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RESEARCH

Effects of penguin guano on spatial behavior of Antarctic marine invertebrate species: an exploratory study

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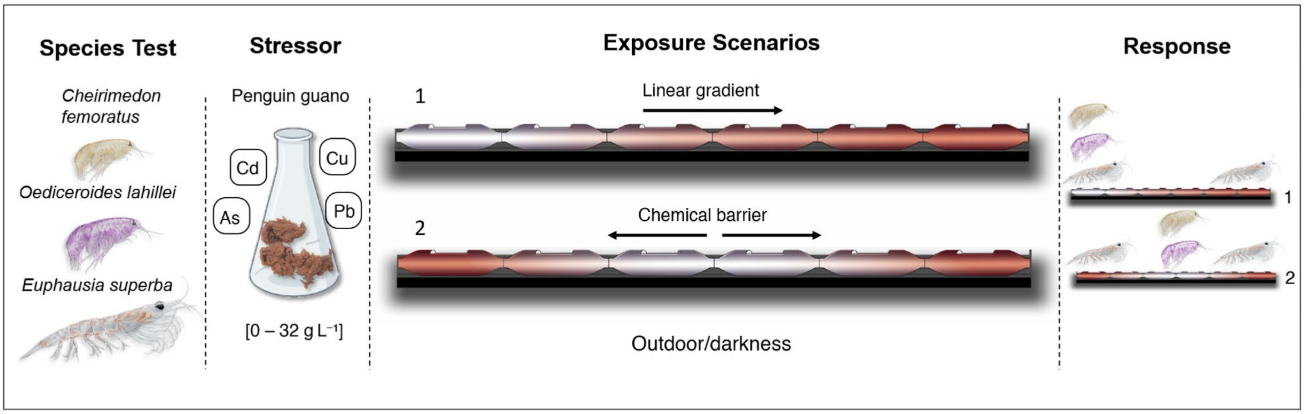
Abstract

Antarctic penguins' guano represents a complex mixture of nutrients and chemical compounds. A large amount of guano produced during the breeding season can flow into seawater, near the colonies, altering the chemical balance of coastal environments. However, information on how guano input may alter marine community structures and dynamics in Antarctic ecosystems remains scarce. This exploratory investigation assesses the influence of guano, as a chemical stressor, on habitat selection (spatial avoidance behavior) in three key Antarctic marine invertebrate species: the amphipods *Cheirimedon femoratus* and *Oediceroides lahillei*, and Antarctic krill *Euphausia superba*. We employed a linear non-forced exposure system simulating a chemically heterogeneous environment, combining: (i) model organisms; (ii) two exposure scenarios, a linear guano gradient and guano as chemical barrier; (iii) guano from two penguin species, Chinstrap and Gentoo; (iv) two light conditions (outdoor and darkness); and (v) two exposure times (5 and 8 h). After 5 h, both amphipod species exhibited significant avoidance responses to guano under the conditions tested. Krill did not show a response related to guano; they consistently aggregated at the system's extremes, reflecting krill's complex spatial dynamics. These findings indicate that guano can alter amphipod distribution by triggering avoidance responses, potentially affecting distribution patterns and leading to localized population declines. As a pilot assessment, this study highlights the need to integrate behavior-based endpoints and non-forced exposure approaches to understand the ecological effects of chemically complex inputs in Antarctic marine ecosystems.

Graphical abstract



Keywords Penguin guano · Spatial behavior · Avoidance · Antarctic marine invertebrates

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Introduction

In Antarctic coastal ecosystems, several parameters exist that shape the biological structure of the flora and fauna landscape, including ice cover, light regime, biological and chemical interactions, and trophic energy transfer (Gutt 2001). Among the biotic factors, the deposition of seabird guano (mainly penguins) during the breeding season leads to the input of a variety of compounds (e.g., nutrients and organic matter) into the water column, representing a significant yet often overlooked driver of local ecological processes. Such biotic disturbances can alter the chemical and biological balance of Antarctic coastal environments, potentially triggering behavioral responses in marine organisms through multiple, non-exclusive mechanisms, including changes in resource availability, chemical cues associated with predation risk, and exposure to complex mixtures of guano-derived compounds (Shatova et al. 2016; Otero et al. 2018; Ratnarajah et al. 2018; Belyaev et al. 2023; García-Veira et al. 2024). Due to these variable conditions, the ecological niche of organisms plays an important role in ecosystem functioning and nutrient cycling (Convey and Peck 2019; Henley et al. 2020).

Penguins represent the predominant seabirds on the Antarctic continent, mainly from the genus *Pygoscelis*: Adélie (*P. adeliae*), Chinstrap (*P. antarcticus*), and Gentoo (*P. papua*) (Espejo et al. 2017). During the breeding season, thousands of pairs of Pygoscelid penguins gather along the continental shores to nest, leading to the deposition of a huge amount of guano (Borboroglu and Boersma 2013). In polar and subpolar regions, their colonies are known to significantly enrich surrounding soils with organic matter, nitrogen, phosphorus, and trace metals (TMs) (Shatova et al. 2016; Wing et al. 2017), influencing the vegetation on land by shaping moss and lichen communities (Ellis 2005). In Antarctic coastal environments, guano can easily enter nearshore waters through runoff, leaching, or weathering processes (Ratnarajah et al. 2018; De La Peña-Lastra 2021). Upon reaching seawater, guano contributes to the pool of macro- and micronutrients, modifying the phytoplankton community structure and enhancing primary production (Shatova et al. 2016, 2017; Otero et al. 2018; Sparaventi et al. 2021). Although these inputs are well documented, the potential ecological impacts of guano-derived compounds (e.g., TMs among others) on marine invertebrates remain unexplored. Penguin guano composition depends on habitat and diet, adding further complexity to its ecological effects. Penguins primarily feed on Antarctic krill *(Euphausia superba*, hereafter krill) (Smichowski et al. 2006), a species known to act as a reservoir of TMs in the euphotic layer of the Southern Ocean (SO) (Tovar-Sánchez et al. 2009). As a result, guano represents a chemically complex input to coastal waters, containing a mixture of nutrients, organic compounds, and trace elements. Guano discharge may therefore alter seawater chemical composition, potentially influencing benthic and pelagic organisms inhabiting nearshore environments. These changes may lead to ecological effects, including avoidance and repellency reactions that influence habitat selection, as guano-derived chemical compounds can trigger stress conditions related to chemical imbalance in marine invertebrates (Hay 2009; Jermacz et al. 2020). However, the effects of penguin guano on animal behavior and interactions remain poorly understood. To date, only one study has reported krill responses under laboratory conditions, using forced guano exposure, showing that penguin guano can alter krill swimming activity and ingestion rates, potentially affecting trophic interactions and energy transfer in Antarctic marine ecosystems (Hellessey et al. 2025). In aquatic systems, behavioral responses such as avoidance or attraction are often mediated by non-toxic primary metabolites released by predators, which function as chemical cues of predation risk. These cues, including compounds derived from predator excreta, can induce strong spatial responses in prey without causing physiological toxicity.

Marine invertebrates play a key role in Antarctic ecosystems and food webs by linking primary producers and higher trophic levels. They serve as a food source for many organisms and are involved in nutrient cycling and the biological carbon pump, facilitating the export flow of particulate organic matter to the seafloor through the production of fecal pellets (Hughes 2012). Amphipods are a group widely used in aquatic ecotoxicological assays due to their abundance and broad distribution across different habitats (Duquesne et al. 2000; Araújo et al. 2016). The gammarid *Cheirimedon femoratus* belongs to one of the most important groups of Antarctic amphipods, the Lysianassoidea (Dauby et al. 2001). It has a circumpolar distribution and is an omnivore and scavenger that feeds on a wide variety of food sources, from detritus to algae and invertebrates (Bregazzi 1972; De Broyer et al. 2007). *Oediceroides lahillei* (Chevreux, 1911) is another ecologically important amphipod species, as it constitutes part of the diet of fish and penguins. It can be found along the islands of the Scotia Arc and extends northward to the sub-Antarctic waters of Tierra del Fuego (De Broyer et al. 2007; Xavier et al. 2017). Krill is a keystone species in the SO, shaping biogeochemical cycles and food webs in the Antarctic ecosystem by acting as the main grazer of phytoplankton and serving as a vital food source for many organisms, such as whales, seals, and penguins (Cavan et al. 2019). Despite their key ecological roles, little is known about the sensitivity of Antarctic marine invertebrates to chemical stressors.

