Clinical pathology of plastic ingestion in marine birds and relationships with blood chemistry

Jennifer L. Lavers\textsuperscript{a,*}, Ian Hutton\textsuperscript{b}, Alexander L. Bond\textsuperscript{a,c}

\textsuperscript{a}Institute for Marine and Antarctic Studies, University of Tasmania, Battery Point, Tasmania, 7004, Australia

\textsuperscript{b}Lord Howe Island Museum, Lord Howe Island, New South Wales, 2898, Australia

\textsuperscript{c}Bird Group, Department of Life Sciences, The Natural History Museum, Tring, Hertfordshire, HP23 6AP, UK

*Corresponding author. E-mail address: Jennifer.Lavers@utas.edu.au (J.L. Lavers)

ABSTRACT: Pollution of the environment with plastic debris is a significant and rapidly expanding threat to biodiversity due to its abundance, durability, and persistence. Current knowledge of the negative effects of debris on wildlife is largely based on consequences that are readily observed, such as entanglement or starvation. Many interactions with debris, however, result in less visible and poorly documented sub-lethal effects, and as a consequence, the true impact of plastic is underestimated. We investigated the sub-lethal effects of ingested plastic in Flesh-footed Shearwaters (\textit{Ardenna carneipes}) using blood chemistry parameters as a measure of bird health. The presence of plastic had a significant negative effect on bird morphometrics and blood calcium levels, and a positive relationship with the concentration of uric acid, cholesterol, and amylase. That we found blood chemistry parameters to be related to plastic pollution is one of few examples to date of the sublethal effects of marine debris and highlights that superficially healthy individuals may still experience the negative consequences of ingesting plastic debris. Moving beyond crude measures, such as reduced body mass, to physiological parameters will provide much needed insight into the nuanced and less visible effects of plastic.
TABLE OF CONTENTS GRAPHIC

KEYWORDS: haematology; seabird health; marine debris; morbidity; plastic pollution
1. INTRODUCTION

Increasing demand for, and production of, plastic products coupled with inadequate waste management and policy contributes to the ongoing and rapidly expanding issue of pollution of our waterways and wildlife. Mass production of small plastic items, such as pellets or ‘nurdles’ combined with the break-up of larger items present in the marine environment for decades has led to an increase in the availability of bite-size pieces for wildlife and a concomitant increase in the frequency of accidental consumption by marine animals in all of the world’s oceans.

The negative consequences resulting from interactions between wildlife and plastic debris are diverse, often visually striking, and can include nutritional deprivation, entanglement, and damage to or obstruction of the gut (e.g., perforations and ulcers). Many of these interactions also include less visible and therefore less well documented effects such as reduced growth and survival rates following ingestion and as a consequence, we are drastically underestimating the true impact of plastic waste on our oceans.

Plastic is inherently toxic and becomes increasingly hazardous over time as it accumulates pollutants from the surrounding marine environment. Once ingested, the absorbed toxins leach into the animal’s blood stream and contribute to neurological, behavioural, and reproductive problems at all levels of biological organization, and in extreme cases, death of individuals. At least 43-100% of the world’s marine mammal, seabird, and turtle species are at risk from the ingestion of plastic and this number is expected to increase as plastic production and studies into its effects also rise. How we manage our waste and understand the true scope and severity of impacts this has on wildlife therefore has broad implications for the health of marine ecosystems.

The Flesh-footed Shearwater (Ardenna carneipes) is one of the most heavily impacted marine vertebrates with regards to plastic pollution, containing a mixture of macro-, micro-, and ultrafine debris. Populations across the species’ range are in decline with the ingestion of plastic implicated in the downward trend due to its negative effect on chick growth and survival and its capacity to act as a vector for contaminants. The mechanisms by which plastic negatively influences shearwater and other wildlife populations are poorly understood, are likely present at the molecular or cellular levels.
of organization, and may not result in organisms’ death, but in their poor health \(^{18,26}\). To better understand the often invisible and sub-lethal effects of ingested plastic waste, we examined the clinical pathology of ingested plastic through blood chemistry and body condition in Flesh-footed Shearwaters.

