Journal of Hazardous Materials 450 (2023) 131090

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

'Plasticosis': Characterising macro- and microplastic-associated fibrosis in seabird tissues

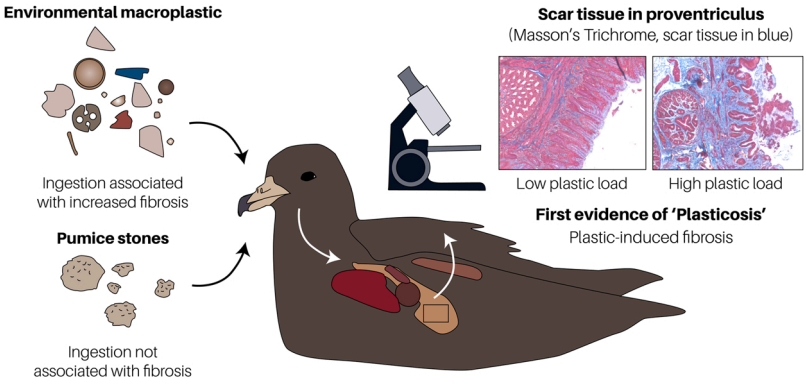
Hayley S. Charlton-Howard [a], Alexander L. Bond [b], Jack Rivers-Auty [c], Jennifer L. Lavers [b,d,e,*]

[a] Institute for Marine and Antarctic Studies, 20 Castray Esplanade, Battery Point, Tasmania 7004, Australia
[b] Bird Group, The Natural History Museum, Akeman Street, Tring, Hertfordshire HP23 6AP, United Kingdom
[c] Tasmanian School of Medicine, University of Tasmania, 17 Liverpool Street, Hobart, Tasmania 7000, Australia
[d] Gulbali Institute, Charles Sturt University, Wagga Wagga, New South Wales 2678, Australia
[e] Esperance Tjaltjraak Native Title Aboriginal Corporation, 11A Shelden Road, 6450 Esperance, Western Australia, Australia

HIGHLIGHTS

- Extensive scar tissue formation was associated with plastic exposure in seabirds.
- Plastic significantly altered collagen prevalence within stomach tissue structures.
- Pathology was caused directly by plastic, rather than natural items, such as pumice.
- First record of plastic-related fibrosis in seabird stomach tissues.
- Evidence for a new plastic-induced fibrotic disease, '*Plasticosis*'.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dr. R Teresa

Keywords:
Flesh-footed Shearwater
Histopathology
Masson's trichrome
Plastic pollution
Scar tissue
Stomach

ABSTRACT

As biota are increasingly exposed to plastic pollution, there is a need to closely examine the sub-lethal 'hidden' impacts of plastic ingestion. This emerging field of study has been limited to model species in controlled laboratory settings, with little data available for wild, free-living organisms. Highly impacted by plastic ingestion, Flesh-footed Shearwaters (*Ardenna carneipes*) are thus an apt species to examine these impacts in an environmentally relevant manner. A Masson's Trichrome stain was used to document any evidence of plastic-induced fibrosis, using collagen as a marker for scar tissue formation in the proventriculus (stomach) of 30 Flesh-footed Shearwater fledglings from Lord Howe Island, Australia. Plastic presence was highly associated with widespread scar tissue formation and extensive changes to, and even loss of, tissue structure within the mucosa and submucosa. Additionally, despite naturally occurring indigestible items, such as pumice, also being found in the gastrointestinal tract, this did not cause similar scarring. This highlights the unique pathological properties of plastics and raises concerns for other species impacted by plastic ingestion. Further, the extent and severity of fibrosis documented in this study gives support for a novel, plastic-induced fibrotic disease, which we define as '*Plasticosis,*'.

* Corresponding author at: Esperance Tjaltjraak Native Title Aboriginal Corporation, 11A Shelden Road, 6450 Esperance, Western Australia, Australia.
E-mail address: jennifer.lavers1@nhm.ac.uk (J.L. Lavers).

https://doi.org/10.1016/j.jhazmat.2023.131090
Received 4 January 2023; Received in revised form 16 February 2023; Accepted 24 February 2023
Available online 26 February 2023

1. Introduction

As a potential geological indicator for the Anthropocene [1], plastic is a ubiquitous and pervasive hallmark of our modern society. Recently the rapid consumption and emission of plastics into the environment has exceeded the 'novel entities' planetary boundary, for both ubiquity in the environment and irreversibility of pollution [2,3]. Plastics and climate change are intrinsically linked [4], with plastic production currently contributing to 4.5% of global greenhouse gas emissions [5], exacerbating current damage to our global environments. Without policy intervention, demand for plastic may double by 2050 [5] and plastic emissions may triple by 2060 [6]. Worryingly, despite current remediation and policy-based efforts, plastic will continue to be emitted and accumulate in the environment for many decades to come [7]. While one study estimated the global marine plastic load between 15 and 51 trillion pieces [8], due to limitations in the detection and collection of smaller fragments, current plastic estimates are vastly underestimated [9,10]. Further, previous studies specifically quantifying plastic load within organisms may be severe underestimations, with novel techniques identifying magnitudes larger plastic burdens that have not previously been detected [11,12]. Such large numbers and tipping points are already difficult to comprehend.

Of growing concern is the increase in smaller plastic fragments being reported and the emerging threat posed by these small particles. From plankton to blue whales (*Balaenoptera musculus)* [13], plastic is thought to impact over 1200 marine species [14]. Though there is debate as to what extent plastics are causing harm to populations or ecosystems, there is growing evidence that the ingestion of plastic leads to long-lasting and diverse consequences for a wide array of fauna [13,15]. Ingestion of large or sharp macroplastic (<5 mm) items can lead to blocked, ulcerated, or perforated digestive tracts [16,17], as well as altered or diminished feeding behaviour, and starvation in severe cases [16–19]. Once ingested, large macroplastics can be fragmented into smaller pieces, categorised as either micro- (1 μm-5 mm) or nanoplastics (<1 μm), through digestion and mechanical grinding [20,21]. These tiny plastic fragments can be absorbed by the digestive tract, transported around the body via the bloodstream [22], and accumulate in tissues and organs [23–26]. Plastics < 20 μm can penetrate most organs, with plastics < 10 μm able to cross cell membranes [27], potentially damaging tissues and intracellular structures [28,29]. Microscopic plastics can also cross both the blood-brain barrier [24,30] and the placenta [31]. While many studies of plastic ingestion focus on non-human animals, a recent paper by Ragusa et al. [32] suggests that due to the ubiquity of plastic and exposure to plastic from birth, it is likely human inhalation and ingestion of plastic is also inevitable. In this light, it is crucial to better understand the impacts of plastic on biota, so that we can also better understand how our own tissues may respond to this pollutant.

Laboratory studies examining plastic ingestion have documented a swathe of negative impacts, including tissue damage [24,33], behavioural changes [34,35], reduced growth [36] and fecundity [37], oxidative stress [38], altered metabolism [39] and transgenerational fitness impacts [40,41]. Combined, the variety of sub-lethal effects of plastic exposure are likely to impact overall fitness or survival of individuals. While plastics can interact with soft tissues in a variety of ways, our understanding of these interactions and sub-lethal impacts is a limited, but rapidly growing field of study [42–44]. This limitation is due, in part, to a reliance on studies that have used laboratory-grade, virgin plastic in their experiments; often spherical polystyrene [45,46]. This does not accurately reflect weathered plastics found in the environment, which are conversely a heterogeneous mix of polymers of different shapes, sizes, and stages of fragmentation [47]. Plastics with irregular size and shape could cause greater cellular damage [47] and are more likely to promote cell death [48]. Weathered environmental plastics exhibit different physical and chemical properties to the plastics used in most laboratory-based studies [49,50], and are more likely to be subject to phagocytosis by cells [51].

While not directly comparable to environmental plastic, laboratory studies have reported that exposure to plastics can cause inflammation of tissues [52,53]. During the resolution of inflammation collagen is deposited by fibroblasts, forming scar tissue, to add strength to damaged tissue while healing [54]. While this scar formation is a natural, often beneficial process associated with tissue repair, excessive scar tissue can become a pathological disease called fibrosis. Fibrosis can impede organ function, contribute to organ failure in severe cases, and is also a symptom of many chronic auto-immune diseases [55]. In response to inflammation, scar tissue may form around persistent inflammatory stimuli, causing severe, chronic problems if the irritant is not removed [55]. Several fibrotic diseases have been linked to this continuous damage-healing process, such as silicosis and asbestosis [56]. As comparably durable compounds, plastics may induce a similar response, where excessive scar tissue formation in response to plastic-induced inflammation may lead to organs becoming fibrotic. While previously documented in laboratory experiments [57], and recently observed within seabirds [26], plastic-induced scarring and fibrosis have not been comprehensively studied in wild animals ingesting environmental plastics.

This study aims to address this knowledge gap by examining if plastics have any impact on scar formation and fibrosis, in Flesh-footed Shearwaters (*Ardenna carneipes*). This species is heavily impacted by plastic pollution, with ~90% of necropsied birds containing ingested plastics [58]. Since 2010, the average body mass and condition of Flesh-footed Shearwaters in one of their major breeding colonies has declined substantially Lavers and Bond, [59]. Exposure to plastic is associated with reduced chick growth and survival [60], and causes altered blood chemistry [61] and extensive tissue damage [26]. While the ingestion of plastic has been implicated in the decline of this species [58], the potential impact of plastics on scar tissue formation has not been thoroughly investigated. Here we provide the first quantification of scar tissue formation due to plastic ingestion in a wild population, and provide evidence for a new plastic-induced fibrotic disease; '*Plasticosis.*'.

2. Materials and methods

2.1. Sample collection

Twenty-one freshly deceased Flesh-footed Shearwater fledglings (80–90 days old) were collected from Lord Howe Island, Australia (31.554°S, 159.085°E) from 28 April – 8 May 2021. These birds were aged due to their strict life history and phenology, as all birds hatch within a very narrow window of 3–5 days in January. These fledglings were collected either from specific beach transects following an unsuccessful fledging attempt (n = 12), from within the breeding colony (n = 8) or following a collision with a motor vehicle (n = 1). Additional samples from birds that exhibited noticeably fibrotic organs during necropsy were collected from 26 April – 10 May 2022 (beach-washed: n = 7, colony: n = 2), for a total of 30 individuals. Birds were already deceased when collected (n = 5) or euthanised under permit due to extremely low body mass (n = 25; See Acknowledgements for permit and animal ethics details). To avoid post-mortem delay, only freshly deceased beach-washed birds were used and were processed within 1 h of collection, while birds that had been euthanised were processed within 5 min of death.

2.2. Morphometrics analysis

Morphometrics of each bird were taken, including body mass (± 10 g, spring balance), wing chord (± 1 mm, flattened, stopped ruler), culmen length (± 0.1 mm, Vernier callipers), and head + bill length (± 0.1 mm Vernier callipers). To minimise plastic contamination of samples, glass or paper laboratory equipment was used where possible, and stainless-steel dissection tools were washed and sterilised with 70%

ethanol between each use.

