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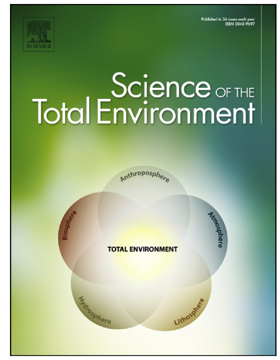

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Urbanity as a source of genotoxicity in the synanthropic Kelp Gull (*Larus dominicanus)*

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Abstract

Increases in human population lead to an increase in urban wastes, which could affect wildlife in several ways. Urban pollutants can affect erythrocytes of birds generating morphological membrane and nuclear anomalies. The Kelp Gull (*Larus dominicanus*) is an opportunistic species, which take advantage of urban environments, thus being highly exposed to environmental pollution. In northeastern Patagonia, the dynamic of the waste management was transformed in the last decade and consequently, gulls changed their movements in response to changes in waste management systems. The food available to the seagulls went from being a mixture of urban/fishing discards until 2015, when this landfill closures, to being domestic urban offerings. In order of evaluating genotoxicity and changes in pollutants exposition due to these changes, we analyzed the frequencies of erythrocytes nuclear abnormalities and micronuclei (ENAs and MN respectively) in 58 blood smears from adults extracted during the non-breeding season in two periods in landfills with different waste compositions: a mixed landfill (ML) in 2013 before closure (n = 24) versus an urban landfill (UL) (n = 34) in 2021. We found that Kelp Gull showed high values of abnormalities with an average of 151.5 /10,000 RBC in comparison with other seabird species. The bud and notched types of ENAs were the most prevalent abnormalities in both sites. We did not find significate differences in the overall abnormality frequency between sites, however we found significant higher frequencies in displaced and tailed types of ENAs in ML. We also found poikilocytosis, as seen previously in other animals exposed experimentally to pollutants such as metals and crude oil. Cellular abnormalities found in the Kelp Gull suggest an exposition of individuals to pollutants in foraging areas. The hemispheric distribution and the synanthropic characteristics of the species denote its importance as a suitable global monitor of genotoxicity.

Keywords: erythrocytes, gulls, landfills, Patagonia, pollution, urban.

1. Introduction

Pollution in urbanized and industrial areas could increase mutagenic rates and induce heritable DNA damage affecting wildlife (Sommers et al., 2004; de Souza et al. 2017; Giraudeau et al., 2020). Detection of nuclear abnormalities in erythrocytes is one of the methods most applied for detecting genotoxicity, generally used as biomarkers of DNA damage (Fenech, 2000; Gomez-Meda et al., 2006; de Lemos et al., 2007). DNA corruption during mitosis might be one of the consequences triggered by pollutants on blood cells (Baesse et al., 2015). Such factors suggest some urban animal species could be considered as good sentinels of pollution because they express the anomalies by pollutants even if their body condition shows no outright change (Santos et al., 2017; de Souza et al., 2017; Csiszar et al., 2019; Brandts et al., 2022).

Human populations are increasing in relation to urban development, with concomitant increases in other factors such as garbage, which can contain polluted discards. Several animal species show population increases in urban settings, (Yirga et al., 2015; Luna et al., 2021) and may consequently be exposed to greater negative effects of urban pollutants (Steigerwald et al., 2015; Katlam et al., 2018). Among vertebrates, mammals and birds may take advantage of urban garbage in many ways (Oro et al., 2013), thus increasing their exposure to several types of threats (Matejczyk et al., 2011; Plaza et al. 2020).

There are many kinds of waste disposal depending on human activities in the environment; landfills focused more specifically on urban activities only, where organic and inorganic remnants can be found by opportunistic species (Ergene et al., 2007; Alimba and Bakare, 2016), or landfills where agricultural discards from rural areas are also found (Brousseau et al., 1997), and/or fisheries discards from coastal activities, which also include port discards (Bertellotti et al., 2001; Yorio and Giaccardi, 2002), thus supplementing the waste from strictly urban settings. In these types of landfills, chemicals and diverse microorganisms could be released into the ground, surface waters, and surrounding air (Vilavert et al., 2012; McIntyre et al., 2022). In urban landfills Persistence Organic Pollutants (POPs) are the most abundant chemical, toxins as brominated flame retardants (PBDE, OBDE, HBB), hexachlorocyclohexane (HCH), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and hexachlorobutadiene (HCBD) are among these chemicals (Weber et al., 2008). In agricultural landfills, agrochemicals, pharmacological and biological hazards are the greatest threats affecting opportunistic wildlife (Battin, 2004; Jayaraj et al., 2016; Plaza et al., 2020), and in landfills associated to fisheries, risks are more related with biological threats such as viruses and bacteria (D'Amico et al., 2018).

