SETAC Europe 34th Annual Meeting 2024

Assessing the Impact of Penguin Guano on the Antarctic Bivalve *Aequiyoldia eightsii*: Effects on Biomarker Responses

Erica Sparaventi[1], M. Laura Martín-Díaz[2], Antonio Tovar-Sánchez[1], Araceli Rodríguez-Romero[1,3]

1. Department of Ecology and Coastal Management, Institute of Marine Sciences of Andalusia, ICMAN (CSIC), Campus Río San Pedro, 11510 Puerto Real, Cádiz, Spain
Email: erica.sparaventi@csic.es; a.tovar@csic.es
2. Chemical Physics Department, University Institute of Marine Research (INMAR), International Campus of Excellence of the Sea (CEI.MAR), University of Cádiz, República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain
Email: laura.martin@uca.es
3. Department of Analytical Chemistry, Faculty of Marine and Environmental Sciences, Institute of Marine Research (INMAR), University of Cádiz, Campus Río San Pedro, Puerto Real, 11510 Cádiz, Spain
Email: araceli.rodriguez@csic.es

INTRODUCTION

Penguin guano contains high concentrations of bioactive Trace Metals (TMs), essential for several biological processes (i.e., Fe, Cu, Zn and Mn). However, high levels of non-biogenic metals (e.g. Cd and Pb) have also been reported (Espejo et al., 2014). *Pygoscelis* spp. represent about 90% of the seabird biomass in the Southern Ocean. During the Antarctic summer huge numbers of penguins reach their colonies on land for nesting activities. Due to melting glaciers or rainwater, sediment ablation leads to guano being discharged into the waters surrounding the colony and affects the TMs pool. However, the potential effects of TMs released from guano on the organisms of the Antarctic ecosystem need to be addressed.

Fig. 1 I The Antarctic continent (A), sampling site in Livingston Island (B), photo of the Johnsons Dock (C).

METHODS

Experimental design

- During Austral summer 2021-22 we carried out an exposure experiment with the Antarctic clam *Aequiyoldia eightsii* to four different Gentoo penguin (*Pygoscelis papua*) guano concentrations. The experiment was conducted outdoors to ensure the natural amount of light, photoperiod and temperature, on Livingston Island (South Shetland Islands) (Fig. 1).
- Guano collection: fresh guano samples were collected manually from the ground, in a Gentoo penguin colony (Argentina Cove, Livingston Island; 62°40′S, 60°24′W) (Fig. 2, A).
- Exposure time: 10 days.
- Treatments: 2 replicates per concentration of guano (see Table 1).
- Chemical analysis: TM content in guano was measured with ICP-MS (iCAP Thermo), see Sparaventi et al., 2021 for the method.

