#### AGU ADVANCING EARTH AND SPACE SCIENCES



## **RESEARCH ARTICLE**

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#### **Key Points:**

- Accumulation of genotoxic damage may partly explain loss of bird diversity in seasonally dry tropical forest (SDTF)
- The bird community of SDTF shows the highest levels of incidence of genotoxic damages in most disturbed areas
- The forest degradation could increase the environmental stress that support the bird community

#### **Supporting Information:**

Supporting Information may be found in the online version of this article.

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# Chronic Degradation of Seasonally Dry Tropical Forests Increases the Incidence of Genotoxicity in Birds

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**Abstract** Multiple studies have shown that exposure to pollutants can increase genotoxic damage in different taxa. However, to our knowledge, the effects of environmental stress have been explored little. In certain stressful ecosystems, such as seasonally dry tropical forests, the combined effects of anthropogenic activities and ongoing global changes can cause an increase in environmental stresses, in turn, may trigger physiological and genetic effects on biodiversity. The present aims to assess changes in the prevalence of genotoxic damage in birds within three states of forest degradation in the Tumbesian Region of Western Ecuador. We used blood samples from 50 bird species to determine the frequency of micronucleus and nuclear abnormalities in erythrocytes. Our results revealed a significant impact of forest degradation on the occurrence probability of micronucleus and nuclear abnormalities at the community level. Localities with higher levels of degradation exhibited higher levels of abnormalities. However, when analyzing the dominant species, we found contrasting responses. While Lepidocolaptes soulevetii showed a reduction in the proportion of nuclear abnormalities from the natural to shrub-dominated localities Troglodytes aedon and Polioptila plumbea showed an increase for semi-natural and shrub-dominated respectively. We concluded that the degradation process of these tropical forests increases the stress of bird community generating genotoxic damage. Bird responses seem to be species-specific, which could explain the differences in changes in bird composition reported in other studies.

**Plain Language Summary** Many studies have shown that pollutants can cause changes in the characteristics of cells, specifically at the level of the nucleus. These changes are known as genotoxic damage and can have serious consequences for individuals. In addition to pollutants, other factors can cause genotoxic damage, such as water deficit or solar irradiation. The degradation of seasonally dry tropical forests can lead to changes in water availability and the amount of irradiation that organisms receive. We conducted a study to evaluate how the environmental changes caused by the degradation of these forests affect the level of genotoxic damage. We evaluated genotoxic damage in 50 bird species inhabiting areas with different levels of degradation. We found that the degradation of these forests increases the level of genotoxic damage at the community level, which could be causing the local extinction of many bird species.

## 1. Introduction

International environmental policies claim for establishment of easy-to-use applicable tools to monitoring the conservation status of ecosystems and the impact of human actions on ecosystem viability and persistence (Sillero et al., 2021). In this sense, biomonitoring of health and performance of sentinel organisms has proven appropriate in many different types of assemblages, including bird communities (Baos et al., 2022; Plaza et al., 2018; Solgi et al., 2020). Different studies worldwide have shown that human activities generate toxic pollutants that alter the physiology of species (Giraudeau et al., 2018). These pollutants are diverse and can affect different processes, including the replication and preservation of DNA integrity generating genotoxic damage (Kontaş & Bostancı, 2020; Louzon et al., 2019; Maya-Mendoza et al., 2018; Vriens et al., 2019).

Genotoxic damage originating from different sources of contaminants has been reported in some locations (de Oliveira et al., 2019; Sordo et al., 2019; Yadav et al., 2019). However, few studies had documented the genotoxic





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damages produced by environmental stress. Although the organisms posses mechanisms to face intrinsic stress factors inherent to their natural history, abrupt changes in the environment can cause imbalances and require emergency adjustments in their physiology or behavior (Blas, 2015), thus causing genotoxic damage (Haro et al., 2017). The adjustments should allow organisms to adapt to the new conditions; otherwise, they would have to move to other environments or face the process of local extinction (Blas, 2015). However, the anthropogenic activities could intensify natural stressors (Tuteja et al., 2001) limiting the capacity of species to adapt to these new conditions (Haro et al., 2017).