2.3. Plastic and pumice analysis

Birds were necropsied, and ingested plastics from the proventriculus and gizzard were dried, counted, weighed, sorted by type and colour, and stored separately, according to the protocols outlined by Provencher et al. [62] and Lavers et al. [58]. Plastics were identified through visual and physical inspection, and inspection under dissection microscopes was used where necessary. Only particles that were visible to the naked eye (~1 mm and above) were counted, thus for the purposes of our study, microplastics are defined as particles 1–5 mm and macroplastics > 5 mm (for additional clarification about size categories, please see Supplementary Methods 1.1). Pumice stones found within the proventriculus and gizzard were similarly weighed, counted, and recorded separately.

2.4. Proventriculus region-based analysis

Tissue samples approximately 1 cm^3 from all individuals were collected from the proventriculus from the inferior (~1 cm above the pyloric sphincter) and superior (~1 cm below the cardiac sphincter) regions and fixed in 10% formalin. Tissue samples were stored in red Eppendorf tubes, which were pre-rinsed in MilliQ water, so any plastic contamination as a result of transport could be easily identified.

2.5. QA/QC

To minimise plastic contamination during all tissue processing and staining protocols in this study, stains were prepared using MilliQ water where necessary, the handling of samples was minimised, and slides were housed in standard slide boxes where plastic exposure from dust or air could be minimised prior to observation. The use of plastic laboratory clothing and equipment was minimised where possible (e.g. use of glass stripettes and tin foil, use of plastic gloves only where necessary, use of cotton laboratory gowns) and surfaces were thoroughly cleaned with ethanol.

2.6. Laboratory procedures and method development

Using a microtome and histological wax, multiple thin histological sections (~5 μm thick) were prepared for each tissue sample, adhered to a glass slide, and deparaffinised through two 3-minute washes in xylene, decreasing concentrations of ethanol (absolute ethanol, 95% ethanol, 70% ethanol; 3 min each, respectively) and 1✖ phosphate-buffered solution.

Initially, an optimisation experiment was conducted with trial tissue slides stained with Nile Red (bathed for 30 min; Sigma Aldritch, U.S.A; [63]) to assess plastic presence within the tissue samples, as well as a tissue-specific Sudan Black counterstain (200 μL for five minutes; Sigma Aldritch, U.S.A; see Supplementary Methods 1.2) to prevent accidental quantification of cell autofluorescence. We attempted to view these samples using fluorescent microscopy, to count and measure visible fluorescing particles which could be categorised as plastic fragments within the tissue. To identify plastics, a visual observation under multiple wavelengths was conducted, as fluorescence of plastics under many channels has been previously reported [64]. Unfortunately, optimisation of this staining technique and the creation of plastic-dosed positive gelatine controls showed this process to be unreliable, as we could not confidently identify plastics within the sample (See Supplementary Methods 1.3, 1.4). It may be that any fluorescence of plastic was smothered by the counterstain, or the small size of the plastics meant the Nile Red did not adhere effectively. As such, we did not continue with this technique.

To assess collagen formation and visually assess tissue health, a Masson's Trichrome procedure was used. While commonly used to identify poor tissue condition and pathology, this technique has been applied only recently to assess plastic-induced collagen formation in laboratory-based rodent studies [65,66]. Tissue slides were first bathed in Bouin's solution (Sigma-Aldritch, U.S.A) overnight at room temperature to enhance stain quality, and Weigert's Iron Hematoxylin (300 μL for 5 min; Sigma-Aldritch, U.S.A) to enhance staining of the nuclei. The samples were then stained with a Masson's Trichrome procedure as per manufacturer's instructions (Sigma-Aldritch, U.S.A; Supplementary Methods 1.5). Slides were then viewed using a Zeiss Axio Lab A1 (Carl Zeiss AG, Oberkochen, Germany). Over 300 photographs were taken of areas along the epithelial surface of the proventriculus at 20✖ magnification using a Zeiss Axiocam 506 colour, ranging from 2 to 15 images per sample depending on section quality. Each photo was processed in AxioVision 4.8.2 software and tissue health was graded semi-quantitatively (Fig. 1).

2.7. Scar tissue severity grading

As collagen is the primary component of scar tissue, elevated collagen prevalence was used as a marker for fibrosis. Samples were graded for severity (grade 0–5) by assessing the presence of excessive collagen formation or tissue damage across the whole sample (Fig. 1), and then specific histological features such as the submucosa and lamina propria within the tubular glands were assessed (Fig. 2). The tubular glands are the long glands responsible for secretion within the proventriculus [67], and within those are the lamina propria, a thin core of collagenous tissue [68]. The submucosa is the loose connective tissue beneath the mucosa and tubular glands [69]. All samples were graded twice by an observer who was blinded to plastic and pumice burden in each bird. Examples of a 'Grade 0' sample and 'Grade 5' sample are given in Fig. 2, and examples of each histological feature used are presented in Figs. 3 and 4. The mean score of those photographs was then used as a grade for the overall severity of scar tissue formation for each individual. Samples from the inferior and superior proventriculus were graded separately for proventriculus region-specific analysis, but were also averaged for an overall individual grade per individual.

2.8. Tubular gland and submucosa scar severity

To further quantify the prevalence of scar tissue formation, both the submucosa and tubular glands were examined separately for overall prevalence of collagen. For the tubular glands, images were cropped around the edges of the glands. A Masson's Trichrome-specific colour thresholding macro was utilised in ImageJ (version 1.53 t, [70]) to assess the percentage of the sample that was composed of collagen (Fig. 5).

2.9. Statistical analysis

All analyses were conducted in Jamovi v2.3 [71], with additional analysis conducted in R v4.1 [72]. Paired student's t-tests were used to compare scar tissue formation between superior and inferior proventriculus samples for the continuous data, and the Friedman test was applied for grade (Supplementary Results 1). As no significant differences were detected, data from all the images (a mean of 6 ± 2.4 images per bird) were combined into a single mean per individual, which was then used for subsequent analyses. For the grade variable, this mean was considered continuous and parametric statistics were applied (after assumption tests). Linear regressions were performed between numerical variables (plastic mass and number, pumice mass and number, body mass, wing chord length, culmen length, head + bill length) with the pathological variables (scar tissue severity grade, submucosa collagen prevalence, tubular gland collagen prevalence) used as dependent variables. Type I sums of squares linear regression analyses were used to investigate the relationship between pumice burden and pathological variables, after adjusting for plastic burden. Linear regressions were

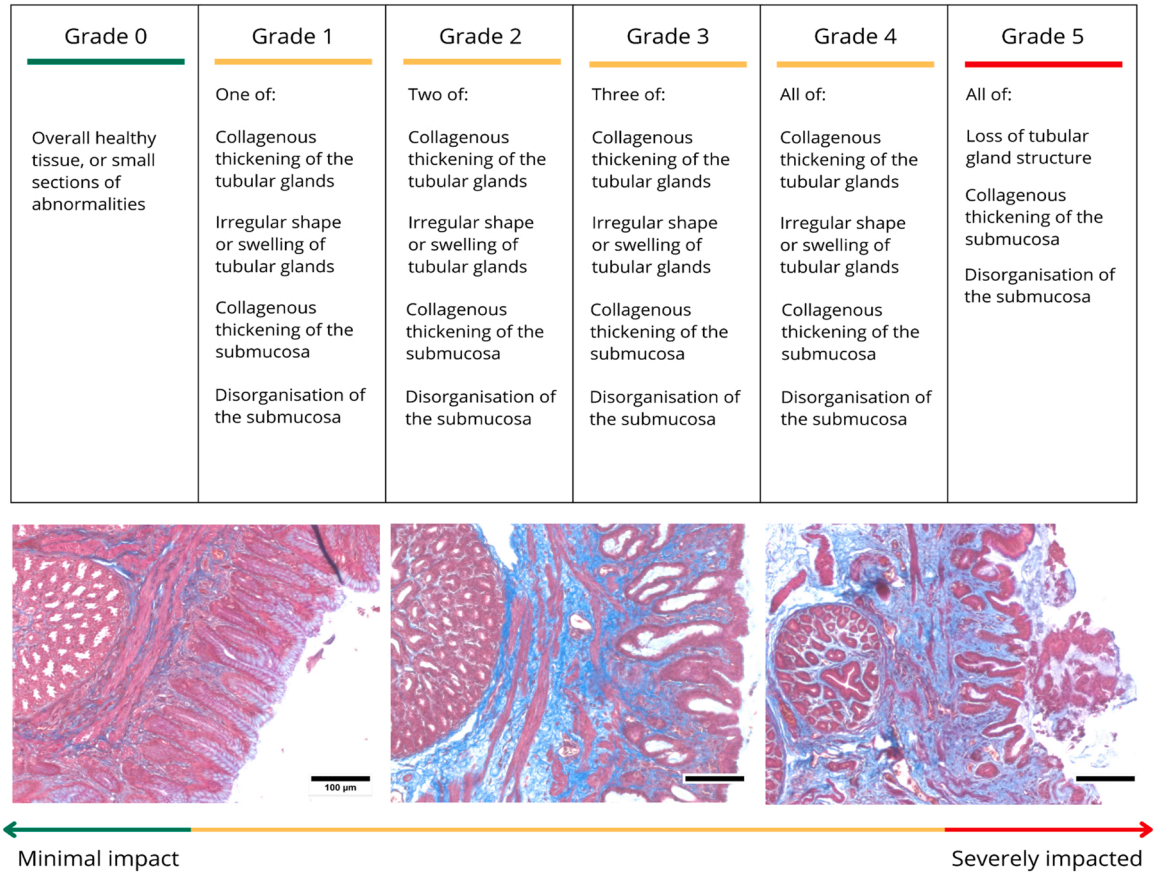


Fig. 1. Grading scheme used to assess the prevalence of collagen formation or tissue damage in Flesh-footed Shearwater proventriculus samples from Lord Howe Island. From left to right, examples of a Grade 0 image, a Grade 3 image, and a Grade 5 image, least to most impacted, respectively. Images taken at 20✖ magnification, scale bar = 100 µm.