2. MATERIALS AND METHODS

2.1. Ingested plastic sampling. Flesh-footed Shearwater fledglings (\(n = 53\)) were captured by hand at night on the colony surface on Lord Howe Island, New South Wales (31.554ºS, 159.085ºE; for more information see Lavers, Hutton and Bond \(^{24}\)) from 1-9 May 2014-2017 when the birds were approximately 90 days of age. Body mass (±10 g) was determined using a spring balance, wing chord (flattened; ±1 mm) using a stopped ruler, and culmen and head + bill length using Vernier callipers (±0.1 mm). Ingested plastic was collected by stomach flushing following procedures outlined by Duffy and Jackson \(^{27}\), and detailed in Lavers, et al. \(^{28}\). In brief, seawater (approx. 150 ml) at ambient temperature was gently pumped into the proventriculus through a tube, thus displacing any ingested items. Once fluid and stomach contents began to flow back up the oesophagus (i.e., once the stomach was filled completely), the bird was inverted over a container to collect anything expelled. Plastic items were dried and weighed to the nearest 0.001 g using an electronic balance.

2.2. Blood sampling. Approximately 0.5 ml of whole blood was collected from the brachial vein of each bird using a 26-gauge needle and stored in lithium heparin vacutainer tubes (Grenier MiniCollect, Austria). A drop of blood was smeared onto a microscope slide immediately after collection and air dried. Samples were refrigerated at 4°C overnight then express posted to IDEXX Laboratory (Rydelmere, NSW, Australia) by airplane and courier the following afternoon (i.e., within 18 hours of collection). At the laboratory, two slides were prepared from each sample using two separate techniques to evaluate haematological parameters. The first technique involved diluting and mixing 20 μl of whole blood with 620 μl of Eosin stain solution. The mixture was then used to fill two chambers of a haemocytometer for manual count of heterophils and eosinophils through a microscope. The second technique required a May-Grünswald/Giemsa stain to perform a differential stain, calculate a total white blood cell count, and
assess morphology of the red blood cells, white blood cells, and platelets. The proportion of different
types of leucocytes (lymphocytes, heterophils, monocytes, eosinophils, and basophils) was assessed
based on an examination of 100 leucocytes under oil immersion (1000× magnification). Blood
components (glucose (mmol/L), urea (mmol/L), calcium (mmol/L), total protein (g/L), albumin (g/L),
globulin (g/L), aspartate aminotransferase (AST; IU/L), creatine kinase (IUt/L), cholesterol (mmol/L),
amylase (IUt/L), glutamate dehydrogenase (GLDH; IUt/L), uric acid (mmol/L), and bile acids (umol/L)) were
measured using an Olympus AU680 chemistry analyser (Beckman-Coulter, Japan). A packed cell volume
(PCV; haematocrit) was obtained by spinning whole blood in heparinised capillary pipettes in a
microhematocrit centrifuge for five minutes. If the PCV was below the reference range for seabirds, a slide
was stained with Methylene blue and a reticulocyte count on the red blood cells was performed. All
QA/QC and analytical procedures were undertaken by IDEXX Laboratories under ISO/IEC 17025
accreditation (No. 10166).

2.3. Statistical methods. Data were tested for normality using Shapiro-Wilk test, and where needed,
log-transformed to improve normality. Parameters were grouped by physiological function: those
associated with the liver (aspartate aminotransferase, glutamate dehydrogenase, bile acids), blood
proteins (total protein, albumen, globulin), those with kidney/renal function (urea, uric acid), and white
blood cell types (numbers of heterophils, lymphocytes, monocytes, eosinophils, and basophils). Other
parameters (glucose, calcium, cholesterol, creatine kinase, amylase) and the number of white blood cell
types were analysed individually.

Relationships between measurements of body condition or blood parameters and plastic
ingestion were explored using a series of univariate (reporting F-statistics) or multivariate linear models
(reporting Wilk’s λ, followed by univariate tests when results were significant). Analyses were done using
R 3.5.1 (R Core Team 2018), differences were considered statistically significant when p < 0.05 and values
are reported as mean ± SD.