Different abiotic stress factors have been studied as triggers of genotoxic damage, which has been well demonstrated in organisms such as plants (Tuteja et al., 2001) and marine species (Przeslawski et al., 2005), but also affects other species (Blaustein & Belden, 2003), including humans (Watts et al., 2019). Different conditions, such as water availability, extreme temperatures, ion-induced toxicity, and radiation represent some of the most important sources of abiotic stress (Kasurinen et al., 2012; Mittler, 2006; Mittler & Blumwald, 2010; Srivastava et al., 2012). Ultraviolet radiation (UV) is one of the most powerful agents that can alter the normal state of life by inducing a variety of mutagens. DNA damage caused by UV radiation can be caused by direct absorption of photon energy or by indirect damage. In indirect damage, endogenous chromophores transfer the energy of the photon to other molecules, which then cause modifications in the DNA (Davies, 1995).

In this study, we used the bird community of the seasonally dry tropical forest (hereafter SDTF) as model organisms to test changes in nuclear abnormalities caused by forest degradation. Birds are considered efficient bioindicators because of their facility to be identified (Price, 2006), their intimate relationship with the ecosystems they inhabit (Roché et al., 2010), for forming part of the various levels of the ecological pyramid in the ecosystems (Chambers, 2008; Francis, 2017), and for several behavioral characteristics, such as a diversified diet and use of different vegetation layers. On the other hand, in the SDTFs the biota is exposed to environmental stress produced by to long periods of drought, high temperatures, and high solar incidence (Espinosa et al., 2012; Jara-Guerrero et al., 2019, 2021; Ordóñez-Delgado et al., 2016). Birds respond to this strong climatic seasonality by changing their behavior and physiology to survive during the dry season (Bullock et al., 1995). The main adaptive strategies of these animals are based on changes in daily activity patterns to reduce water loss or changes in the diet to exploit resources that are not available in dry periods (Bullock et al., 1995). All these strategies allow the adaptation and maintenance of homeostasis (Allostasis processes) (Mcewen & Wingfield, 2010), which involves changes energy use and accumulation of glucocorticoids. These changes can have consequences for health and longevity (Romero et al., 2009).

Climatic stress in this ecosystem is strongly intensified by disturbance processes (Jara-Guerrero et al., 2021). Anthropogenic disturbances in the SDTFs are mainly timber extraction and cattle over-grazing (Espinosa et al., 2011, 2012; Jara-Guerrero et al., 2019). Both actions result in a reduction of forest cover (Jara-Guerrero et al., 2019), with an associated increase in exposure to solar radiation and an increase in temperature. Some works have shown that these changes in microclimatic conditions can act as filters for some sensible species (Jara-Guerrero et al., 2021).

We evaluated the effect of the stress increase as consequence of forest degradation on genotoxic damage of bird community. Two specific goals were proposed in this study: (a) evaluate the effects of forest degradation in the incidence of genotoxic damage at community level and (b) assess whether the patterns of genotoxic damage along a degradation gradient are consistent between species. We assumed that forest degradation causes an increase in drought stress and solar incidence, factors that promote genotoxic damage, so it was expected that in the most disturbed habitats, the proportion of genotoxic damage would be higher. Additionally, we expected a species-specific interspecific responses of the most abundant species due to differences in ecological requirements and physical condition of species (Santos et al., 2017); together with the sum of epigenetic effects. Including transgenerational inheritance associated with mechanisms other than DNA variation that may affect evolutionary dynamics. In the case of birds, it has been seen to be very specific, studies have shown that certain bird species may have specific genetic or epigenetic mechanisms that make them more resilient or sensitive to environmental changes (Frésard et al., 2013).

## 2. Methods

## 2.1. Study Area

This study was carried out from February to August of 2015 in the Zapotillo Canton, Province of Loja, Southwest Ecuador as shown in Figure 1 (reference coordinates 4° 16.601'S, 80° 19.751'W). This area has an altitude range





**Figure 1.** Reference map of the study area showing the extent of the Tumbesian region in Ecuador and Peru (a, b), and the sampling sites (c): natural (green circles), semi-natural (blue diamonds), shrub-dominated (red triangles). The boundary of the Tumbesia La Ceiba Nature Reserve is shown in orange in panel (c).

of 100–540 m above sea level, a tropical equatorial climate, and an average annual temperature of 25.6°C with a warmer period between December and May and a cooler one between June and November (Maldonado, 2002). The fluviometric regime of this region is semi-arid (642 mm annual rainfall) (Espinosa et al., 2018). The climate is characterized by a strong seasonality, where around 75% of the precipitation occurs between January and May (García-Cervigón et al., 2017, 2019).