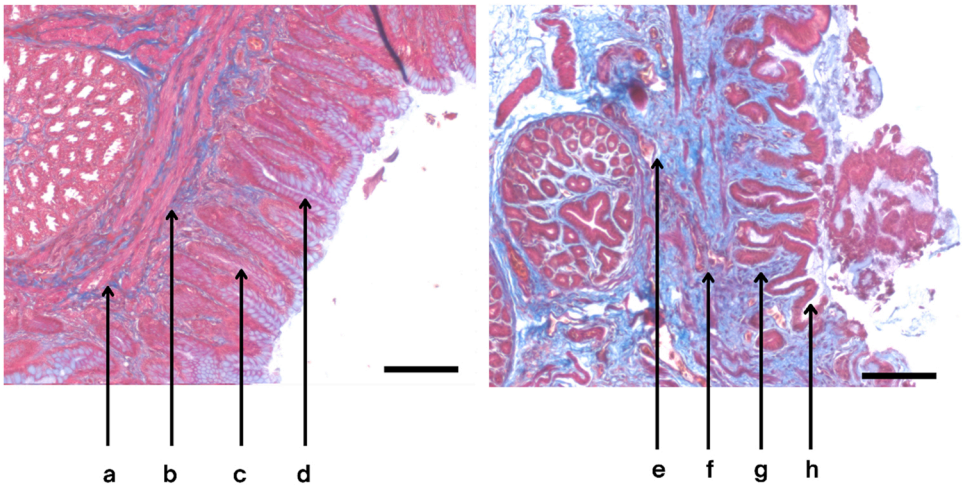


Fig. 2. A Grade 0 proventriculus (left), compared to a Grade 5 proventriculus (right). Note the parallel, organised submucosa (a), minimal collagenous deposition within the submucosa (b), minimal collagenous thickening of the lamina propria within the tubular glands (c), and the long, uniformly shaped tubular glands (d). In comparison, a Grade 5 individual is shown on the right. Note the disorganised submucosa (e), extensive collagen deposition within the submucosa (f), collagenous thickening of the lamina propria within the tubular glands (g), and the loss of tubular gland structure (h). The Grade 0 individual had 1 piece of plastic in its gizzard and proventriculus, while the Grade 5 individual had 170 pieces. Images taken at 20✖ magnification, scale bar = 100 µm.

displayed with 95% confidence intervals. For all analyses, the assumptions of normality and homoscedasticity of the residuals were evaluated graphically using Q-Q plots and residual vs predicted plots, respectively. Box-Cox transformations were applied where necessary. Effects were considered significant when $p < 0.05$. Data are reported as mean ± standard deviation. Superior and inferior proventriculus comparison data are displayed as boxplots with median and interquartile ranges. For each analysis, further statistical detail is in the Supplementary Results 1.

3. Results

3.1. Morphometrics analysis

There was no significant linear relationship between shearwater morphometrics and scar grade severity (body mass: $p = 0.218$, wing chord length: $p = 0.152$, head + bill length: $p = 0.462$, culmen length: $p = 0.237$). There was a significant linear relationship between plastic number and body mass ($p = 0.029$) and wing chord length ($p = 0.026$),

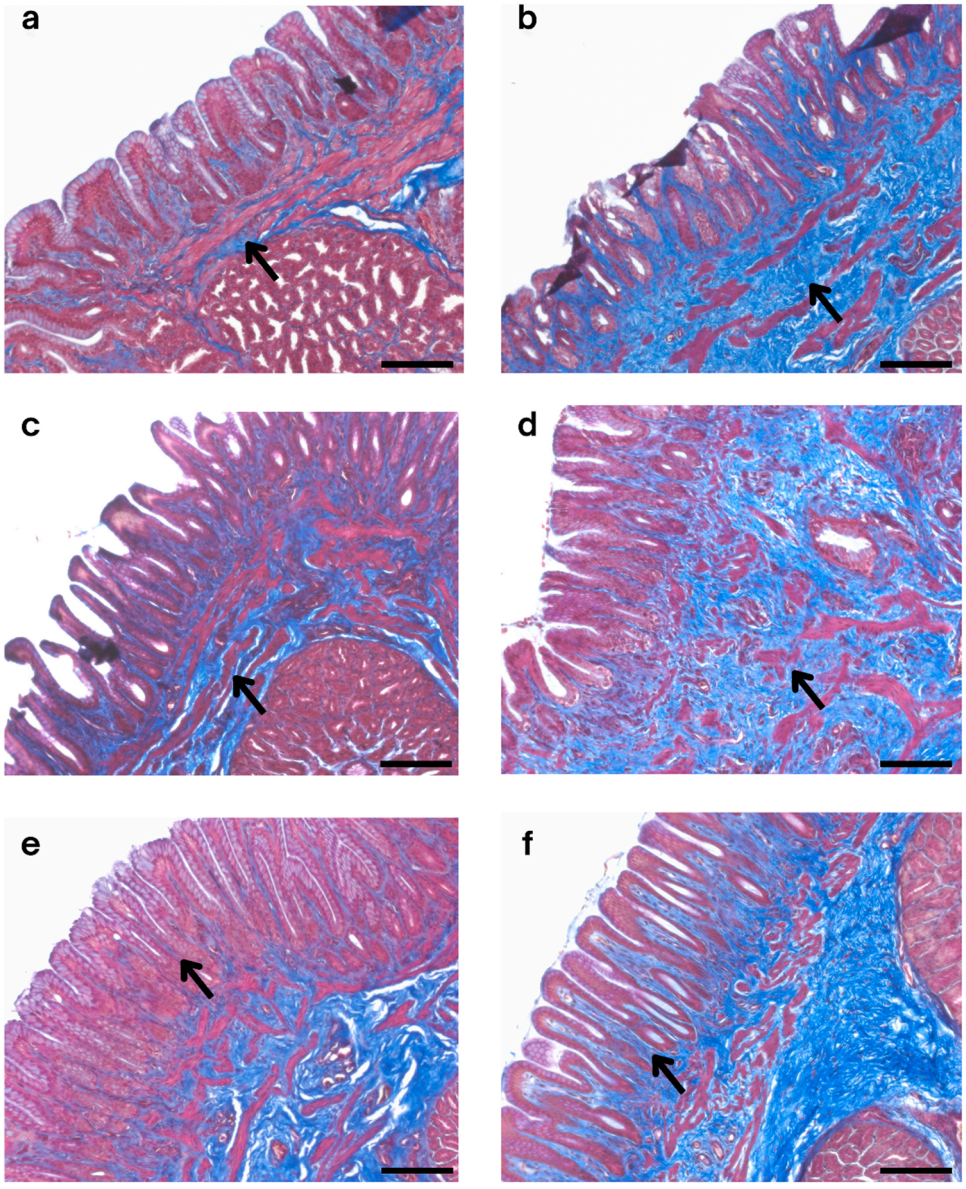


Fig. 3. Examples of grading of individual proventriculus structures. Regular submucosa (a) in comparison to a thickened submucosa (b), an organised submucosa (c) in comparison to a disorganised submucosa (d), and finally minimal collagenous thickening of the lamina propria within tubular glands (e) in comparison to tubular glands with collagenous thickening (f). Images taken at 20✖ magnification, scale bar = 100 μm.

while head + bill length ($p = 0.461$) and culmen length ($p = 0.858$) were not significantly associated. A similar result was observed for plastic mass; wing chord length ($p = 0.032$) was significantly linearly associated, while body mass ($p = 0.067$), head + bill ($p = 0.730$) and culmen length ($p = 0.785$) were not. Shearwater morphometrics did not have a significant linear relationship with collagen prevalence in the submucosa (body mass: $p = 0.635$, wing chord length: $p = 0.788$, head + bill length: $p = 0.463$, culmen length: $p = 0.218$) or the tubular glands (body mass: $p = 0.551$, wing chord length: $p = 0.688$, head + bill length: $p = 0.707$, culmen length: $p = 0.413$).

3.2. Plastic and pumice analysis

Mean fledgling body mass was slightly lower compared to previous years (2021–2022 mean body mass: 266.19 ± 48.85 g; in comparison, 2015–2019 mean body mass: mean 291 ± 98 g; authors' unpublished data) with one individual consuming 12.5% of its body weight in plastic.

The mean number of plastic items ingested per bird was 32 ± 53 pieces (range: 0–202 items; n = 30 birds), with a mean plastic mass of 3.00 ± 5.49 g (range: 0.00–20.61 g). Fledglings in this study on average consumed more pieces of plastic than in previous years, and slightly higher by mass (2015–2019 mean plastic number ingested: 14 pieces, mean plastic mass ingested: 2.73 g; authors' unpublished data). The mean number of pumice pieces per bird was 10 ± 14 stones (range: 0–42 stones), with a mean mass of 1.74 ± 3.00 g (range: 0.00–11.35 g per individual). Characteristics of plastics, such as colour and type of plastic (i.e nurdle, foam, fragment, etc) are given in Supplementary results 2.

3.3. Proventriculus region-based analysis

Scar severity grade did not differ significantly between the inferior and superior proventriculus samples (Supplementary Results 3a: $p = 0.310$). Likewise, scar tissue prevalence in the submucosa and tubular glands was not significantly different between the inferior and superior proventriculus samples (Supplementary Results 3b; Tubular glands: $p = 0.592$, 3c; Submucosa: $p = 0.934$).

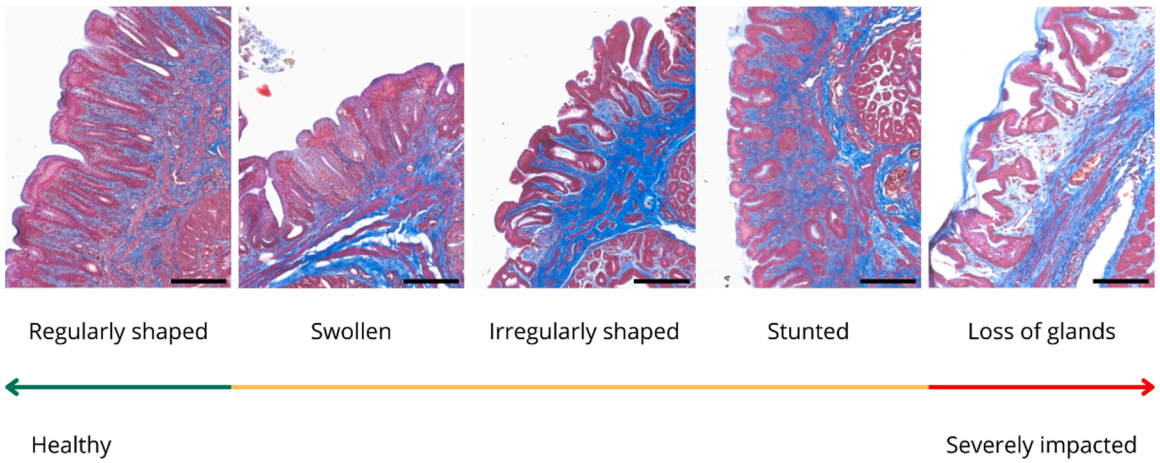


Fig. 4. Examples of tubular gland shapes in proventriculus samples from healthy, regularly shaped tubular glands to severely impacted tubular glands with a loss of structure. Images taken at 20✖ magnification, scale bar = 100 μm.