Behavioral responses, such as avoidance or attraction, represent an early alert mechanism in organisms associated with the perception or exposure to xenobiotic substances, chemical stressors, or alarm cues (i.e., predation risk). In this context, our study aims to explore the ecological effects of penguin guano on marine invertebrate spatial behavior, representing a pilot experimental framework that integrates multiple environmentally relevant scenarios. A non-forced exposure system was employed to simulate chemically heterogeneous environments and to assess spatial avoidance behavior, in response to guano as a chemical stressor (Araújo and Blasco 2018). This system allows the evaluation of organisms' habitat selection (e.g., avoidance or attraction responses) assuming that some organisms can move voluntarily between habitats and escape from potentially hazardous environments or stress conditions (Araújo et al. 2019). Avoidance behavior experiments have been successfully applied to a wide range of marine and freshwater organisms (e.g., crustaceans and fishes), across several ecosystems worldwide, from temperate (Araújo et al. 2019; Islam et al. 2019) to tropical regions (Araújo et al. 2018; Silva et al. 2024) and involving different classes of substances such as pharmaceutical compounds, pesticides, and metals (Moreira-Santos et al. 2019), as well as predator signals (i.e., kairomones) (Araújo et al. 2020).

In this context, this study aims to assess spatial behavior in Antarctic marine invertebrates in response to penguin guano as a complex chemical input under environmentally relevant experimental scenarios. To address this objective, different experimental configurations were tested to assess whether habitat selection patterns were affected by (i) model exposure organism; (ii) the presence of guano, either as a linear gradient or in a configuration simulating a chemical barrier (patchy distribution); (iii) differences between guano derived from Gentoo and Chinstrap penguins, using different concentration ranges (0–32 g L^{-1}); (iv) light conditions (outdoor and dark); and (v) exposure times (5 and 8 h). Additionally, TM concentrations were measured in guano in order to identify chemical components potentially triggering the behavioral responses, although it cannot be ruled out that the behavioral responses of organisms may be associated with the presence of chemical compounds that indicate the presence of a potential predator.

Yet, empirical studies focused on the effect of penguin guano on several Antarctic native invertebrate behaviors have not been conducted so far. The goal was to assess behavioral responses and evaluate which experimental parameters yielded the most ecologically relevant results, providing a methodological baseline for future research. The working hypothesis is that penguin guano can influence the spatial behavior of Antarctic marine invertebrates by altering their habitat selection patterns, acting as an early warning cue indicating unfavorable environmental conditions. This study contributes both to understanding the ecological implications of guano inputs and to refining experimental approaches for evaluating environmental dynamics in polar ecosystems.

Material and methods

Penguin guano and organisms' collection

The sampling of penguin guano and organisms was performed during the Austral summer of 2021/2022 (February-March). Superficial and fresh wet guano samples were collected manually in plastic zip-lock bags from Gentoo penguin at the Argentina Cove and Hannah Point colonies, and from Chinstrap penguin at Miers Bluff colony, on Livingston Island (62°37′S, 60°12′W) and stored frozen at − 20 °C until the experiment setup and the analyses. Specimens of amphipods were collected using a beam trawl (50 × 20 cm) deployed from a zodiac (March 2022), at Johnson Bay, near the Spanish Antarctic Station (BAE Juan Carlos I) on Livingston Island (62°39′S, 60°23′W). Krill individuals were collected at night in the coastal waters of South Bay, near BAE JCI, using a shrimp trap with 5 mm mesh. The trap was deployed on a floating platform and equipped with a torch as a light source to attract krill. Before starting the experiment, the organisms were acclimated for one week in a 400-L tank, with aerated and 1 µm-filtered seawater, without feeding, at natural external conditions and photoperiod (Temperature: 2.1 ± 0.2 °C, salinity: 34‰, pH: 7.87 ± 0.05, dissolved oxygen 90.2 ± 2.8% of saturation).

Experimental design: spatial behavior assays

A linear multi-habitat, non-forced exposure system characterized by six interconnected compartments (Araújo et al. 2020) was employed to simulate two guano concentration gradient scenarios (Fig. 1). Five assays (referred to as assay #1 to assay #5), using three different species (the amphipods *C. femoratus* and O. *lahillei,* and the krill *E. superba),* were conducted to detect avoidance behavior in response to guano, by combining different guano exposure scenarios and environmental conditions. For each assay, guano samples were defrosted and weighed to prepare the treatments. With the connections between adjacent compartments closed by the plugs, guano treatments were added to the system. Subsequently, the organisms were introduced at a density of five individuals in each compartment. Then, the plugs were removed with the aid of an external clamp, to minimize disturbance to the organisms and water. Four replicates were carried out per test. No artificial light sources or food were provided during the assays. We tested a range of guano concentrations from 0 to 32 g L^{-1}. The experimental settings and

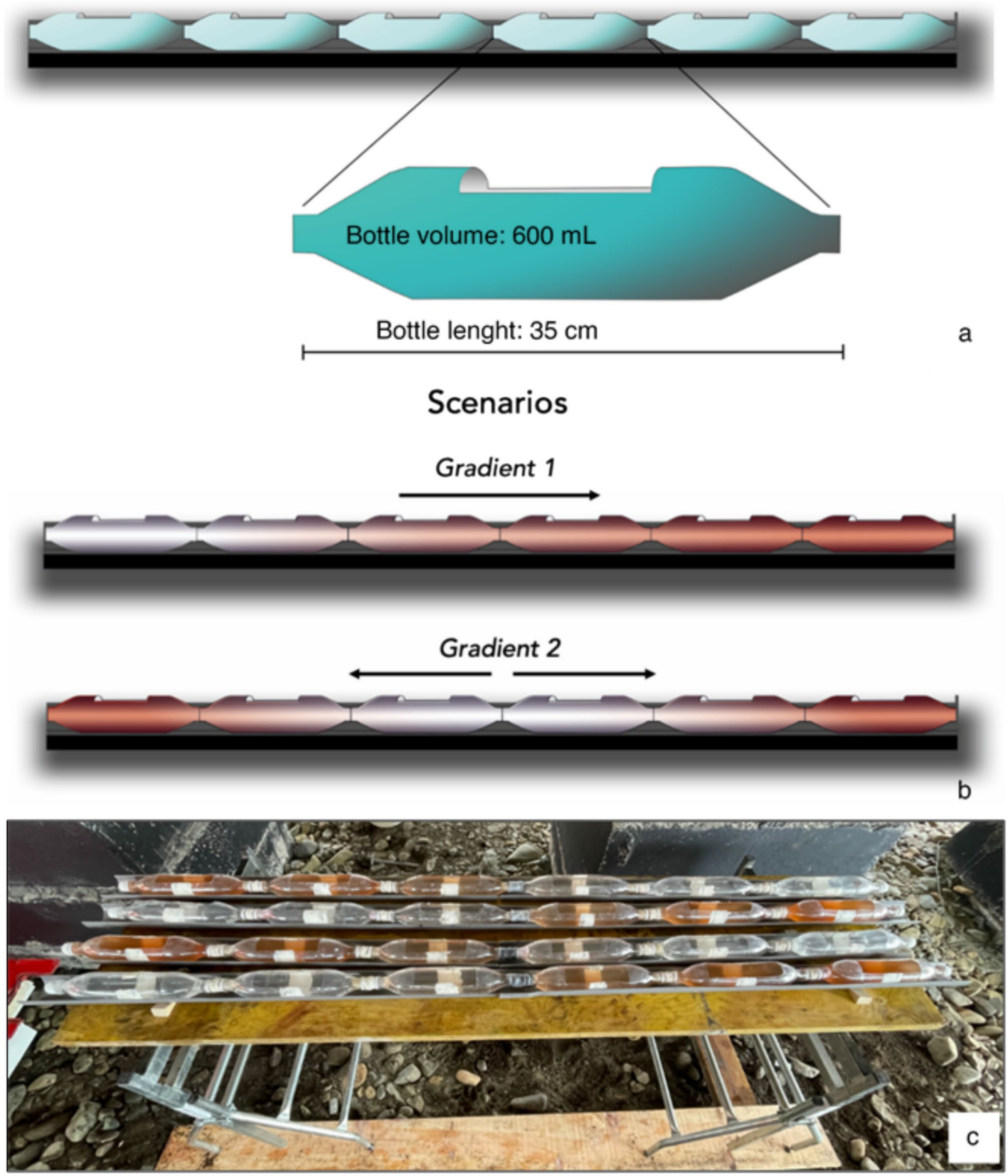

Fig. 1 Schematic representation of the free-choice, non-forced, linear multi-compartmented exposure system used in the spatial behavior assays (total length: 189 cm; total volume: 4200 mL) (**a**); the two exposure scenarios: Gradient 1, a linear guano gradient, and Gradient 2, guano as a chemical barrier (**b**); the system setup in the field (**c**)