In large areas of distribution, SDTF supports chronic disturbances, mainly due to cattle overgrazing and timber extraction (Álvarez-Yépiz et al., 2008; Jara-Guerrero et al., 2019; Méndez-Toribio et al., 2016; Ribeiro-Neto et al., 2016). Because of these pressures, the forest presents various levels of degradation (Jara-Guerrero et al., 2019). Our study was developed in three of the five states proposed by Jara-Guerrero et al., 2019 and consisted of three levels of chronic degradation: (a) natural, (b) semi-natural, and (c) shrub-dominated (Figure 2).

## 2.2. Sampling Design

The sampling points were located in areas away from populated and agricultural zones to avoid urban and agrochemical contamination. The sampling points are located within the Tumbesia La Ceiba Natural Reserve (91.82 km<sup>2</sup>), a private protected area belonging to the NGO Nature & Culture International. This reserve is recognized in Ecuador as an Important Bird Conservation Area for its richness in biodiversity and significant levels of endemism (IBA: EC081) (Freile & Santander, 2005).

Based on previous extensive and detailed characterization of the area (Cueva Ortiz et al., 2019; Jara-Guerrero et al., 2021), we selected three localities within each forest degradation level: (a) natural, (b) semi-natural, and (c) shrub-dominated. This resulted in a total of nine localities, each separated by at least 2 km (Figure 1). In each locality, we conducted three sampling campaigns (February, April, and August - 2015) at three capture points, each separated by 250 m. Three mist nets  $(3 \times 12 \text{ m}; 36 \text{ mm mesh})$  were operated continuously between 06:00 a.m. and 11:00 a.m. for 3 days for each point 135 hr/net. Captured birds were identified using on Ridgely and

Greenfield (2006) and banded with numbered aluminum rings except for hummingbirds, which were marked by clipping 1 mm of a tail feather tip. The taxonomic classification used in this document corresponded Freile and Restall (2018) for Ecuador.

Fieldwork was conducted following the Guidelines for the Use of Wild Birds in Research (Fair et al., 2010) and the Scientific Bird Banding Manual (Pinilla, 2000). And this research was conducted under research permit MAE-DNB-CM-2015-0016 granted by the Ministerio del Ambiente del Ecuador.

#### 2.3. Micronucleus Assay and Nuclear Abnormalities

From each captured bird, a blood sample (10–50 µl) was extracted from the brachial vein (in hummingbirds, the sample was drawn from the medial metatarsal vein) with the aid of a needle and a capillary tube. A drop of blood was smeared on a slide and at once air-dried and later fixed with ethanol (96%). Blood smears were kept at room temperature and protected from moisture and light until staining. Blood smears were stained with a 5% solution of GIEMSA and Sorensen buffer at pH 6.8 for 45 min, rinsed in distilled water, and finally dried at room temperature (Tomás et al., 2005). For detection of micronucleus and nuclear abnormalities, blood smears were scanned with an Olympus optical microscope at 100× magnification using immersion oil; on each slide 10,000 erythrocytes were counted following a double-blind protocol. Micronucleus were identified as round or oval structures with the same focal plane, coloration, and texture and being 1/3 to 1/16 the size of a normal erythrocyte nucleus according to criteria proposed by Quero et al. (2016). Other detected nuclear abnormalities included





Figure 2. Descriptions of the sites sampling. Include vegetation structure, natural regeneration, and the most abundant bird species. Graphical representation of a scale. Modified from Jara-Guerrero, et al. (2019).

nuclear buds (nuclei with an apparent sharp constriction at one end, wherein the buds are between 1/4 and 1/3 the size of the principal nucleus), binucleated cells (two nuclei of the same size and staining), and notched nucleus (a well-defined slit of uniform width extending to an appreciable depth in the nucleus and demarcated by the nuclear envelope) (Quero et al., 2016). The total sum of nuclear abnormalities was used for statistical analyses. Micronucleus were also analyzed separately because they are the most often studied erythrocyte abnormality (see De Mas et al., 2015).