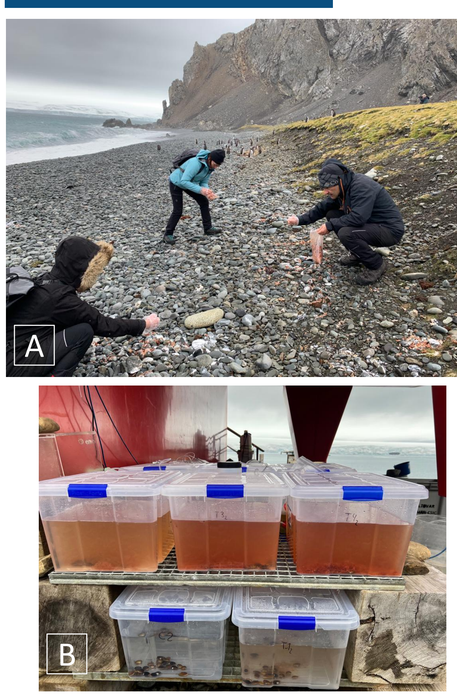


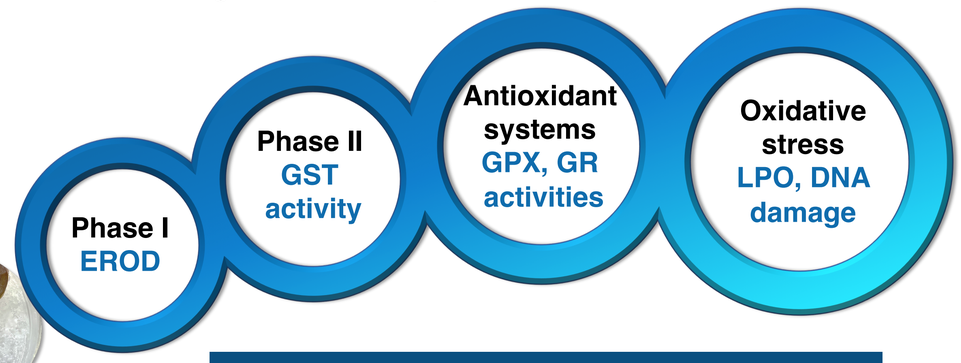
Fig. 2 I Penguin guano sampling in the Argentina Cove (A), exposure experiment, treatments in plastic aquaria (B).

Table 1 I Different concentrations of guano (mg L^{-1}) used for the exposure experiment.

Treatment	Guano concentration (mg L^{-1})
Control	0
T1	300
T2	3300
T3	6700

Biochemical biomarkers

- *A. eightsii* biochemical responses were investigated with a wide panel of antioxidant defences, including Ethoxyresorufin O-deethylase (EROD), Gluthatione S-transferase (GST), Gluthatione peroxidase (GPX), Gluthatione reductase (GR), lipid peroxidation (LPO) and DNA damage determined in the digestive gland.
- Biochemical analysis: 4 specimens were sampled from each aquarium on days 2, 4, 7, 10 (n=5 replicates). Biomarkers were measured using a kinetic microplate reader, Infinite® M200.