3. RESULTS
We found no relationship between the presence, number, or mass of plastics and packed cell volume, white blood cell count, or white blood cell composition (Table 2). Similarly, there was no relationship between any measure of plastic burden and molecules associated with liver function (aspartate aminotransferase, glutamate dehydrogenase, or bile acids; Table 2).

The only significant relationship between plastic burden and measures of kidney function was a positive association between the number of plastic pieces and concentration of uric acid (Figure 1, Table 2). There were negative relationships between plastic presence, number, and mass with blood calcium, and positive relationships between plastic presence and cholesterol, as well as plastic mass and amylase concentration (Table 2).

The presence of plastic had significant negative relationships with all morphometrics, though there was no link between bird size and the number or mass of ingested plastic (Figure 1, Table 2).

4. DISCUSSION

There is scant information on blood composition for free-living birds, especially seabirds, many of which have been identified as threatened species. Flesh-footed Shearwaters have experienced considerable mortality through human actions (e.g., fisheries bycatch) which contributed to a reduction in population numbers in recent decades. Our data therefore provide much needed blood chemistry reference values for Flesh-footed Shearwaters, a species that was recently up-listed to Near Threatened and is declining across its range. Blood chemistry data also provides valuable information on other threats that may affect the health of individuals, including plastic debris.

Blood cell volume (PCV) varied considerably among individual Flesh-footed Shearwaters and is known to be influenced by age or physiological status in other Procellariiforms. The body mass recorded for some shearwater fledglings (180-270 g) was well below the range typically observed in this species (500-750 g), suggesting these birds were in poor condition. This agrees with our visual observations, which indicated these individuals were emaciated and often lethargic. However, PCV values do not reliably reflect condition in some seabird species, even when birds are experimentally handicapped. In light of this, and the variability in blood cell count parameters we observed, it is
unsurprising we failed to detect a relationship between PCV and plastic presence, mass, or number of ingested items (Table 2).

Changes in blood parameters have been evaluated in some seabirds well adapted to seasonal fasting (e.g., penguins; 37). Fasting in birds occurs in three phases with the final phase indicating the depletion of fat reserves, when body proteins are catabolized, resulting in a higher rate of body-mass loss, which is often indicative of a critical limit 38. Low total protein (<25 g/L) is considered a good indicator of starvation in birds, but can also signify chronic disease or stress 39. In Flesh-footed Shearwaters, total protein was only slightly above this threshold (33 ± 10 g/L; Table 1) and was not related to plastic presence, mass, or number of ingested items (Table 2). However, uric acid, cholesterol, and amylase values all increased in response the presence of ingested plastic (Figure 1, Table 2).

Increased production of uric acid is commonly reported in birds during this final phase of fasting as it minimizes water loss while allowing nitrogen to be excreted 37,40. However, high concentrations of uric acid in the blood can also indicate renal failure in birds and contribute to diseases such as kidney stones and diabetes 41. Thus, increased uric acid associated with the ingestion of plastic is potentially problematic. Renal issues are also associated with increases in amylase which plays a key role in digestion in birds 42. We detected a positive relationship between plastic presence and mass and amylase concentration in shearwaters. Similar patterns have been reported in tropical fish where exposure to microplastics significantly increased amylase concentrations 43. While very little has been published on amylase in seabirds, this reinforces the possible role that ingested plastic may play in renal health.

Plasma cholesterol can increase in birds during periods of stress 44, including during short fasting periods 45. However, the concentrations we detected in shearwaters were elevated (12.0 ± 7.0 mmol/L; Table 1) compared with other juvenile seabirds experiencing significant food deprivation (7.5 ± 0.2 mmol/L; 38). This suggests other factors, not just emaciation, may be influencing cholesterol concentrations in this species. The ingestion of plastic could play a key role, with cholesterol levels in Flesh-footed Shearwaters increasing in relation to plastic presence (Table 2). The relationship between ingested plastic and cholesterol is likely complex, as plastic items contribute to emaciation, subsequent stress levels, and exposure to chemicals 6,46. In marine mammals, plasticizers (chemicals directly
associated with plastic) can interfere with cholesterol levels and reproduction by altering transport through the mitochondrial membrane\textsuperscript{47}.