#### 2.4. Statistical Analyses

All statistical analyses were performed with the R environment (R Core Team, 2022). A generalized mixed model with binomial distribution was used to evaluate the effect of forest degradation status in the proportion of occurrence of micronucleus and nuclear abnormalities in erythrocytes of the whole bird community. The forest degradation level was used as explained variable and species was included in the model as a random factor to avoid species-associated effects. We used the level of forest degradation as an explanatory variable, since it has been shown to be associated with changes in plant structure and composition (Jara-Guerrero et al., 2021). We graphically verify that the residuals meet the assumptions by plotting the fitted values against the residuals and verifying that there are no discernible patterns. The model was adjusted using the glmer function of the lme4 package (Bates et al., 2015). Additionally, a logistic regression was adjusted to evaluate the effect of disturbances in occurrence of micronucleus and nuclear abnormalities in erythrocytes in the four most abundant species (Amazilis amazilia, Troglodytes aedon, Lepidocolaptes souleyetii, and Polioptila plumbea). The models were adjusted using the glm function of the base package. Our data is highly imbalanced, with many samples in one category and small numbers in another. To address this, we use the *downSample* function from the *caret* package (Kuhn, 2008) to randomly sample our data set so that all classes have the same frequency as the minority class. This procedure is necessary because algorithms often do not accurately represent the distributive characteristics of complex imbalanced data sets, resulting in unfavorable accuracies across the classes of the data (Haibo & Garcia, 2009). To evaluate the effectiveness of the model in predicting the response variable, we used the chi-square statistic, which measures the difference between the implemented model and the model when only the response variable was included. Finally, for each model, we calculated the Odds ratio at each level of the predictor variable.

## 3. Results

Blood samples were obtained from 396 individuals belonging to 50 bird species in 21 families (Table S1). The average occurrence of micronucleus, including all bird species and sampling areas, was 0.21/10,000 erythrocytes,







Figure 3. The occurrence of the different erythrocyte micronucleus (a) and nuclear abnormalities (b) (per 10,000 erythrocytes) in the five bird species with the highest values for each abnormality and mean occurrence of micronucleus (c) and nuclear abnormalities (d) in relation to the degree of forest disturbance.

and 52% of bird species. We found a great difference in the occurrence of micronucleus the species with highest occurrence, *Claravis pretiosa* and *Sicalis taczanowskii* were the species with the highest occurrence of micronucleus with 2.33/10,000 cells and 2/10,000 cells respectively, presented four times that in the other species (Figure 3a and Table S1). The average occurrence of nuclear abnormalities, including all bird species and sampling areas, was 19.95/10,000 erythrocytes, and all bird species exhibited some type of nuclear abnormalities. The differences between species in the occurrence of nuclear abnormalities were similar between species, *Icterus graceannae* and *Muscigralla brevicauda* were the species with higher frequencies of nuclear abnormalities (51/10,000 cells, Figure 3b and Table S1). A greater accumulation of genetic damage measured by the two types of damage was observed in the shrub-dominated forests. The mean occurrence of micronucleus in bird species changes from 0.1 nuclei in natural habitats to 0.5 in shrub-dominated (Figures 3c and 3d).

Our findings revealed a significant effect of the forest degradation status in the occurrence of cells with micronucleus and with nuclear abnormalities in the birds' blood. Birds living in semi-natural and shrub-dominated forests exhibited a higher frequency of both genotoxic damage compared to birds residing in natural forests (Table 1)

Table 1

Generalized Linear Mixed Models on the Effects of Forest Degradation Status

	Estimate	Std. error	z value	Pr(> z )
Micronucleus				
Intercept	-0.7223	0.2816	-2.5651	0.0103*
Seminatural	0.9479	0.3823	2.4792	< 0.0132*
Shrub-dominated	1.4128	0.4321	3.2697	<0.0011***
Nuclear abnormalities				
Intercept	-0.2096	0.0824	-2.5434	0.0110*
Semi-natural	0.1052	0.0400	2.6293	0.0086***
Shrub-dominated	0.1288	0.0623	2.0666	0.0388*

*Note.* On the probability of occurrence of micronucleus and nuclear abnormalities in blood cells of birds. Statistically significant differences are shown as: \*: p < 0.05, \*\*: p < 0.01, \*\*\*: p < 0.001.