Table 1 Summary of the experimental design, settings, and exposure conditions applied in each behavioral assay

Assay	Species	Exposure scenario	Guano concentrations (g L^{-1})	Penguin species	Light condition	Exposure time (h)
#1	*Cheirimedon femoratus*	Linear guano gradient	0–3	Gentoo	Outdoors	5
#2	*Oediceroides lahillei*	Guano as chemical barrier	32–0–32	Gentoo	Darkness	5
#3	*Euphausia superba*	Linear guano gradient	0–13	Gentoo	Darkness	5 and 8
#4	*Euphausia superba*	Linear guano gradient	0–13	Gentoo	Darkness	5
#5	*Euphausia superba*	Guano as chemical barrier	7–0–7	Chinstrap	Darkness	5

[1] Not all possible combinations of invertebrate species, penguin guano types, and exposure scenarios were tested due to the limited availability of sampled organisms. Experimental scenarios were therefore selected based on ecological relevance and logistical feasibility

exposure conditions for each assay are described in detail in the following sections and resumed in Table 1.

Assay #1

The first assay (#1) was carried out with the amphipod *Cheirimedon femoratus*. The system was placed outdoors to maintain natural temperature and light conditions. The selected scenario was the linear guano gradient, using Gentoo penguin guano, with the following concentration treatments: control (C): 0 g L^{-1}, Treatment 1 (T1): 0.1 g L^{-1}, Treatment 2 (T2): 0.4 g L^{-1}, Treatment 3 (T3):1 g L^{-1}, Treatment 4 (T4): 2 g L^{-1}, Treatment 5 (T5): 3 g L^{-1}. The experimental replicates ($n=4$) were alternated in horizontal orientation to avoid potential lateral stimuli or directional biases in organism behavior (Fig. 1C). The distribution of organisms was recorded after a 5-h exposure period.

Assay #2

Due to the limitation of collecting organisms belonging to the same species of amphipod, a second assay (#2) was conducted with the amphipod *Oediceroides lahillei*. In this case, the system was placed in a closed room next to BAE JCI and kept in complete darkness to ensure that behavioral responses were not affected by light conditions. The selected scenario was the guano as a chemical barrier, with the following guano concentration treatments: T2.1: 32 g L^{-1}, T1.1: 8 g L^{-1}, C.1: 0 g L^{-1}, C.2: 0 g L^{-1}, T1.2: 8 g L^{-1}, T2.2: 32 g L^{-1} of Gentoo penguin guano. The experimental replicates ($n=4$) were alternated to avoid potential biases in the behavior. The distribution of the organisms was recorded after a 5-h exposure period.

Assays #3, #4, and #5

Three assays (#3, #4, and #5) were carried out with krill *Euphausia superba*. In the initial setup, the experimental system was placed outdoors to maintain natural temperature and light orientation. However, due to krill's unexpected behavioral response possibly due to directional light cues, subsequent trials were performed without light to avoid potential interference. The expected behavioral response was spatial avoidance of areas with high guano concentrations; however, this pattern did not occur in the assays. For these assays, the system was placed in a closed room next to BAE JCI and kept in complete darkness to avoid external light interference. Krill behavioral responses were evaluated under both guano scenarios. For assays #3 and #4, the linear guano gradient was tested using the Gentoo guano with the following concentration treatments: C: 0 g L^{-1}, T1: 0.3 g L^{-1}, T2: 2 g L^{-1}, T3: 3 g L^{-1}, T4: 7 g L^{-1}, and T5: 13 g L^{-1}. The experimental replicates ($n=4$) were alternated in horizontal orientation to avoid potential biases in the behavior. In assay #5, guano was tested as a chemical barrier scenario, with the concentrations: T2.1: 7 g L^{-1}, T1.1: 1 g L^{-1}, C.1: 0 g L^{-1}, C.2: 0 g L^{-1}, T1.2: 1 g L^{-1}, T2.2: 7 g L^{-1}, employing Chinstrap penguin guano, as alternative chemical cues. The distribution of organisms was recorded after a 5-h exposure period, except for assay #3, where an initial count was made at 5 h, as in the other assays, and the system was left for up to 8 h to assess a potential delayed response.

Guano trace metal composition

In order to identify potential chemical drivers of responses, TM concentrations were analyzed in guano samples. The TMs (i.e., V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ag, Cd, and Pb) and the metalloid As content in guano samples were analyzed in triplicate by inductively coupled plasma mass spectrometry (ICP-MS, iCAP Thermo) after prior freeze-drying and chemical digestion, in accordance with the SW-846 EPA Method 3051A (USAEPA 1987). Briefly, 0.2 g of guano was digested with 10 mL of Suprapur quality nitric acid at 65%. Samples were diluted to 45 mL with Milli-Q water. Acid digestions were performed in Teflon vessels using a microwave system (MARS5; CEM). Blanks and Certified Reference Material for digestion and analysis were treated like the samples. The accuracy of the analytical procedure was checked using a Certified Reference Material (Lobster hepatopancreas TORT-2) (see Table S1, Supplementary Information). Based on the average TM concentration found in guano samples (μg g^{-1} dry weight) and a guano moisture content of approximately 70% (Ruiz-Gutiérrez et al. 2024), the dissolved TM input in the experimental gradient solutions was calculated considering the range of guano applied for the treatments (0.1 to 32 g L^{-1} wet weight).

Statistical analysis

Organism distributions among experimental compartments were analyzed using generalized linear mixed models (GLMMs) within a frequentist framework. Raw organism counts (rather than proportions) were modeled assuming a negative binomial error distribution to account for overdispersion, compositional constraints, and the blocked experimental design (Brooks et al. 2017).

For each assay, the response variable was the number of organisms observed (Y) in compartment i of replicate j. Models were specified with a log link function as

$$\begin{aligned} Y_{ij} &\sim \mathit{NegBin}\left(\mu_{ij}, \theta\right) \\ \log\left(\mu_{ij}\right) &= \beta_0 + \beta_1 \cdot \log\_conc\_std_{ij} + \alpha_j \\ \alpha_j &\sim \mathit{mathcalN}\left(0, \sigma_\alpha^2\right) \end{aligned}$$

where μ_{ij} is the expected count, θ is the dispersion parameter, β_0 is the population-level intercept, β_1 represents the fixed effect of guano concentration (the primary parameter of interest), and α_j is a random intercept for replicate j, assumed to be normally distributed with mean zero and variance σ^2_a. The inclusion of replicate as a random effect accounts for non-independence among compartments within each experimental unit. Guano concentration was log-transformed to reduce skewness using log("concentration" + 0.01) and then standardized within each assay. To allow comparison of effect sizes across assays with different concentration ranges, the transformed concentration was then standardized within each assay by subtracting the mean and dividing by the standard deviation. This standardization improves numerical stability and facilitates interpretation of concentration-dependent responses across experiments. In assays with barrier designs (assays #2 and #5), compartments sharing identical guano concentrations (e.g., symmetric compartments on either side of a barrier) were assigned the same predictor value. This approach naturally pools information across equivalent treatments while retaining a parsimonious, continuous test of dose-dependent behavioral responses.

Models were fitted using maximum likelihood to allow model comparison and inference using standard information criteria. We specified a negative binomial distribution with a quadratic variance function (nbinom2 in glmmTMB), where $\mathrm{Var}(Y) = \mu + \mu^2/\theta$, which adequately captured the substantial overdispersion observed in the data (variance-to-mean ratios ranging from 1.2 to 4.8 across treatments).

