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Research Paper

The one-two punch of plastic exposure: Macro- and micro-plastics induce multi-organ damage in seabirds

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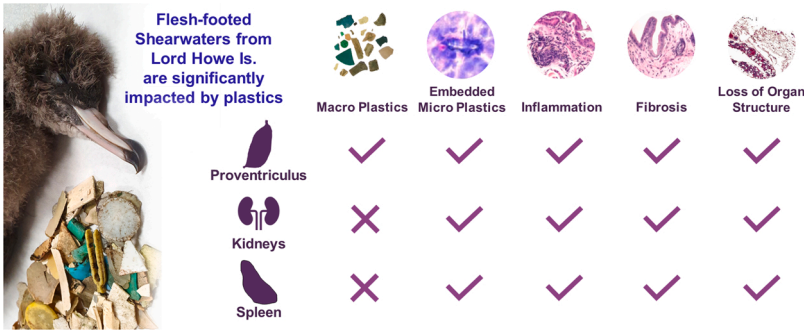
HIGHLIGHTS

- All organs examined (kidney, spleen, proventriculus) had embedded microplastics.
- Microplastics in tissues was correlated with macroplastics in the proventriculus.
- Plastic exposure led to considerable tissue damage.
- Tubular glands and rugae were significantly reduced in the proventriculus.
- Inflammation, fibrosis and loss of organ structure observed in both kidney and spleen.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic pollution in the world's oceans is ubiquitous and increasing. The environment is inundated with microplastics (< 1 mm), and the health effects of these less conspicuous pollutants is poorly known. In addition, there is now evidence that macroplastics can release microplastics in the form of shedding or digestive fragmentation, meaning there is potential for macroplastic exposure to induce direct and indirect pathology through microplastics. Therefore, there is an urgent need for data from wild populations on the relationship between macro- and microplastic exposure and the potential compounding pathological effects of these forms of plastics. We investigated the presence and impact of microplastics in multiple tissues from Flesh-footed Shearwaters *Ardenna carneipes*, a species that ingests considerable quantities of plastics, and used histopathological techniques to measure physiological responses and inflammation from the plastics. All organs examined (kidney, spleen, proventriculus) had embedded microplastic particles and this correlated with macroplastic exposure. Considerable tissue damage was recorded, including a significant reduction in tubular glands and rugae in the proventriculus, and evidence of inflammation, fibrosis, and loss of organ structures in the kidney and spleen. This indicates macroplastics can induce damage directly at the site of exposure, while microplastics can be mobilised throughout the body causing widespread pathology. Collectively, these results indicate the scope and severity of the health impacts of plastic pollution may be grossly underestimated.

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1. Introduction

In the oceans, plastic pollution has increased tenfold since 1980, and without global intervention, the quantity entering the oceans is predicted to be 100–250 million tons annually by 2025 (Jambeck et al., 2015). Current measures at controlling plastics in the environment are inadequate to stem the tide of marine pollution (Borrelle et al., 2020). Plastics pollution, and the associated chemicals, already exceed planetary boundaries (Cousins et al., 2022; Persson et al., 2022), while the toxicity debt, or future impact of plastics used today, are unknown but large (Rillig et al., 2021). Debris contributes to ecological impacts with ingestion of items adding to negative health consequences for humans and wildlife, including cellular-level responses such as inflammation, oxidative stress, altered blood chemistry, and apoptosis (Canesi et al., 2015; Hu and Palić, 2020; Lavers et al., 2019). Up to 400 million tons of heavy metals, solvents, toxic sludge, and other industrial wastes also enter the world's waters annually, including many that are bioaccumulative and toxic, endocrine-disrupting or contribute to neurodevelopmental effects (Encarnação et al., 2019; Kim et al., 2018). Despite this, the global chemical industry's production capacity has nearly doubled in the past two decades (Swift, 2012; UNEP, 2021) and our understanding of consequences to chemical exposure are hampered by a lack of testing for "unknown unknowns" (Sobek et al., 2016).

Rather than being considered a single pollutant, "plastics" encompass numerous polymers and their associated additives with varying degrees of environmental degradation, compounding, recycling, and environmental exposure resulting in highly heterogenous products (Hahladakis et al., 2018; Rochman et al., 2019). Consequently, their effects and organisms' responses cannot be approached in a traditional dose-response framework typically applied to single contaminants (Safe, 1998). And unlike chemical pollutants that have one mode of contamination (e.g., blood absorption of mercury or inhalation of $PM_{2.5}$), ingested plastics have numerous modes of potential harm (from physical damage to genotoxicity) and different scales of action (from the nm to cm scales) across different tissues throughout the gastrointestinal tract (and beyond) where plastics have varying residence times (Ryan, 2015). While this huge complexity has been recognised in the polymer composition of plastics (Rochman et al., 2019), it hasn't yet been a focus of studies looking at modes of harm induction. This may explain, in part, why research into the effects of macroplastic ingestion often produces equivocal results (e.g., Lavers et al., 2022a, 2022b; Puskic et al., 2019). Given these challenges, and absence of appropriate monitoring and reporting mechanisms (Provencher et al., 2017, 2019, 2020), there is an urgent need to document risk and harm posed by the ever-increasing volume of plastic pollution (Lavers et al., 2022a, 2022b).