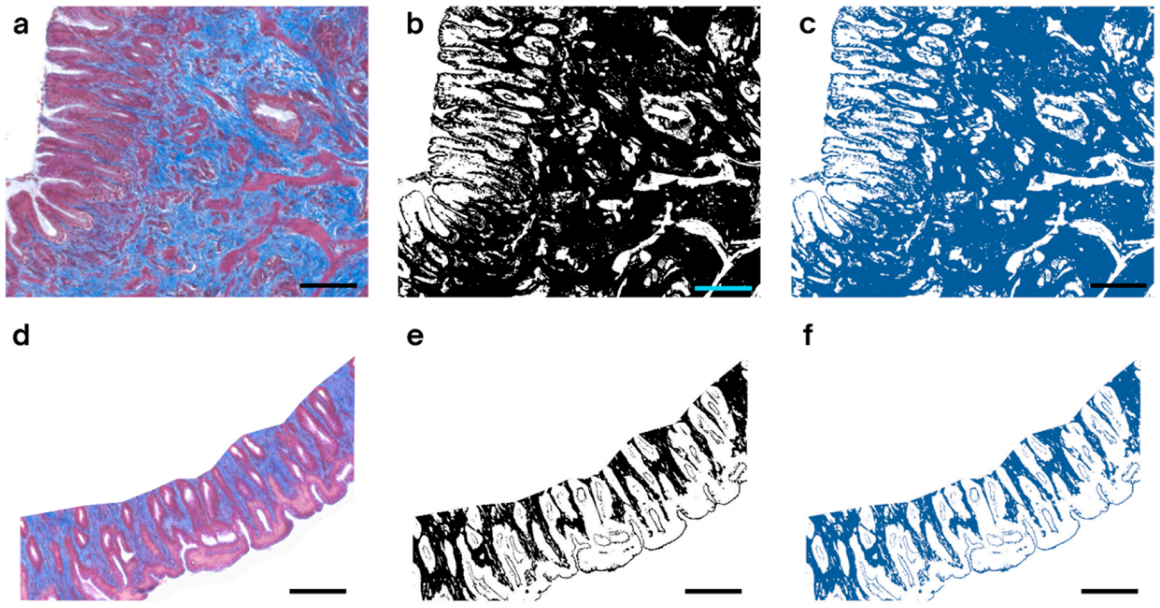


Fig. 5. Examples of ImageJ thresholding results. Shown are the original and cropped images (a, d), results of the ImageJ macro (b, e), and edited images to visualise the percentage of the sample that is blue, denoting collagen within the samples (c, f). Images taken at 20✖ magnification, scale bar = 100 μm.

3.4. Scar tissue severity grading

The amount of plastic and pumice stones found within the proventriculus was colinear (Supplementary Results 4a; mass of both: $r^2 = 0.50$, $p < 0.001$, 4b; mass of plastic and number of stones: $r^2 = 0.66$, $p < 0.001$, 4c; number of plastic and mass of pumice: $r^2 = 0.31$, $p = 0.002$, and 4d; number of both: $R^2 = 0.44$, $p < 0.001$). After correcting for this collinearity, the amount of plastic had a significant linear association with scar severity grade (Fig. 6a; mass: $p < 0.001$, 6b; number of items: $p < 0.001$), while pumice did not significantly explain any additional variation in scar severity (Fig. 6c; mass: $p = 0.234$, 6d; number of stones: $p = 0.121$). The mean scar severity grade within this study was 3 ± 1.

3.5. Tubular gland and submucosa scar severity

Mean collagen prevalence within the tubular glands was 34.18 ± 10.92% (range 13.45–68.02%). Tubular gland collagen prevalence had a significant linear association with plastic mass (Fig. 7a: $p = 0.037$), and plastic number (Fig. 7b: $p = 0.021$), while pumice mass and pumice number did not significantly explain any additional variation in collagen deposition (Fig. 7c: $p = 0.975$, 7d: $p = 0.533$).

Mean collagen prevalence within the submucosa was 46.74 ± 12.63%, (range 10.65–69.87%). Submucosa collagen prevalence was significantly associated with plastic mass (Fig. 8a: $p = 0.022$) and plastic number (Fig. 8b: $p = 0.040$). Pumice mass (Fig. 8c: $p = 0.902$) and pumice number (Fig. 8d: $p = 0.413$) did not significantly explain any additional variation in submucosa collagen deposition.

4. Discussion

We identified significant evidence for widespread plastic-related scar tissue formation in the proventriculus of wild seabirds. We found highly significant relationships between plastic presence, the severity of scar tissue formation, and prevalence of collagen within proventriculus tissue structures, but we did not find such elevated collagen prevalence to be related to the presence of pumice, reinforcing the notion that plastics induce this unique pathology. Scar tissue formation was clear and

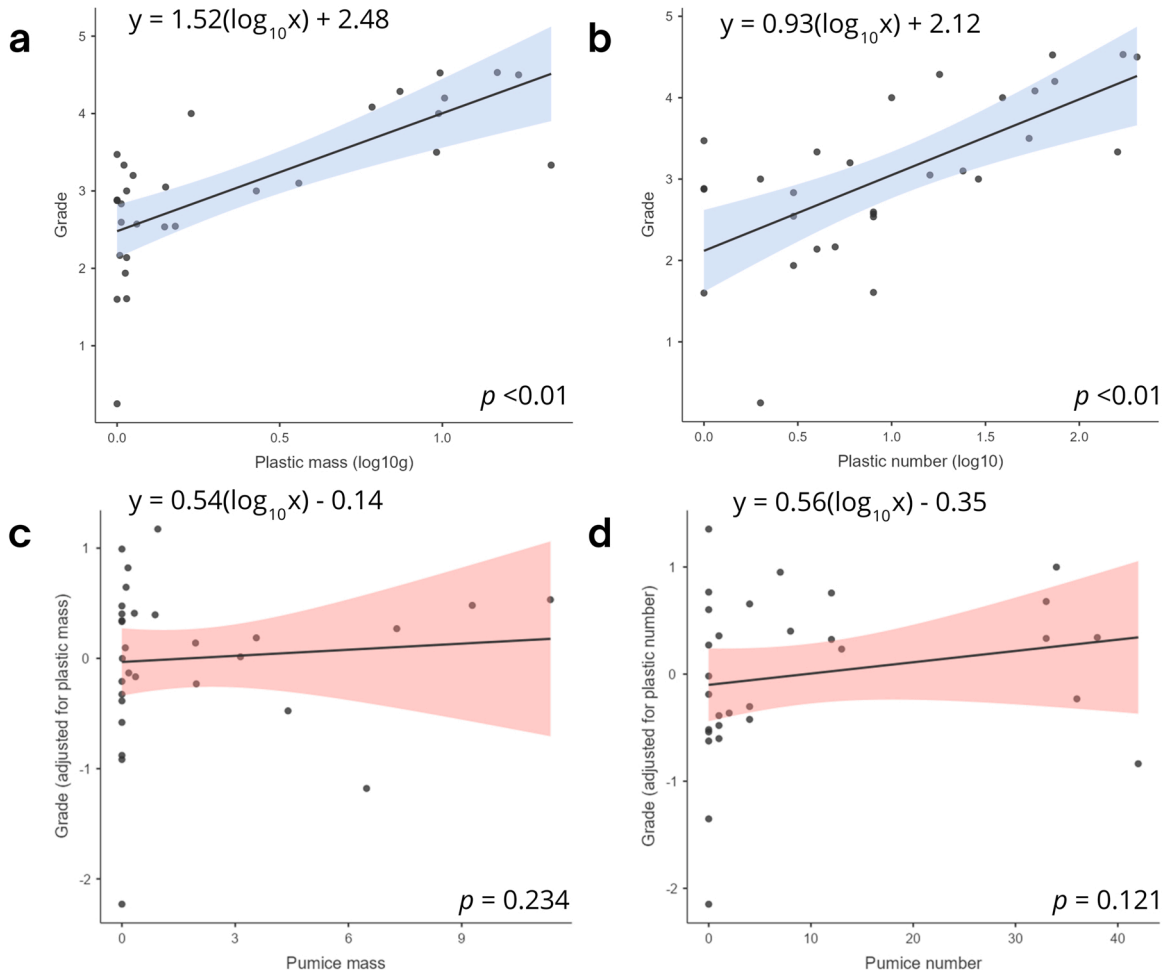


Fig. 6. Linear regression between ingested debris in Flesh-footed Shearwaters and scar tissue severity grade. Plastic mass and plastic number explained a significant proportion of variation in scar tissue severity (6a; plastic mass: $r^2 = 0.37$, $p < 0.001$, 6b; plastic number: $r^2 = 0.33$, $p < 0.001$). Pumice mass and pumice number did not explain any additional variation in scar tissue grade severity (6c; pumice mass: $r^2 = 0.05$, $p = 0.234$, 6d; pumice number: $r^2 = 0.08$, $p = 0.121$). Data analysed with linear regression, 95% CI shown in shaded area, n = 30.

evident in nearly all samples that were assessed, raising greater concerns for the health of the overall shearwater population.

In controlled, laboratory-based experiments, plastic exposure has been linked to markers for fibrosis in the ovaries [65], uterus [66], heart [73,74], and liver [57]. However, these studies have primarily focussed on rodents within a sterile laboratory setting, and the applicability of these studies to 'real-world' scenarios (e.g. free-living organisms) has been questioned [75]. To the best of the authors' knowledge, this is the first study to document and quantify plastic-induced fibrosis in wild organisms.

Like many other bird species, Flesh-footed Shearwaters ingest hard naturally-occurring debris, such as pumice, which is thought to aid in digestion [76,77], and is mostly processed in the gizzard [78]. We found that the amount of plastic and pumice were highly colinear, with individuals consuming large amounts of pumice also having a high prevalence of plastics in their stomach and gizzard (Supplementary Results 4). A similar trend has recently been found for both Flesh-footed Shearwaters, and another shearwater species [79]. It may be that adult birds are aware of a large presence of indigestible material within their stomach and ingest pumice to try to remove the source of this irritation, which is then passed onto fledglings during feedings. Conversely, the high prevalence of plastic within the proventriculus may simply reduce the ability of fledglings to naturally regurgitate pumice stones. Ingested pumice may grind plastics into a small enough form to be safely excreted [21], however, pumice could also exacerbate the situation and cause further damage, creating tiny plastic shards which could become embedded within tissues [26] or be small enough to be absorbed and transferred to the bloodstream [22].

Despite the similarity in the shape and size of some pumice and plastic items, ingested pumice does not contribute to the loss of rugae or tubular glands in the proventriculus, which are both essential to the proper functioning of the stomach [26]. Additionally, the ingestion of pumice was not found to negatively impact the body condition of two species of shearwaters [79]. Our results provide further evidence for the minimal impact of the ingestion of pumice on bird health, as there was no significant association between scar tissue severity grade and the number of pieces or mass of pumice ingested (Fig. 6). In addition, while there was a significant relationship between the prevalence of collagen in the tubular glands and the submucosa, and the amount of plastic ingested, we did not observe this same relationship for pumice (Figs. 7 and 8). The negligible impact of pumice is somewhat unsurprising, as birds have evolved to use pumice as a digestive aid [76,77]; if such pumice caused tissue damage or was detrimental to survival, this would likely not be the case. Further, this suggests the visible scar tissue formed as a direct result of plastic-induced injury along the epithelial surface of the proventriculus, which supports the notion that the ingestion of plastic causes unique physical damage and pathologies that are not created by indigestible, naturally occurring material [26].