Seabirds are commonly used as biomonitors of pollution and ecosystem health (Elliott and Elliott, 2013). Among seabirds, gulls are a group of mostly opportunistic birds, which are known to increase around human populations, likely due in part to the increased food availability due to human waste (Bertellotti et al., 2001). Landfills are key spaces where these groups of seabirds find the energetic requirements for survival and reproduction, driving their population expansion throughout the world (Duhem et al., 2008; Delgado et al., 2021). The Kelp Gull (*Larus dominicanus*) is a large size gull, with adult body mass ranging between 730 and 1,200 g (Torlaschi et al.,

2000) and is widely distributed along the southern hemisphere inhabiting most of South America, Southern Africa, Australia, New Zealand, and Antarctica (BirdLife International, 2022). This gull is an opportunistic and generalist species, which takes advantage of several kinds of anthropogenic food sources (Bertellotti and Yorio, 1999), mainly during its non-breeding stages (Bertellotti et al., 2003; Ludynia et al., 2005; Frixione and Alarcón, 2016), increasing and expanding their populations in several areas throughout the hemisphere (Coulson and Coulson, 1998; Frixione et al., 2012).

For many years Kelp gulls attracted to the urban area of Puerto Madryn in Patagonia (Fig. 1) used to forage over a unique landfill, which concentrated both urban wastes as well as discards from local fisheries (Bertellotti and Yorio, 2000; Lisnizer et al., 2011). Over many years as a new policy around waste management was implemented, this dual-functioning landfill was closed in 2015. Now, fishery discards are processed for use in composting, and therefore the fisheries waste in the local landfill is greatly diminished. On the other hand, the urban waste is deposited in a single landfill that gathers the domestic garbage produced by the five most populated cities of northeastern Chubut, Patagonia (www.argentina.gob.ar/sites/default/files/provincia_de_chubut.pdf). These waste management modifications led to a change in the food available for Kelp gulls. In the past most of the gulls of the area fed on the mixed urban/fisheries landfill, whereas now they feed on the new urban-waste only landfill located 30 km south from Puerto Madryn (Fig. 1) (Bertellotti, in progress). Thus, over the last 10 years, the food resources available to the seagulls went from being a mixture of urban/fishing discards, to being mostly domestic urban offerings.

Here, we will compare the presence and frequency of erythrocyte nuclear abnormalities in the Kelp Gull, evaluating and contrasting the genotoxicity in a polluted environment during two periods of time separated by a significant change of the dynamics and distribution of waste management and therefore food availability: that of the previous mixed urban/fisheries waste landfill to the now strictly domestic urban one.

2. Material and methods

As part of a monitoring program on Kelp Gull populations responses to waste management and distribution, we randomly captured and sampled 34 adult Kelp Gull of unknown sex at the mixed waste landfill (hereafter ML) located near the port and industrial city of Puerto Madryn, Chubut, Patagonia Argentina, in 2013 before the landfill was closed due to changes in waste management policies (42°46′ S; 64° 54′ W; Fig. 1). Then, we randomly captured 21 Kelp Gull adults of unknown sex in the newer urban only landfill during 2021 (hereafter UL) (42°02’ S; 65°11’ W; Fig. 1). All captures were conducted during the non-breeding season. During breeding (October-January) Kelp gulls congregate along the northeastern Patagonian coast foraging in the areas directly around breeding colonies (Kasinski et al., 2021). Once the breeding process is finished, gulls could move to urban areas in continental spots where several kinds of discards are available (Frixione et al., 2022). Therefore, both spots of capture congregated the major number of adults during non-breeding stage of the study area in each year of sampling.