Seabirds offer an excellent opportunity to investigate ingestion as many species are large, long-lived, site faithful, and can be readily accessed (Burger and Gochfeld, 2004). However, most studies have focused on investigating the presence/absence of debris items and we must move beyond simply documenting towards understanding the effects of plastics exposure on individuals and populations (Lavers et al., 2021; Provencher et al., 2017). For example, countless studies have demonstrated small quantities of ingested plastics in diverse seabird species (Cartraud et al., 2019; Ryan, 2008), while others have consistently highlighted that only a handful of species are heavily impacted (Lavers et al., 2021; van Franeker and Law, 2015). In contrast, a single study of seabird blood chemistry suggests the ingestion of one plastic item may lead to detrimental consequences (Lavers et al., 2019). Although two studies have investigated the physiological consequences of plastic ingestion in a laboratory setting (Roman et al., 2019; Ryan, 1988), both focused on seabird surrogates (i.e., poultry). These physiological studies also used plastic shapes/types or concentrations that were not environmentally relevant and used naïve (virgin) plastics that had not been exposed to the marine environment (e.g., no biofilm, no UV degradation). Finally, most of the available research currently assumes that for plastic exposure to matter, animals need to consume relatively large quantities. However, new data suggests exposure to micro- and nano-particles is not related to the number or mass of macro-plastics recorded in bird stomachs with species containing small numbers of visible (macro) plastics having elevated concentrations of very small particles (Keys et al., 2022; Mylius et al., 2022).

Small particles (i.e., micro- and nano-plastics; < 1 mm) are likely to result in detrimental effects, most of which have not yet been documented in wildlife, as they can enter the blood stream, placenta, and lungs in humans where they contribute to disruption of regulatory pathways (Leslie et al., 2022; Pauly et al., 1998; Ragusa et al., 2021). Once circulating in the blood, particles may also be transferred to the liver, kidney and other organs involved in detoxification, and once embedded, may lead to inflammation, scarring or other tissue damage. Thus, identifying and recording sublethal effects is vitally important for understanding the extent of the impact plastic debris has on biota. Histopathology is a common method for documenting the health and condition of tissues, yet few studies have applied this technique to better understand sublethal effects from plastic exposure. A handful of controlled experiments have documented a range of histological maladies in fish and rodents exposed to plastics under laboratory conditions, including necrosis and inflammation of the liver, reproductive, digestive, and respiratory tissues (Amereh et al., 2020; Hamed et al., 2022; Hsieh et al., 2021; Xia et al., 2020). In many instances, risk of harm was directly related to the dose and size of plastic particle, with smaller particles in higher concentrations causing the most tissue damage. However, no data are available for wild, free-living populations.

Here we investigate the pathological impacts and tissue responses to late-stage lethal plastic exposure through histology of tissues collected from free-living seabirds. Specifically, we examined the physiological response of seabird gastrointestinal tissue to the presence of ingested macro- and microplastics to determine the effects and potential consequences of particle size and location within the body. Finally, we compare our findings for plastic with the ingestion of other hard, indigestible but naturally occurring items to demonstrate the stomach's inability to cope with this diverse and harmful class of particles.

2. Materials and methods

2.1. Field collection

Fledgling Flesh-footed Shearwaters (*Ardenna carneipes*) were collected from Lord Howe Island, Australia (31.53°S, 159.07°E). Birds were euthanised under permit after an unsuccessful fledging attempt, having been nest-bound and provisioned by both parents for the preceding ~ 90 days. Flesh-footed Shearwaters have high rates of plastic ingestion (Lavers et al., 2021), and severely affected birds are unable to regurgitate plastics prior to departing for the sea (Bond et al., 2021). Deceased birds were processed within five minutes of euthanasia.

Birds used for this study were randomly selected on a gradient of plastic exposure, ensuring that there was a selection of birds that had not ingested any plastics (i.e., control birds; $n = 8$). We weighed and measured birds as described in Lavers et al. (2021), namely measuring mass using a spring scale (± 5 g), flattened wing chord with a stopped ruler (± 1 mm), and exposed culmen (± 0.1 mm) and head+bill (± 0.1 mm) using Vernier callipers.

Plastic items > 1 mm recovered from the gizzard and proventriculus (kept separate; hereafter "macroplastics") were washed, dried, and weighed to the nearest 0.0001 g using an electronic balance. Items were then sorted by type following the categories outlined by Provencher et al. (2017, industrial pellets, user plastic, foam, threads, sheet plastic, and other), except for plastic strapping, which more closely resembled plastic fragments in our study system.