We assessed model adequacy using simulated residuals from the DHARMa package (Hartig 2022), which provides diagnostics that are the frequentist analog to Bayesian posterior predictive checks, ensuring the model captures key data features (overall distribution, variance structure, and zero frequency). The concentration-dependent avoidance was assessed by testing H_0:$\beta_1 = 0$ against H_A:$\beta_1 < 0$ using Wald tests from the fitted model. Evidence for avoidance was inferred when the estimated concentration effect was negative and statistically supported ($p < 0.05$). To facilitate biological interpretation, standardized effect estimates were back-transformed to the original concentration scale. Specifically, the expected multiplicative change in organism counts associated with a doubling of guano concentration was calculated as

$$\text{Effect} = \exp\left(\beta_1 \cdot \frac{\log(2)}{SD(\log\_conc)}\right).$$

For example, an effect of 0.4 indicates counts are reduced to 40% of the control level (a 60% reduction) when concentration doubles. Effect sizes are reported together with 95% confidence intervals to convey estimation. Post hoc analyses were conducted using estimated marginal means (EMMs) obtained with the emmeans package (Lenth 2023). These were used to visualize dose–response relationships, compare predicted and observed responses across concentrations, perform treatment-versus-control contrasts using Dunnett-style comparisons on the ratio scale, and explore differences between adjacent concentration levels to identify potential response thresholds. No multiplicity adjustments were applied to confidence intervals. Following recommendations for ecological and environmental studies, we focus on effect sizes and their associated uncertainty rather than dichotomous significance thresholds. Consistent patterns observed across multiple independent assays provide robust support for the biological interpretations without reliance on conservative alpha-level corrections (Rothman 1990). All statistical analyses were conducted in R version 4.3.2 using glmmTMB (v1.1.8), DHARMa (v0.4.6), emmeans (v1.10.0), and broom.mixed (v0.2.9). Figures were produced using ggplot2 (v3.4.4).

Statistical differences in TMs among the three guano samples used in the assays have been analyzed using the SPSS (V29) statistical package. After testing for normality using the Shapiro–Wilk test, significant differences were assessed by means of the nonparametric analysis of variance, the Kruskal–Wallis test. The significance level was set at $p < 0.05$.

Results

A total of five behavioral assays were carried out under different exposure scenarios and environmental conditions to test the hypothesis that penguin guano inputs can act as a chemical driver, influencing the spatial distribution and habitat selection of key Antarctic marine invertebrate species.

Amphipod spatial behavior responses

The average percentages of organisms obtained in the assays with the amphipods (#1 and #2) are shown in Fig. 2. The results showed that both amphipod species detected and significantly avoided guano under both exposure scenarios, in both light conditions. Their distribution across compartments was influenced by the presence of guano in the conditions tested in both assays. Amphipods exhibit strong concentration-dependent avoidance. Both *C. femoratus* (assay #1) and *O. lahillei* (assay #2) showed highly significant negative concentration effects ($\beta_1 = -0.89$, 95% CI: [−1.21, −0.58] and $\beta_1 = -1.15$, 95% CI: [−1.52, −0.79], respectively; both $p < 0.001$, one-tailed tests). When guano concentration doubles, expected counts in treated compartments decrease to 40–45% of baseline control levels (95% confidence intervals exclude 1.0 in both assays), representing 55–60% reductions in amphipod abundance. The magnitude of this avoidance response was remarkably consistent between the two amphipod species despite differences in body size and ecology: *C. femoratus* (body length 15–20 mm, epibenthic scavenger) and *O. lahillei* (body length 8–12 mm, tube-dwelling deposit feeder) both exhibited similarly strong negative slopes. This avoidance pattern was independent of experimental design (linear gradient in assay #1 vs. chemical barrier in assay #2) and light conditions (outdoor natural light vs. complete darkness), indicating robust chemosensory-mediated habitat selection that operates consistently across environmental contexts. Post hoc contrasts revealed that amphipod avoidance was evident at every concentration tested. In *C. femoratus*, count ratios relative to control ranged from 0.58 (95% CI: [0.41, 0.82]) at 0.1 g L^{-1} to 0.18 (95% CI: [0.11, 0.28]) at 3 g L^{-1}, with all five treatment levels significantly different from control (all $p < 0.01$). In *O. lahillei*, the barrier design produced even more extreme responses: count ratios of 0.06 (95% CI: [0.03, 0.13]) at 8 g L^{-1} and 0.03 (95% CI: [0.01, 0.07]) at 32 g L^{-1}, indicating near-complete exclusion from high-concentration compartments. Across both species, 80–95% of individuals were recovered from control compartments (0 g L^{-1}), demonstrating that amphipods can detect and actively avoid even subtle guano cues across a broad concentration range.

Krill spatial behavior responses

In contrast, *E. superba* showed no significant concentration effects across four independent assays (#3–5 h, #3–8 h, #4, #5; all $p > 0.05$, one-tailed tests). Rather than responding to chemical gradients, krill exhibited consistent aggregation at system extremities (compartments 1 and 6), with 60–70% of organisms at edges regardless of guano concentration or compartment position (Fig. 3). This spatial pattern was reproducible across independent trials and persisted even when high-concentration compartments (13 g L^{-1} in assays #3–4, 7 g L^{-1} in assay #5) occupied edge positions. For example, in assay #3–5 h, mean counts at edges were 10.3 ± 2.1 (compartment 1) and 9.8 ± 1.9 (compartment 6),

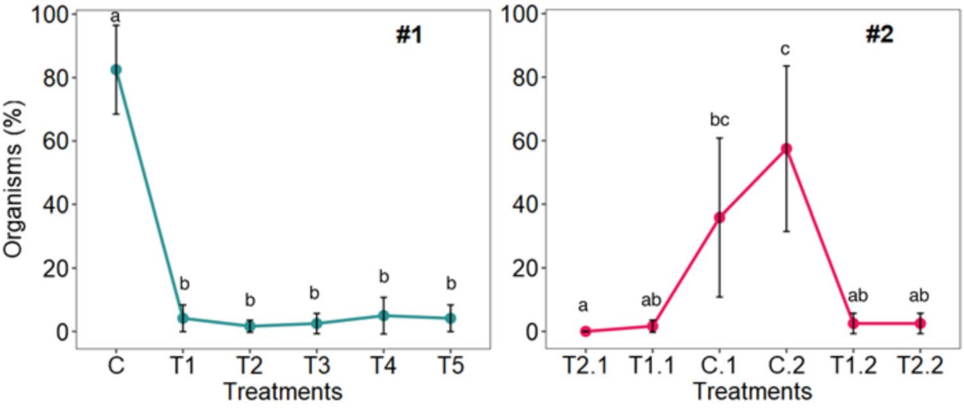

Fig. 2 Mean (and standard deviations) percentages of organisms counted in the different compartments after 5 h exposure to guano concentration gradients; assays #1 with *Cheirimedon femoratus* and #2 with *Oediceroides lahillei*. The different colors indicate the two different gradient scenarios and light conditions, #1 in green (C:0, T1: 0.1, T2: 0.4, T3:1, T4:2, T5:3 g L^{-1}; outdoors) and #2 in red (T2.1: 32, T1.1: 8, C.1: 0, C.2: 0, T1.2: 8, T2.2: 32 g L^{-1}; darkness). Statistically significant differences ($p < 0.05$) among treatments are represented by different letters