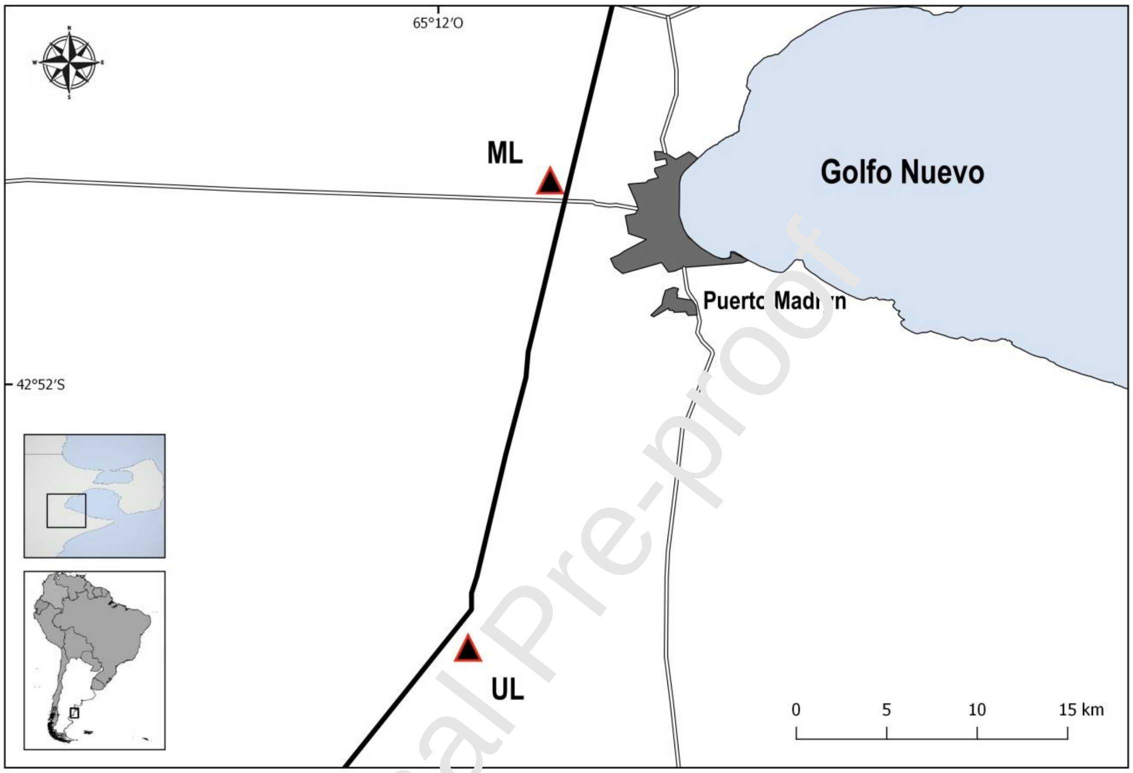

Figure 1: Sites of captures: mixed landfill, (ML) and urban landfill (UL) located in Puerto Madryn and surroundings, in northeastern Patagonia, Argentina.

Birds were captured using a canon net and maintained in shaded cages until blood samples were taken. Individuals were classified as adults considering plumage, beak, and leg colors (Bertellotti and Yorio, 2000). Blood samples were obtained from the ulnar vein and a thin blood smear was made with a fresh blood drop (Blanco et al., 2001). Each smear was fixed in ethanol, stained with Tinción 15 (Biopur; D'Amico et al., 2018), and photographed under a 100x magnification objective (oil inmersion, LeicaDM500) (Baesse et al., 2015). Erythrocyte counts were processed with the software ImageJ 1.52 s. Photomicrographs were first converted into 8-bit black-and white images, and erythrocytes were identified and counted using threshold parameters for circularity and size (pixel^2). An average of 2,110 erythrocytes per bird was counted. Slides were coded, randomized, and blind-scored by a single observer to reduce experimental bias (de Souza et al., 2017). Nuclear shape abnormalities were classified as micronucleus (MN), nuclear anomalies (ENAs) including bud, segmented, notched nuclei, tailed nuclei, polymorphic, enucleated (Oudi et al., 2019), displaced nuclei (de Souza et al., 2017) or “other” nuclear unclassified shape abnormalities (Fig. 2). Additionally, we also counted an abnormal shape expressed on the membrane of erythrocytes named poikilocytosis, a condition which has been registered in fish and seabirds (among others) exposed to pollution (Leighton, 1985; Marenkov et al., 2021). We calculated the prevalence and the frequency of MN and ENA types in terms of number per 10,000 cells (Schmid, 1975). As nuclear abnormalities are developed in short periods of time related to the short life span of bird erythrocytes (28–45 days, Rodnan et al., 1957), this characteristic makes blood cells as a good biomarker of recent exposure to pollutants. The same procedures were applied for poikilocytosis.

2.1. Statistical Analyses

To evaluate the frequencies of anomalies between years of sampling we used a GLM with negative binomial distribution taking into account statistical overdispersion (Crawley, 1993). We evaluated differences between years for the global frequencies of anomalies and for each kind of nuclear anomaly.

