used methods to achieve this are micronuclear and comet assays (Adam et al., 2010; Falcão et al., 2020; Lima et al., 2019; Pinheiro et al., 2013). These protocols are based on detecting the fragmentation of genetic material at differing degrees of magnitude: macrolesions and microlesions, respectively (Bolognesi & Cirillo, 2014; Fenech et al., 2016; Olive et al., 1990). Additionally, the quantification of nuclear morphological alterations is a third protocol category which aims to identify genetic toxicity through the identification and quantification of nuclear malformations due to the presence of environmental disturbances (Carrasco et al., 1990; Souza & Fontanetti, 2006; Sula et al., 2020). The Sergeant-major (Abudefduf saxatilis) is a very common fish in the natural pools of Porto de Galinhas and is one of the main visitation attractions in this environment, thus this species is a good sentinel for testing environmental quality (Leão & Araújo, 2017).

In this study, the impact of tourism on a tropical beach (Porto de Galinhas – Brazil) was evaluated using parameters of genomic damage, comparing periods of intense human movement on this beach and a pristine region, as well as the lockdown period caused by the COVID-19 pandemic.

**Material and methods**

**Biological material sampling**

A total of ten *Abudefduf saxatilis* specimens were collected monthly from one of the natural pools in Porto de Galinhas (Ipojuca, Pernambuco, Brazil; Fig. 1) between July/2017 and June/2018 (with tourism), as well as during pandemic restrictions (June to August 2020 – without tourism). Regarding the size and weight of the fish, such measurements were not carried out, to remain the heterogeneity of the sample and reflects the population of the animals, and therefore, a response closer to reality, but adult animals were collected. We decided to collect samples from only one pool due to the occurrence of impacts related to trampling and intense visitation, including bathing. All samples were collected during low tide (variation between 0.0 and 0.5) during the morning. For the control sample, ten specimens of the sample species were collected on Tamandaré beach (PE – control), located at a distance of 58.7 km from Porto de Galinhas, in June 2018. The samples in Tamandaré were collected in a natural pool as parameters, situated at a distance of 2km from the beach zone, where touristic visits are not permitted as this region is an environmentally protected area.

**Environmental data collection**

When the biological material was collected, the water temperature in degrees Celsius (°C) was measured using a rod thermometer at a depth of 1m in the pool. Monthly rainfall data was also obtained from the Agência Pernambucana de Águas e Clima (http://www.apac.pe.gov.br). The number of monthly visits to the study natural pool by raft was obtained from the Associação de Jangadeiros da Praia de Porto de Galinhas – Secretaria do Meio Ambiente do Município de Ipojuca (Pernambuco, Brazil). The monthly hotel occupancy rate in Porto de Galinhas was obtained from this same association.

**Genotoxicity analyses**

Using the blood of the sampled specimens, we obtained genomic damage data using a micronucleus assay (MN) [sensu Heddle (1973) and Schmid (1975), with some modifications], comet assay (CA)
[sensu Singh et al (1988) with some modifications], and erythrocyte nuclear morphological alterations (ENAS) (Carrasco et al., 1990).

**Micronucleus assay and nuclear morphological alterations**

Approximately 0.5 ml of peripheral blood was collected from each animal during capture. Using a single drop of blood from each animal, a blood smear was made on slides in order to perform dry microscopy. The slides were then numbered, dated, and identified and were posteriorly maintained at room temperature. In the laboratory, the slides were fixed in absolute methanol for approximately 5 min and were then washed in distilled water and stained using Giemsa (absolute – approximately 2 min). Once dried at room temperature, the slides were analyzed using an optical microscope, at magnification of 100X. A total of 3000 cells (erythrocytes) were observed for each animal, of which normal and micronucleated cells were counted.

For the nuclear morphological alteration analyses, the same slides were used for the micronuclei analyses, following the methodology described above. Approximately 3000 cells were counted for each individual and were discriminated based on Carrasco et al.’s (1990) classification. Thus, nuclear morphological alterations were counted concurrently with micronucleated cells.

**Comet assay**

The CAs were performed seasonally during the dry and rainy periods which are characteristic of the climate in the study region. The dry season occurred from September to February. The rainy season was concentrated between May and July. The months of April and August are considered transition months, and in this study, April was included in the dry period and August was included in the rainy season. The month of June 2020, corresponding to the pandemic period, was also included in this analysis, and samples in Tamandaré were used as reference parameter. The seasonal aspect of this methodology greatly

![Fig. 1 Map showing the locations of Porto de Galinhas and Tamandaré beaches (State of Pernambuco Brazil). A Porto de Galinhas Beach and the natural pool where the animals were collected. B Rafts in the natural pool, i.e., sample area. C The sample species Abudefduf saxatilis. D Blood sample collection of Abudefduf saxatilis. Source: Author](image-url)
contributed to the analysis of the different types of possible damage caused to the DNA molecule, in terms of the potential differential availability of genotoxic agents during both seasons, as well as the absence of tourism on Porto de Galinhas beach.