2.2. Necropsy and tissue preparation

In the research lab on Lord Howe Island, approximately 1 cm^2 each

of kidney, spleen, and proventriculus (inferior to the cardiac sphincter) tissue was removed from each bird via necropsy. Stainless steel scissors and scalpels were washed with 70 % ethanol between each use and paper and glass were used whenever possible to prevent contamination.

Each dissected proventriculus was photographed in the field using a Samsung S10 mobile phone using the macro setting, and the number of rugae across the widest section (inferior proventriculus) or at the proximal end to the cardiac sphincter (superior proventriculus) were counted along a superimposed straight line and converted to the number of rugae/cm. They were then weighed on an electronic balance (± 0.01 g), dried for 72 h at 60 °C, and weighed again to obtain the moisture content.

All tissues were washed in phosphate buffered saline and fixed in 4 % paraformaldehyde (0.1 M phosphate buffer + 4 % paraformaldehyde) for 24 h before storing in phosphate buffer for transport to the histology lab at the University of Tasmania School of Medicine.

2.3. Histology

Tissues were mounted in paraffin, sectioned using a microtome to 5 μm thickness, and mounted on glass slides. Slides were deparaffinized in two 10-minute washes of xylene and hydrated with decreasing concentrations of ethanol (two 5-min washes in absolute ethanol, one 2-minute wash in 95 % ethanol, and one 2-minute wash in 70 % ethanol), and then briefly washed with distilled water. They were then stained for 1 min with haematoxylin and placed under running tap water for 5 min, differentiated in 1% acid alcohol (0.5 % HCl and 70 % ethanol) for 30 s followed by 1 min under running tap water. Next, they were blued with 0.2 % ammonia water or saturated lithium carbonate solution for 30–60 s and washed in running tap water for 5 min followed by 10 brief immersions in 95 % ethanol. They were then counterstained with eosin phloxine B solution for 3 min, dehydrated with three washes of ethanol (one at 95 % and two in absolute ethanol) for 5 min each before final fixing in two 5-minute immersions in xylene and mounting with a xylene-based mounting medium.

Slides were examined using a Zeiss Axiolab 5 (Carl Zeiss AG, Oberkochen, Germany) with an Axiochem 506 colour. Images were taken on Axiovision LE64 at 10 × and 20 × magnification. When embedded microplastics were detected, images were taken at 40 × magnification.

For sections of the proventriculus, we counted and measured the tubular glands in 20 × images using ImageJ (Collins, 2007), resulting in a mean (± SD) number and tubular gland depth (μm) for each individual bird (Fig. 1d).

We examined 2–3 different sections per tissue per individual and quantified the number of embedded microplastics/μm^2. The pathology (fibrosis, loss of histological structure and inflammation) of each slide was then scored qualitatively from 0 to 5, with 5 being the most severe (Fig. S1 and Table S1). All slides were scored by one observer (JRA) who was blind to the plastic contents measured in the field.

Microplastic identification was performed microscopically. Particles were identified visually using the following criteria: 1) the particle must be in the focal plane of the tissue, 2) the particle must not appear organic (e.g., no internal complexity, consistent appearance through focal planes, consistent high light refraction around the border of the particle, inorganic colour – for example, consistent red across the particle, and inorganic shape – for example, high eccentricity and low complexity such as a microfibre), 3) the particle must appear to have been embedded in living tissue (i.e., not introduced during processing) and have induced a response visible in the histological section such as particulates surrounded in fibrotic tissue, inflammation or a granuloma structure, 4) the particle must not be stained, as haematoxylin and eosin do not stain non-polar substances such as plastic. Particles identified in this way are hereafter referred to as microplastics, however, we acknowledge that this method has limitations including an unknown rate of false negatives and false positives.

2.4. Statistical analysis

We analysed the relationships between bird morphometrics, macroplastics (total mass), and tissue responses (percent water content, inferior and superior proventriculus rugae/cm, proventriculus tubular gland count and depth) using multi-level general linear models with individual as a random variable using the lme4 package (Bates et al., 2015). To model causal relationships between macroplastics, microplastics, and tissue pathology a mediation analysis was performed with macroplastic as the primary explanatory variable, microplastic as the mediator variable and tissue pathology as the dependent variable. This analysis was performed with the mediation packages utilising the bootstrap method with 1000 simulations (Tingley et al., 2014). For all analyses, residuals were evaluated for normality and homoscedasticity graphically with Q-Q plots and residual vs fitted plots, respectively. In the event of the violation of these assumptions a Box-Cox transformation was performed utilising the MASS package (Venables and Ripley, 2002). All analyses were conducted in R 4.0.5 (R Core Team, 2022) in RStudio 1.4.1106, and effects were considered significant when $p < 0.05$. Detailed results and statistical outputs are provided in the Supplementary information.

