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# Early-life exposure to plastics is associated with health parameters and biomarkers in southern giant petrels

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ABSTRACT

The sub-lethal effects of plastics and their associated chemicals on wildlife are poorly understood, primarily due to the lack of reliable indicators. We used southern giant petrels (*Macronectes giganteus,* SGP), key scavengers and predators in southern seas, to assess whether early-life exposure to plastics was associated with health parameters. We examined relationships between plastic ingestion ($\geq$ 1 mm) and biomarkers of enzymatic activities (AChE acetylcholinesterase, BChE butyrylcholinesterase and GST glutathione S-transferase) and conventional health parameters (haematological, biochemical and morphometric data) in SGP chicks from colonies in Chubut, Argentina. During 2022 and 2023, plastics were detected in 71.4% of chicks (1–14 items, mean mass = 0.2510 g, representing < 0.1% of chick body mass). In 30-day-old chicks, plastic ingestion was associated with nutritional parameters (cholesterol, total solids, packed cell volume, calcium, body mass and body condition index), metabolism (enzymes aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine phosphokinase) and immune response (eosinophil percentage). Associations with biomarkers (AChE, BChE, and GST) were observed but lost significance when accounting for inter-annual variation. By fledging (~90 days), most relationships weakened or disappeared, while GST activity and calcium levels emerged as the only variables associated with plastic ingestion. It is unclear whether these associations reflect compensatory physiological responses to oxidative challenge, contaminant exposure, or developmental/ontogenetic adjustments. Our results highlight the value of multi-indicator approaches for detecting subtle physiological variation associated with plastic ingestion. Importantly, the pervasive threat of plastic pollution warrants investigating whether early-life exposure influences post-fledging survival and fitness.

## 1. Introduction

Plastic pollution in the ocean is one of the pressing environmental challenges of the 21st century, accentuated by the rate of plastic production exceeding the world's ability to deal with it (United Nations Environment Programme, 2021). Evidence of wildlife interaction with plastics is abundant (Kühn and Van Franeker, 2020; Puskic et al., 2020). Seabirds, typically top predators in marine food webs, are widely recognized as sentinels of marine plastic pollution (Wilcox et al., 2015; Phillips and Waluda, 2020; Phillips et al., 2023; Carrasco et al., 2025). The evidence of the negative effects of plastics on seabirds is largely based on readily observable outcomes primarily associated with larger debris (e.g., entanglement and gut obstruction or perforation; Pierce et al., 2004; Phillips et al., 2010; Senko et al., 2020). However, many plastic-wildlife interactions also involve less visible and therefore poorly documented sub-lethal effects, suggesting that the overall impact on seabird health is likely underestimated (Puskic et al., 2020).

Plastic ingestion, including micro-, meso-, and macroplastics (1–5 mm, 5–25 mm, > 25 mm respectively) has been extensively documented in seabirds worldwide, and recent research has increasingly focused on understanding its physiological and health impacts (Puskic et al., 2020). In addition, plastic-associated chemicals (i.e., additives and chemicals adsorbed onto plastics) have been detected in seabird tissues (Tanaka et al., 2013; Hardesty et al., 2015; Tanaka et al., 2020; Padula et al., 2020; Sühring et al., 2022; Vanstreels et al., 2023; Collard et al., 2024), although their physiological consequences in free-living

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seabirds remain poorly understood (Alam et al., 2025). These chemicals can induce a broad range of chronic and sub-lethal disorders in marine organisms, including endocrine disruption, neurotoxicity, hepatic stress, metabolic dysfunction, and altered antioxidant responses (Teuten et al., 2009; Hirai et al., 2011; Rochman et al., 2013; Prokić et al., 2019; Puskic et al., 2020 and references therein). However, this knowledge largely derives from controlled laboratory studies, while data from free-ranging vertebrates, such as seabirds, remain scarce (Lavers et al., 2019; Roman et al., 2019a, 2020a; Serafini et al., 2024; Veríssimo et al., 2024).

The insufficient understanding of sub-lethal effects from plastic ingestion and exposure to associated pollutants responds primarily to the lack of known harm-specific indicators, the difficulty of applying them to free-living wildlife, and the influence of species-specific responses. In the last two decades, several ecotoxicological studies have used biochemical biomarkers to evaluate the sub-lethal effects of chemical pollutants, including those derived from plastics, on living organisms (Vasseur and Cossu-Leguille, 2003; Prokić et al., 2019). Experimental studies have shown that microplastics and their chemical additives can alter enzymatic activities related to antioxidant defense (e.g., catalase CAT, glutathione S-transferase GST, and glutathione reductase GR); metabolism (e.g., lactate dehydrogenase LDH); as well as metabolism of xenobiotic and endogenous molecules (butyrylcholinesterase BChE and acetylcholinesterase AChE) across a range of taxa, including aquatic organisms (Prokić et al., 2019; Xu et al., 2025). However, their applicability to free-living animals is unknown (Porcino et al., 2022; Kaur and Kumar, 2024). Recently, Veríssimo et al. (2024) reported that high concentrations of plastic additives (flame retardants, i.e. MeO-BDEs) were associated with decreased cholinesterase activity in adult yellow-legged gulls (*Larus michahellis*), providing field-based evidence of neurotoxic effects. Nevertheless, the toxicological responses of organisms to pollutants are complex, as multiple confounding factors and exposure pathways can influence individual responses (Hagger et al., 2006). To address this variability, multi-biomarker approaches are increasingly used to assess the impacts of environmental stressors on organisms *in situ* (Van der Oost et al., 2003; Hagger et al., 2006; Serafini et al., 2026).

In ecotoxicological studies, biochemical biomarkers can provide early indication of physiological disturbance at the molecular or enzymatic level, whereas broader physiological and health-related parameters may reflect more integrated organismal responses. In the context of plastic exposure, physiological parameters can provide complementary insights into the overall health status of exposed individuals (Lavers et al., 2019; Mejia et al., 2024; Puskic et al., 2025). For example, plastic ingestion may not only trigger toxic effects; it may also lead to dietary dilution (Machovsky-Capuska et al., 2019; Senko et al., 2020). The presence of inorganic and space-occupying material within the gastrointestinal tract can induce a false sense of satiation, limiting food intake (McCauley and Bjorndal, 1999) and potentially leading to starvation (Pierce et al., 2004). Consequently, plastic ingestion in seabirds has been associated with reduced body mass, growth rate, and size (Ryan, 1988; Lavers et al., 2014; Roman et al., 2019a; Colvin et al., 2025), decreased fat deposition (Auman et al., 1997), and altered blood chemistries related to nutritional condition in chicks (e.g., calcium, uric acid and cholesterol levels; Lavers et al., 2019). Parameters including haematology and plasma biochemistries, along with morphometric measurements (e.g., body mass or size-adjusted body mass), are widely used as indicators of physiological, nutritional, and overall health status in birds (Uhart et al., 2003; Gallo et al., 2019). Although these parameters are maintained within a relatively narrow homeostatic range (Kaneko et al., 2008), they can respond sensitively to nutritional or anthropogenic stressors (Milner et al., 2003). However, some alterations may remain masked by compensatory mechanisms until organisms reach a critical physiological threshold (Alonso-Alvarez and Ferrer, 2001). In addition, physiological responses may depend on exposure duration and cumulative burden and therefore may not necessarily reflect the same temporal window as the stressor itself, highlighting the importance of integrative, multi-indicator approaches when assessing sub-lethal health impacts on wildlife (Puskic et al., 2024).