For this methodology, the blood collection procedure was performed concurrently and using the same method as was used for the micronucleus assay and the nuclear morphological alteration collections. Slides for microscopy were prepared with agarose (1.5%—1.5 g of agarose in 100 ml of PBS solution) following the procedures proposed by Singh et al. (1988). A total of 100 cells were analyzed for each individual. The evaluation was carried out according to tail length which was categorized into four classes: 0 – no apparent damage; 1 – little damage; 2 – apparent damage; 3 – intermediate damage; 4 – maximum damage. The levels of DNA damage were compared between the seasonal (dry and rainy) and the pandemic samples and damage indices and frequencies were established. The data collected by the comet assay were used to check whether the DNA microlesions showed a significant difference from one seasonal period to another and if both were significantly different from the control. Indicating whether the study site was more impacted than the control and if we observed greater trends in genomic perturbation in different seasonal periods.

Statistical analyses

The general averages of genomic damage (micronuclei and nuclear morphological alterations) for each study period (control, with tourism and without tourism) were calculated and proportionality indices were established for the following comparisons: control vs. with tourism, control vs. without tourism and with tourism vs. without tourism. These proportionality indices indicated the level of genomic damage observed in each of the comparisons described above.

For each sample, the number of micronucleated cells and the number of nuclear morphological alterations were counted, and the rates and frequencies of damage were determined by the comet assay. These values were compared using a one-way analysis of variance (data log (x + 1) transformed), considering beaches (Porto de Galinhas and Tamandaré) and tourism (presence or absence) as predictor variables. Tukey’s tests were performed when the ANOVA results were significant (Zar, 2010). Additionally, one-way permutational multivariate analyses of variance (PERMANOVA) (Software: PRIMER 7) (Anderson, 2001) were applied to compare the whole nuclear alteration (MN + ENAs) (data log(x + 1) transformed – Euclidian distance), with beaches (Porto de Galinhas and Tamandaré) and tourism (presence or absence) as predictor variables. A canonical analysis of principal coordinates (CAP) (Anderson & Willis, 2003) was used to assess multivariate patterns of nuclear alterations (based on resemblance matrices prepared using Euclidian distance with data log(x + 1) transformed) across beaches and sampling date.

Distance-based linear models (DistLM) were used to identify which environmental characteristics (temperature, rainfall rate, hotel occupancy, and number of raft trips) were predictors for the nuclear alterations. The best models in DistLM were chosen using a forward routine with 9999 permutations based on AIC selection criterion. Analyses and tests were run in PRIMER 7 + PERMANOVA (Anderson et al., 2008), and a 5% significance level was used for all tests.

**Results**

The highest values for rainfall precipitation, water temperature, hotel occupancy rate (%), and number of rafts in the natural pool were observed in April 2018 (534.4 mm), March 2018 (31 °C), January 2018 (89.06% of hotel rooms occupied), and January (2018) (25,583 boats). The water temperature (24 °C) and precipitation (6 mm) were the lowest in August and November 2017. Hotel occupancy rate was the lowest during the lockdown period (June/2020: 11%; July/2020: 11.9%; and August/2020: 12.3%). During this period, no visitation to the pool by raft was observed (Table 1).

Our results indicate a variation in the average observed genomic damage in *Abudefduf saxatilis* erythrocytes that were collected and measured monthly in Porto de Galinhas. In relation to the presence of micronucleated cells, the lowest micronuclei average (4.6) was observed during the month of August 2020 and the highest average (32.2) in October 2017 (Table 1). The lowest average nuclear morphological alterations occurred in July 2020 (20.9) and the highest average was in October 2017 (112.2). All the observed values are summarized in Table 1.
The proportionality indices for the general averages of micronucleated cells and nuclear morphological alterations in *Abudefduf saxatilis* during the study period (Porto de Galinhas de July/17 to June/18), without tourism, Porto de Galinhas June–Aug/20, period of restricted human mobility, and control (Tamandaré Beach).

### Table 1
Average values for rainfall, water temperature, hotel occupancy rate, number of raft visitations to the pool, and number of micronucleated cells and cells with nuclear morphological alterations in *Abudefduf saxatilis*. With tourism (Porto de Galinhas de July/17 to June/18), without tourism (Porto de Galinhas June–Aug/20, period of restricted human mobility), and control (Tamandaré Beach).