All the statistical analyses were carried out using the "MASS", "car" software and for graphics the "ggplot2" package of the R version 4.0.1 (R Core Development Team, 2020).

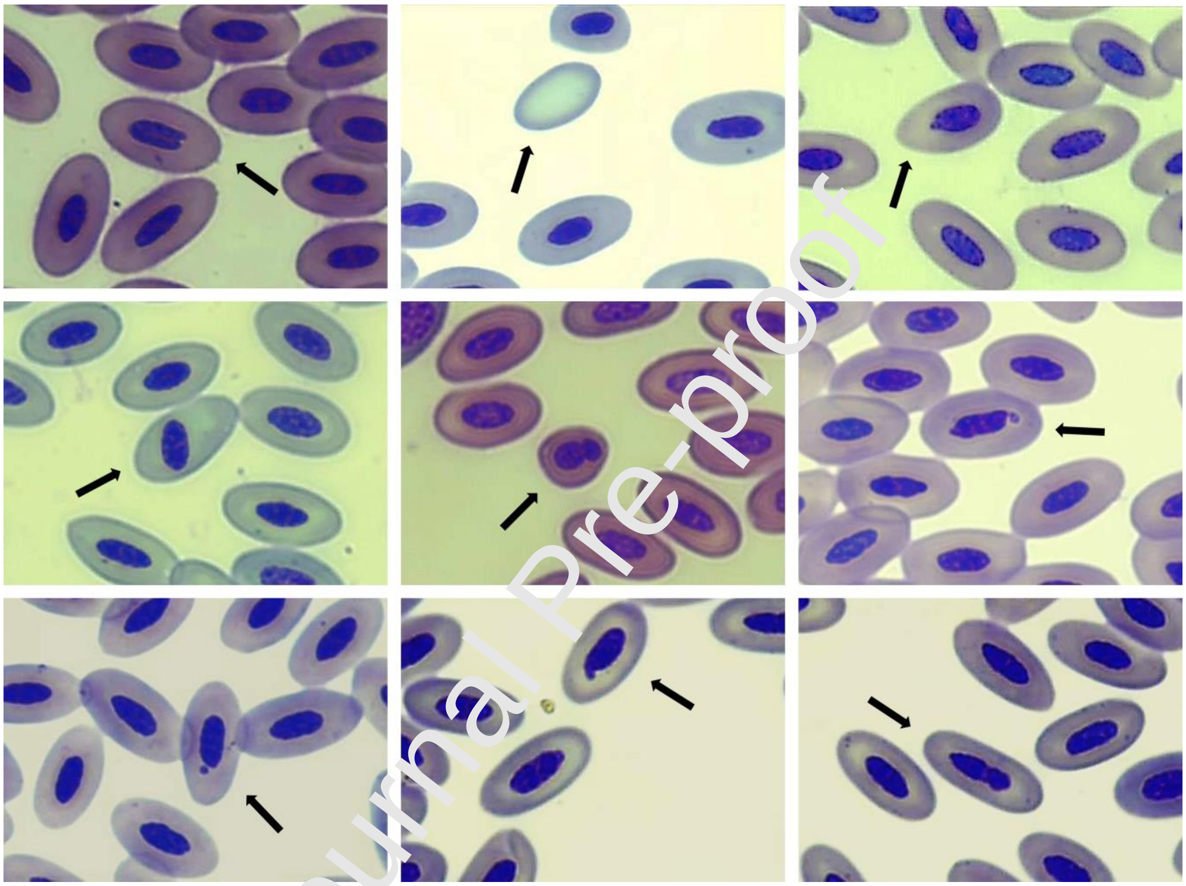

Figure 2: Nuclear abnormalities found in erythrocytes of the Kelp Gull: A) notched, B) enucleated, C) bud, D) displaced, E) other (polymorphic: segmented and reduced size), F), other, G) micronucleus, H) tailed, and I) segmented.

3. Results

We registered nuclear abnormalities in every individual, with the bud (97.1%) and notched (96.4%) traits being the most prevalent abnormalities (Table 1). For both sites combined, we found an average of 151.5 nuclear anomalies per 10,000 erythrocytes (range: 24.5–424.5, SD 87.6), with 161.8 nuclear anomalies in ML (range: 29.4–337.1, SD 79.9) and 136.7 in UL (range: 24.5–424.5, SD 97.3).