Calcium levels in Flesh-footed Shearwater fledgling blood (2.3 ± 0.2 mmol/L; Table 1) were substantially lower than other juvenile seabirds (10.1-12.2 mmol/L; Table 3) and the reference range for putative healthy, uncontaminated Northern Fulmars \textit{(Fulmarus glacialis}; 6.1 mmol/L; \textsuperscript{48}). Many factors can influence calcium levels, including gastrointestinal diseases that contribute to malabsorption, hypoparathyroidism, and pancreatitis\textsuperscript{49}. The cause of the low calcium in Flesh-footed Shearwaters is unclear, however, exposure to petroleum-based chemicals has been shown to reduce plasma calcium levels in many waterbirds\textsuperscript{50, 51}. Seabirds with reduced body fat also exhibit lower blood calcium concentrations\textsuperscript{48}. Reduced blood calcium was associated with the ingestion of plastic in Flesh-footed Shearwaters (Table 2), which is attributed to poor fat reserves and increased chemical exposure\textsuperscript{6}.

In contrast to previous work\textsuperscript{6}, we did not find significant relationships between the amount of plastic and bird morphometrics. Rather, simply the presence of plastic had a negative relationship with body mass, wing length, culmen, and head+bill length. Though our sample size is small (n = 38), it does span the range of severity of plastic ingestion\textsuperscript{6, 52}. This suggests that such effects are likely to be complex, influenced by inter-annual variation, and could imply a threshold over which additional plastic has little additional effect. Understanding this mechanism is fundamental to the question “how much plastic is bad”, which is key for establishing relevant policy targets.

To date, studies of the effect of plastic debris on wildlife have been largely confined to quantifying plastic burden (e.g., Provencher, et al.\textsuperscript{53}), or relating that burden to gross measures of body condition, such as mass or chick size (e.g., Lavers, Bond and Hutton\textsuperscript{6}). Though these studies remain important, increasingly, the potential for sublethal impacts on birds’ physiology is of concern\textsuperscript{54}, including the presence of toxic chemicals\textsuperscript{6, 15, 55}, harmful microbiota\textsuperscript{56, 57}, and the ubiquity of small particles which can be distributed throughout the digestive tract\textsuperscript{21, 58}. Such relationships have been recently described in marine invertebrates and fish\textsuperscript{59, 60}, but few data exist for seabirds. This is concerning as birds can compensate for physiological impairments caused by disease or other stressors\textsuperscript{49} meaning they can...
appear healthy for a period of time, which can be misleading when undertaking visual assessments of health and condition or using superficial measures, such as body size.

While quantifying population-level impacts of plastic ingestion on wildlife remains an important goal, this will continue to be challenging given the myriad threats faced by seabirds, and as we have demonstrated, the often hidden sublethal effects plastic may be having on wildlife. In seabirds, which spend very little time on land or at breeding colonies in the course of their annual cycle, most mortality occurs at sea where the causes are often unknown and only manifest in the failure of an individual to return in subsequent years. Crucially, mortality of juvenile pre-breeding seabirds, which have deferred maturity and may not begin breeding until 5-10 years old, is poorly studied owing to the logistical challenge of measuring survival and determining the fates of individuals away from breeding sites. Consequently, we cannot know at present how, or indeed whether, the physiological challenges faced by fledging birds, such as we measured, might affect demographic rates and the survival of individuals. However, the application of clinical pathology to wildlife conservation (e.g., Dagleish, et al.) has the potential to inform management and conservation action, including through the elucidation of the presence and magnitude of pressures on individuals. Moving beyond crude measures such as mortality, or reduced body mass to physiological parameters will help us understand the nuanced effects of plastic ingestion and other, cumulative stressors.

ACKNOWLEDGMENTS

Detached Foundation, L. Bryce, Living Ocean, and Trading Consultants Ltd generously provided funding for this project. Samples were collected with the permission of the Lord Howe Island Board (LHIB02/14 & 06/16) and New South Wales Office of Environment and Heritage (SL100169) under the approval of University of Tasmania Animal Ethics (A13836 & A14130). Analytical and logistical support was provided by the Australian Bird and Bat Banding Scheme, J. Benjamin, P. Clive, B. Doneley, B. & I. Fulton, J. Harris (Mayfair Veterinary Clinic), S. Jaensch, P. Sharp, S. Stuckenbrock, and V. Wilkinson (Zoological Society of London). Comments from three anonymous reviewers improved earlier drafts.