| Month/Year | Rainfall (mm) | Water temperature (°C) | Hotel occupation rate (%) | Number of boats | Micronucleated cells | Nuclear morphological alterations |
|------------|---------------|------------------------|---------------------------|-----------------|---------------------|----------------------------------|
| Control    | 496.8         | 25                     | NA                        | NA              | 9.9                 | 41.5                             |
| Jul/17     | 496.8         | 25                     | 73.86                     | 13,989          | 13.4                | 84.2                             |
| Aug/17     | 55.5          | 24                     | 64.06                     | 8780            | 18.9                | 107.5                            |
| Sep/17     | 124.7         | 25                     | 74.39                     | 10,153          | 16.1                | 130.0                            |
| Oct/17     | 40.3          | 27                     | 71.86                     | 12,245          | 32.2                | 112.8                            |
| Nov/14     | 6.0           | 28                     | 68.79                     | 11,599          | 17.0                | 72.5                             |
| Dec/17     | 60.9          | 29                     | 75.49                     | 15,601          | 18.0                | 63.7                             |
| Jan/18     | 130.4         | 30                     | 89.06                     | 25,583          | 31.4                | 65.5                             |
| Feb/18     | 191.3         | 30                     | 82.26                     | 13,844          | 20.0                | 49.2                             |
| Mar/18     | 242.5         | 31                     | 70.45                     | 14,017          | 18.3                | 106.2                            |
| Apr/18     | 534.4         | 29                     | 72.67                     | 9454            | 21.6                | 65.2                             |
| May/18     | 221.3         | 28                     | 63.95                     | 8326            | 21.9                | 28.3                             |
| Jun/18     | 87.3          | 28                     | 65.96                     | 7123            | 12.3                | 38.0                             |
| Averages   | 182.62        | 27.83                  | 72.73                     | 12,559.9        | 20.09               | 76.92                            |
| Jun/20     | 368.4         | 27                     | 20.0                      | 0.0             | 11.0                | 27.6                             |
| Jul/20     | 163.3         | 27                     | 25.5                      | 0.0             | 11.9                | 20.9                             |
| Aug/20     | 123.2         | 28.5                   | 33.5                      | 0.0             | 4.6                 | 46.7                             |
| Averages   | 218.3         | 27.5                   | 26.33                     | 0.0             | 9.16                | 31.73                            |

The analysis of variance of the number of micronucleated erythrocytes observed during the study months showed statistically significant differences ($p < 0.0001$) (Fig. 2) when compared to the control in the months of October 2017 and January 2018.

### Table 2
Proportionality indices of the general averages of micronucleated cells and nuclear morphological alterations of *Abudefduf saxatilis* during the study period.

| Period                  | Micronucleated cells | Nuclear morphological alterations |
|-------------------------|----------------------|-----------------------------------|
|                         | Control              | With tourism                      | Without tourism       |
| Average                 | 99.0                 | 193.17                            | 91.67                 |

#### Micronucleated cells

|                     | Proportionality index |
|---------------------|-----------------------|
| Control             | 1.95                  | 1.08                              |
| With tourism        | 2.11                  |                                   |
| Without tourism     |                       | 2.99                              |

#### Nuclear morphological alterations

|                     | Proportionality index |
|---------------------|-----------------------|
| Control             | 316.0                 | 945.08                           | 315.33               |
| With tourism        | 2.99                  | 1.00                             | 2.99                 |
| Without tourism     |                       |                                   |                      |
Tukey’s a posteriori test confirmed these statistical differences (Table 3). According to the ANOVA and Tukey’s test, there were highly significant statistical differences in micronucleated cells when comparing the lockdown period to the months with the highest averages of micronucleated cells (32.2; 31.4; 21.6; and 29.9 in October/17, January/18; and April and May/18, respectively) (Table 3).

Statistically significant differences (ANOVA and Tukey) between the values of nuclear morphological alterations, counted monthly in the animals’ erythrocytes, were mainly observed between the lockdown period and the months of July to October 2017 and March 2018 (Fig. 3, Table 4).

For the canonical analysis of principal coordinates (CAP) (Fig. 4), it was possible to observe the separation of the samples collected during periods with tourism and samples collected when this activity was restricted and in the control area. There was an observed overlap of genomic damage during the periods without tourism and the control area, as well as a partial overlap between the genomic damage of the control area and the periods with tourism. Subtle overlaps were observed between the genomic damage of all the study conditions. The CAP explained 95% (m:9) of the relationships and classified 71% of samples. The first canonical axis (δ₁ = 0.61) demonstrates the strongest correlation with the hypothesis of the existence of two principal sample groups, where the first is a group with active tourist activities and the second is a group with restricted tourism and the control area. The variables of genomic damage that were strongly related to the separation of samples were the following: micronuclei, blebbed nucleus, vacuolated nucleus, nuclear bud, lobed nucleus, all with higher values in the fish samples collected during the period with tourism.

When comparing the number of micronuclei, there was a significant difference between the period with tourism and the control and the periods with tourism and without tourism. However, the comparison between the period without tourism and the control did not present a statistically significant difference. The same pattern was repeated for the set of total nuclear alterations (MN + ENAs) (Table 5).