We found no significate differences in total frequencies of nuclear anomalies between locations (GLM: $Z = -1.08$, $p = 0.2$). However, when anomalies were considered individually, we found significant differences for displaced (GLM: $Z = -3.7$, $p < 0.0005$) and tailed anomalies (GLM, $Z = -3.8$, $p < 0.0005$), being both more frequent in the ML (Figure 3). All other specific anomalies showed no significant differences between locations.

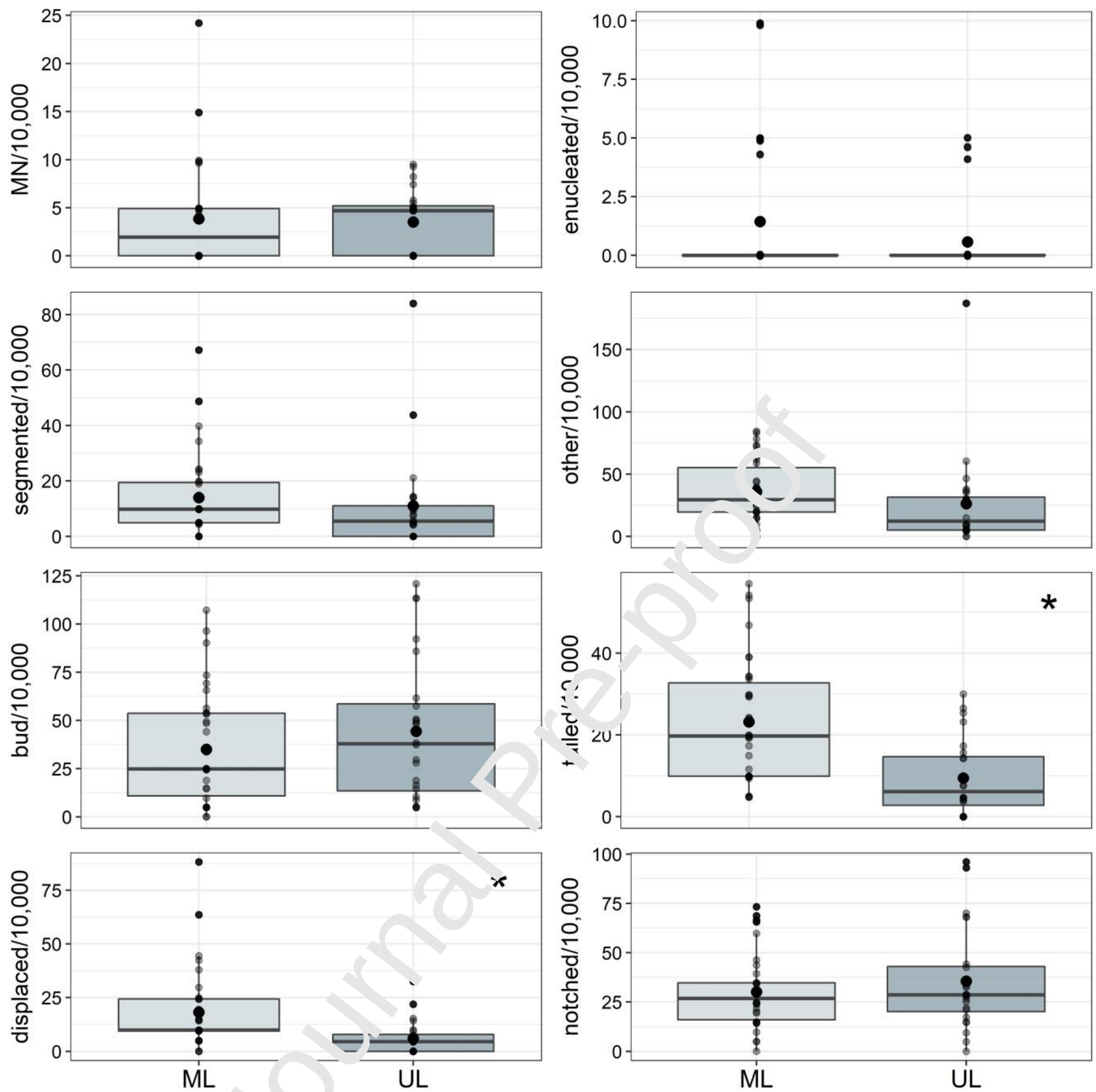

Figure 3: Frequencies of nuclear anomalies (micronucleus, enucleated, segmented, other, bud, tailed, displaced, and notched) by 10,000 erythrocytes in Kelp Gull adults in ML and UL in northeastern Chubut, Argentina. ("*" = significant differences)

We detected a clear pattern of poikilocytosis in 75.8% of individuals, being similar both study sites (ML: 76.4%; UL: 75%). The mean frequencies of these malformed erythrocytes were 13.9/10,000 RBC, also similar between sites (ML: 14.4/10,000 RBC; UL: 13.2/10,000 RBC).