Our study focused on the proventriculus as this organ acts as a 'containment vessel' where plastic is held until it is regurgitated,

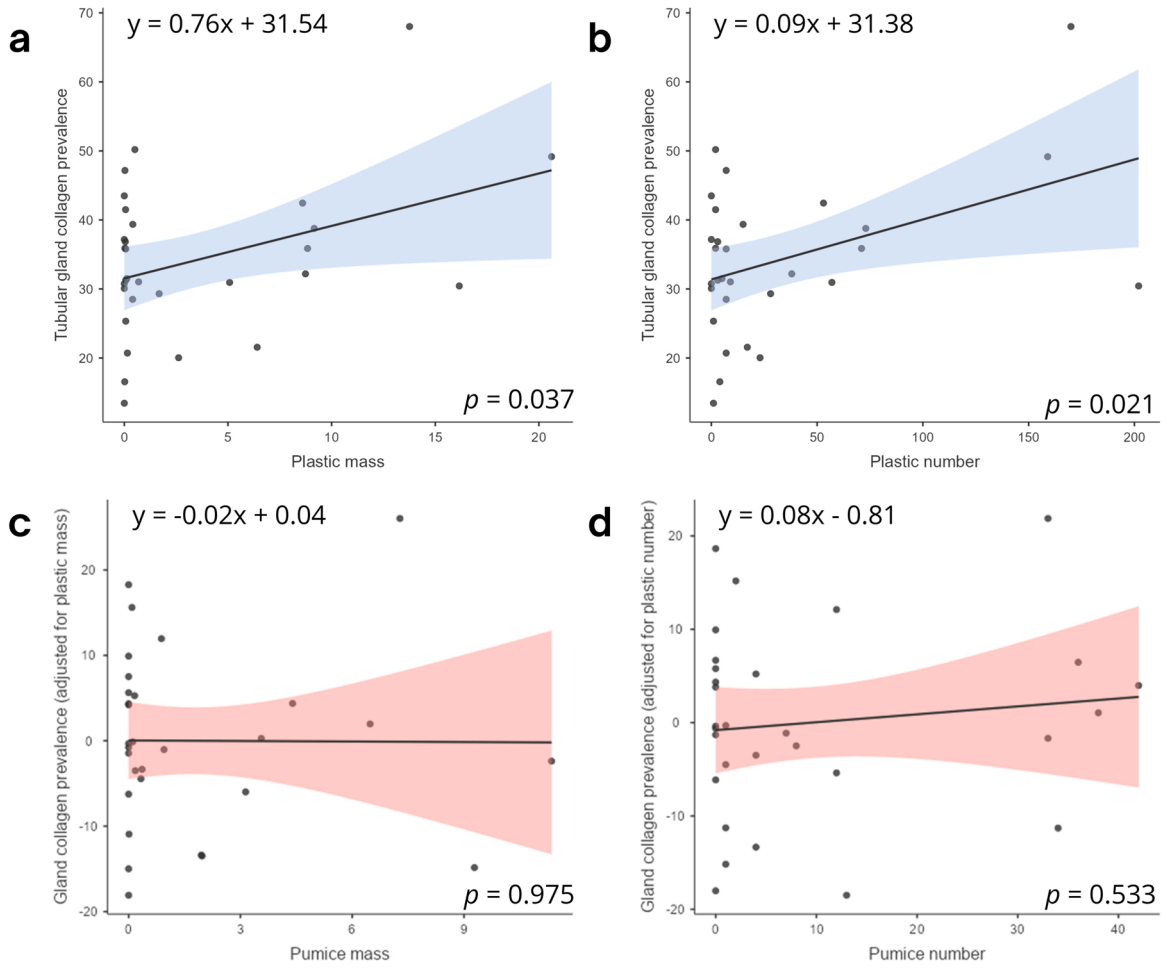


Fig. 7. The linear relationships between ingested debris in Flesh-footed Shearwaters and prevalence of collagen within the tubular glands. Collagen prevalence was had a significant linear relationship with plastic mass and plastic number (7a; plastic mass: $r^2 = 0.15$, $p = 0.037$, 7b; plastic number: $r^2 = 0.18$, $p = 0.021$), but pumice mass and pumice number did not significantly explain any additional glandular collagen deposition (7c; pumice mass: $r^2 = <0.01$, $p = 0.975$, 7d; pumice number: $r^2 = 0.01$, $p = 0.533$). Data analysed with linear regression, 95% CI shown in shaded area, n = 30.

absorbed, or excreted [21,80]. It is thus likely to be the first and potentially most impacted internal organ in relation to plastic exposure and is thus crucial to study. The severity of scar tissue formation was widespread and uniform across the whole proventriculus, with no significant difference between superior and inferior regions in overall scar severity grade, or scar tissue prevalence in the submucosa or tubular glands (Supplementary Results 3).

Most birds displayed at least three elements of scar tissue formation, including significant thickening of the lamina propria within the tubular glands and collagenous deposition within the submucosa. Worryingly, despite some individuals having low levels of ingested plastic within their proventriculus or gizzard, some of these pathologies were still recorded. There are several explanations for this observation. Firstly, at the end of the fledgling period when birds are approximately 90 days old, shearwater chicks are able to regurgitate hard, indigestible items [81]. This once-off event could potentially eliminate some or all of the plastic that had been ingested, impairing our ability to quantify exposure, but leaving behind inflammation, scarring, and other irreversible damage. Alternatively, previous studies involving this same species have demonstrated that ingestion of a single piece of plastic is sufficient to alter blood chemistry parameters [61], and cause rugae loss in the proventriculus [26]. The level of damage caused by one plastic piece may be affected by the morphologies of the plastic pieces themselves; one irregularly shaped, sharp item may have the potential to cause as much injury as numerous rounded, 'softer' plastic items [47,48]. Additionally, the size and chemical composition of the plastics themselves may affect the prevalence of inflammation [82]. These factors may help to explain the scarring observed in this study, even in birds with a comparatively low plastic burden to their peers. Additionally, the presence of undetectable micro- and nanoplastic fragments embedded within the proventriculus tissues, rather than larger ingested fragments damaging the external surface, may be causing inflammation and subsequent scarring. While not within the scope of this study, such microscopic plastic pieces have been documented within proventriculus tissues in Flesh-footed Shearwaters and were shown to cause significant inflammation and tissue damage to multiple organs [26], which could similarly lead to significant scarring.

Shearwater body morphometrics (body mass, wing chord length, culmen length, and head + bill length) were not significantly associated with scar grade severity or collagen prevalence in the submucosa and tubular glands. In contrast, wing chord length had a significant linear relationship with both plastic number and mass, while body mass was significantly associated with plastic number. This suggests some differences in body morphometrics may be attributed to the presence of plastic, but not associated with the formation of scar tissue specifically. Reduced growth rates and subsequent body size as a result of plastic ingestion have been documented in this shearwater species previously [58,60], but are rarely reported in other species [83,84]. It is likely that plastic induces a swathe of sub-lethal effects which we were not able to capture in this study, such as introducing toxic chemical pollutants [85], changing gene expression [35], disrupting metabolism [86], or causing tissue dysfunction [87]. Instead of being the driving factor behind

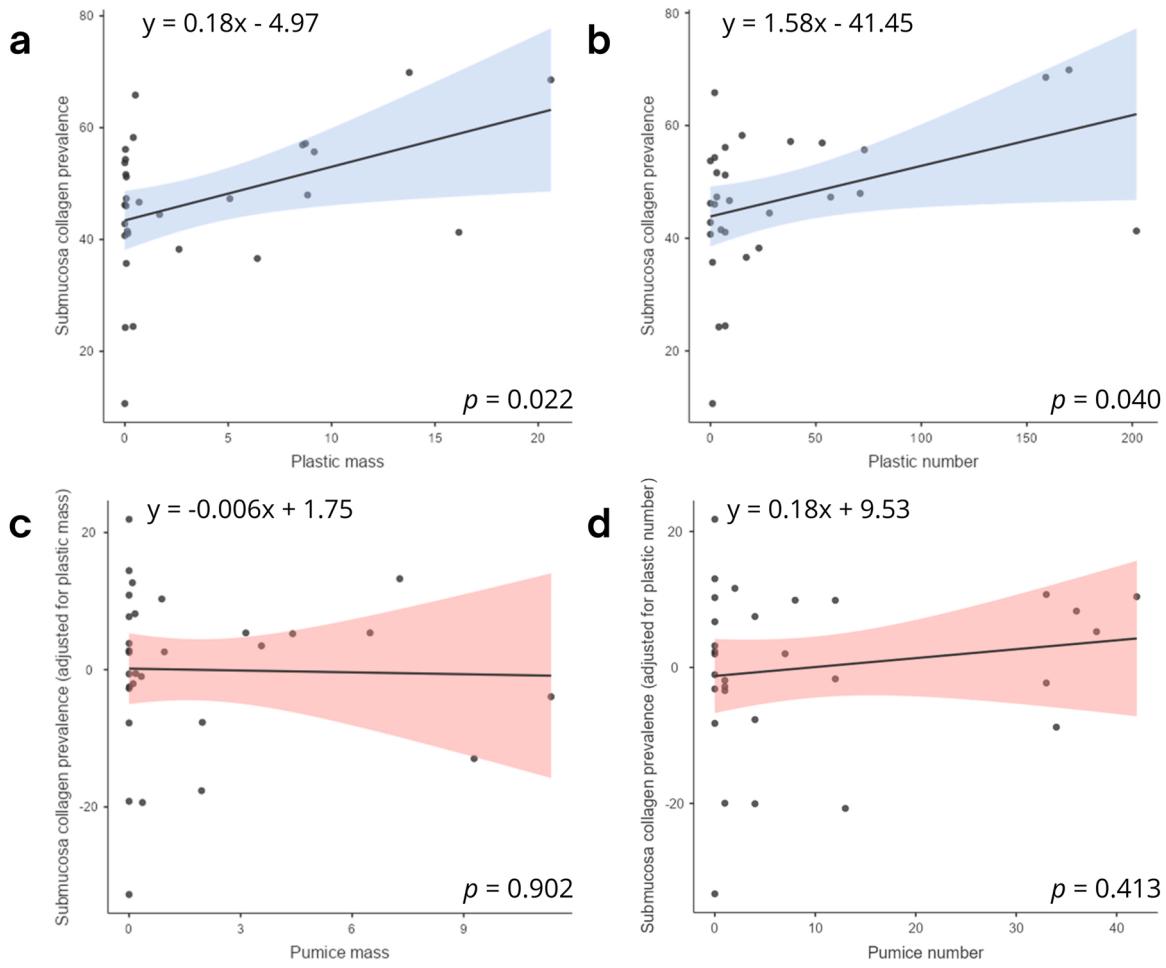


Fig. 8. The linear relationships between ingested debris in Flesh-footed Shearwaters and prevalence of collagen within the submucosa. Collagen prevalence had a significant linear relationship with plastic mass and plastic number (8a; plastic mass: $r^2 = 0.17$, $p = 0.022$, 8b; plastic number: $r^2 = 0.14$, $p = 0.040$), but pumice mass and pumice number did not explain any additional variation in submucosa collagen deposition (8c; pumice mass: $r^2 = <0.01$, $p = 0.902$, 8d; pumice number: $r^2 = 0.02$, $p = 0.413$). Data analysed with linear regression, 95% CI shown in shaded area, n = 30.

reduced body morphometrics, scarring is likely to be a contributing co-factor alongside these additional sub-lethal impacts.