REFERENCES
1. Rochman, C. M.; Browne, M. A.; Halpern, B. S.; Hentschel, B. T.; Hoh, E.; Karapanagioti, H. K.; Rios-Mendoza, L. M.; Takada, H.; Teh, S.; Thompson, R. C., Policy: Classify plastic waste as hazardous. *Nature* **2013**, *494*, (7436), 169-171.

2. Borrelle, S. B.; Rochman, C. M.; Liboiron, M.; Bond, A. L.; Lusher, A.; Bradshaw, H.; Provencher, J. F., Opinion: Why we need an international agreement on marine plastic pollution. *Proc. Nat. Acad. Sci.* **2017**, *114*, (38), 9994-9997.

3. Thompson, R. C.; Olsen, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.; McGonigle, D.; Russell, A. E., Lost at sea: where is all the plastic? *Science* **2004**, *304*, 838.

4. Browne, M. A.; Crump, P.; Niven, S. J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R., Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ. Sci. Technol.* **2011**, *45*, (21), 9175-9179.

5. Gall, S. C.; Thompson, R. C., The impact of debris on marine life. *Mar. Pollut. Bull.* **2015**, *92*, 170-179.

6. Lavers, J. L.; Bond, A. L.; Hutton, I., Plastic ingestion by Flesh-footed Shearwaters (*Puffinus carneipes*): Implications for chick body condition and the accumulation of plastic-derived chemicals. *Environ. Pollut.* **2014**, *187*, 124-129.

7. Ryan, P. G., Entanglement of birds in plastics and other synthetic materials. *Mar. Pollut. Bull.* **2018**, *135*, 159-164.

8. Lavers, J. L.; Sharp, P. B.; Stuckenbrock, S.; Bond, A. L., Entrapment in plastic debris endangers hermit crab populations. *PNAS in review*.

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

10. Ryan, P. G., Effects of ingested plastic on seabird feeding: evidence from chickens. *Mar. Pollut. Bull.* **1988**, *19*, (3), 125-128.

11. Browne, M. A.; Niven, S. J.; Galloway, T. S.; Rowland, S. J.; Thompson, R. C., Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. *Curr. Biol.* **2013**, *23*, (23), 2388-2392.

12. Lithner, D.; Larsson, Å.; Dave, G., Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* **2011**, *409*, (18), 3309-3324.

13. Holmes, L. A.; Turner, A.; Thompson, R. C., Adsorption of trace metals to plastic resin pellets in the marine environment. *Environ. Pollut.* **2012**, *160*, 42-48.

14. Lee, H.; Shim, W. J.; Kwon, J. H., Sorption capacity of plastic debris for hydrophobic organic chemicals. *Sci. Total Environ.* **2014**, *470-471*, 1545-1552.

15. Tanaka, K.; Takada, H.; Yamashita, R.; Mizukawa, K.; Fukuwaka, M.; Watanuki, Y., Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Mar. Pollut. Bull.* **2013**, *69*, (1), 219-222.

16. Lavers, J. L.; Bond, A. L., Ingested plastic as a route for trace metals in Laysan Albatross (*Phoebastria immutabilis*) and Bonin Petrel (*Pterodroma hypoleuca*) from Midway Atoll. *Mar. Pollut. Bull.* **2016**, *110*, (1), 493-500.

17. Bouland, A. J.; White, A. E.; Lonabaugh, K. P.; Varian-Ramos, C. W.; Cristol, D. A., Female-biased offspring sex ratios in birds at a mercury-contaminated river. *J. Avian Biol.* **2012**, *43*, (3), 244-251.

18. Rochman, C. M.; Browne, M. A.; Underwood, A. J.; van Franeker, J. A.; Thompson, R. C.; Amaral-Zettler, L. A., The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. *Ecol. 2016*, 97, 302-312.

19. Künn, S.; Bravo Rebollo, E. L.; van Franeker, J. A., Deleterious effects of litter on marine life. In *Marine Anthropogenic Litter*, Bergmann, M.; Gutow, L.; Klages, M., Eds. Springer International Publishing: 2015; pp 75-116.