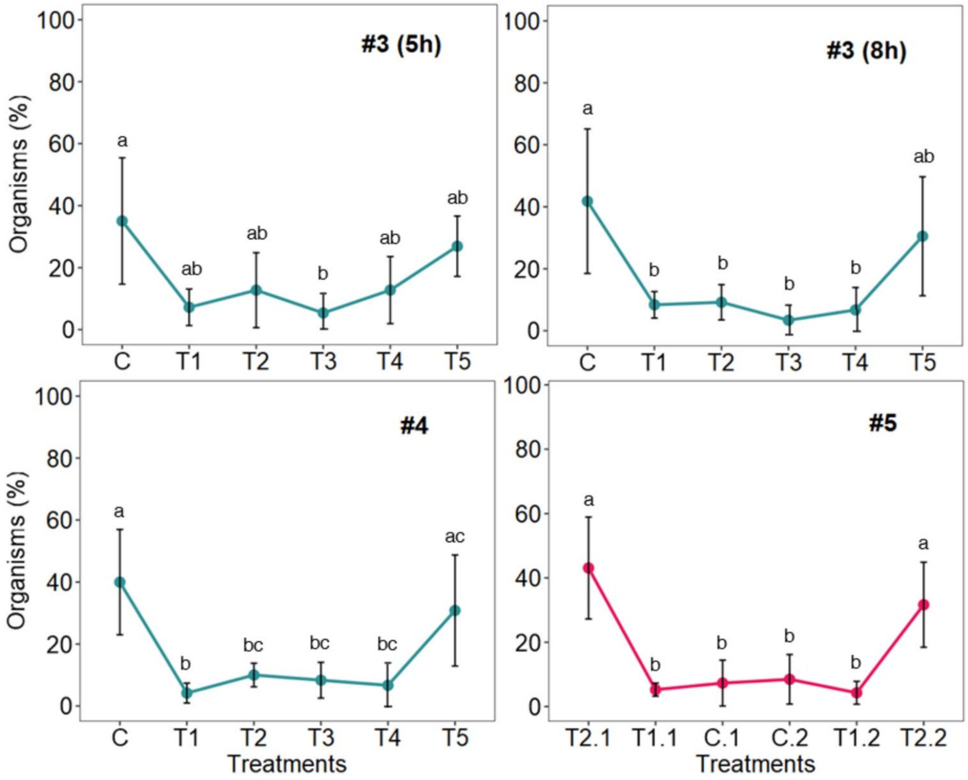

Fig. 3 Mean (and standard deviations) percentages of organisms (krill) counted in the different compartments after 5 and 8 h of exposure to guano concentration gradients (assays #3, #4 and #5), all conducted in darkness. The different colors indicate the two different gradient scenarios: #3 and #4 in green (C: 0, T1: 0.3, T2: 2, T3: 3, T4: 7, T5: 13 g L^{-1}) and #5 in red (T2.1: 7, T1.1: 1, C.1: 0, C.2: 0, T1.2: 1, T2.2: 7 g L^{-1}). Statistically significant differences (p < 0.05) among treatments are represented by different letters

compared to interior compartments ranging from 3.2 to 5.8 (all $p<0.01$ for edge vs. interior comparisons). This edge preference did not vary with concentration (concentration × position interaction: $F_{5,118}=0.83$, $p=0.53$). Concentration effect estimates ranged from $\beta_1=-0.12$ to $+0.18$, with 95% confidence intervals spanning zero in all cases, indicating that the data are consistent with no systematic relationship between guano concentration and krill counts. This null result was highly reproducible: assay #4, conducted as an independent replicate of assay #3–5 h, yielded $\beta_1=+0.12$ (95% CI: [−0.20, +0.44], $p=0.46$), confirming that the absence of avoidance is a real biological pattern rather than a statistical artifact of low power. Krill behavioral response remained invariant across multiple experimental test conditions. Extending exposure time from 5 to 8 h (assays #3–5 h vs. #3–8 h) produced no change in spatial distribution ($\beta_1=-0.06$ vs. −0.04; difference $p=0.89$). Both linear gradient (assays #3, #4) and chemical barrier (assay #5) configurations yielded flat dose–response curves, ruling out the possibility that krill avoid only extreme concentration contrasts. Also substituting Chinstrap penguin guano for Gentoo guano (assay #5 vs. #3/#4) had no effect on spatial behavior ($\beta_1=+0.18$ vs. −0.06; difference $p=0.31$). Treatment vs. control contrasts confirmed the absence of systematic patterns: across all krill assays, count ratios ranged from 0.7 to 1.4 (all 95% CIs spanning 1.0), with no concentration showing consistent deviation from control levels.

Guano trace metal concentrations

Trace metal concentrations (μg g^{-1} dry weight) and recoveries (%) for the Certified Reference Material (Lobster hepatopancreas TORT-2), and analytical detection limits (DL) are shown in Table S1 (Supplementary Information). The levels of TMs (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb) and the metalloid As in guano samples used for the assays are indicated in Table 2. Overall, Fe (943–1405 μg g^{-1} dw), Cu (137–237 μg g^{-1} dw), and Zn (166–250 μg g^{-1} dw) were the TMs with the highest average concentrations, followed by the other TMs, following this stoichiometry: Fe > Zn > Cu > Ag > Mn > As > V > Ni > Mo > Cd > Pb > Cr > Co. Significant differences were found in TM levels (e.g., Cu, Zn, Mo, Ag, Cd, and Pb) among guano samples used for the assays. The estimated TM inputs derived by guano gradient are summarized in Table S3 (Supplementary Information).

Discussion

Penguin guano is a complex mixture that influences Antarctic terrestrial and marine ecosystems, serving as a source of nutrients (nitrogen, phosphorus, and essential TM) (Shatova et al. 2016; Wing et al. 2017; Belyaev et al. 2023), as well as acting as a chemical stressor. Once it reaches seawaters, guano can dissipate as a plume along a dilution gradient or be distributed in patches, leading to chemical barriers (Araújo et al. 2016).

These distribution patterns were simulated using a linear, non-forced, multi-compartment exposure system to assess spatial avoidance behavior in native Antarctic marine invertebrates. This exploratory study assessed the behavioral responses of two amphipod species (*C. femoratus* and *O. lahillei*) and the Antarctic krill (*E. superba*), under two scenarios considering guano as a potential factor affecting habitat selection. While previous ecotoxicological experiments have been conducted with Antarctic organisms (Duquesne et al. 2000; King and Riddle 2001; Sfiligoj et al. 2015), the present approach, using a non-forced exposure system, provides a complementary perspective by allowing organisms to freely redistribute in response to heterogeneous chemical conditions. Despite logistical constraints, this design offers insight into how individuals may respond to guano inputs under conditions that better approximate the natural environment (Kawaguchi et al. 2010). Furthermore, our findings highlight the importance of identifying suitable model organisms, as behavioral sensitivity and response patterns differed markedly among taxa. This approach contributes to a better understanding of ecological dynamics, thereby enhancing the assessment of ecological responses to stress conditions related to chemical cues, in this unique and sensitive ecosystem.

Amphipods

Assays #1 and #2 showed that guano exposure significantly affected amphipod habitat selection dynamics. After 5 h of exposure, individuals fled from guano and preferred the clean compartments, in both scenarios, regardless of the guano concentrations used and the light conditions. The wide range of TM concentrations used among guano treatments suggests that avoidance was triggered in amphipods independently of the TM levels present in the treatments. All tested guano concentrations, from 0.1 to 32 g L^{-1}, were enough to trigger amphipod avoidance responses. This prevents us from establishing a direct link between guano TM levels and habitat selection. This response probably followed an all-or-nothing pattern, potentially influenced by the capacity of the organisms to detect an adverse environment, or by the perception of guano as a predator cue. However, the bioavailability of guano-derived TMs depends on several factors, such as complexation, adsorption, and high organic matter content, which can reduce the toxicity of free metal ions (Gutiérrez et al. 2023; García-Veira et al. 2024). Organic ligands from krill digestion and phytoplankton exudates contribute to this mitigating effect (Rial et al. 2016). A similar absence of direct correlation between preference behavior and contamination levels was previously reported in habitat selection studies with *Danio rerio* in two Ecuadorian rivers, which was related to the non-linear distribution of contamination in the river (Araújo et al. 2018).

Several studies employing non-forced, multi-compartment exposure systems have highlighted that stressors related to TMs, particularly copper (Cu), can induce spatial avoidance behavior, in aquatic organisms. High Cu concentrations limited population connectivity in the estuarine shrimp *Palaemon varians* (Salvatierra et al. 2022), while Cu gradients reduced habitat colonization in *Danio rerio* despite food availability (Islam et al. 2019). Similarly, exposure to high concentrations of metal mixtures induced avoidance responses in *Daphnia magna* (González et al. 2023). Although we have attempted to explain avoidance by the presence of TMs, the observed behavioral responses could be related to the perception of guano-derived chemical cues, including potential predator-risk related metabolites, rather than with stress conditions. Such responses could be mediated by waste products or other metabolites present in guano (known as kairomones) (Poulin et al. 2018). For example, uric acid, the main component of seabird guano, could also contribute to the observed repellency response in amphipods (Benoit et al. 2008), highlighting the role of predator cues in shaping spatial behavior of invertebrate organisms. In this context, predation risk represents a driver of crustacean populations, influencing both their dynamics and

Table 2 Trace metal concentrations (μg g^{-1} dry weight) in Gentoo (*Pygoscelis papua*) and Chinstrap (*Pygoscelis antarcticus*) penguin guano ($n = 3$), collected on Livingston Island in the Austral summer 2021/2022 (February-March), used in five (#1 - #5) spatial behavior assays