We found a pattern of increasing probability of occurrence of micronuclei as forest degradation increases. In the semi-natural forests, the micronucleus were 2.6 times higher (with a 95% confidence interval of 1.25–5.64) compared to natural forests, while in shrub-dominated forests the micronucleus were 4.11 times higher (with a 95% confidence interval of 1.77–10.17) than those found in natural forests (Figure 4a). For nuclear abnormalities this pattern was slightly different, semi-natural forests and shrub-dominated forests had respectively 1.11 (with a 95% confidence interval of 1.03–1.20) and 1.14 (with a 95% confidence interval of 1.01–1.28) times more occurrence of nuclear abnormalities than natural forest (Figure 4b).

The results from the generalized linear models indicate that forest degradation status has varying effects on the occurrence of nuclear abnormalities in different bird species. For *Amazilis amazilia*, no significant differences were observed in the occurrence of nuclear abnormalities between the different forest types (Table 2, Figure 5a). However, in *Troglodytes aedon* and *Polioptila plumbea*, there is a significant increase in the occurrence of nuclear abnormalities in shrub-dominated forests compared to natural forests (Table 2). In semi-natural forest increase about 1.3 times the occurrence of





Figure 4. Odds Ratio of micronucleus frequency and nuclear abnormalities frequency in bird species sampled according to the three forest degradation states in the Tumbesia La Ceiba Nature Reserve Loja-Ecuador. Statistically significant differences are shown as: \*: p < 0.05, \*\*: p < 0.01, \*\*\*: p < 0.001.

nuclear abnormalidades and about 1.6 times in shrub-dominated forest for both species (Figures 5b and 5d). Conversely, in *Lepidocolaptes souleyetii*, the presence of nuclear abnormalities decreases in both semi-natural and shrub-dominated forests (Table 2), the occurrence of nuclear abnormalities reduced in 0.6 and 0.7 times, respectively (Figure 5c).

#### Table 2

Generalized Linear Models of the Effects of Forest Degradation Status on the Occurrence of Nuclear Abnormalities in Erythrocytes of the Four Most Abundant Bird Species

	Estimate	Std. z	z value	Pr(> z )		
Amazilis amazilia						
Intercept	-0.0705	0.0862	-0.8182	0.4132		
Semi-natural	0.0958	0.0997	0.9597	0.3372		
Shrub-dominated	0.0470	0.2334	0.2013	0.8404		
Troglodytes aedon						
Intercept	-0.2708	0.1036	-2.6118	< 0.0090		
Semi-natural	0.2607	0.1321	1.9738	<0.0484*		
Shrub-dominated	0.4744	0.1354	3.5031	< 0.0005***		
Lepidocolaptes souleyetii						
Intercept	0.2357	0.1064	2.2145	< 0.0268*		
Semi-natural	-0.4066	0.1509	-2.6942	< 0.0071		
Shrub-dominated	-0.3334	0.1662	-2.0056	<0.0449*		
Polioptila plumbea						
Intercept	-0.2952	0.1327	-2.2242	< 0.0261*		
Semi-natural	0.3246	0.1713	1.8944	0.0582		
Shrub-dominated	0.4580	0.1670	2.6945	<0.0007***		

*Note*. Statistically significant differences are shown as: \*: <0.05, \*\*: <0.01, \*\*\*: <0.001.

## 4. Discussion

Multiple studies have shown that the degradation process in SDTFs generates a significant loss of plant and animal biodiversity (see Avila-Cabadilla et al., 2009; Espinosa et al., 2011; Falcão et al., 2018; Gillespie et al., 2000; Jara-Guerrero et al., 2021; Shahabuddin & Kumar, 2006; Valle et al., 2021; Williams-Linera & Lorea, 2009; Zúñiga-González et al., 2000). In the case of plants, it has been proposed that the loss of diversity occurs because of increased stress caused by a reduction in tree coverage, which implies an increase in drought and solar incidence (Jara-Guerrero et al., 2021). In the case of animals, the mechanism that generates the loss of diversity is less clear, but it is assumed that the changes may be due to a higher difficulty of the individuals to exploit the most degraded areas (Ordóñez-Delgado et al., 2016; Valle et al., 2021). However, according to our results, another possible explanation for the loss of biodiversity is DNA damage. Accumulation of DNA damage can be an important event capable of influencing the survival, reproduction and perpetuation of species (Kim et al., 2019; Xavier et al., 2019). In our study, we found a strong effect of the level of forest degradation in the occurrence of nuclear damage indicating an elevated risk associated with reduced vegetation coverage. We assume that the increased exposure to UV and thermic stress resulting from forest degradation would be causing the DNA damage. Detrimental effects of UV radiation on erythrocytes and DNA damage have been recognized for decades (Müller et al., 1996). On the other hand, studies have shown that thermal stress induces DNA damage and nuclear abnormalities, including micronucleus formation (Devyatkin et al., 2006; González-Acevedo et al., 2016).