4. Discussion

Nuclear abnormalities have been studied in both the field and in controlled laboratory experiments in several groups of animals (Cavalcante et al., 2008; Morita et al., 2016; Zapata et al., 2016; Suarez-Rodriguez et al., 2017; Santovito et al., 2020). In birds there is a broad spectrum of genotoxicity research conducted in passerines (Baesse et al., 2015; Souto et al., 2018), raptors (Frixione and Rodríguez Estrella, 2020; Stocker et al., 2022), and aquatic birds (Barata et al., 2010; Quirós et al., 2008), among others. Genotoxicity in seabirds has been scarcely studied, although there are some studies registering baseline values of MN and ENAs in several species (De Mas et al., 2015; Oudi et al., 2019).

Studies of nuclear anomalies in erythrocytes on seabirds were conducted mainly in Antarctic penguins. For instance, an average of 16.2 and 31.3 ENAs /2,000 RBC has been registered for adults and chicks respectively of Adelie penguins (*Pygoscelis adeliae*). Barbosa et al. (2013) found 19.10 ENAs/10,000 RBC, and Olmastroni et al. (2020) found no more than 20 ENAs/10,000 RBC in Adelie penguins. In Gentoo penguins (*Pygoscelis papua*), an average of 14.6 and 15.3 ENAs/2,000 RBC has been registered for adults and chicks, respectively (D'Amico et al., 2016). In other scavenging species as the South Polar Skua (*Stercorarius maccormicki*) contrasting lower values were found in Antarctica (0.71 ENAs/10,000 RBC) (Kursa and Besrukov 2008).

In gulls, ENAs records are scarce; for example, the Lesser Black-backed Gull (*Larus fuscus*) showed no more than 50 ENAs/10,000 RBC (Santos et al., 2020) and in

Audouin's Gull (*Ichthyaetus audouinii*) captured in Italy and Tunisia, mean values of ENAs were between 33.3 and 66.6/10,000 RBC (Borghesi 2016). In our study, micronuclei evidenced to be lower than other anomalies; however, the mean frequency of micronuclei is the highest ever registered for a seabird in the southern hemisphere (3.7/10,000 RBC). In comparison with other scavenging species of the southern hemisphere (South Polar Skua: 0.07 MN/10,000 RBC) (Kursa and Bezrukov, 2008), the current study showed higher frequencies in both type of landfills (ML, 3.8 and UL, 3.5 MN/10,000 RBC). Other studies on the Laridae family of the Tunisian coastal areas showed similar frequencies in a highly contaminated industrial area (3.6 MN/10,000 RBC) (Oudi et al., 2019).

The present study is the first showing erythrocytes abnormalities in the synanthropic Kelp Gull, evidencing high frequencies of ENAs in the species, being more than 160 ENAs/10,000 RBC in ML, registering the highest values in literature on seabirds. The bud and notched were the most abundant types of ENAs in Kelp Gull from both landfills (Table 1). It is reported that toxic metals could induce abnormal chromosome segregation, attached chromosome and gene amplifications during S phase of the cell cycle, attributed to the formation of notch and bud nuclei (Tolbert et al., 1982; Shimizu et al., 1998, 2000). Increase of ENAs observed may lead to increase genetic imbalance, which is the basis of carcinogenesis (Mix, 1986; Guha and Khuda-Bukhsh, 2003). Therefore, high values of ENAs found in Kelp Gull could be not unexpected values for the species, considering their landfill foraging areas during nonbreeding.