RESULTS

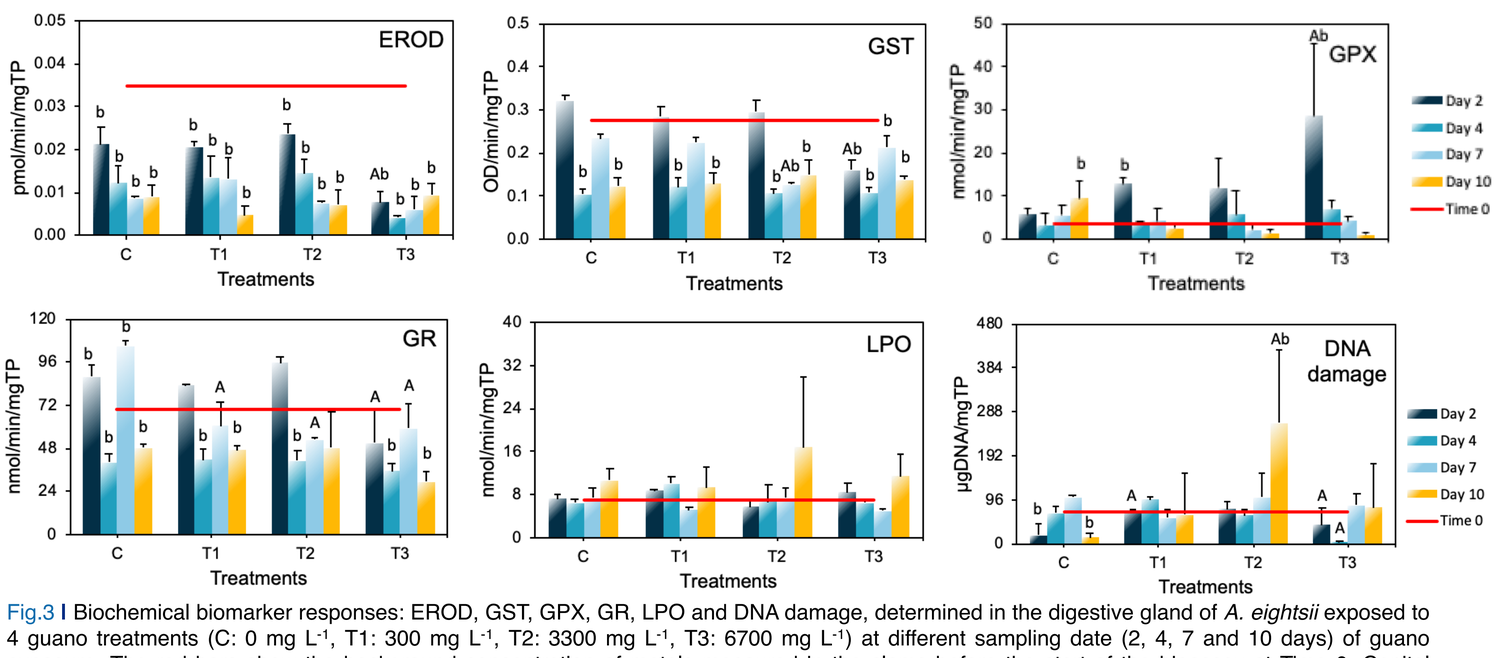


Fig.3 I Biochemical biomarker responses: EROD, GST, GPX, GR, LPO and DNA damage, determined in the digestive gland of *A. eightsii* exposed to 4 guano treatments (C: 0 mg L^{-1}, T1: 300 mg L^{-1}, T2: 3300 mg L^{-1}, T3: 6700 mg L^{-1}) at different sampling date (2, 4, 7 and 10 days) of guano exposure. The red bars show the background concentration of metals measured in the clams before the start of the bioassay at Time 0. Capital letters indicate significant differences (p value < 0.05) between the control (C) and the guano treatments (T1, T2 and T3) at the same sampling date (2, 4, 7, 10). Lowercase letters indicate significant differences (p value < 0.05) between Time 0 and guano treatments (T1, T2 and T3).

Table 2 I Gentoo penguin guano TM concentrations in µg g^{-1} (n=3).

µg g^{-1}	Ni	Cu	Zn	Cd	Pb	As
Time 0	1.74±0.09	185.7±4.6	167.6±1.0	1.091±0.004	0.94±0.07	10.23±0.30
Day 2-4	1.92±0.06	194±11	203.4±6.7	1.07±0.02	1.02±0.07	11.13±0.40
Day 7	1.63±0.12	180±13	213±12	1.24±0.06	1.21±0.11	7.44±0.44

MAIN FINDINGS

- After exposure to penguin guano, the biomarker responses showed different patterns:

  ⇨ For all the treatments, a decrease in antioxidant enzyme activities was observed when increasing the guano concentration;
  ⇨ In some of them (e.g EROD, GST and GR) the decrease was significant (p<0.05);
  ⇨ A decrease in antioxidant enzyme activities has also been observed with time, with significant differences with Time 0;
  ⇨ LPO and DNA damage increased with guano concentrations and time. Nevertheless, it was significant only for DNA damage on day 2 in T1, T3, day 4 in T3 and day 10 in T2.

- In this work are provided novel and relevant information since, for the first time, the levels and the response of biomarkers as an effect to penguin guano exposure on the Antarctic benthic clam *A. eightsii* has been investigated. However, a clear stress response to guano exposure was not observed.
- Inhabiting in a glacier dock, clams of this environment may be routinely exposed to a natural environment with high concentrations of TMs.
- Currently, analyses of TM concentrations in the soft tissues of *A. eightsii* are ongoing, with the aim of integrating biomarker responses with TMs bioaccumulation.
- Further efforts could involve guano exposure experiments with species from different levels on the Antarctic trophic chain and with extended exposure times.

REFERENCES

- Espejo, W., Celis, J.E., González-Acuña, D., Jara, S., Barra, R., 2014. Concentration of trace metals in excrements of two species of penguins from different locations of the Antarctic Peninsula. Polar Biol. 37 (5), 675–683. https://doi.org/10.1007/s00300014-1468-z.
- Sparaventi, E., Rodríguez-Romero, A., Barbosa, A., Ramajo, L., & Tovar-Sánchez, A. (2021). Trace elements in Antarctic penguins and the potential role of guano as source of recycled metals in the Southern Ocean. Chemosphere, 285. https://doi.org/10.1016/j.chemosphere.2021.131423

ACKNOWLEDGEMENT

This research is a contribution of the projects PIMETAN (RTI2018-098048-B-I00), DICHOSO (PID2021-125783OB-I00) and Grant EQC2019-005721-P funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe". E. Sparaventi is supported by the Spanish FPI grant (Ref: PRE2019-089679). This research is part of the POLARCSIC research initiative. We thank the hospitality of the Spanish Antarctic Stations "Juan Carlos I" and the transportation by the BIO Hespérides. We also thank the logistic support by the Marine Technological Unit of CSIC.