It is important to note that the precise mechanism underlying the formation of nuclear anomalies is still not fully understood. However, these anomalies play a complementary role to the frequency of micronucleus (Güner & Muranli, 2011). Prior to the formation of micronucleus, it has been reported

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**Figure 5.** Odds Ratio of micronucleus frequency and nuclear abnormalities frequency in the four most common bird species sampled, according to the three forest degradation states. Statistically significant differences are shown as: \*: <0.05, \*\*: <0.01, \*\*\*: <0.001. Photographs (a, c): J. Córdova, (b): M. J. Balcázar, (d): F. Castillo.

that nuclear anomalies arise as a defense response, aiming to mitigate more severe damages such as double-strand breakage and the generation of additional harm to the organism (Frixione & Rodríguez-Estrella, 2020). Our research findings align with this phenomenon, as we observed a higher risk of nuclear anomalies and a lower risk of micronucleus in the semi-natural locality, while the opposite pattern emerged in the shrub-dominated location. This suggests that areas with higher stress exhibit fewer nuclear aberrations but a higher incidence of micronucleus. Furthermore, it has been noted that nuclear anomalies are more prevalent in individuals captured in regions where native vegetation cover has been diminished due to agricultural land use change (Frixione & Rodríguez-Estrella, 2020; Souto et al., 2018).

We found interspecific variations in the response to forest degradation. The species that exhibited the highest damage in terms of micronucleus and nuclear abnormalities are not the most suitable examples for inferring results regarding forest degradation, as these species were not distributed throughout the entire study area. Our data show the same variability reported for other species in different studies, given by the characteristics of the species and their growth conditions (Bolognesi & Hayashi, 2011; M. Drahulian et al., 2018; Silveira et al., 2022; Souto et al., 2018). One of the limitations of the study is the fact that we cannot establish that the effects of system degradation affect finding different types of birds, which meant that we could only monitor only four species in the three localities. Nevertheless, the information on genotoxic damage gathered in the present study will be useful for biomonitoring the effects of ongoing forest degradation on the health of birds.

Further research is needed to better understand the underlying mechanisms behind the genotoxic damage and how different bird species may uniquely respond to forest degradation. With an integrated approach and a deeper understanding of these dynamics, we can develop more effective strategies to conserve our valuable forest ecosystems and their associated species.

Understanding these mechanisms is especially important in seasonally dry tropical forests, where conservation and research efforts have been limited (Espinosa et al., 2012) and climate change may dramatically increase the

stresses to which these species are subjected. Therefore, any research that contributes to the knowledge of the dynamics of these ecosystems is a valuable contribution for strengthening its management and undertaking future conservation (Armijos-Ojeda et al., 2021).

In conclusion, our results support the idea that the loss of diversity due to forest degradation can be partially explained by the accumulation of genotoxic damage. Those species that are more sensitive to micronucleus accumulation end up disappearing rapidly from degraded areas. These findings underscore the importance of conserving natural forest ecosystems to protect biodiversity and prevent potential genotoxic effects on wildlife. In this sense, biomonitoring of micronucleus and nuclear abnormalities from blood samples from sensitive species, such as birds (together with other measures such as evaluation of the impact of territorial fragmentation on different species), can be a very useful tool for a better knowledge of the actions that need to be taken to ensure the conservation of these fragile ecosystems.

## **Conflict of Interest**

The authors declare no conflicts of interest relevant to this study.

## **Data Availability Statement**

Data from the nuclear and micronucleus and abnormalities nuclear data measurements used in this study are available at: https://data.mendeley.com/datasets/hb8czx2jxn/1. For the taxonomic recognition of the species in the area, the information available at: https://www.museum.lsu.edu/~Remsen/SACCBaseline.htm was used.

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