3. Results and discussion

We found pathologically significant tissue- and cellular-level sublethal effects of ingested plastics in free-living seabirds. Birds with ingested plastics had a higher inflammatory response, greater deterioration of the stomach lining, higher tissue damage scores across multiple organs, and a greater density of embedded micro- and nano-plastics in the proventriculus, spleen, and kidneys, compared to birds with little to no ingested macroplastics. Together, these demonstrate the negative and insidious impacts of plastic pollution on wildlife that have hitherto gone unnoticed.

We observed significant inflammation of the proventriculus in birds that had ingested plastic, particularly the inferior region where most plastics are located (Fig. 1). This presented as oedema, erythema (redness) and the loss of tissue structure on the epithelial surface of the proventriculus (Fig. 1c-iii). Oedema was quantified through water content and plastic presence was associated with significantly higher water content in both the superior and inferior regions of the proventriculus (Fig. 1a). This response was proportional to plastic load (g; Fig. 1d). It seems likely this inflammation was due to damage caused by the presence of sharp or obtrusive plastics that physically damaged the epithelial surface of the proventriculus, leading to significantly increased water content, or swelling, in the surrounding tissues (Fig. 1a, d). However, other large naturally occurring items which are commonly consumed by Flesh-footed Shearwaters, and other seabirds, such as pumice and squid beaks, were not associated with swelling (Fig. 1c-ii and d), suggesting there are unique pathological properties of plastic items.

Ingested plastic also resulted in a significant loss of rugae (Fig. 1b) which play a critical role in digestion and absorption of nutrients through increasing the surface area and allowing the expansion of the proventriculus (Gussekloo, 2006). The loss of rugae was extremely sensitive to plastic presence, with even a single piece of plastic inducing substantial loss (Fig. 1f). This suggests that this loss is not due to the plastic stretching the stomach, which is also caused by the ingestion of pumice and squid beaks (Hindwood, 1946), but does not induce the same loss in rugae (Fig. 1c-ii, e). Thus, rugae loss is not a general response to large hard items but reinforces the idea that the responses demonstrated here are specific to plastics.

Macro-anatomical responses such as oedema, erythema, and rugae loss are often associated with changes at the cellular level (Whitehead et al., 1972). Therefore, we performed histological analyses utilising H&E (haematoxylin and eosin) staining. From this we observed significant loss of tubular gland depth (μm) and count (per μm) in the plastic