Assay	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Mo	Ag	Cd	Pb
#1 (Gentoo)	4.2	0.8	36.3	1126	0.6	1.7	137.4^{a}	166^{a}	9.9^{a}	1.6^{a}	28.2^{a}	0.85^{a}	0.99^{a}
SD	1.1	0.2	6.1	331	0.1	0.1	8.2	11	0.6	0.1	2.4	0.04	0.06
#2, #3, #4 (Gentoo)	4.9	0.9	51	1405	0.7	2.0	237.3^{b}	250.1^{b}	7.9^{b}	1.90^{b}	55.1^{b}	1.5^{b}	0.99^{a}
SD	2.2	0.2	13	595	0.2	0.2	7.8	0.2	0.2	0.04	0.2	0.1	0.05
#5 (Chinstrap)	3.4	0.73	31.5	943	0.58	1.8	216.1^{c}	201.9^{c}	9.9^{a}	1.5^{a}	45.2^{c}	1.4^{b}	1.2^{b}
SD	0.4	0.04	1.7	116	0.04	0.1	2.6	5.3	0.2	0.1	1.7	0.1	0.1
Average	4.2	0.8	40	1158	0.6	1.8	197	206	9.3	1.7	43	1.3	1.1
SD	0.7	0.1	10	233	0.1	0.2	53	42	1.2	0.2	14	0.4	0.1

Different lowercase letters indicate significant differences among guano sample trace metal concentrations ($p < 0.05$). Gentoo penguin guano used in assay #1 was collected from the Argentina Cove colony; Gentoo guano used in assays #2, #3, and #4 derived from the Hannah Point colony; and Chinstrap guano used in assay #5 was obtained from the Miers Bluff colony spatial distribution patterns (Araújo et al. 2020). According to this, previous studies have demonstrated that water-borne predator-related risk cues, including excretory products, can strongly influence prey behavior, physiology, and reproduction (Bell et al. 2019). For example, such chemically mediated signals induced stress responses that led to a decreased consumption of oysters by mud crabs (Weissburg and Beauvais 2015; Weissburg et al. 2016).

Amphipods thus represent suitable model organisms for investigating ecological processes in Antarctic benthic communities, as their behavior can reflect changes in environmental quality and chemical stimuli. In particular, previous studies used *C. femoratus* in several feeding repellency and palatability bioassays with bryozoan chemical defense compounds (Núñez-Pons et al. 2012; Figuerola et al. 2013). Their results demonstrated that *C. femoratus* was sensitive to chemical cues, showing an avoidance response to bryozoan defensive compounds, in accordance with our results. In a recent study by Jermacz et al. (2020), two species of temperate amphipods exposed to short-term predator cues showed an increased respiration rate and antioxidant defense as a response to stress. Behavioral responses are among the earliest endpoints for organisms associated with exposure to perceptible xenobiotic substances or chemical stressors. The spatial displacement in response to contamination or chemical stimuli is of great ecological concern, as it can lead to the reduction of habitable areas, alter migratory patterns and compromise community stability (Araújo and Blasco 2018 and the references therein). Moreover, the repellent effect of a guano gradient could lead to a loss of local amphipod populations, and the patchy distribution could cause the isolation of the populations, or decrease the connectivity among populations. This may increase the susceptibility of the displaced population. Moreover, shifts in amphipod spatial distribution in Antarctic coastal ecosystems may have cascading effects through the food web and ecosystem functioning. As a key contributor to nutrient cycling, organic matter processing, and energy transfer, localized declines in amphipod populations or redistribution could alter key biogeochemical processes (e.g., carbon export, sediment mixing, and oxygenation) (Kürzel et al. 2025). Acting as a food source for invertebrate and upper trophic levels, the spatial arrangement of amphipods in alternative areas may enhance prey availability for higher trophic levels, potentially modifying predator populations and trophic and competitive interactions among species in a relatively simple food web dominated by a few key taxa and life cycles tightly constrained by seasonality (Convey and Peck 2019; Jermacz et al. 2020; Rodriguez et al. 2022; Minutoli et al. 2025).

Krill

Unlike amphipods, Antarctic krill did not show avoidance behavior in response to guano exposure. Instead, a consistent spatial pattern was observed under all test conditions, as individuals tended to aggregate at the system extremities across all treatments, guano scenarios, and exposure times. This pattern indicates that, under the conditions tested, guano-derived chemical cues did not override endogenous or ecological drivers of krill spatial behavior under the tested conditions. The observed pattern suggests that the tendency to seek shelter or stability at the edges of the system prevailed over any possible effects of guano-derived chemical cues.

Antarctic krill exhibit complex social and spatial dynamics driven by schooling behavior, environmental cues, and habitat associations (Hamner and Hamner 2000; Nicol 2003; Young et al. 2024). Their tendency to aggregate may reflect shelter-seeking behavior or internal synchronization rather than responses to external chemical inputs. Krill behavior is also known to be shaped by environmental cues, such as light, current, and food availability (Kawaguchi et al. 2010; Weissburg et al. 2019). In natural settings, the close association between krill and sea ice, which provides food and protection, may further explain this aggregation pattern (Meyer et al. 2017).

A recent study by Hellessey et al. (2025) found that krill can detect guano at a concentration of 0.1 $\mu g\ L^{-1}$ under forced exposure, showing increased swimming speed, and reduced feeding ingestion rates. The contrast with the present findings likely reflects differences in experimental design and exposure regime. Forced systems with few individuals can amplify stress responses and limit movement, whereas non-forced systems allow organisms to choose the most suitable environment (Araújo and Blasco 2018). The persistence of aggregation at the system extremities in trials, regardless of treatment or light conditions, indicates a spatial behavior driven mainly by endogenous or ecological factors (e.g., synchronized movements, internal rhythms and shelter-seeking) rather than guano-derived cues. However, we cannot rule out that it may partly reflect constraints imposed by the experimental system, rather than a response to guano-derived chemical cues. In fact, trials conducted in darkness, both with and without guano, confirmed that this pattern persisted, suggesting that light and guano were not primary drivers of the response. This highlights species-specific differences in behavioral responsiveness and sensitivity to chemical stressors and underscores the importance of matching experimental design to species-specific dynamic patterns and ecology when assessing chemically mediated stress responses. To address uncertainties regarding krill responses to guano-derived chemical stress, future studies should employ new alternative experimental non-forced systems (e.g., the Heterogeneous Multi-Habitat Assay System, HeMHAS, from Salvatierra et al. 2022). Such approaches would allow a more reliable evaluation of krill sensitivity while minimizing artifacts related to confinement and spatial restrictions.

Overall, our results indicate that penguin guano can influence the spatial distribution of Antarctic amphipods, while krill responses appear dominated by species-specific behavioral traits. These findings support the use of non-forced exposure systems to investigate chemically mediated spatial behavior in Antarctic marine invertebrates. Penguin guano, as a naturally occurring and chemically complex input, provides a valuable natural model to investigate how enrichment of organic matter and TMs with ecological cues interacts to shape habitat selection, connectivity, and community dynamics in Antarctic nearshore ecosystems (Otero et al. 2018; Ratnarajah et al. 2018; Belyaev et al. 2023; García-Veira et al. 2024). Similar studies should be conducted on more diverse taxa using non-forced exposure approaches to assess habitat selection in marine invertebrates under realistic conditions. Understanding how these species respond to penguin guano helps explain how coastal food webs function, as changes in species distribution, can affect nutrient cycling and community structure.

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Author contributions ES, EGO, ARR and ATS: conceptualization, planning and designing of the research. ES, EGO, FB and ATS: field and laboratory work. ES: chemical and data analyses, together with ARR. ES: drafted the manuscript with contributions from EGO, FB, ARR and ATS. ATS: investigation, visualization and funding acquisition. All the authors read, commented and approved this manuscript.

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Competing interests The authors declare no competing interests.