Procellariiform birds, especially albatrosses and petrels, are particularly prone to ingest plastic because they feed on small prey and garbage discarded from ships at the water surface, where floating plastics tend to accumulate (Ryan, 2008; Copello and Quintana, 2009; Avery-Gomm et al., 2012; Roman et al., 2019b, 2020b; Kühn et al., 2021). In the western South Atlantic, plastics in stomach contents of these species have been mainly reported in stranded and bycaught birds off Brazil (Petry et al., 2007; Barbieri, 2009; Colabuono et al., 2009; Tourinho et al., 2010; Tavares et al., 2017; Petry and Benemann, 2017; Baes et al., 2024), and Uruguay (Jiménez et al., 2015; Muñoz et al., 2023), and to a lesser extent in bycaught birds and breeding colonies from southernmost areas, including the Patagonian coast of Argentina, and the Falkland Islands (Islas Malvinas) (Copello and Quintana, 2003; Copello et al., 2008; Gallo et al., 2024). However, despite numerous records of ingestion, studies addressing the association between chronic plastic exposure and health parameters and biomarkers remain extremely scarce. To date, the only study in the region was conducted on juvenile Manx shearwaters (*Puffinus puffinus*) found dead along the Brazilian coast; although plastic debris was detected in 29% of the birds, no significant associations were found between plastic ingestion and the biomarkers assessed (Serafini et al., 2024). This finding highlights the complexity of linking ingested plastics to biological responses in wild seabirds and underscores the need for studies in other species and developmental contexts to determine whether plastic ingestion is associated with measurable health effects.

In this context, the southern giant petrel (SGP, *Macronectes giganteus*) represents an ideal model species. It is a key marine scavenger and top predator, long-lived (up to approximately 40 years of age; Foote et al., 2011), and produces a single-egg clutch, with hatching being highly synchronous and concentrated between late December and early January (Copello and Quintana, 2009). Moreover, the species shows the highest plastic ingestion levels among procellariiform seabirds in the western South Atlantic (Gallo et al., 2024). In addition, intergenerational plastic transfer from adults to chicks through regurgitation has been frequently documented in this species (Copello and Quintana, 2003; Copello et al., 2008). This study investigated the relationship between plastic ingestion and a suite of biomarkers, as well as conventional health and body condition parameters during early life in southern giant petrels. For this, during the chick rearing period we 1) assessed plastic ingestion in chicks by collecting voluntary regurgitates; 2) estimated body condition through morphometric measurements of chicks; 3) determined biomarkers, haematology and plasma biochemistry parameters in chick blood samples; and 4) evaluated the relationship between plastic ingestion metrics (occurrence, number of items, plastic mass, and volume) and the selected biomarkers and parameters.

## 2. Methods

### 2.1. Study area and sample collection

Samples were obtained at Arce and Gran Robredo islands, Chubut, Argentina (Fig. 1) in 2022 and 2023, respectively, during the SGP chick-rearing period (January-March). Most (~80%) of the Argentine SGP breeding population is found on these two nearby islands (Quintana et al., 2006). Each year we randomly selected 30 nests, collected blood samples, took morphometric measurements (n = 30), and voluntary regurgitates (n = 12 and 24 for 2022 and 2023, respectively) from ~30-day-old chicks ("young chicks", January). When chicks were ~90 days old ("pre-fledging", March) we recaptured them, collected blood samples, took morphometric measurements (n = 26 and 23 for 2022 and 2023, respectively), and voluntary regurgitates (n = 4 and 21 for 2022 and 2023, respectively). Some of the chicks initially sampled at 30 days did not survive to this stage (2022: 5/30; 2023: 7/30), resulting in slightly smaller sample sizes for pre-fledging individuals. In addition,

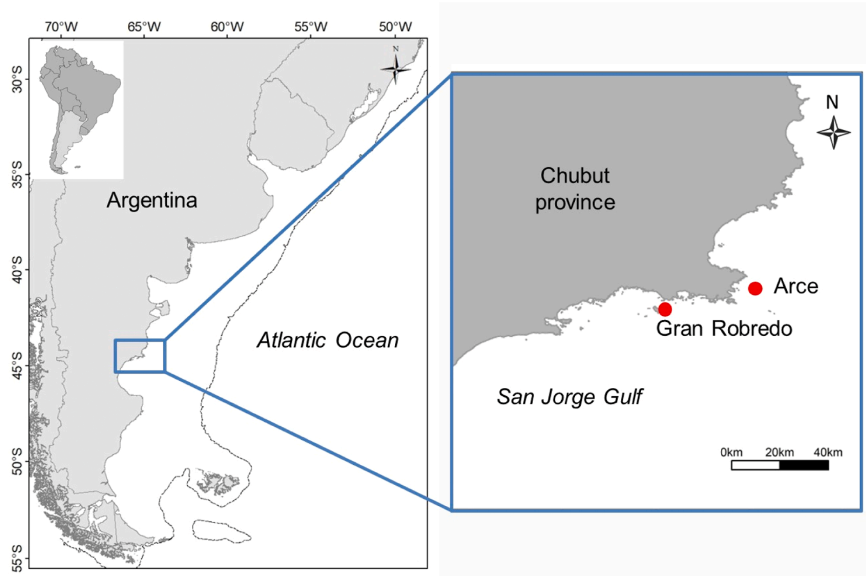


**Fig. 1.** Sampling sites: Arce and Gran Robredo islands, Chubut province, Argentina.

randomly selected control "pre-fledging" chicks were weighed and sampled for regurgitates in both years (2022 = 10, six regurgitated; 2023 = 17, all regurgitated) to evaluate the effects of repeated sampling on body mass and plastic ingestion. In 2023, control chicks were also blood sampled using the same protocol applied to recaptured chicks, increasing the number of physiological and biochemical observations available for the pre-fledging age class. We determined our first sampling point at ~30 days to avoid disturbance in the colony when most chicks are hatching and in need of constant parental care. Chick age was estimated based on the highly synchronous hatching chronology characteristic of this population (see above), together with morphometric development criteria described by Copello and Quintana (2009). Our resampling at ~90 days of age was scheduled to occur 1–2 weeks before fledging (see Copello and Quintana, 2009), aiming to represent fully grown, yet still dependent, chicks.

Regurgitates were obtained by up-ending birds over a plastic container and gently massaging the stomach and throat (Uhart et al., 2020). Blood samples (range 1–3 ml, < 0.5% of body mass) were obtained from the tarsal or jugular vein using heparinized syringes and 25 or 27 G x 1-inch needles, then stored in vacutainer tubes and kept cool on ice until processing within six hours after collection according to Gallo et al. (2019). Following venipuncture, sampled chicks received Ringer-Lactate solution (3 ml/kg) by subcutaneous injection in the groin, to compensate for loss of fluids and electrolytes and avoid dehydration.

In the field laboratory, blood collected in Vacutainer tubes was used to prepare two blood smears and fill two capillary tubes per sample. Blood smears were fixed with methanol and stained with Giemsa. Capillary tubes were centrifuged at 1000 *G*, and packed cell volume (PCV) was visually estimated using a micro-haematocrit ruler and expressed as percentage. Total plasma solids (TS, g/l) were measured using a temperature-compensated refractometer (Schulco, Toledo, Ohio, USA) calibrated on site. The remaining blood was centrifuged at 1000 *G* for 20 min, and plasma was stored frozen (−20°C) until laboratory analysis, which was typically conducted within 30 days of sample collection. Morphometric measurements (bill length and depth, tarsus length, body mass) were obtained using digital calliper (0.01 mm) and spring scales (2500 g, 5 kg and 10 kg), respectively.

### 2.2. Detection and quantification of plastic ingestion

Plastic ingestion was quantified using the methods described in detail by Gallo et al. (2024). Briefly, regurgitates were passed through a 1 mm mesh sieve, and retained materials were rinsed, air dried, and examined under a stereomicroscope. Visual identification followed previously published criteria designed to minimize misclassification of smaller debris items (1–5 mm; Gallo et al. 2024). When necessary, complementary confirmation methods (e.g., hot needle test and selective UV fluorescence) were used to verify suspected plastic particles. Plastic ingestion was characterised using four metrics: occurrence (presence/absence), number of items, plastic mass, and plastic volume. Plastic items were counted and classified as either industrial pellets or user plastics (Provencher et al., 2017), the latter differentiated into hard fragments, sheet, thread, foam, and other plastic items (e.g., cigarette filters, rubber, balloons, etc.). Each item was weighed using a precision scale (± 0.0001 g), photographed on a 1 mm grid background, and its length measured using Software "ImageJ" (Schneider et al., 2012). Plastics were classified into three categories according to their longest dimension (1–5 mm, 5–25 mm, > 25 mm; GESAMP, 2019). Volumes were estimated by multiplying item mass by an average density value for each material type (Roman et al., 2019c).