20. Worm, B.; Lotze, H. K.; Jubienville, I.; Wilcox, C.; Jambeck, J. R., Plastic as a persistent marine pollutant. *Annu. Rev. Environ. Resour.* **2017**, *42*, (1), 1-26.

21. Lavers, J. L.; Stivaktakis, G.; Hutton, I.; Bond, A. L., Detection of ultrafine plastics ingested by seabirds using tissue digestion. *Mar. Pollut. Bull.* **2019**, *142*, 470-474.
22. Lavers, J. L., Population status and threats to Flesh-footed Shearwaters (Puffinus carneipes) in South and Western Australia. *ICES J. Mar. Sci.* 2015, 72, 316-327.

23. Jamieson, S. E.; Waugh, S. M., An assessment of recent population trends of Flesh-footed Shearwaters (Puffinus carneipes) breeding in New Zealand. *Notornis* 2015, 62, (1), 8-13.

24. Lavers, J. L.; Hutton, I.; Bond, A. L., Changes in technology and imperfect detection of nest contents impedes reliable estimates of population trends in burrowing seabirds. *Glob. Ecol. Conserv.* 2019, 17, e00579.

25. Bond, A. L.; Lavers, J. L., Trace element concentrations in feathers of Flesh-footed Shearwaters (Puffinus carneipes) from across their breeding range. *Arch. Environ. Contam. Toxicol.* 2011, 61, (2), 318-326.

26. Avio, C. G.; Gorbí, S.; Milan, M.; Benedetti, M.; Fattorini, D.; D’Errico, G.; Pauletto, M.; Bargelloni, L.; Regoli, F., Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environ. Pollut.* 2015, 198, 211-222.

27. Duffy, D. C.; Jackson, S., Diet studies of seabirds: a review of methods. *Waterbirds* 1986, 9, 1-17.

28. Lavers, J. L.; Hutton, I.; Bond, A. L., Ingestion of marine debris by Wedge-tailed Shearwaters (Ardenna pacificus) on Lord Howe Island during 2005-2018. *Mar. Pollut. Bull.* 2018, 133, 616-621.

29. Shapiro, S. S.; Wilk, M. B., An analysis of variance test for normality (complete samples). *Biometrika* 1965, 52, 591-611.

30. BirdLife International, IUCN Red List for birds species factsheet: Ardenna carneipes. In 2018.

31. Reid, T.; Tuck, G. N.; Hindell, M. A.; Thalmann, S.; Phillips, R. A.; Wilcox, C., Nonbreeding distribution of Flesh-footed Shearwaters and the potential for overlap with North Pacific fisheries. *Biol. Cons.* 2013, 166, 3-10.

32. Edwards, D. B.; Mallory, M. L.; Forbes, M. R., Variation in baseline haematology of Northern Fulmars (Fulmarus glacialis) in the Canadian high Arctic. *Comp. Clin. Path.* 2006, 14, (4), 206-209.

33. Work, T. M., Weights, hematology, and serum chemistry of seven species of free-ranging tropical pelagic seabirds. *J. Wildl. Dis.* 1996, 32, (4), 643-657.

34. Marchant, S.; Higgins, P. J., *Handbook of Australian, New Zealand, and Antarctic birds. Volume 1: ratites to ducks*. Oxford University Press: Melbourne, 1990.

35. Brié, J.; Célérier, A.; Maurel, L.; Bonadonna, F., New haematological data in Cory’s Shearwater, Calonectris diomedea (Aves, Procellariiformes). *Ital. J. Zool.* 2011, 78, (3), 279-286.

36. Navarro, J.; González-Solís, J., Experimental increase of flying costs in a pelagic seabird: effects on foraging strategies, nutritional state and chick condition. *Decol.* 2007, 151, (1), 150-160.

37. Cherel, Y.; Robin, J.-P.; Maho, Y. L., Physiology and biochemistry of long-term fasting in birds. *Can. J. Zool.* 1988, 66, (1), 159-166.