References

Araújo CVM, Blasco J (2018) Spatial avoidance as a response to contamination by aquatic organisms in nonforced, multicompartmented exposure systems: a complementary approach to the behavioral response. Environ Toxicol Chem 38(2):312–320. https://doi.org/10.1002/etc.4310

Araújo CVM, Moreira-Santos M, Ribeiro R (2016) Active and passive spatial avoidance by aquatic organisms from environmental stressors: a complementary perspective and a critical review. Environ Int 92–93:405–415. https://doi.org/10.1016/j.envint.2016.04.031

Araújo CVM, Griffith DM, Vera-Vera V, Jentzsch PV, Cervera L, Nieto-Ariza B, Salvatierra D, Erazo S, Jaramillo R, Ramos LA, Moreira-Santos M, Ribeiro R (2018) A novel approach to assessing environmental disturbance based on habitat selection by zebra fish as a model organism. Sci Total Environ 619–620:906–915. https://doi.org/10.1016/j.scitotenv.2017.11.170

Araújo CVM, González-Ortegón E, Pintado-Herrera MG, Biel-Maeso M, Lara-Martín PA, Tovar-Sánchez A, Blasco J (2019) Disturbance of ecological habitat distribution driven by a chemical barrier of domestic and agricultural discharges: an experimental approach to test habitat fragmentation. Sci Total Environ 651:2820–2829. https://doi.org/10.1016/j.scitotenv.2018.10.200

Araújo CVM, Pereira KC, Sparaventi E, González-Ortegón E, Blasco J (2020) Contamination may induce behavioural plasticity in the habitat selection by shrimps: a cost-benefits balance involving contamination, shelter and predation. Environ Pollut. https://doi.org/10.1016/j.envpol.2020.114545

Bell ATC, Murray DL, Prater C, Frost PC (2019) Fear and food: effects of predator-derived chemical cues and stoichiometric food quality on *Daphnia*. Limnol Oceanogr 64(4):1706–1715. https://doi.org/10.1002/lno.11145

Belyaev O, Sparaventi E, Navarro G, Rodríguez-Romero A, Tovar-Sánchez A (2023) The contribution of penguin guano to the Southern Ocean iron pool. Nat Commun 14(1):1781. https://doi.org/10.1038/s41467-023-37132-5

Benoit JB, Lopez-Martinez G, Philips SA, Elnitsky MA, Yoder JA, Lee RE, Denlinger DL (2008) The seabird tick, *Ixodes uriae*, uses uric acid in penguin guano as a kairomone and guanine in tick feces as an assembly pheromone on the Antarctic Peninsula. Polar Biol 31(12):1445. https://doi.org/10.1007/s00300-008-0485-1

Borboroglu P, Boersma PD (2013) Penguins: natural history and conservation

Bregazzi PK (1972) Habitat selection of *Cheirimedon femoratus* (Pfeffer) and *Thryphosella kergueleni* (Miers) (Crustacea: Amphipoda). In: British Antarctic Survey Bulletin, vol 31. Br Antarct Surv Bull, pp 21–31

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9(2):378–400

Cavan EL, Belcher A, Atkinson A, Hill SL, Kawaguchi S, McCormack S, Meyer B, Nicol S, Ratnarajah L, Schmidt K, Steinberg DK, Tarling GA, Boyd PW (2019) The importance of Antarctic krill in biogeochemical cycles. Nat Commun 10(1):1–13. https://doi.org/10.1038/s41467-019-12668-7

Convey P, Peck LS (2019) Antarctic environmental change and biological responses. Sci Adv 5(11):eaaz0888. https://doi.org/10.1126/sciadv.aaz0888

Dauby P, Scailteur Y, Chapelle G, De Broyer C (2001) Potential impact of the main benthic amphipods on the eastern Weddell Sea shelf ecosystem (Antarctica). Polar Biol 24(9):657–662. https://doi.org/10.1007/s003000100265

De Broyer C, Lowry JK, Jazdzewski K, Robert H (2007) Catalogue of the Gammaridean and Corophiidean Amphipoda (Crustacea) of the Southern Ocean with distribution and ecological data. Bulletin De L'institut Royal des Sciences Naturelles De Belgique, Biologie 1(77):1–325 ((**In the) Census of Antarctic Marine Life: Synopsis of the Amphipoda of the Southern Ocean**)

De La Peña-Lastra S (2021) Seabird droppings: effects on a global and local level. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.142148

Duquesne S, Riddle M, Schulz R, Liess M (2000) Effects of contaminants in the Antarctic environment—potential of the gammarid amphipod crustacean *Paramorea walkeri* as a biological indicator for Antarctic ecosystems based on toxicity and bioacccumulation of copper and cadmium. Aquat Toxicol 49(1–2):131–143. https://doi.org/10.1016/S0166-445X(99)00067-3

Ellis JC (2005) Marine birds on land: a review of plant biomass, species richness, and community composition in seabird colonies. Plant Ecol 181:227–241. https://doi.org/10.1007/s11258-005-7147-y

Espejo W, Celis JE, Gonzalez-Acuña D, Banegas A, Barra R, Chiang G (2017) A global overview of exposure levels and biological effects of trace elements in penguins. Rev Environ Contam Toxicol 245:1–64. https://doi.org/10.1007/398_2017_5

Figuerola B, Núñez-Pons L, Moles J, Avila C (2013) Feeding repellence in Antarctic bryozoans. Naturwissenschaften 100(11):1069–1081. https://doi.org/10.1007/s00114-013-1112-8

García-Veira D, Sukekava CF, Sparaventi E, Navarro G, Huertas IE, Tovar-Sánchez A, Laglera LM (2024) A first estimation of the role of penguin guano on copper cycling and organic speciation in Antarctic coastal waters. Sci Total Environ 912:169266. https://doi.org/10.1016/j.scitotenv.2023.169266

González MP, Cordero-de-Castro A, Salvatierra D, Kholssi R, Fernandes MN, Blasco J, Araújo CVM, Pereira CDS (2023) Multi-level biological responses of *Daphnia magna* exposed to settleable atmospheric particulate matter from metallurgical industries. Aquat Toxicol 263:106692. https://doi.org/10.1016/j.aquatox.2023.106692

Gutiérrez GR, Sparaventi E, Corta BG, Tovar-Sánchez A, Viguri Fuente JR (2023) Penguin guano trace metals release to Antarctic waters: a kinetic modelling. Sci Total Environ 902:166448. https://doi.org/10.1016/j.scitotenv.2023.166448

Gutt J (2001) On the direct impact of ice on marine benthic communities, a review. Polar Biol 24(8):553–564. https://doi.org/10.1007/s003000100262

Hamner WM, Hamner PP (2000) Behavior of Antarctic krill (*Euphausia superba*): schooling, foraging, and antipredatory behavior. Can J Fish Aquat Sci 57(3):192–202. https://doi.org/10.1139/f00-195

Hartig F (2022) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.6. https://CRAN.R-project.org/package=DHARMa

Hay ME (2009) Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. Annu Rev Mar Sci 1(1):193–212. https://doi.org/10.1146/annurev.marine.010908.163708

Hellessey N, Weissburg M, Fields DM (2025) Penguin guano suppresses the grazing rate and modifies swimming behavior in Antarctic Krill (*Euphausia superba*). Front Mar Sci 12:1508287. https://doi.org/10.3389/fmars.2025.1508287

Henley SF, Cavan EL, Fawcett SE, Kerr R, Monteiro T, Sherrell RM, Bowie AR, Boyd PW, Barnes DKA, Schloss IR, Marshall T, Flynn R, Smith S (2020) Changing biogeochemistry of the Southern Ocean and its ecosystem implications. Front Mar Sci. https://doi.org/10.3389/fmars.2020.00581

Hughes R (2012) Antarctic ecosystems an extreme environment in a changing world (Vol. 53).