### 2.3. Biomarkers and parameters

Body condition indices were calculated as the residuals of the regression between the body mass and body size (i.e., "scores" of the first component of a principal component analysis, considering bill length and depth and tarsus length), where positive values indicate individuals heavier than expected for their structural size and negative values indicate individuals lighter than expected (Schulte-Hostedde et al., 2005). Body condition was the only morphometric-derived variable included in subsequent statistical analyses. Biomarkers of toxicity (enzymes AChE, BChE and antioxidant defense enzyme GST), together with health status parameters (haematology and biochemistries), were measured in plasma samples. Enzymatic activities were analysed at Laboratorio de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional Del Litoral (FBCB-UNL, Santa Fe); methodological details are provided in Supplementary File S1. Briefly, AChE and BChE activities were determined colorimetrically following the procedure of Ellman et al. (1961), whereas GST activity was determined by spectrophotometry according to Habig et al. (1974), with adaptations from Habdous et al. (2002). Plasma biochemistries were measured via automated analyzer (Abbot Architect Plus ci4100) in a commercial laboratory (Biomadryn, Puerto Madryn) and included cholesterol, triglycerides, glucose, uric acid, total protein, albumin, calcium, and enzymes lactate dehydrogenase, aspartate aminotransferase, creatinine phosphokinase and alkaline phosphatase. Haematological parameters (differential leukocyte counts, heterophils/lymphocyte ratio and leucocyte counts per field or "leucocyte density", in addition to PCV and TS) were determined according to Gallo et al. (2019).

### 2.4. Statistical analysis

The arithmetic mean and standard deviation of the total number, mass, and volume of plastic items were calculated separately for (a) all regurgitates examined (abundance) and (b) only regurgitates containing plastics (intensity). The frequency of occurrence of plastics (FO; number of samples with plastics/total number of samples analysed) was also calculated. Chi-square and Kruskal-Wallis tests were used to compare plastic ingestion metrics, biomarkers, and physiological parameters by age class, year and survival (i.e., young chicks that survived vs. those that did not survive until fledging). Fisher's exact tests were applied to compare the proportions of each plastic category (by type, colour, and size) between years.

To assess the effect of repeated sampling on study chicks, and whether removal of plastics during the first sampling could influence estimates of plastic accumulation in pre-fledging chicks, we compared body mass and plastic ingestion in recaptured chicks with previously unsampled "control" chicks. Chi-square and Kruskal-Wallis tests were used to assess the significance of these differences.

Relationships between plastic ingestion metrics (presence/absence, total number, mass and volume of items; response variables), biomarkers, and physiological parameters (explanatory variables) were analysed for each sampling group separately (~30 and 90-day-old chicks) using Generalized Linear Models (GLMs) following van Franeker and Mejiboom (2002). This approach allows for the evaluation of associations between plastic ingestion and multiple biomarkers and physiological parameters simultaneously, providing a more integrative perspective than single-variable analyses. Biomarkers were evaluated as a separate group, whereas physiological parameters were grouped according to their biological function (i.e., liver function, renal function, protein and muscle catabolism, biomarkers, nutritional status, general condition and immune function, see Tables S1-S2). Spearman's rank correlation analyses among explanatory variables were used to assess potential collinearity and identify redundant variables within each group. When significantly correlated variables represented overlapping physiological information (e.g., total protein and albumin, lymphocyte counts and H/L ratio), they were evaluated in separate candidate models to avoid redundancy (see Tables S1-S2). Mass and volume data were left-skewed requiring logarithmic transformation and the addition of a small value to deal with zero values as $\ln(x + 0.001)$ (van Franeker and Meijboom, 2002). Plastic abundance (number of items) was modelled using Poisson error distribution, frequency of occurrence of plastics (presence / absence) with binomial response (logistic regression), and ln-transformed plastic mass and volume with Gaussian linear models. When significant interannual differences were detected in a given plastic ingestion metric, year was included as a covariate in the corresponding models. Because the colonies are geographically close (i.e., 45 km), the SGPs from both colonies are known to forage over broadly overlapping areas and are similarly exposed to marine plastic pollution (Blanco et al., 2022), colony identity was not included as an explanatory factor in the analyses. Model selection followed a stepwise procedure based on Akaike's Information Criterion (AIC). Statistical analyses were conducted using R v4.1.1 (R Core Team, 2021), and significance level was 0.05.

## 3. Results

### 3.1. Plastic ingestion

Regurgitates were recovered from 84 chicks, 60 of them (FO = 71.4%) contained plastics, ranging from 1 to 14 items (mean = 3.38), with an average mass of 0.2510 g and volume of 0.2278 $cm^3$. The total mass of plastics recovered from each regurgitate relative to chick body mass was < 0.1% across age classes and year (mean = 0.0088%, range = 0.0003–0.0977%). The frequency of occurrence, abundance, and intensity of the different plastic ingestion metrics (number of items, mass and volume) by year and age class (young vs. pre-fledging chicks) are detailed in Table 1. Differences in plastic ingestion metrics between years and age classes were mostly not significant (all $P > 0.05$). However, the abundance of plastic items in young chicks varied between years, being higher in 2023 (KW = 3.922, $P = 0.047$). In 2022, total plastic mass varied with age class, with higher values in pre-fledging chicks (KW = 4.108, $P = 0.043$). No significant differences in any plastic ingestion metric were detected between young chicks that survived to independence and those that did not in both seasons (all $P > 0.05$).

Size of items recovered (n = 206) was: 1–5 mm (33%), 5–25 mm (40%), and > 25 mm (27%). No industrial pellets were detected, and user plastics were mainly composed of foam (42%) and membranes (bags and packaging, 40%), followed by rigid fragments (9%), other types (5%), and filaments (3%). Brown/tan was the most frequent colour (45%), followed by transparent (20%), white/cream (18%), blue and black/grey (5% each), green, red/pink (3% each) and yellow (<1%). Size and some categories of colours and types of plastic items varied between years (Table S1).

Assessment of differences between treatment and control chicks prior to fledging showed that although the FO, number, and mass of items were slightly higher in control chicks (except mass of items in 2022, which was higher in treatment chicks), differences were not significant ($P > 0.05$).

### 3.2. Biomarkers and physiological parameters

The results for biomarkers and haematology, biochemistries, and morphometric parameters for chicks of each age class are summarized in Tables 2 and 3 (2022 and 2023, respectively). Considering years separately, most parameters varied between age classes. However, no significant differences were found for biomarkers BChE and GST or body condition index in either year, nor for lymphocytes, calcium and aspartate aminotransferase in 2023 (Tables 2–3). Several parameters indicative of nutritional status and metabolism, including triglycerides, cholesterol, total protein, albumin, and glucose, increased at pre-fledging in both years. Conversely, uric acid, LDH and CPK activities were lower in pre-fledging chicks than in young chicks (Tables 2–3). In addition, no significant differences were detected in any of the evaluated parameters (all $P > 0.05$) between young chicks that survived until fledging and those that did not in both years.

Considering age classes separately, all biomarkers and most of the physiological parameters varied between years for young chicks, whereas pre-fledging chicks showed comparatively less interannual variation (Table 4). Additionally, no significant differences ($P > 0.05$) were found between the body mass of recaptured (2022 = 4.340 ± 0.691 g, 2023 = 4.190 ± 0.411 g) and control chicks (2022 = 4.215 ± 0.694 g, 2023 = 4.018 ± 0.534 g). Based on these findings, control chicks were included in the GLMs exploring the relationships between plastic ingestion and selected biomarkers and physiological parameters in pre-fledging chicks.