The linear models based on distance (DistLM) (Table 6) and adjusted between genomic damage and environmental variables indicated that the nuclear alteration with the greatest correlation with environmental variables was the number of...
Table 3  Comparisons of Tukey’s test between the number of micronuclei in *Abudeafduf saxatilis* erythrocytes, collected in Porto de Galinhas during the monitoring (Jul/17–Jun/18) and lockdown (Jun/20–Aug/20) periods in relation to the Control. Significance level 0.05

| Samples | Control | Jul/17 | Aug/17 | Sep/17 | Oct/17 | Nov/17 | Dec/17 | Jan/18 | Feb/18 | Mar/18 | Apr/18 | May/18 | Jun/18 | Jun/20 | Jul/20 | Aug/20 |
|---------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Control | 1       | 0.87732| 0.99534| 0.00118| 0.98213| 0.94392| 0.00143| 0.8613 | 0.9255 | 0.51165| 0.46604| 1      | 1      | 1      | 1      | 0.99917|
| Jul/17  | 0.99874 | 1      | 0.01643| 0.99999| 0.99985| 0.02052| 0.99677| 0.99967| 0.93819| 0.91854| 1      | 1      | 1      | 1      | 0.8951 |
| Aug/17  | 1       | 0.32957| 1      | 0.00118| 0.99999| 0.99985| 0.02052| 0.99677| 0.99967| 0.91854| 1      | 1      | 1      | 1      | 0.99917|
| Sep/17  | 0.08796 | 1      | 1      | 0.10948| 0.99999| 0.99972| 0.99997| 0.99997| 0.99997| 0.99997| 0.99997| 0.99997| 0.99997| 0.99997| 0.99997|
| Oct/17  | 0.1416  | 0.22727| 1      | 0.64296| 0.25876| 0.71745| 0.75762| 0.00755| 0.00284| 0.00284| 1.7E−05| 0.71654| 0.71654| 0.71654| 0.71654|
| Nov/17  | 1       | 0.17465| 1      | 0.99985| 0.99997| 0.9998 | 0.9997 | 0.9997 | 0.9997 | 0.9997 | 0.9997 | 0.9997 | 0.9997 | 0.9997 | 0.9997 |
| Dec/17  | 0.27608 | 1      | 1      | 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999| 0.99999|
| Jan/18  | 0.71654 | 0.71654| 0.78956| 0.82546| 0.00937| 0.00348| 0.00937| 0.00348| 0.00937| 0.00348| 0.00937| 0.00348| 0.00937| 0.00348| 0.00937|
| Feb/18  | 1       | 1      | 1      | 0.98462| 0.9401 | 0.97545| 0.97545| 0.97545| 0.97545| 0.97545| 0.97545| 0.97545| 0.97545| 0.97545| 0.97545|
| Mar/18  | 1       | 0.99999| 0.9967 | 0.97693| 0.99352| 0.24235| 0.24235| 0.24235| 0.24235| 0.24235| 0.24235| 0.24235| 0.24235| 0.24235| 0.24235|
| Apr/18  | 1       | 0.84745| 0.67971| 0.8019 | 0.03987| 0.81387| 0.63479| 0.76384| 0.03284| 0.96312| 1      | 1      | 1      | 1      | 1      |
| May/18  | 1       | 0.93352| 0.97693| 0.76384| 0.03284| 0.96312| 1      | 1      | 1      | 1      | 0.99352| 0.97693|
micronucleated cells (micronuclei) and was mainly influenced by the quantity of rafts in the natural pool in Porto de Galinhas. In turn, the total number of nuclear morphological alterations and the set of total nuclear alterations (micronucleus + nuclear morphological alterations), as well as the number of rafts, were influenced by temperature.

The comet assay presented a significant statistical difference (ANOVA and Tukey $p < 0.001$) for seasonal genomic damage (dry, rainy, and lockdown period) for the comparisons between these periods and the control group, in relation to the damage indices (Fig. 5, Table 7).

The difference in damage frequency (comet assay) between the dry and rainy periods was not statistically significant (ANOVA and Tukey). Nonetheless, both differed in relation to the control. However, the frequency of genomic damage observed during the lockdown period did not differ in relation to either the seasonal or control periods (Fig. 6, Table 8).

### Discussion

Biodiversity is the main source of functionality in an ecosystem and depends on a variety of interactions between ecosystem constituents, including humans, to maintain balance. Any disturbance that results in the disruption of this balance can cause serious and irreparable damage to nature (Nazir et al., 2021). Nonetheless, the environment suffers constant variations and changes which are inherent to a dynamic and nonlinear system and is mainly shaped by socioecological interactions. The anthropogenic exploitation of natural resources has caused intense disturbances to nature, compromising biodiversity and affecting not only the natural balance of the ecosystem, but also negatively impacting human life. Transposing the limits of this dynamic can result in significant consequences at different spatial and temporal scales, making it challenging to understand and predict impacts, not only for scientists, but also for society as a whole (Cheval et al., 2020; Nazir et al., 2021).
Table 4 Comparisons between the number of nuclear morphological alterations in *Abudesfah saxatilis* erythrocytes, collected in Porto de Galinhas during the monitoring (Jul/17–Jun/18) and lockdown (Jun/20–Aug/20) periods in relation to the control. Significance level 0.05