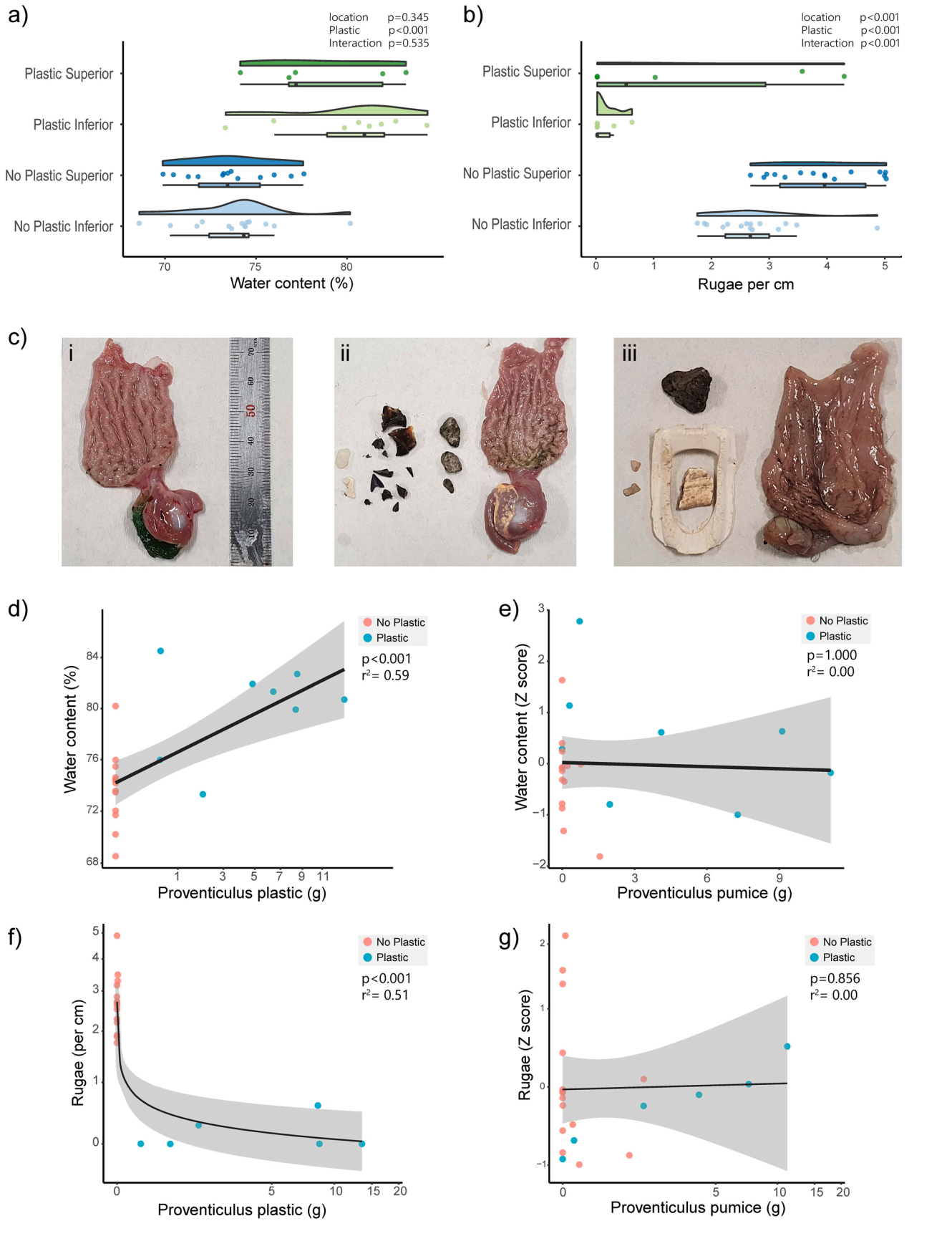

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Fig. 1. Plastic in the proventriculus is associated with inflammation and changes in organ morphology in the Flesh-footed Shearwater. Water content within the proventriculus was significantly higher in birds with plastic present in both the superior and inferior regions of the tissue (**a**); this increase in water content was proportional to mass of plastic (grams) found in the proventriculus (**d**) and was not observed with the natural particulate matter of ingested pumice after adjusting for plastic content (**e**). Water content was quantified through wet weight – dry weight. There was a significant loss of rugae in the proventriculus in plastic exposed birds most notably in the inferior region of the proventriculus (**b, c**), the loss was proportional to mass of plastic found in the proventriculus (**f**) and was not observed with the natural particulate matter of ingested pumice after adjusting for plastic content (**g, c–i**). Data was analysed with multi-level linear modelling with bird ID as the random variable. (**a, b**) Shown are rain cloud plots with box plot (median and interquartile ranges). (**d, f**) Box-Cox transformed linear regression of water content and rugae per cm, respectively, with 95 % confidence interval (grey). (**e, g**) Linear regression of water content and rugae per cm, respectively, after adjusting for plastic content, with 95 % confidence interval (grey).

exposed animals (Fig. 2). This was particularly evident in the inferior region of the proventriculus where the plastic items would naturally settle. Tubular glands play an essential role in the production of mucus that protects the lining of the proventriculus, produce fluids that aid in digestion, and are critical to sterilisation of the digestive environment (e.g., protect the body against infection and parasites; Klasing, 1999).