**Table 1**
Data summary for plastics (all types combined) found in regurgitates from southern giant petrel (*Macronectes giganteus*) chicks in Arce and Gran Robredo islands, 2022−2023. Sample size (n), frequency of occurrence (FO, no. samples with plastic/no. samples). For number of items (no. items), mass (g) and volume ($cm^3$) we calculated: abundance (arithmetic mean ± SD for all samples examined) and intensity (arithmetic mean ± SD for samples containing plastic). The plastic mass relative to chick body mass was expressed as percentage (%). Young chicks: ~30-day-old chicks sampled in January, pre-fledging chicks: ~90-day-old chicks sampled in March.

| | | | Plastic ingestion | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Abundance | | | Intensity | | | |
| | n | FO | no. items | mass (g) | volume ($cm^3$) | no. items | mass (g) | volume ($cm^3$) | % |
| **Total** | **84** | **71%** | **2.38 ± 2.77** | **0.1760 ± 0.5056** | **0.1606 ± 0.4628** | **3.38 ± 2.75** | **0.2510 ± 0.5894** | **0.2278 ± 0.5346** | **0.0088** |
| 2022 | 22 | 64% | 1.68 ± 1.86 | 0.7018 ± 0.9349 | 0.3300 ± 0.8365 | 2.64 ± 1.69 | 0.5747 ± 1.1328 | 0.5316 ± 1.0065 | 0.0146 |
| 2023 | 62 | 74% | 2.60 ± 2.94 | 0.1309 ± 0.2186 | 0.1005 ± 0.1910 | 3.50 ± 2.92 | 0.1774 ± 0.2381 | 0.1329 ± 0.2051 | 0.0070 |
| **Young** | | **72%** | **2.97 ± 3.53** | **0.1059 ± 0.1685** | **0.0775 ± 0.1343** | **4.12 ± 3.54** | **0.1466 ± 0.1831** | **0.1074 ± 0.1481** | **0.0095** |
| 2022 | 12 | 50% | 1.50 ± 1.93 | 0.0504 ± 0.0772 | 0.0568 ± 0.0817 | 3.00 ± 1.67 | 0.1008 ± 0.0839 | 0.1137 ± 0.0833 | 0.0056 |
| 2023 | 24 | 83% | 3.71 ± 3.93 | 0.1337 ± 0.1947 | 0.0879 ± 0.1547 | 4.45 ± 3.90 | 0.1604 ± 0.2035 | 0.1055 ± 0.1644 | 0.0107 |
| **Pre-fledging*** | | **71%** | **1.89 ± 1.82** | **0.2600 ± 0.6700** | **0.2266 ± 0.5954** | **2.68 ± 1.61** | **0.3729 ± 0.7765** | **0.3200 ± 0.6886** | **0.0083** |
| 2022 | 10 | 80% | 1.90 ± 1.85 | 0.7442 ± 1.3196 | 0.6760 ± 1.1758 | 2.56 ± 1.77 | 0.9098 ± 1.4287 | 0.8451 ± 1.2705 | 0.0213 |
| 2023 | 38 | 68% | 1.89 ± 1.84 | 0.1291 ± 0.2353 | 0.1084 ± 0.2124 | 2.77 ± 1.58 | 0.1911 ± 0.2659 | 0.1584 ± 0.2419 | 0.0043 |

* Includes regurgitates from control chicks (2022 = 6, 2023 = 17)

**Table 2**
Summary of biomarkers and physiological parameters for southern giant petrel (*Macronectes giganteus*) chicks at Arce Island, 2022. Values are expressed as mean ± SD. Young chicks: ~30-day-old chicks sampled in January, pre-fledging chicks: ~90-day-old chicks sampled in March (includes 26 recaptures).

| | 2022 | | | |
|---|---|---|---|---|
| | Young chicks | Pre-fledging chicks | Variation | – |
| **Sample size (no.)** | 30 | 26 | | |
| **Body condition** | | | | |
| Body mass (g) | 1.845 ± 0.380 | 4.340 ± 0.691 | | |
| Body condition index | $1.1853^{-08}$±0.3456 | $-1.1538^{-08}$±0.5996 | KW = 0.20, P = 0.657 | |
| **Biomarkers** | | | | |
| **Toxicity** | | | | |
| Acetylcholinesterase (μmol/min/ml) | 1.2840 ± 1.2683 | 1.5599 ± 1.5882 | KW = 15.94, **P < 0.001** | ↑ |
| Butyrylcholinesterase (μmol/min/ml) | 0.3644 ± 0.3269 | 0.3946 ± 0.3945 | KW = 3.07, P = 0.079 | |
| **Antioxidant defense** | | | | |
| Glutathione-S-transferase (μmol/min/ml) | 0.1486 ± 0.1505 | 0.1424 ± 0.1372 | KW = 1.42, P = 0.233 | |
| **Physiological Parameters** | | | | |
| **Haematology** | | | | |
| Packed cell volume (%) | 27.5 ± 3.8 | 38.7 ± 3.8 | KW = 39.1, **P < 0.001** | ↑ |
| Total solids (g L−1) | 5.5 ± 0.3 | 6.0 ± 0.3 | KW = 25.34, **P < 0.001** | ↑ |
| Leucocyte density (cells/field) | 0.88 ± 0.51 | 1.83 ± 0.75 | KW = 23.31, **P < 0.001** | ↑ |
| Eosinophils (%) | 5.90 ± 6.41 | 8.38 ± 4.97 | KW = 6.06, **P = 0.014** | ↑ |
| Lymphocytes (%) | 62.37 ± 18.80 | 52.35 ± 11.38 | KW = 6.96, **P < 0.001** | ↓ |
| Heterophils (%) | 29.65 ± 17.66 | 35.73 ± 10.88 | KW = 7.23, **P = 0.007** | ↑ |
| Monocytes (%) | 2.08 ± 1.81 | 3.54 ± 2.87 | KW = 3.94, **P = 0.047** | ↑ |
| Heterophil/Lymphocyte | 0.52 ± 0.46 | 0.79 ± 0.44 | KW = 9.83, **P = 0.002** | ↑ |
| **Plasma biochemistries and enzymes** | | | | |
| Triglycerides (mg/dl) | 174.00 ± 47.16 | 220.04 ± 98.82 | KW = 4.96, **P = 0.026** | ↑ |
| Cholesterol (mg/dl) | 304.57 ± 55.83 | 355.69 ± 86.56 | KW = 4.81, **P = 0.028** | ↑ |
| Total protein (g/dl) | 3.26 ± 0.33 | 3.84 ± 0.48 | KW = 20.63, **P < 0.001** | ↑ |
| Albumin (g/dl) | 1.13 ± 0.09 | 1.20 ± 0.10 | KW = 6.10, **P = 0.013** | ↑ |
| Calcium (mg/dl) | 10.16 ± 0.51 | 10.48 ± 0.42 | KW = 4.65, **P = 0.031** | ↑ |
| Glucose (g/dl) | 243.27 ± 16.35 | 257.65 ± 19.38 | KW = 9.25, **P = 0.002** | ↑ |
| Uric acid (mg/dl) | 6.58 ± 2.81 | 4.96 ± 2.32 | KW = 5.87, **P = 0.015** | ↓ |
| Aspartate aminotransferase (U/l) | 107.33 ± 100.82 | 120.04 ± 62.07 | KW = 23.27, **P < 0.001** | ↑ |
| Alkaline phosphatase (U/l) | 311.40 ± 86.28 | 250.40 ± 39.69 | KW = 10.32, **P = 0.001** | ↓ |
| Lactate dehydrogenase (U/l) | 410.30 ± 147.12 | 258.12 ± 62.07 | KW = 20.79, **P < 0.001** | ↓ |
| Creatinine phosphokinase (U/l) | 314.30 ± 161.49 | 237.42 ± 149.30 | KW = 7.35, **P = 0.007** | ↓ |

### *3.3. Relationships between plastic ingestion, biomarkers and physiological parameters*

Plastic ingestion, especially the number of ingested items, was significantly associated with variation in several biomarkers and physiological parameters in chicks (Figs. 2–3; see detailed results in Tables S2−3). In young chicks, plastic ingestion was positively associated with hepatic enzymes alkaline phosphatase (plastic number: P = 0.006; Fig. 2A) and lactate dehydrogenase (plastic number: P < 0.001, plastic mass: P = 0.037; Fig. 2C-D), and negatively associated with aspartate aminotransferase (plastic number: P = 0.002; Fig. 2B). Creatinine phosphokinase, an enzyme linked to muscle catabolism, was positively related to plastic ingestion (occurrence: P = 0.045, plastic number P = 0.008; Fig. 2E-F). The number of plastic items was negatively related to parameters reflecting physical and nutritional condition, including cholesterol (P = 0.010), total solids (P = 0.009), and body condition index (P = 0.049). Conversely, it was positively related to packed cell volume (P = 0.002) and calcium levels (P = 0.004) (Fig. 2G-K). In addition, the percentage of eosinophils, an indicator of immune response, also showed a positive relationship with plastic item numbers (P = 0.025; Fig. 2L). Although not statistically significant when including year as covariate, AChE and BChE activities tended to be positively associated with plastic item numbers, while GST showed a negative trend (Table S2).