| Samples       | Control | Jul/17 | Aug/17 | Sep/17 | Oct/17 | Nov/17 | Dec/17 | Jan/18 | Feb/18 | Mar/18 | Apr/18 | May/18 | Jun/18 | Jun/20 | Jul/20 | Aug/20 |
|---------------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Control       | 0.18943 | 0.00312| 0.00001| 0.00154| 0.60931| 0.90448| 0.86058| 0.99993| 0.00411| 0.8686 | 1      | 1      | 1      | 1      | 1      | 0.99997|
| Jul/17        | 0.99445 | 0.40824| 0.96867| 1      | 0.99861| 0.99952| 0.91317| 0.99697| 0.99942| 0.12031| 0.39281| 0.10858| 0.03672| 0.74414|
| Aug/17        | 0.99615 | 1      | 0.82881| 0.48849| 0.56341| 0.18386| 1      | 0.55086| 0.00151| 0.01162| 0.00129| 0.00027| 0.04619|
| Sep/17        | 0.99988 | 0.09487| 0.02139| 0.0297 | 0.00546| 0.9931 | 0.02814| 5.7E−05| 0.00071| 4.7E−05| 7E−06  | 0.00059|
| Oct/17        | 0.67424 | 0.33115| 0.39569| 0.11114| 1      | 0.38456| 0.00074| 0.00587| 0.00063| 0.00013| 0.03011|
| Nov/17        | 1       | 1      | 0.99812| 0.86596| 1      | 0.4721 | 0.8437 | 0.44377| 0.21519| 0.98475|
| Dec/17        | 1       | 0.99999| 0.54249| 1      | 0.81638| 0.9853 | 0.79354| 0.52997| 0.99985|
| Jan/18        | 0.99998 | 0.61761| 1      | 0.75511| 0.97262| 0.72922| 0.45585| 0.99949|
| Feb/18        | 0.21387 | 0.99998| 0.9994 | 1      | 0.99913| 0.98498| 1      | 1      | 1      | 0.9996 |
| Mar/18        | 0.60516 | 0.00202| 0.01495| 0.00173| 0.00037| 0.06943|
| Apr/18        | 0.76588 | 0.97517| 0.74044| 0.46802| 0.99958|
| May/18        | 1       | 1      | 1      | 0.9996 |
| Jun/18        | 1       | 0.99984| 1      |
| Jun/20        | 1       | 0.99938|
| Jul/20        | 0.98475|
| Aug/20        | 1      | 1      | 0.9996 |
The documented environmental impacts of tourism are still limited and inconclusive. Studies have generally only considered the impact of transport on the environment and are usually not related to the hospitality and service industries, which also have impacts on the environment (Nagaj & Žuromskaitė, 2021). The limited existing scientific approaches to the impacts of tourism on biota can be categorized as direct and indirect effects. In terms of direct effects, methodological approaches generally take behavioral, biochemical, and physiological consequences to organisms into consideration (Lamine et al., 2019; Lynch et al., 2019; Smith et al., 2021). For indirect effects, approaches are based on responses to different concentrations of specific contaminants or population densities of determined organisms, for example.

### Table 5
Comparisons (PERMANOVA) between the periods with and without tourism and the control area in relation to the number of micronuclei and total genomic damage (micronuclei + nuclear morphological alterations)

| Micronuclei | Source of variation | df | SS   | MS   | Pseudo- \( F \) | P (Perm) | Unique permutations |
|-------------|---------------------|----|------|------|-----------------|---------|--------------------|
|             | Sampling occasion   | 2  | 57.59| 28.79| 16.41           | 0.001   | 999                |
| Residuals   |                     | 153| 268.46| 1.76 |                 |         |                    |
| Average distance between/within sampling occasions | With tourism | Without tourism | Control |
|            | With tourism        | 1.58 |       |       |                 |         |                    |
|            | Without tourism     | 1.83*| 1.24  | 1.25  |                  |         |                    |
|            | Control             | 1.79*| 1.25  | 1.35  |                  |         |                    |
| Whole nuclear alterations | Source of variation | df | SS   | MS   | Pseudo- \( F \) | P(Perm) | Unique permutations |
|             | Sampling occasion   | 2  | 330.42| 165.21| 8.27           | 0.001   | 999                |
| Residuals   |                     | 153| 3055.5| 19.97 |                 |         |                    |
| Average distance between/within sampling occasions | With tourism | Without tourism | Control |
|            | With tourism        | 6.52 |       |       |                 |         |                    |
|            | Without tourism     | 6.09*| 3.63  | 4.05  |                  |         |                    |
|            | Control             | 5.93*| 3.94  | 4.05  |                  |         |                    |
which may vary due to tourism activities (Gül & Griffen, 2018; Lazcano et al., 2020; Soares et al., 2020). The unprecedented results, directly associated with tourist visitation and genomic damage in *A. saxatilis* presented in this study, will contribute to the understanding of the impacts of tourism on beach ecosystems.