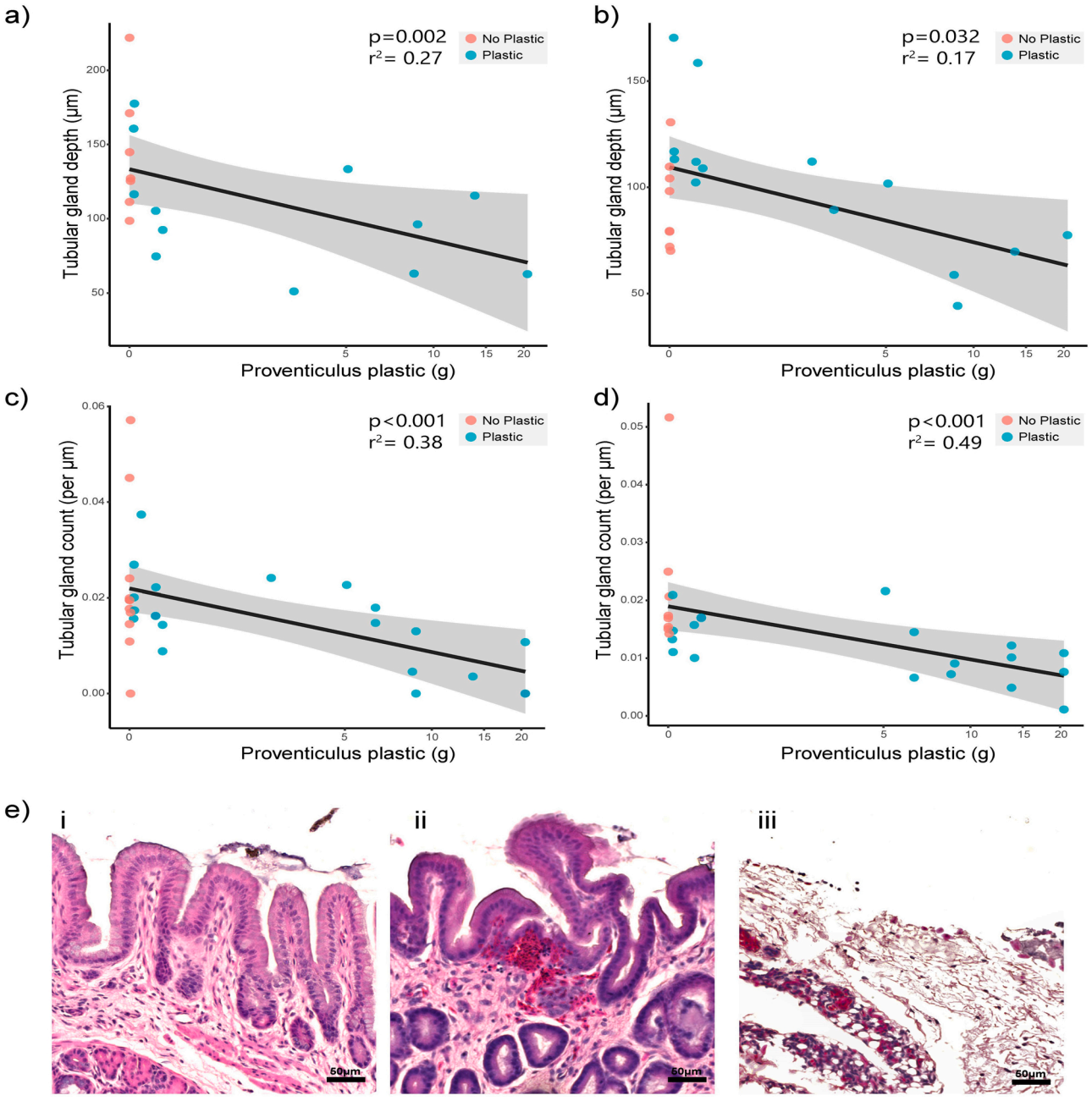

Fig. 2. Plastic ingestion is associated with tissue damage of the proventriculus including reduced size and number of the tubular glands. a and b) Proventriculus plastic burden (grams) is correlated with a significant loss of tubular gland depth (µm) in both the superior (**a**) and inferior (**b**) regions of the proventriculus. **c**) and **d**) Proventriculus plastic burden is correlated with a significant loss of tubular gland depth number in both the superior (**a**) and inferior (**b**) regions of the proventriculus. **e**) Example images of proventriculus histology in birds with low (**i**), medium (**ii**) and high (iii) plastic burden (**g**). (**a–d**) Box-Cox transformed linear regression with 95 % confidence interval (grey).

Shearwaters with moderate plastic burden (Fig. 2e-ii) had reduced tubular gland structure, while birds with high plastic burden showed almost no signs of tubular structures (Fig. 2c) and had extensive tissue fibrosis (Fig. 2e-iii). Loss of these structures would affect digestive fluid production which has been shown to influence nutrient absorption, in particular vitamin B12 (Wolf et al., 1950) and calcium (O'Connell et al., 2005). This provides one potential mechanism for our previous observation of a negative correlation between ingested plastic and plasma calcium concentrations (Lavers et al., 2019). Similar inflammatory responses have also been observed in the digestive tract of fish exposed to polyethylene microplastics (5–1000 μm; Lu et al., 2016; Varó et al., 2021). This suggests that inflammation and malabsorption is a robust pathology observed across clades and should be expected in all vertebrates and indeed humans with ingested plastics (Hernandez et al., 2019; Leslie et al., 2022; Samandra et al., 2022).

The mass of macroplastics located within the proventriculus was significantly correlated with the density of microplastics (particles/mm^3; Fig. 3a, e, i) in seabird tissues suggesting the mass of visible plastics recorded in the proventriculus can act as a proxy for the number of microplastics detected in the tissues (Fig. 3c, g, k). This result suggests that the visual method of microplastic identification in histological sections (see Methods) is valid for approximating microplastic exposure, as substantial levels of particle misidentification would prevent the discovery of the correlation with macroplastic exposure. Tissue was also scored by a blinded observer for pathology on a scale of 0–5, with the pathology score significantly correlated with proventriculus plastic mass (Fig. 3b, f, j; Fig. S1 and Table S1). Microplastic quantity in the tissues also correlated with the tissue damage score (Fig. 3c, k), however, this pattern was less obvious in the kidney (Fig. 3e). A mediation analysis to evaluate the relative contribution of macro- and microplastics to tissue damage, with the hypothesis that a proportion of tissue damage induced by macroplastics is through the release of microplastic following digestive fragmentation and shedding (Dawson et al., 2018; Ryan, 2015), revealed that macroplastics are likely the primary cause of