Conversely, pre-fledging chicks showed fewer significant relationships. GST activity (P = 0.049) and calcium levels (P = 0.016) were

**Table 3**
Summary of biomarkers and physiological parameters for southern giant petrel (*Macronectes giganteus*) chicks sampled at Gran Robredo Island, 2023. Values are expressed as mean ± SD. Young chicks: ~30-day-old chicks sampled in January, pre-fledging chicks: ~90-day-old chicks sampled in March (includes 23 recaptures and 17 control chicks).

| | 2023 | | | |
|---|---|---|---|---|
| | **Young chicks** | **Pre-fledging chicks** | **Variation** | – |
| **Sample size (no.)** | 30 | 40 | | |
| **General body condition** | | | | |
| Body mass (g) | 1.476 ± 0.2428 | 4.116 ± 0.4632 | | |
| Body condition index | 0 ± 0.1895 | $-1.0957^{-08}$±0.3520 | KW = 0.02, P = 0.877 | |
| **Biomarkers** | | | | |
| **Toxicity** | | | | |
| Acetylcholinesterase (μmol/min/ml) | 1.8996 ± 0.2697 | 1.6440 ± 0.4887 | KW = 6.65, **P = 0.01** | ↓ |
| Butyrylcholinesterase (μmol/min/ml) | 0.4241 ± 0.0840 | 0.5255 ± 0.5953 | KW = 0.572, P = 0.449 | |
| **Antioxidant defense** | | | | |
| Glutathione-S-transferase (μmol/min/ml) | 0.1097 ± 0.0219 | 0.1052 ± 0.0282 | KW = 0.97, P = 0.324 | |
| **Physiological Parameters** | | | | |
| **Haematology** | | | | |
| Packed cell volume (%) | 33.3 ± 4.1 | 38.3 ± 3.6 | KW = 19.8, **P < 0.001** | ↑ |
| Total solids (g L−1) | 3.9 ± 0.6 | 4.7 ± 0.5 | KW = 32.48, **P < 0.001** | ↑ |
| Leucocyte density (cells/field) | 1.46 ± 0.75 | 1.49 ± 0.56 | KW = 0.46, P = 0.99 | |
| Eosinophils (%) | 2.27 ± 3.45 | 13.31 ± 9.30 | KW = 34.22, **P < 0.001** | ↑ |
| Lymphocytes (%) | 44.03 ± 13.58 | 48.68 ± 13.96 | KW = 1.71, P = 0.192 | |
| Heterophils (%) | 52.60 ± 13.13 | 34.52 ± 12.28 | KW = 23.65, P **< 0.001** | ↓ |
| Monocytes (%) | 1.10 ± 1.29 | 3.49 ± 2.00 | KW = 27.09, P **< 0.001** | ↑ |
| Heterophil/Lymphocyte | 1.41 ± 0.79 | 0.83 ± 0.51 | KW = 11.89, P **< 0.001** | ↓ |
| **Plasma biochemistries and enzymes** | | | | |
| Triglycerides (mg/dl) | 123.28 ± 40.51 | 272.55 ± 122.28 | KW = 40.74, P **< 0.001** | ↑ |
| Cholesterol (mg/dl) | 270.29 ± 43.27 | 316.60 ± 70.81 | KW = 9.72, **P = 0.002** | ↑ |
| Total protein (g/dl) | 3.60 ± 0.50 | 4.25 ± 0.41 | KW = 27.17, **P < 0.001** | ↑ |
| Albumin (g/dl) | 1.38 ± 0.14 | 1.48 ± 0.12 | KW = 8.20, **P = 0.004** | ↑ |
| Calcium (mg/dl) | 10.51 ± 0.40 | 10.53 ± 0.30 | KW = 0.08, P = 0.783 | |
| Glucose (g/dl) | 248 ± 17 | 248 ± 15 | KW = 0.01, P = 0.973 | |
| Uric acid (mg/dl) | 5.82 ± 2.24 | 4.56 ± 2.29 | KW = 6.90, **P = 0.009** | ↓ |
| Aspartate aminotransferase (U/l) | 108.72 ± 38.85 | 108.30 ± 29.09 | KW = 0.99, P = 0.319 | |
| Alkaline phosphatase (U/l) | 318.90 ± 63.42 | 450.83 ± 98.99 | KW = 28.81, P **< 0.001** | ↑ |
| Lactate dehydrogenase (U/l) | 816.04 ± 260.78 | 503.98 ± 168.61 | KW = 25.79, P **< 0.001** | ↓ |
| Creatinine phosphokinase (U/l) | 582.41 ± 379.05 | 408.70 ± 163.25 | KW = 3.30, P = 0.069 | |

**Table 4**
Results of statistical analyses evaluating variability of biomarkers and physiological parameters for southern giant petrel (*Macronectes giganteus*) chicks between years for each age class (young chicks: ~30-day-old chicks sampled in January, pre-fledging: ~90-day-old chicks sampled in March).

| | 2022 vs. 2023 | | | |
|---|---|---|---|---|
| | **Young chicks** | | **Pre-fledging chicks** | |
| **Sample size (no.)** | 60 | | 66 | |
| Body mass (g) | KW = 16.49, **P < 0.001** | ↓ | KW = 2.46, P = 0.117 | |
| Body condition index | KW = 0.11, P = 0.734 | | KW = 0.01, P = 0.903 | |
| Acetylcholinesterase (μmol/min/ml) | KW = 38.65, **P < 0.001** | ↑ | KW = 3.46, P = 0.063 | |
| Butyrylcholinesterase (μmol/min/ml) | KW = 8.10, **P = 0.004** | ↑ | KW = 0.06, P = 0.799 | |
| Glutathione-S-transferase (μmol/min/ml) | KW = 26.11, **P < 0.001** | ↓ | KW = 22.92, **P < 0.001** | ↓ |
| Packed cell volume (%) | KW = 20.94, **P < 0.001** | ↑ | KW = 0.20, P = 0.654 | |
| Total solids (g L−1) | KW = 38.76, **P < 0.001** | ↓ | KW = 44.95, **P < 0.001** | ↓ |
| Leucocyte density (cells/field) | KW = 12.96, **P < 0.001** | ↑ | KW = 3.13, P = 0.077 | |
| Eosinophils (%) | KW = 9.10, **P = 0.003** | ↓ | KW = 4.26, **P = 0.039** | ↑ |
| Lymphocytes (%) | KW = 15.25, **P < 0.001** | ↓ | KW = 1.59, P = 0.207 | |
| Heterophils (%) | KW = 22.91, **P < 0.001** | ↑ | KW = 0.05, P = 0.818 | |
| Monocytes (%) | KW = 5.03, **P = 0.025** | ↓ | KW = 0.50, P = 0.481 | |
| Heterophil/Lymphocyte | KW = 25.11, **P < 0.001** | ↑ | KW = 0.389, P = 0.533 | |
| Triglycerides (mg/dl) | KW = 14.26, **P < 0.001** | ↓ | KW = 5.22, **P = 0.022** | ↑ |
| Cholesterol (mg/dl) | KW = 6.03, **P = 0.014** | ↓ | KW = 4.69, **P = 0.030** | ↓ |
| Total protein (g/dl) | KW = 8.01, **P = 0.005** | ↑ | KW = 12.97, **P < 0.001** | ↑ |
| Albumin (g/dl) | KW = 32.97, **P < 0.001** | ↑ | KW = 40.42, **P < 0.001** | ↑ |
| Calcium (mg/dl) | KW = 6.03, **P = 0.014** | ↑ | KW = 0.64, P = 0.425 | |
| Glucose (g/dl) | KW = 1.93, P = 0.165 | | KW = 4.98, **P = 0.026** | ↓ |
| Uric acid (mg/dl) | KW = 1.16, P = 0.282 | | KW = 0.72, P = 0.397 | |
| Aspartate aminotransferase (U/l) | KW = 4.88, **P = 0.027** | ↑ | KW = 3.07, P = 0.080 | |
| Alkaline phosphatase (U/l) | KW = 1.00, P = 0.317 | | KW = 44.37, **P < 0.001** | ↑ |
| Lactate dehydrogenase (U/l) | KW = 30.95, **P < 0.001** | ↑ | KW = 39.52, **P < 0.001** | ↑ |
| Creatinine phosphokinase (U/l) | KW = 15.30, **P < 0.001** | ↑ | KW = 22.38, **P < 0.001** | ↑ |