Despite the undeniable socioeconomic importance of beach tourism, this activity has had a strong impact on coastal zones, where beaches act as a source of indispensable natural resources (Bhat et al., 2021; Doris, 2020; Loizia et al., 2021; Zambrano-Monserrate et al., 2020). As such, due to its large coral reef extension with a high diversity of organisms and since it is one of the most frequented tourist points along the Brazilian coast, Porto de Galinhas beach has suffered from high anthropic pressure (Araújo et al., 2018). In addition to its high level of urbanization, elevated levels of annual tourism visitation (Table 1) have also impacted this environment, as shown by the results of genomic damage obtained here which were independent of climactic season (dry/rainy) (Figs. 5, 6, Tables 7 and 8).

Through the quantification of genomic damage in *A. saxatilis* cells, two distinct scenarios can be observed from the results of the canonical analysis of principal coordinates (Fig. 4). The first scenario encompasses the highest observed frequency of genomic damage (both for micronuclei and nucleated morphological alterations), which corresponds to the annual period (July/2017 to June/2018) of tourist access to Porto de Galinhas beach. The second scenario presents the lowest frequencies of damage found in the erythrocytes of the study animals

### Table 6 Linear models based on distance adjusted between genomic damage and environmental variables

| Micronuclei–sequential tests for stepwise model ($r^2=0.59$) | Variable | AIC | Pseudo-$F$ | $P$ | Prop$^1$ | res.df$^2$ |
|-------------------------------------------------------------|----------|------|----------|-----|---------|-----------|
| Number of rafts                                             | −11.5    | 18.726 | $<0.01$  | 0.59 | 13      |
| Nuclear morphological alterations—sequential tests for stepwise model ($r^2=0.52$) | Variable | AIC | Pseudo-$F$ | $P$ | Prop$^1$ | res.df$^2$ |
| Number of rafts                                             | −22.9    | 8.66  | 0.01     | 0.41 | 13      |
| Temperature                                                 | −23.94   | 2.99  | 0.12     | 0.11 | 12      |
| Whole nuclear alterations—sequential tests for stepwise model ($r^2=0.44$) | Variable | AIC | Pseudo-$F$ | $P$ | Prop$^1$ | res.df$^2$ |
| Temperature                                                 | 34.83    | 4.31  | $<0.01$  | 0.28 | 13      |
| Number of rafts                                             | 32.47    | 4.05  | $<0.01$  | 0.21 | 12      |

### Table 7 Comparisons between the damage indices (comet assay) of *Abudefduf saxatilis* erythrocytes collected in Porto de Galinhas during the dry, rainy, and lockdown periods in relation to the control. Significance level, 0.05

| Samples          | Control | Dry      | Rainy    | Lockdown |
|------------------|---------|----------|----------|----------|
| Control          | 0.000159* | 0.000159* | 0.000159* |
| Dry              | 0.024611* | 0.000159* |
| Rainy            | 0.000211* |
| Lockdown         |          |          |          | 0.000159* |

**Fig. 5** Analysis of variance (ANOVA) of the damage indices (Comet assay) observed in *Abudefduf saxatilis* erythrocytes collected monthly in Porto de Galinhas, in relation to the control and the lockdown period period (Art created in softstat ESTATISTICA V6)
collected in Tamandaré beach (control region) and during the period with no tourism activities (lockdown – June to August 2020). The results of the Proportionality Indices (IP) between the general averages of the micronucleated cells and those with nuclear morphological alterations (Table 2) indicate an impact of approximately double the genomic damage for nuclear morphological alterations (IP = 2.99) and twice as many micronucleated cells (IP = 1.95) when comparing the period with tourism activity on Porto de Galinhas beach and the control region. When comparing the periods with and without tourism (lockdown), considerable proportionality indices were also observed, with values higher than 2, both for the micronucleated cell averages (IP = 2.11) and for the nuclear morphological alterations (IP = 2.99).

In general, the quantification of genomic damage in A. saxatilis allowed for the observation of two distinct scenarios. One scenario showed that the highest frequencies both for MNs and ENAs occurred during the period with tourism in the reef environment of Porto de Galinhas. Whereas the other, characterized by the pandemic lockdown, revealed lower frequencies of both genomic damage categories. According to Marshall et al. (2014), beaches with a high frequency of tourists during the year, as is the case in Porto de Galinhas, present a large variety of environmental stressors, which include solid waste, human activities, and odors. It should be noted that bathing is allowed in the pool where the fish were collected, thus there is a greater level of interaction between humans and animals which can cause stress to the resident animals. Adam et al. (2011) demonstrated the direct relationship between stress and the frequency of MNs in cells of Rattus novergicus during changes in circadian cycle, temperature, and noise. Thus, in addition to human presence, these other parameters may have contributed to the stress of the animals and consequently, resulting in an increase in the expression of genomic damage.