The scarring evident in this histopathological analysis may have severe consequences. Firstly, tubular glands are essential in the secretion of mucus to protect the epithelium [88], as well as the production of pepsinogen, hydrochloric acid, and intrinsic factor, which are crucial for the digestion and absorption of proteins and nutrients [89,90]. In cases where these glands are damaged, such as in chronic gastritis, a decrease in hydrochloric acid production can result in de-acidification of the stomach, which can lead to increased susceptibility to infection or parasites [91,92]. As environmental plastics have been noted to be vectors for pathogens and diseases [93,94], this could be especially detrimental. Additionally, a lack of mucus production can lead to further injury and atrophy of the stomach, while a failure to secrete intrinsic factor can also lead to a decrease in vitamin B12 absorption [95]. This in turn can cause anaemia, as red blood cells fail to mature in the absence of vitamin B12 [96,97]. Loss of tubular glands, or reduced function because of excessive collagen formation within the lamina propria, may thus influence the ability of shearwaters to maintain their gastric health and effectively absorb nutrients. It is assumed that this plastic-induced fibrosis is caused by plastic items repeatedly injuring the tissue. However, in some cases, vitamin deficiency can also lead to fibrosis and impaired tissue function [98]. While likely not the leading factor, the extent of plastic-related scar tissue may be further exacerbated by nutrient deficiencies, caused by the repeated ingestion of plastic over nutritious food items.

Additionally, collagen deposition within the submucosa may also negatively impact survival. In other pathologies where the stomach wall is thickened by scar tissue formation, such as in gastric linitus plastica, the stomach can become rigid and reduced in size [99], which reduces overall stomach volume and can interfere with peristalsis [100]. Scar tissue formation also can disrupt blood supply, which can cause further tissue damage and dysfunction, and even organ failure [55]. Additionally, many individuals in this study exhibited the formation of disorganised collagen formation, which is often a feature of scar tissue, although scar tissue morphology can be highly variable [101]. Dense irregular connective tissue has reduced flexibility [101,102], further contributing to a potential decrease in stomach elasticity. Plastic exposure also induces a loss of stomach rugae, which are essential for allowing the stomach to expand [26]. Any additional stiffness in the stomach because of scar tissue formation may have severe consequences, especially in the case of the Shearwater fledglings assessed in this study. Chicks and fledglings can often go several days between provisioning by parents, with an increased duration between feedings as chicks age [103]. A reduction in stomach capacity could thus have negative implications, as chicks and fledglings may have a reduced ability to ingest the amount of food necessary to sustain themselves between feedings. The notion that plastic ingestion can lead to a reduced feeding rate in birds is an established one [19], however the presence of extensive scar tissue within the proventriculus, and subsequent restriction in stomach capacity, may further compound the consequences of plastic ingestion.

Fibrotic diseases caused by foreign particles are not uncommon. Both

asbestosis and silicosis are marked by long-term inflammation and subsequent scar tissue formation as a result of exposure to asbestos fibres and crystalline silica dust, leading to tissue damage and impairment [56]. Plastic exposure within the proventriculus causes inflammation [26], and for the individuals studied here, plastic exposure in the proventriculus is chronic; satisfying the requirement for a 'persistent inflammatory stimuli'. Additionally, this study has demonstrated that the presence of plastic can cause significant fibrosis, leading to extensive reorganisation and potential loss of function in proventriculus tissues. In line with the terms silicosis and asbestosis, as a similar fibrotic response to foreign materials, this pathology should be defined as '*plasticosis*'. The term '*plasticosis'* was briefly introduced nearly 30 years ago; narrowly defining it as the breakdown of plastic components within metal joint replacement devices [104]. We argue that the term *'plasticosis'* is more appropriately defined as the inflammation and fibrosis in response to plastic presence. On these grounds, we propose '*plasticosis*': a fibrotic disease developed in response to plastic exposure.

Plastic-induced fibrosis is a relatively recent discovery, with only a handful of studies being published within the last two years, and it has not been formally classified [57,65,66,73,74]. However, it is important to note that this *'plasticosis*' is not limited to controlled, laboratory studies where plastic ingestion was deliberate and forced; our study demonstrates the capacity of plastic to cause severe pathology in free-living organisms foraging naturally. Future study is recommended to assess whether similar fibrosis can be identified in the array of wildlife species documented to ingest plastic, and whether extensive scarring found in juveniles is chronic or resolves itself during adulthood. Future research is also recommended to examine whether plastic-induced scar tissue formation is also documented in other organs, and whether it is primarily caused by macroplastics, such as in this study, or by the intrusion of microscopic plastic fragments into tissues.

5. Conclusions

As plastic emissions continue to grow and plastic pollution becomes increasingly prevalent in all environments globally, it is likely that exposure of all organisms to plastic is inevitable. Further, the ingestion of plastic has far-reaching and severe consequences, many of which we are only just beginning to fully document and understand. Building on recent literature documenting plastic-induced fibrosis in a controlled laboratory setting, this study clearly demonstrates the ability of plastic to directly induce severe, organ-wide scar tissue formation or '*plasticosis*' in wild, free-living animals, which is likely to be detrimental to individual health and survival. The scar tissue formation evident within the shearwater proventriculus tissues also highlights the unique pathological properties of plastic, as the damage was significantly linked to plastic ingestion, but not the ingestion of natural abrasive materials like pumice. The results of this study thus lend support for the creation of a novel, plastic-induced fibrotic disease, '*plasticosis*'. Scar tissue formation documented here is widespread and likely chronic, and has led to potentially irreversible changes in tissue structure and function, which has been previously unrecorded. Due to the potential impacts of plastic on the health of wildlife, and humans by extension, our results thus highlight the urgent need to continue to strengthen our knowledge of the sub-lethal impacts of this diverse pollutant.

Environmental implication

Research into plastic impacts is a rapidly growing field of study. Significant behavioural, physiological, and pathological impacts have been documented, with an urgent call to further understand the sub-lethal impacts of plastic exposure under environmentally-relevant conditions. This research found severe, widespread fibrosis and subsequent tissue damage in wild birds due to plastic exposure which was not documented for the ingestion of similarly abrasive natural materials, such as pumice. Due to the extent of evident scar tissue formation, here we describe the first instance of plastic-induced fibrosis in wild animals and propose a new pathology 'Plasticosis'.

CRediT authorship contribution statement

Hayley Charlton-Howard: Methodology, Formal analysis**,** Writing – original draft, **Dr Alexander L Bond:** Resources, Funding acquisition**, Dr Jack Rivers-Auty:** Conceptualisation, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition, **Dr Jennifer L Lavers:** Conceptualisation**,** Resources**,** Writing – review & editing, Supervision, Funding acquisition.

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 will be uploaded to Figshare once the paper is published.

Acknowledgements

The authors acknowledge the traditional custodians of the land and waters where this paper was written, the muwinina people of nipaluna (Hobart, Australia), and celebrate the role that Indigenous people have played as the first scientists and conservationists of this land. Our thanks to the Pure Ocean Fund, the Natural History Museum, the Detached Cultural Organization, and Lynton Mortensen (Ocean Plastic Relay - Day of 30) for generously providing funding for this research. Logistical and field assistance was provided by the Australian Bird and Bat Banding Service, Lord Howe Island community, and the Two Hands Project (Paul Sharp and Silke Stuckenbrock). Special thanks to Adrift Lab members, Megan Grant and Alix de Jersey, for their invaluable assistance with sample collection. Samples were collected under permit from the Lord Howe Island Board (permit no. 07/18) under the approval of the University of Tasmania Animal Ethics Committee (permit no. A18480) and New South Wales Office of Environment & Heritage (licence no. SL100619). Thanks to two anonymous reviewers who provided feedback to improve earlier drafts.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131090.

References

[1] Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., et al., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. https://doi.org/10.1016/j.ancene.2016.01.002.

[2] Persson, L., Carney Almroth, B.M., Collins, C.D., Cornell, S., de Wit, C.A., Diamond, M.L., et al., 2022. Outside the safe operating space of the planetary boundary for novel entities. Environ Sci Technol 56, 1510–1521. https://doi.org/10.1021/acs.est.1c04158.

[3] Villarrubia-Gómez, P., Cornell, S.E., Fabres, J., 2018. Marine plastic pollution as a planetary boundary threat – The drifting piece in the sustainability puzzle. Mar Policy 96, 213–220. https://doi.org/10.1016/j.marpol.2017.11.035.

[4] Lavers, J.L., Bond, A.L., Rolsky, C., 2022. Far from a distraction: plastic pollution and the planetary emergency. Biol Conserv 272, 109655. https://doi.org/10.1016/j.biocon.2022.109655.

[5] Stegmann, P., Daioglou, V., Londo, M., van Vuuren, D.P., Junginger, M., 2022. Plastic futures and their CO_2 emissions. Nature 612, 272–276. https://doi.org/10.1038/s41586-022-05422-5.

[6] Lebreton, L., Andrady, A., 2019. Future scenarios of global plastic waste generation and disposal. Palgrave Commun 5. https://doi.org/10.1057/s41599-018-0212-7.

[7] Borrelle, S.B., Ringma, J., Law, K.L., Monnahan, C.C., Lebreton, L., McGivern, A., et al., 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369, 1515–1518. https://doi.org/10.1126/science.aba3656.

[8] van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., van Franeker, J.A., et al., 2015. A global inventory of small floating plastic debris. Environ Res Lett 10, 124006. https://doi.org/10.1088/1748-9326/10/12/124006.

[9] Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J.R., et al., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. Environ Pollut 265, 114721. https://doi.org/10.1016/j.envpol.2020.114721.

[10] Mitrano, D.M., Wick, P., Nowack, B., 2021. Placing nanoplastics in the context of global plastic pollution. Nat Nanotechnol 16, 491–500. https://doi.org/10.1038/s41565-021-00888-2.

[11] Keys, B.C., Grant, M.L., Rodemann, T., Mylius, K.A., Pinfold, T.L., Rivers-Auty, J., et al., 2023. New methods for the quantification of ingested nano- and ultrafine-plastics in seabirds. Environ Sci Technol 57, 310–320. https://doi.org/10.1021/acs.est.2c06973.

[12] Mylius, K.A., Lavers, J.L., Woehler, E.J., Rodemann, T., Keys, B.C., Rivers-Auty, J., 2023. Foraging strategy influences the quantity of ingested micro- and nanoplastics in shorebirds. Environ Pollut 319, 120844. https://doi.org/10.1016/j.envpol.2022.120844.

[13] Kühn, S., van Franeker, J.A., 2020. Quantitative overview of marine debris ingested by marine megafauna. Mar Pollut Bull 151, 110858. https://doi.org/10.1016/j.marpolbul.2019.110858.

[14] Santos, R.G., Machovsky-Capuska, G.E., Andrades, R., 2021. Plastic ingestion as an evolutionary trap: Toward a holistic understanding. Science 373, 56–60. https://doi.org/10.1126/science.abh0945.

[15] Salerno, M., Berlino, M., Mangano, M.C., Sarà, G., 2021. Microplastics and the functional traits of fishes: A global meta-analysis. Glob Chang Biol 27, 2645–2655. https://doi.org/10.1111/gcb.15570.