positively related to the number of ingested plastics (Fig. 3A-B, Table S3). Although the body condition index exhibited a marginally non-significant negative relationship with plastic ingestion (P = 0.055; Fig. 3C), physiological parameters indicative of nutritional status and metabolism that were associated with plastic ingestion in young chicks (Fig. 2; i.e., cholesterol, total solids, and lactate dehydrogenase), showed

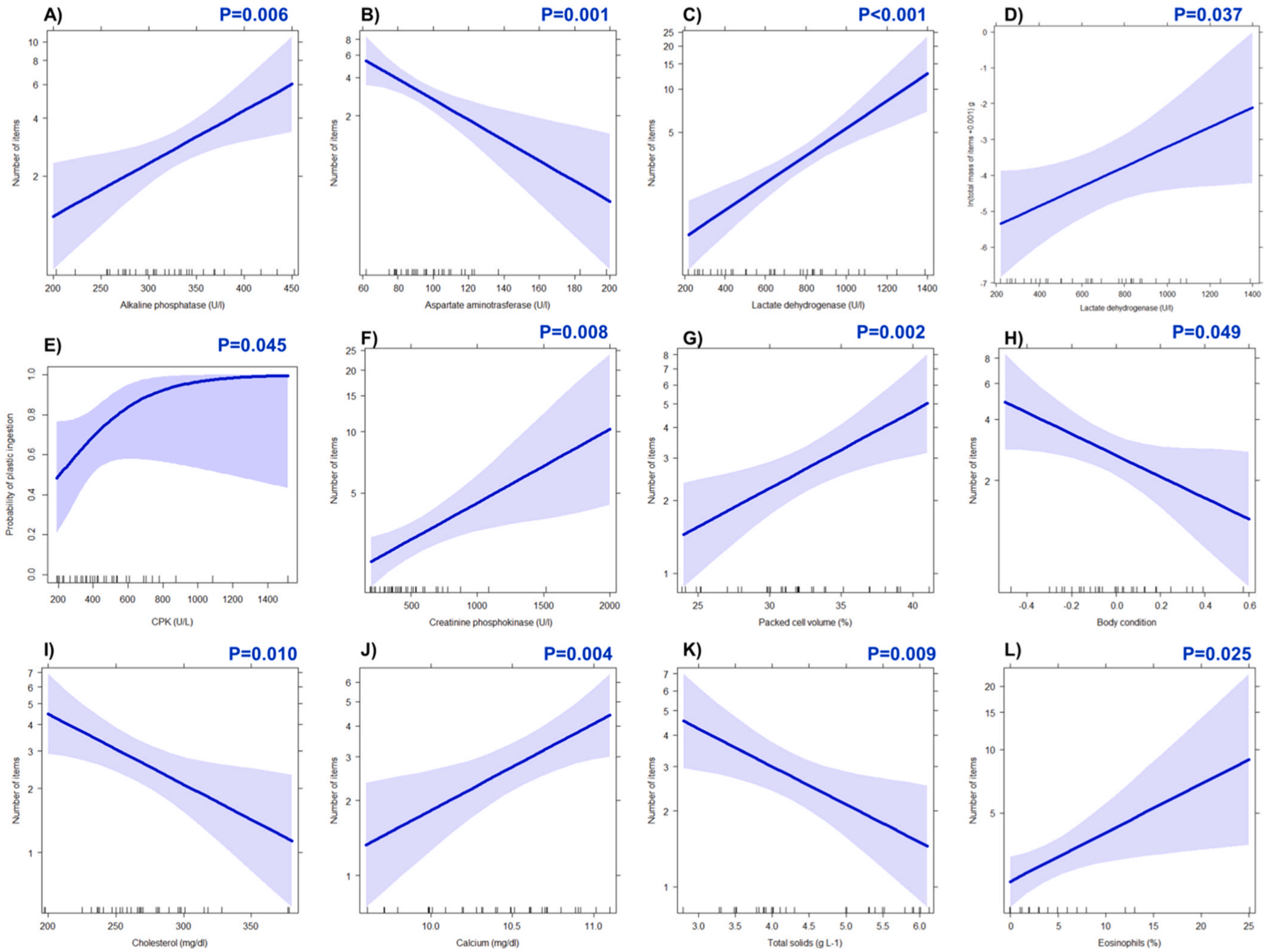


**Fig. 2.** Significant relationships between physiological parameters indicating liver function (A-D), muscle catabolism (E-F), general body condition (G-H), nutritional status (I-K), immune function (L), and plastic ingestion metrics (presence/absence, number, and ln-transformed total mass of items) in southern giant petrel (*Macronectes giganteus*) young chicks (~30-days-old). Blue lines and shaded areas represent best-fitting model-predicted means and 95% confidence intervals. Black rugs along the margins of the plots represent the data distribution used to produce the model. Plastic abundance (number of items) was modeled using generalized linear models with a Poisson error distribution, plastic occurrence (presence/absence) with binomial response (logistic regression), and ln-transformed plastic mass with linear models. We added 0.001 g to the total plastic masses to account for zeroes before ln-transformation. Year was included as covariable for models with no. items.

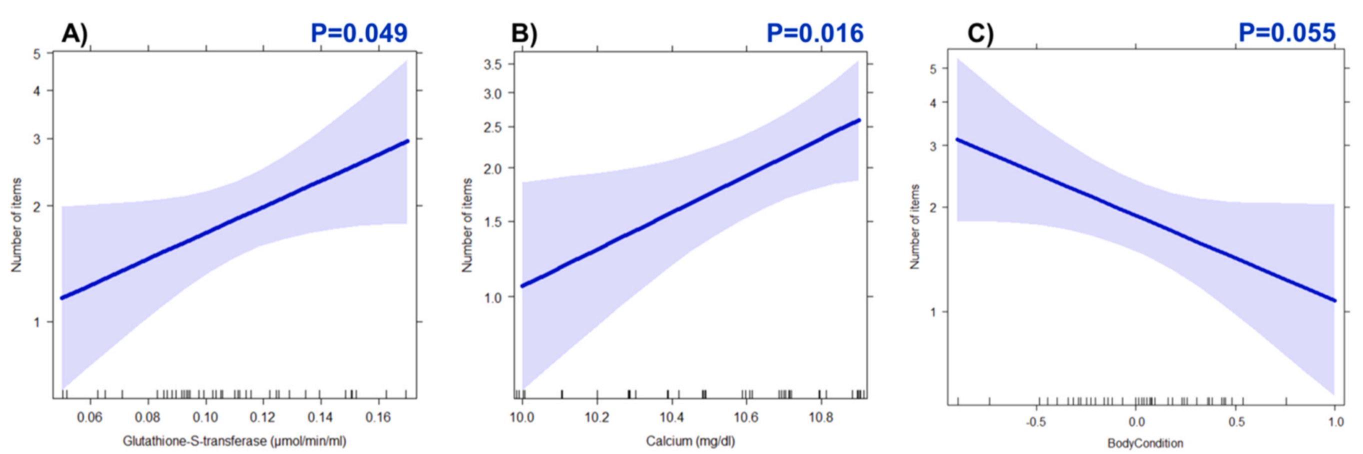


**Fig. 3.** Significant relationships between glutathione-S-transferase (GST) activity (A), calcium levels (B), body condition index (C), and plastic ingestion (number of items) in southern giant petrel (*Macronectes giganteus*) pre-fledging chicks (~90-days-old). Blue lines and shaded areas represent best-fitting model-predicted means and 95% confidence intervals. Black rugs along the margins of the plots represent the data distribution used to produce the model. Plastic abundance was modeled using generalized linear models with Poisson error distribution.

an age-related shift by fledging, with cholesterol and total solids increasing and lactate dehydrogenase decreasing significantly by fledging (Tables 2–3).