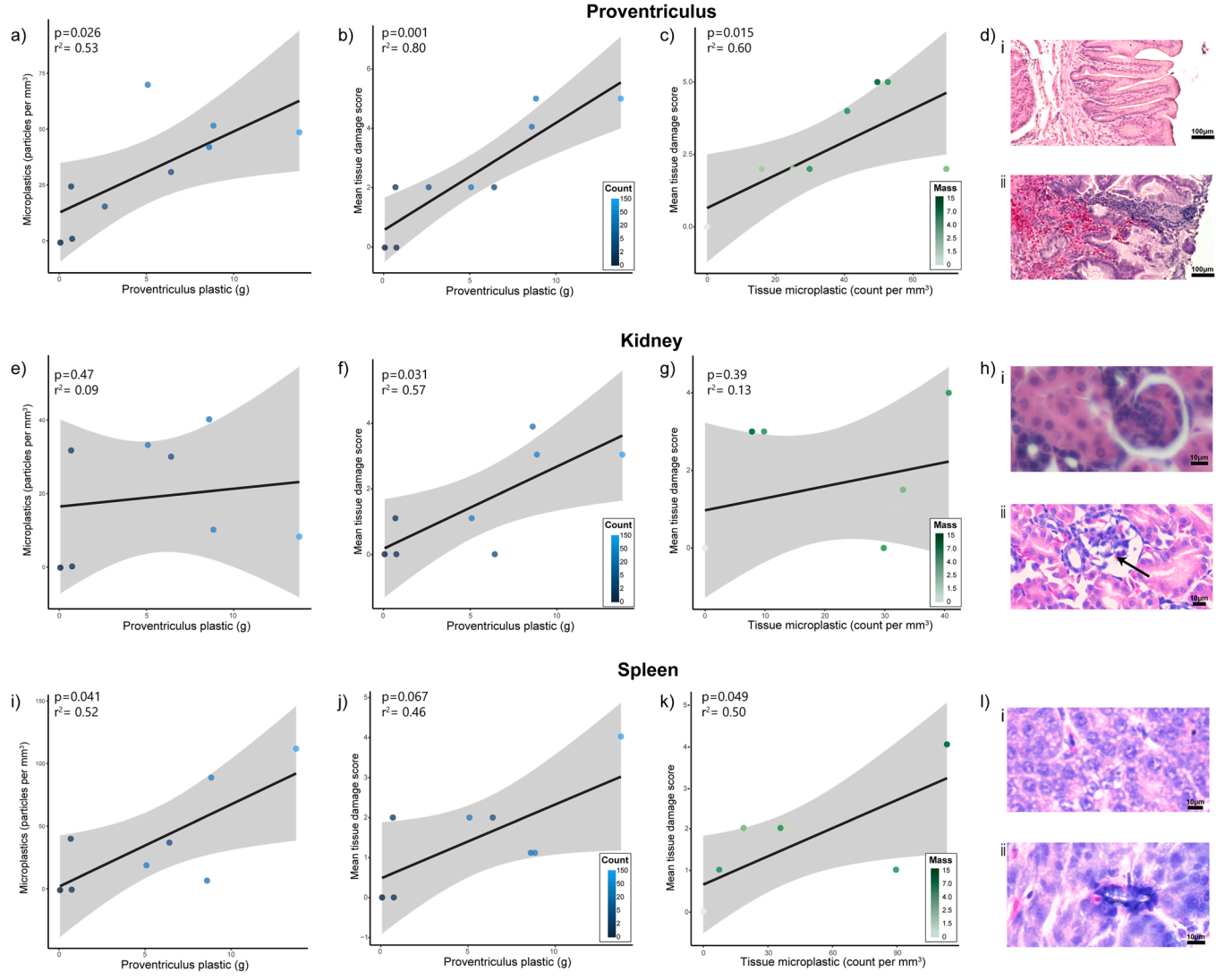

Fig. 3. The mass of proventriculus macroplastics (> 1 mm) correlates with organ microplastics (< 1 mm) and organ pathology. a, e & I) The correlation between the mass of proventriculus macroplastics (grams) with tissue microplastics identified through histology in the proventriculus **(a)**, kidney **(e)** and spleen **(i)**. **b, f & j)** The correlation between the mass of proventriculus macroplastics with mean tissue pathology score (0–5) in the proventriculus **(b)**, kidney (f) and spleen **(j)**. **c, g & k)** The correlation between the tissue microplastics with mean tissue pathology score (0–5) in the proventriculus **(c)**, kidney **(g)** and spleen **(k)**. **d, h & l)** Histological examples of healthy tissue **(i)** and tissue pathology **(ii)** in the proventriculus (**d**, inflammation and haemorrhage; scalebar 100 μm), kidney (**h**, collapsed glomerus around microplastic (arrow); scalebar μm) and spleen (**l**, inflammation surrounding microplastic; scalebar 10 μm). Shown are Box-Cox transformed linear regressions with 95 % confidence intervals (grey), black-blue coloured points displaying proventriculus macroplastic count, grey-green coloured points displaying proventriculus macroplastic mass (g).