When compared both landfills ML and UL, displaced and tailed nuclei showed significate higher frequencies in gulls from ML, and most of the anomalies showed a similar pattern (see Table 1). Misshapen nuclei indicating nuclei displacement were registered in captive fish and birds exposed to toxic concentrations of metals in a controlled experiment (Gill and Pant, 1985; Romero et al., 2009; Marenkov et al., 2021). Experimental studies in Australian parakeets (*Melopsittacus undulates*) also showed higher frequencies of ENAs in individuals exposed to industrial pollutants and found the displaced nuclei as the most frequent nuclear anomaly (de Souza et al., 2017). These results could be related to the fact of gulls fed on significantly different and more diverse waste at the ML, until 2015 when it closed (Bertellotti and Yorio, 1999, 2000; Yorio and Giaccardi, 2002; Bertellotti et al., 2003). Pollutants from the industrial port and urban areas and from the mixed landfills could contain contaminated products that may potentially affect the DNA of gulls as showed in their nuclear erythrocyte shape (Baesse et al., 2015). Soil and air pollution, metals from industrial waste and pollution from port activities in urban areas have been widely recognize as carcinogenic (Cachot et al., 2006; Singh et al., 2011; Sharifuzzaman et al., 2016; Pan et al. 2018). In fact, some reports showed that organisms of urbanized coastal sites close to this study area evidenced morphological and other kind of anomalies caused by marine pollution: gastropods imposex (Bigatti et al., 2009), exoskeletal asymmetries in crabs (Lezcano et al., 2014) and cellular nuclear abnormalities in mussels (Izquierdo et al., 2003). In Norway, for example, high DNA damage has been registered in gulls of urban areas caused by genotoxic agents of these polluted environments (Keilen et al. 2022).

We found high percentages of poikilocytosis in Kelp Gull in both ML and UL, and detected kidney shaped erythrocytes, which was similar to the “convolutioned” erythrocytes as called by Leighton in 1985 found in the Herring Gull (*Larus argentatus*) exposed to crude oil. Poikilocytosis could be formed by a mutagenic result affecting microtubules formation, and proteins such as spectrin and actin that are associated with the inner surface of the plasmalemma (Leighton, 1985). These erythrocyte shapes were also identified in fish experimentally exposed to metals (Marenkov et al., 2021), and several researchers have shown poikilocytosis due to metals exposure in fish (Naskar and Amhad, 2005; Joni et al., 2006; Tomova et al., 2008). As mentioned before, the feeding areas of Kelp Gull led them to be exposed to metals coming from industrial waste. Therefore, it is possible that the novel poikilocytosis found in our study is associated with this related exposition to the metal polluted area, where gulls fed on quite often.

Further studies should extend research related to blood anomalies and polluted environments, and using Kelp Gull as a model of study would be informative, as the ecological characteristics and the wide distribution of the species across the southern hemisphere is unique. Future research also should expand efforts to conduct further toxicological analyses in other seabirds of Patagonia, especially in gulls, closely related to our species. In particular, as synanthropic species are quite resilient (i.e., tolerant to human presence and disturbances), they may serve as model species for study of genotoxicity problems at a global scale.

5. Conclusions

Synanthropic Kelp gulls evidenced high frequencies of nuclear abnormalities, being the highest measured in gulls and in seabirds in general. The occurrence and frequencies of nuclear abnormalities are considered high and differed between type of landfills, due to the change from mixed landfill (urban, fisheries to urban only). Kelp gulls showed poikilocytosis which has previously been shown to be related to pollutant exposure. The Kelp Gull is proposed as a good monitoring species for coastal areas of the southern hemisphere in accordance with its ecology and distribution.

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Permits

Field permits were obtained from the environmental agency Dirección de Fauna y Flora Silvestre, Chubut.

Declaration of competing interest

The authors declare there are no competing interests.

CRediT authorship contribution statement

Marcelo Bertellotti, Verónica L. D'Amico and Martín G. Frixione conceived the idea and design. Martín G. Frixione conducted the analyses of ENAs and wrote the paper. Verónica L. D'Amico, Miguel A. Adami and Marcelo Bertellotti organized fieldwork, collected data, and prepared the blood smears for lab analyses. Marcelo Bertellotti and Verónica L. D'Amico contributed substantial materials, resources, and funding for field and laboratory work.