## 4. Discussion

Our results confirm that plastic ingestion is a common threat to SGP chicks, as most are fed plastic by their parents throughout the rearing period (Copello and Quintana, 2003; Copello et al., 2008). We found significant associations between plastic ingestion and several biomarkers and blood parameters, particularly in young chicks, indicating that ingested plastics may be linked to measurable physiological alterations during early development. However, these associations were not uniform across developmental stages and weakened or disappeared by fledging, suggesting that responses to plastic ingestion may vary with age, growth demands, metabolic activity, nutritional buffering, and/or detoxification capacity. These findings should be interpreted with caution, since plastic items recorded at sampling provide an estimate of current gastrointestinal burden, whereas biomarkers and blood parameters may integrate prior exposure, recent nutritional status, developmental stage, and cumulative physiological stress. Because plastic retention times and the timing of physiological responses remain unknown in this species, delayed or cumulative effects cannot be excluded. Further research is needed to identify the mechanisms underlying these age-related responses and to determine whether early physiological alterations have long-term consequences for health, survival and recruitment. This is particularly relevant because experimental evidence from other birds suggests that some effects of plastic ingestion may be reversible by adulthood (Roman et al., 2019a), whereas comparable longitudinal evidence is lacking for seabirds.

A large proportion (71%) of chicks in this study were fed plastic items by their parents, matching records from the same population two decades ago (Copello and Quintana, 2003; Copello et al., 2008). Although the frequency of plastic ingestion in SGP chicks appears to have remained stable over the past two decades, this indicator alone may not fully capture changes in plastic exposure. Changes in plastic mass, item composition, polymer type, and colour may occur even when frequency of occurrence remains relatively constant, providing a more sensitive indication of shifts in exposure intensity and, importantly, composition (Provencher et al., 2017). Indeed, only limited interannual variation in plastic ingestion metrics was observed during the present study, yet some differences in item size, colour, and plastic categories were detected between years (Table S1). Thus, non-invasive monitoring via collection of boluses post-fledging at breeding colonies, coupled with characterization of plastic composition (item type, colour, mass, and polymer identity through FTIR spectroscopy), can provide a practical approach for tracking long-term trends in plastic ingestion and changing risks to this SGP population (Bond et al., 2021). This is particularly important given projected increases in global and South-West Atlantic plastic pollution (Ryan et al., 2019; Eriksen et al., 2023) and the overlap between SGP foraging areas and plastic accumulation zones (Blanco et al., 2022).

Based on the confirmed high exposure to plastics, for the first time, we evaluated the association between biomarkers, physiological parameters and plastic ingestion in SGP chicks under parental care. The dose-response and time-effects of plastics on avian biomarkers of toxicity and health parameters are not well understood (Chambers et al., 2002; Hagger et al., 2006). Recently, Puskic et al. (2025) building on previous studies (Bond and Lavers, 2011; Lavers et al., 2021), suggested that a plastic load of at least 1–3% body mass can reduce body condition in pre-fledgling shearwaters. Conversely, Colvin et al. (2025) suggested that negative effects on body condition may occur at considerably lower plastic loads (~ 0.1% of mean fledgling body mass) in the same species. However, both studies were based mainly on dead birds prior to fledging, limiting comparisons with our assessment of "apparently healthy" living chicks at two developmental stages. Of note, plastic loads estimated from regurgitates represent conservative estimates of exposure rather than total retained plastic burdens (Provencher et al., 2019). Despite this limitation, mean plastic mass represented only 0.0088% of chick body mass across age classes and years, approximately one order of magnitude lower than the threshold proposed by Colvin et al. (2025) as potentially detrimental. Nevertheless, plastic ingestion was detected at both developmental stages evaluated, and several biomarkers and parameters showed relationships with the number of plastic items ingested. Notably, plastic volume was not associated with any physiological parameter or biomarker, whereas plastic occurrence (presence/absence) and plastic mass showed only limited associations (Table S2). Whether these associations represent transient early-life physiological responses or are sufficient to affect long-term fitness remains uncertain and warrants further longitudinal investigation.

Associations were particularly noticeable in younger chicks, in which parameters related to metabolism (enzymes aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine phosphokinase), nutritional status and general condition (cholesterol, total solids, packed cell volume, calcium, body mass and body condition index), as well as immune response (eosinophil percentage), showed significant relationships especially with the number of plastic items ingested (Fig. 2, Table S2). Such findings suggest that these parameters may serve as early indicators of sub-lethal effects of plastic ingestion in SGP chicks under 30-days old. Our results are consistent with those obtained in fledging Flesh-footed shearwaters (*Ardenna carneipes*) on Lord Howe Island, Australia, by Lavers et al. (2014, 2019). They showed a significant relationship between ingested plastic (FO > 80%, comparable to our findings in SGP, yet with higher average total mass and number of items, ie., 2.7 g and 17 items, respectively; see Table 1) and some nutritional status and condition parameters (i.e., cholesterol, uric acid, calcium, body mass, and body size). In shearwaters, these associations may arise because parents ingest more plastic and low-quality food during early chick-rearing, when the pressure to feed their chicks almost daily is highest, resulting in the delivery of smaller and/or less nutritious meals and ultimately affecting chick nutritional status and growth (Puskic et al., 2025). Accordingly, in SGP variation in prey nutritional composition (e.g., lipid and protein content) may also have contributed to some of the physiological variation observed among chicks and represents a potential confounding factor that was not directly evaluated in this study (Roman et al., 2019c; Puskic et al., 2025; Colvin et al., 2025). Variation in parental provisioning and chick diet can influence chick growth and physiological condition in seabirds and may therefore confound or buffer associations between plastic ingestion and health parameters (Kitaysky et al., 2006; Ibarra et al., 2022). In SGPs, available information from the early 2000s indicates no variation in prey composition during chick development (Copello et al., 2008); however, potential age-related changes in provisioning rate, meal size, or energetic quality remain unknown. Future studies using non-invasive remote monitoring such as high-resolution cameras could quantify parental provisioning and attendance patterns, thereby helping disentangle parental compensatory mechanisms from physiological responses potentially associated with plastic ingestion.

In extreme situations, reduced meal quality or dietary dilution associated with plastic ingestion (i.e., non-nutritious plastic items physically occupy gastrointestinal space, limiting the actual volume available for real food) could lead to malnutrition and starvation in chicks, supporting an indirect negative plastic-nutrition relationship (Roman et al., 2020c). In our study, outlier values of plasma uric acid and glucose (parameters often associated with fasting in birds; see Alonso-Alvarez and Ferrer, 2001) were observed at first sampling in three chicks (Figure S1). All had ingested plastics, and two of them died before reaching the pre-fledging stage. However, these and the other non-surviving young chicks did not differ significantly from survivors in plastic ingestion or in any of the other haematological, biochemical, or morphometric parameters evaluated. For context, these outlier values still fell within the reference range previously reported for apparently healthy adult SGP, in which plastic ingestion was not assessed (Uhart et al., 2003). Nevertheless, age-related variation in haematological and biochemical parameters has been documented in several avian species, and reference values for SGP chicks are currently unavailable. In addition, chick body mass in this study was also comparable to that recorded in previous breeding seasons, when plastic ingestion was monitored but physiological parameters were not evaluated (Copello and Quintana, 2009). Together, these observations suggest that biologically relevant responses may occur in a subset of individuals without producing detectable differences between age-groups. Future studies with larger sample sizes and repeated assessments of individuals may help better characterize this individual variability and identify subtle responses that could otherwise remain undetected.