proventriculus pathology (explains 81.5 % of the variation in the model; see Section 1.4 of the Supplementary information). This was supported by the macro-scale of the pathology with bleeding and scar formation commonly observed (Fig. 3d). In contrast, the mediation analysis indicated 43 % of the association of macroplastic burden and spleen pathology is likely mediated through microplastics. This is supported by the fact that spleen pathology mostly occurred proximal to observed microplastics within the tissue (Fig. 3l-ii). These microplastics were typically surrounded by leucocytes suggesting inflammation. Activated innate immune cells release reactive oxygen species (ROS) which can cause further tissue damage. This mechanism of tissue damage is support by Cunha et al. (2022) which demonstrated that plastic consumption correlated with ROS.

The mediation analysis found that kidney pathology was predominately caused by proventriculus macroplastics which explains the poor correlation between microplastics and kidney tissue pathology (Fig. 3g). This suggests the proventriculus pathology induced physiological stress on the birds which caused kidney pathology independent of the microplastics embedded in the tissue (Fig. 3h-ii). These stresses likely include malnutrition, malabsorption and dehydration which are known to induce kidney damage (Benabe and Martinez-Maldonado, 1998; Roncal-Jimenez et al., 2015). This hypothesis also explains our previous observations of plastic burden correlating with stunted growth (reduced body weight, head+bill length, bill, and wing length; Lavers et al., 2014, 2019a, 2019b, 2021). Interestingly, the damaged glomeruli and fibrosis observed in the kidney (Fig. 3h-ii) provide a mechanism for our previous observation of heightened uric acid in plastic impacted Flesh-footed Shearwaters (de Nobrega et al., 2020; Lavers et al., 2019).

The pathological consequences we describe here could also help explain some of the broader patterns observed in seabird populations affected by large amounts of macro- and microplastic debris, including slower development (Lavers et al., 2014), nutritional stress (Lavers et al., 2019), and poor body condition (Lavers et al., 2021). The ability for chicks to efficiently absorb nutrients is clearly compromised, even in instances where the parent birds continue to provision with high quality food. And in cases where chicks can rid themselves of macroplastics by casting a bolus (Bond et al., 2021), the physiological damage to the proventriculus has already been done, and embedded plastics will remain. These two factors are likely to impair recovery, thus, it is not the amount of plastics present at the time of sampling, but rather an individuals' history of ingestion that must be considered.

4. Conclusion

Importantly, the consequences of plastic ingestion are not confined to a single event. Rather, exposure occurs across different tissues and at different temporal and spatial scales. Ingested macroplastics can puncture the gastrointestinal tract (Carey, 2011), have clearly detrimental effects on the proventriculus (Fig. 1), and are absorbed into the proventricular lining (Fig. 3a). Some are passed to the gizzard, where they are further broken up (Lavers et al., 2019a, 2019b) before passing to the intestines where they can be taken up in the spleen and kidneys during the generation of nitrogenous waste (Fig. 3e, i). Lastly, some can be expelled in the guano (Bourdages et al., 2021; de Souza et al., 2022; Keys et al., 2022). Thus, the correlation between macroplastics contained within the proventriculus and microplastics embedded in organs suggest that a single macroplastic ingestion event may have long-last repercussions on animal health through the release of inflammatory microplastics through shedding and/or digestive fragmentation. The macroplastics can also induce direct substantial pathology of the digestive tract, while the microplastics cause pathology throughout the organs of the body through inflammation and other mechanisms. Collectively, our research highlights the complex and interacting nature of plastics as a health hazard for wildlife and emphasises the critical need for drastic plastic pollution mitigation strategies.

CRediT authorship contribution statement

Jack Rivers-Auty: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft. **Alexander L. Bond**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft. **Megan L. Grant**: Investigation, Methodology, Writing – original draft. **Jennifer L. Lavers**: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft.

Environmental implications statement

Pollution of our environment with plastics is increasingly recognised as one of the key threats to ecosystem health. Understanding the diversity of impacts, including those that are difficult to quantify, is critical in the face of the global biodiversity crisis. Here we show considerable and widespread damage to tissues of wild birds – a consequence that's been widely assumed to occur, but not demonstrated, and resulting from direct exposure to nano-plastics. We link these observations to documented declines in bird health and condition and suggest current estimates of the scope and severity of plastic pollution impacts on wildlife are grossly underestimated.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jennifer Lavers, Jack Rivers-Auty, Alexander Bond reports financial support was provided by Pure Ocean Fund.

Data Availability

The data generated in this study are provided in the manuscript or via the IMAS Data Portal.

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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.jhazmat.2022.130117.

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