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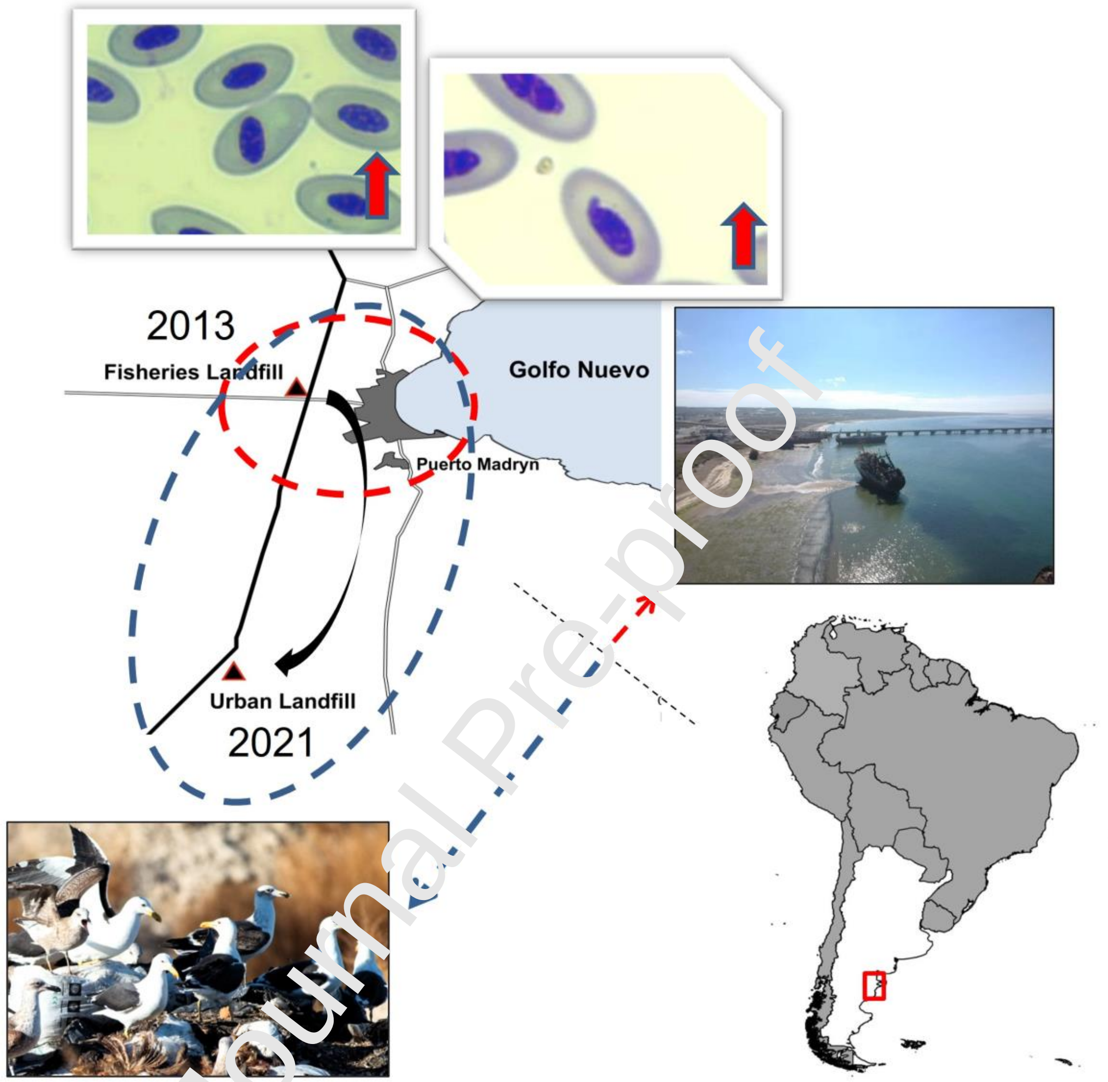
Table 1: Mean frequencies of nuclear anomalies by 10,000 erythrocytes in Kelp Gull adults captured in mixed landfill (ML) and urban landfill (UL) in northeastern Chubut, Argentina. (SE in parentheses)

	notched	segmented	displaced	bud	tailed	enucleated	other	MN	total
ML	30.1 (3.2)	14.0 (2.5)	18.2 (3.2)	35.0 (4.9)	23.2 (2.5)	1.4 (0.4)	36.1 (4.2)	3.8 (0.9)	161.8 (13.7)
UL	35.6 (5.2)	11.0 (3.7)	6.0 (1.6)	44.4 (7.5)	9.5 (1.9)	0.6 (0.3)	26.2 (7.7)	3.5 (0.6)	136.7 (19.8)
mean	32.4 (2.8)	12.8 (2.1)	13.1 (2.1)	38.9 (4.2)	17.5 (1.9)	1.1 (0.3)	32.0 (4.0)	3.7 (0.6)	151.5 (11.5)

Graphical abstract



Highlights

-Erythrocytes abnormalities were analyzed considering changes in the urban waste management in the study area

-Gulls evidenced high frequencies of nuclear abnormalities and poikilocytosis

-Changes in waste management showed a diminished occurrence on some types of nuclear abnormalities