Islam MA, Blasco J, Araújo CVM (2019) Spatial avoidance, inhibition of recolonization and population isolation in zebrafish (*Danio rerio*) caused by copper exposure under a non-forced approach. Sci Total Environ 653:504–511. https://doi.org/10.1016/j.scitotenv.2018.10.375

Jermacz Ł, Nowakowska A, Kletkiewicz H, Kobak J (2020) Experimental evidence for the adaptive response of aquatic invertebrates to chronic predation risk. Oecologia 192(2):341–350. https://doi.org/10.1007/s00442-020-04594-z

Kawaguchi S, King R, Meijers R, Osborn JE, Swadling KM, Ritz DA, Nicol S (2010) An experimental aquarium for observing the schooling behaviour of Antarctic krill (*Euphausia superba*). Deep Sea Res Part II Top Stud Oceanogr 57(7–8):683–692. https://doi.org/10.1016/j.dsr2.2009.10.017

King CK, Riddle MJ (2001) Effects of metal contaminants on the development of the common Antarctic sea urchin *Sterechinus neumayeri* and comparisons of sensitivity with tropical and temperate echinoids. Mar Ecol Prog Ser 215:143–154. https://doi.org/10.3354/meps215143

Kürzel K, Hammock CP, Pitusi V, Brix S, Lörz AN (2025) Species distribution modelling of benthic amphipod crustaceans in the deep North Atlantic under climate change. Sci Rep 15(1):39581. https://doi.org/10.1038/s41598-025-26442-x

Lenth RV (2023) emmeans: estimated marginal means, aka least-squares means. R package version 1.10.0. https://CRAN.R-project.org/package=emmeans

Meyer B, Freier U, Grimm V, Groeneveld J, Hunt BPV, Kerwath S, King R, Klaas C, Pakhomov E, Meiners KM, Melbourne-Thomas J, Murphy EJ, Thorpe SE, Stammerjohn S, Wolf-Gladrow D, Auerswald L, Götz A, Halbach L, Jarman S, Yilmaz NI (2017) The winter pack-ice zone provides a sheltered but food-poor habitat for larval Antarctic krill. Nat. Ecol. Evol. 1(12):1853–1861. https://doi.org/10.1038/s41559-017-0368-3

Minutoli R, Guglielmo L, Bonanno A, Genovese S, Ferreri R, Di Paola D, Grillo M, Guglielmo Y, Swadling KM, Granata A, Aronica S (2025) Diversity and spatial distribution of pelagic amphipods in the Western Ross Sea and the Pacific sector of the Southern Ocean. Front Mar Sci 12:1633246. https://doi.org/10.3389/fmars.2025.1633246

Moreira-Santos M, Ribeiro R, Araújo CVM (2019) What if aquatic animals move away from pesticide-contaminated habitats before suffering adverse physiological effects? A critical review. Crit Rev Environ Sci Technol 49(11):989–1025. https://doi.org/10.1080/10643389.2018.1564507

Nicol S (2003) Krill and currents-physical and biological interactions influencing the distribution of *Euphausia superba*. Ocean Polar Res 25(4):633–644. https://doi.org/10.4217/OPR.2003.25.4.633

Núñez-Pons L, Rodríguez-Arias M, Gómez-Garreta A, Ribera-Siguán A, Avila C (2012) Feeding deterrency in Antarctic marine organisms: bioassays with the omnivore amphipod *Cheirimedon femoratus*. Mar Ecol Prog Ser 462(Gutt 2000):163–174. https://doi.org/10.3354/meps09840

Otero XL, De La Peña-Lastra S, Pérez-Alberti A, Ferreira TO, Huerta-Diaz MA (2018) Seabird colonies as important global drivers in the nitrogen and phosphorus cycles. Nat Commun. https://doi.org/10.1038/s41467-017-02446-8

Poulin RX, Lavoie S, Siegel K, Gaul DA, Weissburg MJ, Kubanek J (2018) Chemical encoding of risk perception and predator detection among estuarine invertebrates. Proc Natl Acad Sci U S A 115(4):662–667. https://doi.org/10.1073/pnas.1713901115

Ratnarajah L, Nicol S, Bowie AR (2018) Pelagic iron recycling in the Southern Ocean: Exploring the contribution of marine animals. Front Mar Sci 5:1–9. https://doi.org/10.3389/fmars.2018.00109

Rial D, Santos-Echeandía J, Álvarez-Salgado XA, Jordi A, Tovar-Sánchez A, Bellas J (2016) Toxicity of seabird guano to sea urchin embryos and interaction with Cu and Pb. Chemosphere 145:384–393. https://doi.org/10.1016/j.chemosphere.2015.11.064

Rodriguez ID, Marina TI, Schloss IR, Saravia LA (2022) Marine food webs are more complex but less stable in sub-Antarctic (Beagle Channel, Argentina) than in Antarctic (Potter Cove, Antarctic Peninsula) regions. Mar Environ Res 174:105561. https://doi.org/10.1016/j.marenvres.2022.105561

Rothman KJ (1990) No adjustments are needed for multiple comparisons. Epidemiology 1(1):43–46

Ruiz-Gutiérrez G, Sparaventi E, Corta BG, Tovar-Sánchez A, Viguri Fuente JR (2024) Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater. Sci Total Environ 948:174684. https://doi.org/10.1016/j.scitotenv.2024.174684

Salvatierra D, Rodríguez-Ruiz Á, Cordero A, López-Doval J, Baldó F, Blasco J, Araújo CVM (2022) Experimental evidence of contamination driven shrimp population dynamics: susceptibility of populations to spatial isolation. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2022.153225

Sfiligoj BJ, King CK, Candy SG, Mondon JA (2015) Determining the sensitivity of the Antarctic amphipod *Orchomenella pinguides* to metals using a joint model of survival response to exposure concentration and duration. Ecotoxicology 24(9):583–594. https://doi.org/10.1007/s10646-014-1406-4

Shatova O, Wing SR, Gault-Ringold M, Wing L, Hoffmann LJ (2016) Seabird guano enhances phytoplankton production in the Southern Ocean. J Exp Mar Biol Ecol 483:74–87. https://doi.org/10.1016/j.jembe.2016.07.004

Shatova OA, Wing SR, Hoffmann LJ, Wing LC, Gault-Ringold M (2017) Phytoplankton community structure is influenced by seabird guano enrichment in the Southern Ocean. Estuar Coast Shelf Sci 191:125–135. https://doi.org/10.1016/j.ecss.2017.04.021

Silva DCVR, Silva GC, Siqueira-Silva DH, Bazzan T, Silva HTL, Silva MCR, Bérgamo AL, Freitas JVR, Porfírio AEP, Pelegrini GH, Araújo CVM (2024) A multi-evidence approach in an Amazonian river based on land use, water quality, histopathological effects and habitat selection behavior in fish. Chemosphere 361:142492. https://doi.org/10.1016/j.chemosphere.2024.142492

Smichowski P, Vodopivez C, Muñoz-Olivas R, María Gutierrez A (2006) Monitoring trace elements in selected organs of Antarctic penguin (*Pygoscelis adeliae*) by plasma-based techniques. Microchem J 82(1):1–7. https://doi.org/10.1016/j.microc.2005.04.001

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. https://doi.org/10.1016/j.chemosphere.2021.131423

Tovar-Sánchez A, Duarte CM, Hernández-León S, Sañudo-Wilhelmy SA (2009) Impact of submarine hydrothermal vents on the metal composition of krill and its excretion products. Mar Chem 113(1–2):129–136. https://doi.org/10.1016/j.marchem.2009.01.010

USAEPA (1987) Epa_An overview of sediment quality in the United.pdf

Weissburg M, Beauvais J (2015) The smell of success: the amount of prey consumed by predators determines the strength and range of cascading non-consumptive effects. PeerJ 3:e1426. https://doi.org/10.7717/peerj.1426

Weissburg M, Poulin RX, Kubanek J (2016) You are what you eat: a metabolomics approach to understanding prey responses to diet-dependent chemical cues released by predators. J Chem Ecol 42(10):1037–1046. https://doi.org/10.1007/s10886-016-0771-2

Weissburg MJ, Yen J, Fields DM (2019) Phytoplankton odor modifies the response of *Euphausia superba* to flow. Polar Biol 42(3):509–516. https://doi.org/10.1007/s00300-018-02440-w

Wing SR, Wing LC, Shatova OA, Van Hale R (2017) Marine micronutrient vectors: seabirds, marine mammals and fishes egest high concentrations of bioactive metals in the subantarctic island ecosystem. Mar Ecol Prog Ser 563:13–23. https://doi.org/10.3354/meps11978

Xavier JC, Trathan PN, Ceia FR, Tarling GA, Adlard S, Fox D, Edwards EWJ, Vieira RP, Medeiros R, Broyer CD, Cherel Y (2017) Sexual and individual foraging segregation in Gentoo penguins *Pygoscelis papua* from the Southern Ocean during an abnormal winter. PLoS ONE 12(3):e0174850. https://doi.org/10.1371/journal.pone.0174850

Young EF, Thorpe SE, Renner AHH, Murphy EJ (2024) Environmental and behavioural drivers of Antarctic krill distribution at the South Orkney Islands: a regional perspective. J Mar Syst 241:103920. https://doi.org/10.1016/j.jmarsys.2023.103920

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