While variation in physiological parameters in seabirds can be multifactorial, some biomarkers can still indicate toxic exposure (Prokić et al., 2019). In this study, biomarkers of toxicity and antioxidant defense (i.e., the enzymes AChE, BChE and GST, respectively) showed subtle associations with the number of plastic items ingested in younger chicks, although none remained significant when inter-annual variation in plastic abundance was taken into account. This pattern suggests that multiple concurrent environmental stressors not measured in our study, including possible co-exposure to other contaminants (Serafini et al., 2024; Xu et al., 2025), may have masked any plastic-specific enzymatic response. Additionally, as detoxification pathways are still developing in young chicks, enzyme activities may exhibit transient or non-specific fluctuations, reflecting physiological immaturity rather than a direct toxic effect. Nevertheless, the responses of these biomarkers to plastic ingestion and possible exposure to associated contaminants remain poorly understood in wild species, underscoring the need for further research. Controlled *in vitro* studies using plasma samples or hepatic tissue extracts exposed to environmentally relevant plastic-associated chemicals could help validate the responsiveness and specificity of these or other biomarkers (Solé et al., 2021, 2022a, 2022b; Omedes et al., 2022).

Overall, rather than overt toxicity, our results revealed associations between plastic ingestion and nutritional parameters of young chicks which weakened as chicks developed. Although chicks were fed plastic by their parents at both developmental stages evaluated, and the relative plastic mass to body mass ratio remained stable, only GST activity and plasma calcium levels were associated with the number of plastic items ingested at fledging. Moreover, some physiological parameters indicative of nutritional status and metabolism that showed negative relationships with plastic ingestion in younger chicks, reverted at pre-fledging (cholesterol and total solids increased, while lactate dehydrogenase decreased). Likewise, other parameters reflecting good nutritional status and metabolism (e.g., triglycerides, cholesterol, total protein, albumin, and glucose) also increased by the time of fledging compared with values at 30 days of age, suggesting an overall improvement in chick condition as development progressed. This pattern may reflect compensatory/regulatory responses that modulate the health of chicks and/or physiological ontogenetic changes during development. In addition, because regurgitates and physiological parameters may reflect different temporal windows of exposure and response, the mechanisms underlying these associations remain difficult to disentangle.

In summary, GST activity and plasma calcium levels were the only variables that showed association with plastic ingestion across both age classes, although the strength and direction of these associations differed between developmental stages. GST plays a crucial role in detoxification and neutralization of reactive oxygen species (ROS), which are harmful by-products generated under conditions of oxidative challenge (Walker et al., 2012; Attademo et al., 2021). In SGP chicks, GST exhibited contrasting patterns between age classes, showing a weak negative relationship with number of plastic items ingested by young chicks (a trend that became significant when removing "year" as a covariate) whereas GST activity increased slightly at fledging. This pattern may indicate a shift from transient enzymatic inhibition to compensatory up-regulation with age and/or chronic exposure to plastics. Increases in GST activity are commonly interpreted as a compensatory antioxidant response in marine organisms exposed to pollutants in experimental settings, including microplastics and plastic-associated chemicals (Prokić et al., 2019; Roman et al., 2019a). Thus, the higher GST activity observed in fledging chicks with more ingested plastic items may reflect activation of antioxidant pathways under continued plastic exposure. Likewise, calcium has also been linked to oxidative balance; experimental studies in passerine nestlings show that higher calcium availability can enhance antioxidant defenses, stimulating enzymes such as GST and modulating ROS dynamics (Espín et al., 2017; Sánchez-Virosta et al., 2019). Although GST activity and plasma calcium levels were not correlated with each other within age classes in this study, the concurrent positive associations between GST activity and plasma calcium levels with plastic ingestion may reflect complementary physiological adjustments associated with moderate oxidative challenge. However, mechanistic interpretation remains limited because direct oxidative stress markers were not measured in this study. These findings underscore the need to incorporate such indicators (e.g., reactive oxygen metabolites, antioxidant capacity assays) in future assessments to confirm whether plastic ingestion contributes to redox imbalance in SGP chicks. Although GST variation has been reported across life stages in aquatic organisms (Tierbach et al., 2018), comparable information is lacking for seabirds. A recent study on juvenile Manx Shearwaters (*Puffinus puffinus*) stranded dead along the Brazilian coast reported no significant association between plastic ingestion (FO = 29%, lower than in SGPs) and molecular and biochemical toxicity biomarkers, including GST (Serafini et al., 2024). Interpretation of GST responses in free-living birds remains challenging due to confounding intrinsic factors (species-specific detoxification mechanisms, ontogenic changes in enzyme activity, and potential dose-time-dependent responses) as well as extrinsic factors (environmental variability, co-exposure to other pollutants) all of which are difficult to control in wild populations (Prokić et al., 2019; Santos et al., 2019; Serafini et al., 2024). Notwithstanding, our results raise the possibility that physiological adjustments occurring during development may help buffer some of the associations between plastic ingestion and health indicators observed at earlier developmental stages.

Physiological responses observed in this study may not only reflect the ingestion of plastic particles but also exposure to plastic-derived chemicals (Tanaka et al., 2013; Rochman, 2016; Shigeishi et al., 2025). Direct evidence shows that additives can leach from ingested plastics and accumulate in seabird tissues (Tanaka et al., 2020); while the contribution of plastics as vectors of environmental pollutants (e.g., POPs) appears negligible compared with dietary uptake (Ryan et al., 1988; Tanaka et al., 2013; Herzke et al., 2016; Provencher et al., 2018; Tanaka et al., 2018; Roman et al., 2020a). Nonetheless, *in vitro* experiments indicate that stomach oil can significantly enhance the leaching of highly-chlorinated PCBs from ingested plastics in petrels (Van Hassel et al., 2024). These findings underscore that quantifying ingested plastics alone provides an incomplete picture of risk. Because adsorption and leaching dynamics largely depend on polymer type (Jiménez-Skrzypek et al., 2021), and polymer composition was not determined in the present study, future studies should integrate polymer characterization with assessment of plastic-associated chemicals (both additives and adsorbed onto plastics) to better understand the mechanisms underlying physiological and biomarker responses. More broadly, integrating biomarkers from multiple physiological pathways, contaminant burdens, omics approaches (including transcriptomics and proteomics), polymer characterization, and long-term demographic monitoring will be necessary to identify sensitive and reproducible indicators of sub-lethal effects of plastic exposure in seabirds (reviewed by Serafini et al. 2026).

## 5. Conclusions

This study shows that plastic ingestion remains widespread among SGP chicks and was associated with several biomarkers and physiological parameters, particularly during early development. Although plastic loads were low relative to body mass, the observed associations suggest that even moderate early-life exposures may be linked to subtle physiological variation. Most relationships weakened or disappeared by fledging, indicating that responses may be stage-dependent and potentially influenced by growth, nutritional status, compensatory mechanisms, or differences between the timing of plastic ingestion and measurable physiological responses. Within this framework, GST activity and plasma calcium warrant further evaluation as complementary parameters in broader multi-indicator approaches to detect sub-lethal effects of plastic exposure in seabirds.

The long-term significance of these early-life associations remains unresolved. Future studies should combine repeated health assessments, plastic characterization, contaminant analyses, and post-fledging monitoring to determine whether early-life plastic exposure affects survival, recruitment or fitness.

## CRediT authorship contribution statement

**Luciana Gallo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Flavio Quintana:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Andrés M. Attademo:** Writing – review & editing, Methodology, Investigation, Data curation. **Marcela M. Uhart:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Conceptualization.

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## Permits

This study was conducted under permits from the Administración de Parques Nacionales, Chubut (No. 1369), Dirección de Fauna y Flora Silvestre Provincia de Chubut (79/22-DFyFS-MAGIyC), Subsecretaría de Conservación y Áreas protegidas Provincia de Chubut (76/22-SsCyAP). This study was approved by the Institutional Committee for the Use of Experimental Animals of CCT-CENPAT, CONICET (CICUAE Protocol No. 005/21).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2026.120628.

## Data Availability

Data will be made available on request.

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