[16] R.H. Day, D.H.S. Wehle, F. Coleman, Ingestion of plastic pollutants by marine birds, National Oceanic and Atmospheric Administration, 1985.

[17] Pierce, K.E., Harris, R.J., Larned, L.S., Pokras, M.A., 2004. Obstruction and starvation associated with plastic ingestion in a Northern Gannet *Morus bassanus* and a Greater Shearwater *Puffinus gravis*. Mar Ornithol 32, 187–189.

[18] S. Kühn, E.L. Bravo Rebolledo, J.A. van Franeker, Deleterious effects of litter on marine life, in: M. Bergmann, L. Gutow, M. Klages (Eds.), Marine Anthropogenic Litter, Springer International Publishing, Cham, 2015: pp. 75–116. https://doi.org/10.1007/978–3-319–16510-3_4 (accessed April 4, 2022).

[19] Ryan, P.G., 1988. Effects of ingested plastic on seabird feeding: Evidence from chickens. Mar. Pollut. Bull 19, 125–128. https://doi.org/10.1016/0025-326X(88)90708-4.

[20] Lavers, J.L., Stivaktakis, G., Hutton, I., Bond, A.L., 2019. Detection of ultrafine plastics ingested by seabirds using tissue digestion. Mar Pollut Bull 142, 470–474. https://doi.org/10.1016/j.marpolbul.2019.04.001.

[21] Nania, T.G., Shugart, G.W., 2021. Are plastic particles reduced in size in seabirds' stomachs. Mar Pollut Bull 172, 112843. https://doi.org/10.1016/j.marpolbul.2021.112843.

[22] Hwang, J., Choi, D., Han, S., Jung, S.Y., Choi, J., Hong, J., 2020. Potential toxicity of polystyrene microplastic particles. Sci Rep 10, 7391. https://doi.org/10.1038/s41598-020-64464-9.

[23] Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci Rep 7, 46687. https://doi.org/10.1038/srep46687.

[24] Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.-A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci Rep 7, 11452. https://doi.org/10.1038/s41598-017-10813-0.

[25] Pitt, J.A., Kozal, J.S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., et al., 2018. Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*). Aquat Toxicol 194, 185–194. https://doi.org/10.1016/j.aquatox.2017.11.017.

[26] Rivers-Auty, J., Bond, A.L., Grant, M.L., Lavers, J.L., 2023. The one-two punch of plastic exposure: macro- and micro-plastics induce multi-organ damage in seabirds. J Hazard Mater 442, 130117. https://doi.org/10.1016/j.jhazmat.2022.130117.

[27] Campanale, C., Massarelli, C., Savino, I., Locaputo, V., Uricchio, V.F., 2020. A detailed review study on potential effects of microplastics and additives of concern on human health. Int J Environ Res Public Health 17, 1212. https://doi.org/10.3390/ijerph17041212.

[28] Canesi, L., Ciacci, C., Fabbri, R., Balbi, T., Salis, A., Damonte, G., et al., 2016. Interactions of cationic polystyrene nanoparticles with marine bivalve hemocytes in a physiological environment: Role of soluble hemolymph proteins. Environ Res 150, 73–81. https://doi.org/10.1016/j.envres.2016.05.045.

[29] Geiser, M., Rothen-Rutishauser, B., Kapp, N., Schürch, S., Kreyling, W., Schulz, H., et al., 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect 113, 1555–1560. https://doi.org/10.1289/ehp.8006.

[30] Shan, S., Zhang, Y., Zhao, H., Zeng, T., Zhao, X., 2022. Polystyrene nanoplastics penetrate across the blood-brain barrier and induce activation of microglia in the brain of mice. Chemosphere 298, 134261. https://doi.org/10.1016/j.chemosphere.2022.134261.

[31] Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., et al., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ Int 146, 106274. https://doi.org/10.1016/j.envint.2020.106274.

[32] Ragusa, A., Notarstefano, V., Svelato, A., Belloni, A., Gioacchini, G., Blondeel, C., et al., 2022. Raman microspectroscopy detection and characterisation of microplastics in human breastmilk. Polymers 14, 2700. https://doi.org/10.3390/polym14132700.

[33] Hsieh, S.-L., Wu, Y.-C., Xu, R.-Q., Chen, Y.-T., Chen, C.-W., Singhania, R.R., et al., 2021. Effect of polyethylene microplastics on oxidative stress and histopathology damages in *Litopenaeus vannamei*. Environ Pollut 288, 117800. https://doi.org/10.1016/j.envpol.2021.117800.

[34] Chae, Y., An, Y.-J., 2020. Nanoplastic ingestion induces behavioral disorders in terrestrial snails: trophic transfer effects via vascular plants. Environ Sci: Nano 7, 975–983. https://doi.org/10.1039/C9EN01335K.

[35] Pedersen, A.F., Meyer, D.N., Petriv, A.-M.V., Soto, A.L., Shields, J.N., Akemann, C., et al., 2020. Nanoplastics impact the zebrafish (*Danio rerio*) transcriptome: Associated developmental and neurobehavioral consequences. Environ Pollut 266, 115090. https://doi.org/10.1016/j.envpol.2020.115090.

[36] Jeong, C.-B., Kang, H.-M., Lee, M.-C., Kim, D.-H., Han, J., Hwang, D.-S., et al., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod *Paracyclopina nana*. Sci Rep 7, 41323. https://doi.org/10.1038/srep41323.

[37] Lee, K.-W., Shim, W.J., Kwon, O.Y., Kang, J.-H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. Environ Sci Technol 47, 11278–11283. https://doi.org/10.1021/es401932b.

[38] Hu, M., Palić, D., 2020. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. Redox Biol 37, 101620. https://doi.org/10.1016/j.redox.2020.101620.

[39] Mattsson, K., Ekvall, M.T., Hansson, L.-A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environ Sci Technol 49, 553–561. https://doi.org/10.1021/es5053655.

[40] Luo, T., Wang, C., Pan, Z., Jin, C., Fu, Z., Jin, Y., 2019. Maternal polystyrene microplastic exposure during gestation and lactation altered metabolic homeostasis in the dams and their F1 and F2 offspring. Environ Sci Technol 53, 10978–10992. https://doi.org/10.1021/acs.est.9b03191.

[41] Yu, C.-W., Luk, T., Liao, V., 2021. Long-term nanoplastics exposure results in multi and trans-generational reproduction decline associated with germline toxicity and epigenetic regulation in Caenorhabditis elegans. J Hazard Mater 412, 125173. https://doi.org/10.1016/j.jhazmat.2021.125173.

[42] Bucci, K., Tulio, M., Rochman, C.M., 2020. What is known and unknown about the effects of plastic pollution: A meta-analysis and systematic review. Ecol Appl 30, e02044. https://doi.org/10.1002/eap.2044.

[43] Peng, L., Fu, D., Qi, H., Lan, C.Q., Yu, H., Ge, C., 2020. Micro- and nano-plastics in marine environment: Source, distribution and threats — A review. Sci Total Environ 698, 134254. https://doi.org/10.1016/j.scitotenv.2019.134254.

[44] Ugwu, K., Herrera, A., Gómez, M., 2021. Microplastics in marine biota: A review. Mar Pollut Bull 169, 112540. https://doi.org/10.1016/j.marpolbul.2021.112540.

[45] Dong, C.-D., Chen, C.-W., Chen, Y.-C., Chen, H.-H., Lee, J.-S., Lin, C.-H., 2020. Polystyrene microplastic particles: In vitro pulmonary toxicity assessment. J Hazard Mater 385, 121575. https://doi.org/10.1016/j.jhazmat.2019.121575.

[46] Hou, J., Lei, Z., Cui, L., Hou, Y., Yang, L., An, R., et al., 2021. Polystyrene microplastics lead to pyroptosis and apoptosis of ovarian granulosa cells via NLRP3/Caspase-1 signaling pathway in rats. Ecotoxicol Environ Saf 212, 112012. https://doi.org/10.1016/j.ecoenv.2021.112012.

[47] Lambert, S., Scherer, C., Wagner, M., 2017. Ecotoxicity testing of microplastics: Considering the heterogeneity of physicochemical properties. Integr Environ Assess Manag 13, 470–475. https://doi.org/10.1002/ieam.1901.

[48] Danopoulos, E., Twiddy, M., West, R., Rotchell, J.M., 2022. A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells. J Hazard Mater 427, 127861. https://doi.org/10.1016/j.jhazmat.2021.127861.

[49] Andrady, A.L., 2011. Microplastics in the marine environment. Mar Pollut Bull 62, 1596–1605.

[50] Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., et al., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc Lond B Biol Sci 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

[51] Ramsperger, A.F.R.M., Narayana, V.K.B., Gross, W., Mohanraj, J., Thelakkat, M., Greiner, A., et al., 2020. Environmental exposure enhances the internalization of microplastic particles into cells. eabd1211 Sci Adv 6. https://doi.org/10.1126/sciadv.abd1211.

[52] Li, B., Ding, Y., Cheng, X., Sheng, D., Xu, Z., Rong, Q., et al., 2020. Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. Chemosphere 244, 125492. https://doi.org/10.1016/j.chemosphere.2019.125492.

[53] Umamaheswari, S., Priyadarshinee, S., Bhattacharjee, M., Kadirvelu, K., Ramesh, M., 2021. Exposure to polystyrene microplastics induced gene modulated biological responses in zebrafish (*Danio rerio*). Chemosphere 281, 128592. https://doi.org/10.1016/j.chemosphere.2020.128592.

[54] I.R. Williams, Fibroblasts, in: P.J. Delves (Ed.), Encyclopedia of Immunology, Elsevier, Oxford, 1998: pp. 905–909. https://doi.org/10.1006/rwei.1999.0237.

[55] Wynn, T.A., Ramalingam, T.R., 2012. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 18, 1028–1040. https://doi.org/10.1038/nm.2807.

[56] Wagner, G.R., 1997. Asbestosis and silicosis. Lancet 349, 1311–1315. https://doi.org/10.1016/S0140-6736(96)07336-9.

[57] Shen, R., Yang, K., Cheng, X., Guo, C., Xing, X., Sun, H., et al., 2022. Accumulation of polystyrene microplastics induces liver fibrosis by activating cGAS/STING pathway. Environ Pollut 300, 118986. https://doi.org/10.1016/j.envpol.2022.118986.

[58] Lavers, J.L., Hutton, I., Bond, A.L., 2021. Temporal trends and interannual variation in plastic ingestion by Flesh-footed Shearwaters (*Ardenna carneipes*) using different sampling strategies. Environ Pollut 290, 118086. https://doi.org/10.1016/j.envpol.2021.118086.

[59] Lavers, J.L., Bond, A.L., 2022. Long-term decline in fledging body condition in Flesh-footed Shearwaters (*Ardenna carneipes*). ICES. J. Mar. Sci. [accepted].

[60] Lavers, J.L., Bond, A.L., Hutton, I., 2014. Plastic ingestion by Flesh-footed Shearwaters (*Puffinus carneipes*): Implications for fledgling body condition and the accumulation of plastic-derived chemicals. Environ Pollut 187, 124–129. https://doi.org/10.1016/j.envpol.2013.12.020.