Table 8 Comparisons between the damage frequencies (comet assay) in Abudefduf saxatilis erythrocytes, collected in Porto de Galinhas during the dry, rainy, and lockdown periods in relation to the control. Level of significance 0.05

| Samples       | Control | Dry    | Rainy  | Lockdown |
|---------------|---------|--------|--------|----------|
| Control       | 0.003261* | 0.003261* | 0.132370 |
| Dry           | 1.000000 | 0.429941 |        |
| Rainy         |         | 0.429941 |        |
| Lockdown      |         |        |        |          |

The damage proportionality indices undoubtedly indicate that tourism has an impact on this beach. These results are also corroborated by the fact that when comparing the control region and the period without tourism the values were close to 1 for the occurrence of both types of damage (micronuclei IP = 1.08; nuclear morphological alterations IP = 1.00) and thus had relatively no impact. The highest frequencies of micronucleated cells occurred in October/2017 and January/2018. Although tourists visit Porto de Galinhas beach throughout the
year, as demonstrated by the hotel occupancy indices (Table 1), the peak period occurred during the months of September to April, which includes the months mentioned above. Therefore, these months experienced a high level of tourist visitation. It is worth noting that due to its geographical insertion in the Metropolitan Region of Recife (Capital of the state of Pernambuco), Porto de Galinhas beach also receives intense visitation from inhabitants of the surrounding region. This factor was not included in the hotel occupancy rate calculations, since these tourists do not tend to stay overnight and usually return to their residence at the end of the afternoon. This may be related to the incidence of micronucleated cells observed in the months of April and May 2018, where there were long weekends (Easter, Worker’s Day, and Corpus Christi) which act as periods of high resident movement to beaches.

In January/2018, there was a greater hotel occupancy rate (89.06%), greater number of rafts (25,583) visiting the natural pools of Porto de Galinhas, and significantly more micronucleated cells. These results clearly indicate that tourist activity has an impact on this beach, and also suggest a strong relationship between genomic damage and environmental variables such as number of rafts and temperature. The micronucleus was the most highly correlated genomic damage parameter with the effect of tourism and environmental variables (Tables 5 and 6) and was thus the principal biomarker which supported the robustness of the analyses. Since the number of micronucleated cells was mainly related to the number of rafts that visited the natural pool during the study period, this correlation indicates a high impact of tourist presence on the environment.

Anthropogenic sources of environmental stress can affect beach environments to different extents and in different ways (Araújo et al., 2018). Araújo et al. (2018) observed that Porto de Galinhas beach had a greater quantity of litter (plastic, food remains, wood (wood skewers), and cigarette butts) from its users than other beaches with greater levels of urbanization. Dovzhenko et al. (2020) observed a high degree (1.5–2 times) of DNA damage (Comet assay) in the gill cells and digestive glands of mussels (Mytilus trossulus) exposed to fragments of plastic in natura compared to a control group. Since cigarette butts have a filter, they retain more than 7000 chemical compounds present in cigarettes (Mansouri et al., 2020). Among these are heavy metals (aluminum, zinc, lead, selenium, chromium, nickel and cadmium etc.), nicotine, ethyl phenol, ammonia, formaldehyde, butane, acrylonitrile, toluene, benzene, alkalioid, cyanide, and asbestos most of which have a genotoxic effect (Al-Saleh et al., 2020; Gökalp et al., 2020; Laio et al., 2019; Salem et al., 2018; Thirunavukkarasu et al., 2020; Yamin et al., 2020; Zafra-Lemos et al., 2021; Zhang et al., 2020). The origin of the majority of these chemical compounds in cigarettes come from pesticides, insecticides, herbicides, and fungicides used in tobacco culture (Lee, 2012), which also present genotoxic potential (Amaeze et al., 2020; Oliveira et al., 2020). The disposal of these pollutants attached to the end of cigarettes into the environment occurs through leaching and in the case of beaches, the cigarettes reach the sea and are carried away by the movement of the waves (Freiberg, 2014; Kadir & Sarani, 2015; Lee, 2012). Food remains can also be polluting sources of heavy metals and pesticides (Kopp et al., 2018) and can therefore contribute to the observation of DNA damage in exposed organisms.

In addition to all these environmental disturbance agents, sunscreen represents one of the principal sources of TiO2 and ZnO nanoparticles found in coastal environments (Schiavo et al., 2018) with high genotoxic potential (Di Giampaolo et al., 2021; Kukla et al., 2021). Thus, all the compounds mentioned above can contribute to the genotoxic effects observed in A. saxatilis erythrocytes collected in Porto de Galinhas.