[61] Lavers, J.L., Hutton, I., Bond, A.L., 2019. Clinical pathology of plastic ingestion in marine birds and relationships with blood chemistry. Environ Sci Technol 53, 9224–9231. https://doi.org/10.1021/acs.est.9b02098.

[62] Provencher, J., Bond, A., Avery-Gomm, S., Borrelle, S., Bravo Rebolledo, E., Lavers, J., et al., 2017. Quantifying ingested debris in marine megafauna: A review and recommendations for standardization. Anal Methods 9, 1454–1469. https://doi.org/10.1039/c6ay02419j.

[63] Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep 7, 44501. https://doi.org/10.1038/srep44501.

[64] Shruti, V.C., Pérez-Guevara, F., Roy, P.D., Kutralam-Muniasamy, G., 2022. Analyzing microplastics with Nile Red: Emerging trends, challenges, and prospects. J Hazard Mater 423, 127171. https://doi.org/10.1016/j.jhazmat.2021.127171.

[65] An, R., Wang, X., Yang, L., Zhang, J., Wang, N., Xu, F., et al., 2021. Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. Toxicology 449, 152665. https://doi.org/10.1016/j.tox.2020.152665.

[66] Wu, H., Xu, T., Chen, T., Liu, J., Xu, S., 2022. Oxidative stress mediated by the TLR4/NOX2 signalling axis is involved in polystyrene microplastic-induced uterine fibrosis in mice. Sci Total Environ 838, 155825. https://doi.org/10.1016/j.scitotenv.2022.155825.

[67] Hall, J.E., Guyton, A.C., 2011. Guyton and Hall textbook of medical physiology, 12th ed. Saunders,Elsevier.

[68] J.G. Betts , P. Desaix , E. Johnson , J.E. Johnson , O. Korol , D. Kruse et al. , Anatomy and physiology, 2nd ed., OpenStax College, Rice University, Texas, United States of America, 2021.

[69] Sayrafi, R., Aghagolzadeh, M., 2020. Histological and histochemical study of the proventriculus (Ventriculus glandularis) of common starling (*Sturnus vulgaris*). Anat Histol Embryol 49, 105–111. https://doi.org/10.1111/ahe.12495.

[70] Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al., 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.

[71] The Jamovi project, Jamovi, (2022). https://www.jamovi.org.

[72] R. Core Team, R: A Language and environment for statistical computing., (2021). https://cran.r-project.org.

[73] Li, Z., Zhu, S., Liu, Q., Wei, J., Jin, Y., Wang, X., et al., 2020. Polystyrene microplastics cause cardiac fibrosis by activating Wnt/β-catenin signaling pathway and promoting cardiomyocyte apoptosis in rats. Environ Pollut 265, 115025. https://doi.org/10.1016/j.envpol.2020.115025.

[74] Lin, P., Tong, X., Xue, F., Qianru, C., Xinyu, T., Zhe, L., et al., 2022. Polystyrene nanoplastics exacerbate lipopolysaccharide-induced myocardial fibrosis and autophagy in mice via ROS/TGF-β1/Smad. Toxicology 480, 153338. https://doi.org/10.1016/j.tox.2022.153338.

[75] Abolins, S., King, E.C., Lazarou, L., Weldon, L., Hughes, L., Drescher, P., et al., 2017. The comparative immunology of wild and laboratory mice, *Mus musculus domesticus*. Nat Commun 8, 14811. https://doi.org/10.1038/ncomms14811.

[76] J.P. Gionfriddo, L.B. Best, Grit use by birds: a review., in: Current Ornithology, Springer Sciemce+Business Media, New York, 1999: pp. 89–148.

[77] Klasing, K.C., 1999. Avian gastrointestinal anatomy and physiology. Semin Avian Exot Pet Med 8, 42–50. https://doi.org/10.1016/S1055-937X(99)80036-X.

[78] Schwenk, K., Rubega, M., 2005. Diversity of vertebrate feeding systems. In: Physiological and Ecological Adaptations to Feeding in Vertebrates. Science Publishers, Enfield, NH, pp. 1–41.

[79] J.L. Lavers, A.L. Bond, Pumice ingestion in seabirds: interannual variation, and relationships with chick growth and plastic ingestion, Mar. Biol. [In Review] (2022).

[80] Ryan, P.G., 2015. How quickly do albatrosses and petrels digest plastic particles. Environ Pollut 207, 438–440. https://doi.org/10.1016/j.envpol.2015.08.005.

[81] Bond, A.L., Hutton, I., Lavers, J.L., 2021. Plastics in regurgitated Flesh-footed Shearwater (*Ardenna carneipes*) boluses as a monitoring tool. Mar Pollut Bull 168, 112428. https://doi.org/10.1016/j.marpolbul.2021.112428.

[82] Wright, S.L., Kelly, F.J., 2017. Plastic and human health: A micro issue. Environ Sci Technol 51, 6634–6647. https://doi.org/10.1021/acs.est.7b00423.

[83] Cousin, H.R., Auman, H.J., Alderman, R., Virtue, P., 2015. The frequency of ingested plastic debris and its effects on body condition of Short-tailed Shearwater (*Puffinus tenuirostris*) pre-fledging chicks in Tasmania, Australia. Emu 115, 6–11. https://doi.org/10.1071/MU13086.

[84] Lavers, J.L., de Jersey, A.M., Jones, N.R., Stewart, L.G., Charlton-Howard, H.S., Grant, M.L., et al., 2022. Ingested plastics in beach-washed Fairy Prions *Pachyptila turtur* from Tasmania. Mar Pollut Bull 184, 114096. https://doi.org/10.1016/j.marpolbul.2022.114096.

[85] Provencher, J.F., Malaisé, F., Mallory, M.L., Braune, B.M., Pirie-Dominix, L., Lu, Z., 2022. 44-Year retrospective analysis of ultraviolet absorbents and industrial antioxidants in seabird eggs from the Canadian Arctic (1975 to 2019. Environ Sci Technol. https://doi.org/10.1021/acs.est.2c05940.

[86] Zhao, Y., Bao, Z., Wan, Z., Fu, Z., Jin, Y., 2020. Polystyrene microplastic exposure disturbs hepatic glycolipid metabolism at the physiological, biochemical, and transcriptomic levels in adult zebrafish. Sci Total Environ 710, 136279. https://doi.org/10.1016/j.scitotenv.2019.136279.

[87] Jin, Y., Lu, L., Tu, W., Luo, T., Fu, Z., 2019. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. Sci Total Environ 649, 308–317. https://doi.org/10.1016/j.scitotenv.2018.08.353.

[88] S.W.S. Gussekloo, Feeding structures in birds, in: Feeding in Domestic Vertebrates: From Structure to Behaviour, CABI Publishing, Wallingford, UK, 2006: pp. 14–19.

[89] Engevik, A.C., Kaji, I., Goldenring, J.R., 2020. The physiology of the gastric parietal cell. Physiol Rev 100, 573–602. https://doi.org/10.1152/physrev.00016.2019.

[90] Nielsen, M.J., Rasmussen, M.R., Andersen, C.B.F., Nexø, E., Moestrup, S.K., 2012. Vitamin B12 transport from food to the body's cells—a sophisticated, multistep pathway. Nat Rev Gastroenterol Hepatol 9, 345–354. https://doi.org/10.1038/nrgastro.2012.76.

[91] Bateman, D.N., Colin-Jones, D., Hartz, S., Langman, M., Logan, R.F., Mant, J., et al., 2003. Mortality study of 18 000 patients treated with omeprazole. Gut 52, 942–946.

[92] Mowat, C., Williams, C., Gillen, D., Hossack, M., Gilmour, D., Carswell, A., et al., 2000. Omeprazole, *Helicobacter pylori* status, and alterations in the intragastric milieu facilitating bacterial N-nitrosation. Gastroenterology 119, 339–347. https://doi.org/10.1053/gast.2000.9367.

[93] Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C., 2021. Oceanic hitchhikers – Assessing pathogen risks from marine microplastic. Trends Microbiol 29, 107–116. https://doi.org/10.1016/j.tim.2020.06.011.

[94] Maquart, P.-O., Froehlich, Y., Boyer, S., 2022. Plastic pollution and infectious diseases. Lancet Planet Health 6, e842–e845. https://doi.org/10.1016/S2542-5196(22)00198-X.

[95] Wolf, D.E., Wood, T.R., Valiant, J., Folkers, K., 1950. VII. Vitamin B12 and the intrinsic factor. Proc Soc Exp Biol Med 73, 15–17. https://doi.org/10.3181/00379727-73-17560.

[96] Briani, C., Dalla Torre, C., Citton, V., Manara, R., Pompanin, S., Binotto, G., et al., 2013. Cobalamin deficiency: Clinical picture and radiological findings. Nutrients 5, 4521–4539. https://doi.org/10.3390/nu5114521.

[97] Stabler, S.P., 2013. Vitamin B12 deficiency. New Engl J Med 368, 149–160. https://doi.org/10.1056/NEJMcp1113996.

[98] Shi, Y., Liu, T., Yao, L., Xing, Y., Zhao, X., Fu, J., et al., 2017. Chronic vitamin D deficiency induces lung fibrosis through activation of the renin-angiotensin system. Sci Rep 7, 3312. https://doi.org/10.1038/s41598-017-03474-6.

[99] Pohl, J., 2013. Gastric linitis plastica. Video J Encycl GI Endosc 1, 172–173. https://doi.org/10.1016/S2212-0971(13)70071-2.

[100] S. Jafferbhoy, H. Shiwani, Q. Rustum, Managing gastric linitis plastica, Sultan Qaboos Univ. Med. J. 13 (2013) 451–453.

[101] Verhaegen, P.D.H.M., Van Zuijlen, P.P.M., Pennings, N.M., Van Marle, J., Niessen, F.B., Van Der Horst, C.M.A.M., et al., 2009. Differences in collagen architecture between keloid, hypertrophic scar, normotrophic scar, and normal skin: An objective histopathological analysis. Wound Repair Regen 17, 649–656. https://doi.org/10.1111/j.1524-475X.2009.00533.x.

[102] Stockhausen, K.E., Qwamizadeh, M., Wölfel, E.M., Hemmatian, H., Fiedler, I.A.K., Flenner, S., et al., 2021. Collagen fiber orientation is coupled with specific nano-compositional patterns in dark and bright osteons modulating their biomechanical properties. ACS Nano 15, 455–467. https://doi.org/10.1021/acsnano.0c04786.

[103] Thalmann, S.J., Lea, M.-A., Hindell, M., Priddel, D., Carlile, N., 2010. Provisioning in Flesh-Footed Shearwaters (*Puffinus carneipes*): Plastic foraging behavior and the implications for increased fishery interactions. Auk 127, 140–150. https://doi.org/10.1525/auk.2009.09158.

[104] P.A. Campbell, G. Chun, N. Kossovsky, H.C. Amstutz, Histological analysis of tissues suggests that "metallosis" may really be "plasticosis," Transactions of the 38th Ann. Meeting of the Orthop. Res. Soc. 17 (1992) 393.