Significant statistical differences were also observed for nuclear morphological alterations (Fig. 3, Table 4). The origin of such alterations is based on the inefficiency of the extrusion mechanisms in eliminating damaged DNA fragments from the nucleus (where micronuclei probably originated), which remain attached to the nuclear envelope (Seriani et al., 2011). Oxidative stress, as a consequence of reactive oxygen species (ROS), produced by environmental disturbances, can also cause the formation of nuclear morphological alterations. According to Morina et al. (2013), such oxidative stress can change the permeability of the nuclear envelope, increasing the susceptibility of the nucleus to create erythrocytes with nuclear anomalies. Thus, nuclear morphological alterations with statistically significant differences observed in the months of July to October 2017 and March 2018 add to the genotoxic effects caused by the impact of
tourism in Porto de Galinhas. This is also sustained by the canonical analysis of principal coordinates (Fig. 4), PERMANOVA (Table 5), and linear models based on distance (Table 6). Therefore, we suggest the complementarity of both methodological approaches, i.e., the analysis of micronucleated cells and nuclear morphological alterations, for diagnosing environmental disturbances.

The impact of tourism seasonality in Porto de Galinhas was evaluated by DNA microlesions and quantified by the comet assay. Although microlesion frequencies were statistically significant in relation to the control and the lockdown period (Fig. 6, Table 8), no significant differences were observed between the two seasons (dry and rainy). However, when the damage indices were considered, significant differences were found between the two seasons and in relation to the control and lockdown period (Fig. 5, Table 7). During the dry period (with an average rainfall of 166.31 mm; average temperature 28.62 °C, average hotel occupancy rate equal to 74.75%, and average number of rafts in the natural pool equal to 14,062), there was a greater expression of microlesions in the animals’ DNA in relation to rainfall (average rainfall equal to 251.22 mm, average temperature of 26.25 °C, average hotel occupancy equal to 66.95%, and average number of rafts in a natural pool equal to 9554). According to Gerić et al. (2018), environmental variables such as UV ray incidence and higher temperatures, such as those found during the hottest period of the year in Porto de Galinhas (water temperature variation between 25 and 31 °C), can induce a greater expression of damage observed in the comet assay, as well as increasing the basal expression level (baseline) of such damage. This is in accordance with the results presented by animals in Porto de Galinhas. However, when analyzing these results, taking into account the periods with and without tourism (Figs. 5, 6, Tables 7 and 8), both the dry and rainy periods differed in terms of the expression of damage in relation to both the control and lockdown period, indicating impacts caused by intense tourist presence on this beach, independent of climactic season.

The lockdown period should be considered with greater attention, since this period, which has been named *anthropause* (Rutz et al., 2020) can unequivocally contribute to the understanding of interactions between humans and relative wildlife, in this case, the presence of tourists on beaches (Coll, 2020; Soto et al., 2021). Unlike the unprecedented negative effects on society and the economy, the lockdown has helped to repair some environmental damage (Shakil et al., 2020). It is undeniable that the COVID-19 pandemic has resulted in the improvement of environmental quality in terms of air, water, and sound pollution (Bhat et al., 2021; Koohdarag & Ahadi Ravoshti, 2020).

As previously mentioned, the restrictions to human movement and social distancing established worldwide, in an attempt to combat the COVID-19 pandemic, impacted the tourism sector, with beach tourism being one of the most affected sectors. The absence of tourists on beaches resulted in notable changes to these ecosystems as described by many researchers (Zambrano-Monserrate et al., 2020; Zielinski & Botero, 2020; Ormaza-González et al., 2021; Edward et al., 2021; Loizia et al., 2021). According to Mousazadeh et al. (2021), tourist restriction on beaches has resulted in the evident reduction of pollutants in the water and as a consequence, many animals have returned to these habitats. Soto et al. (2021) observed notable positive changes in relation to biological compounds and a reduction in anthropogenic stressors (pollution, noise, human activities and user density) in 29 urban beaches in seven Latin American countries.

All the comparisons established here between the periods with and without tourism (control and lockdown) on Porto de Galinhas beach, in relation to genomic damage for all three methodologies (micronucleus, nuclear morphological alterations, comet assay) showed a notable reduction in genotoxic effects in *A. saxatilis* cells in the absence of tourists. The overlap observed in the canonical analysis of principal coordinates (Fig. 4) during the lockdown period and the control region, both with the absence of tourists, indicated a negative effect of tourism on this beach. It is worth highlighting that the lockdown period presented lower average micronucleated cells (9.16) and nuclear morphological alterations (31.73) both in relation to the control (9.9 and 41.5, respectively) and for the period with tourism (20.09 and 76.925, respectively). Thus, the elimination of anthropogenic disturbance effects, as observed during the lockdown period, can be considered as the closest condition (baseline) to natural environmental conditions.
Conclusions

The results presented here fill the current gap in the literature with regard to quantifying the impact of tourism on beaches, especially in relation to the current pandemic. The lowest incidence of genomic damage in A. saxatilis erythrocytes was observed during the lockdown period, suggesting that the absence of tourists can result in the resumption of more natural environmental conditions. On the contrary, intense human presence in this ecosystem can compromise its natural balance, as the negative effects measured by genomic damage show. Therefore, the measures presented here will contribute to the development of sustainable behaviors for the use of the environment.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval The research was approved by the SISNAMA (National System of Environment) and Ipojuca Environment Secretary to collect the bioindicators.

Conflict of interest The authors declare no competing interests.

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