38. Totzke, U.; Fenske, M.; Hüppop, O.; Raabe, H.; Schach, N., The influence of fasting on blood and plasma composition of Herring Gulls (Larus argentatus). *Physiol. Biochem. Zool.* 1999, 72, (4), 426-437.

39. Lewandowski, A. H.; Campbell, T. W.; Harrison, G. J., Chapter 13: Clinical chemistries. In *Clinical avian medicine and surgery*, WB Saunders: Philadelphia, 1986; pp 192-200.

40. Alonso-Alvarez, C.; Ferrer, M.; Viñuela, J.; Amat, J. A., Plasma chemistry of the Chinstrap Penguin Pygoscelis antarctica during fasting periods: a case of poor adaptation to food deprivation? *Polar Biol.* 2003, 26, (1), 14-19.

41. Krogdahl, Å.; Sell, J. L., Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. * Poult. Sci.* 1989, 68, (11), 1561-1568.

42. Duke, G. E., Alimentary canal: Secretion and digestion, special digestive functions, and absorption. In *Avian Physiology*, 4th Edition ed.; Sturkie, P. D., Ed. 1986.

43. Wen, B.; Zhang, N.; Shi-Rong, J.; Chen, Z.; Gao, J.; Liu, Y.; Liu, H.; Xu, Z., Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. *Aquat. Toxicol.* 2018, 195, 67-76.

44. Harvey, S.; Phillips, J. G.; Rees, A.; Hall, T. R., Stress and adrenal function. *J. Exp. Zool.* 1984, 232, (3), 633-645.

45. Jeffrey, D. A.; Peakall, D. B.; Miller, D. S.; Herzberg, G. R., Blood chemistry changes in food-deprived Herring Gulls. *Comp. Biochem. Physiol. A* 1985, 81, (4), 911-913.
46. Auman, H. J.; Ludwig, J. P.; Giesy, J. P.; Colborn, T., Plastic ingestion by Laysan Albatross chicks on Sand Island, Midway Atoll, in 1994 and 1995. In Robertson, C. J. R.; Gales, R., Eds. Surrey Beaty & Sons: Chipping Norton, 1998; pp 239-244.
47. Mathieu-Denoncourt, J.; Wallace, S. J.; de Solla, S. R.; Langlois, V. S., Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species. Gen. Comp. Endocrinol. 2015, 219, 74-88.
48. Newman, S. H.; Platt, J. F.; White, J., Hematological and plasma biochemical reference ranges of Alaskan seabirds: their ecological significance and clinical importance. Waterbirds 1997, 20, (3), 492-504.
49. Vogelnest, L., Part I: Haematology and biochemistry. In Avian Clinical Pathology, Proceedings of the Avian Medicine Conference in Veterinary Science: University of Sydney, 1991; pp 243-313.
50. Seiser, P. E.; Duffy, L. K.; David M., A.; Roby, D. D.; Golet, G. H.; Litzow, M. A., Comparison of Pigeon Guillemot, Cepphus columba, blood parameters from oiled and unoiled areas of Alaska eight years after the Exxon Valdez oil spill. Mar. Pollut. Bull. 2000, 40, (2), 152-164.
51. Coon, N. C.; Dieter, M. P., Responses of adult Mallard Ducks to ingested south Louisiana crude oil. Environ. Res. 1981, 24, 309-314.
52. Hutton, I.; Carlile, N.; Priddel, D., Plastic ingestion by Flesh-footed Shearwaters, Puffinus carneipes, and Wedge-tailed Shearwaters, Puffinus pacificus. Pap. Proc. R. Soc. Tas. 2008, 142, (1), 67-72.
53. Provencher, J. F.; Bond, A. L.; Hedd, A.; Montevoci, W. A.; Muzaffar, S. B.; Courchesne, S. J.; Gilchrist, H. G.; Jamieson, S. E.; Merkel, F. R.; Falk, K.; Durinck, J.; Mallory, M. L., Prevalence of marine debris in marine birds from the North Atlantic. Mar. Pollut. Bull. 2014, 84, (1-2), 411-417.
54. Puskic, P.; Lavers, J. L.; Adams, L. A.; Grünenwald, M.; Hutton, I.; Bond, A. L., Exploring the sub-lethal effects of plastic ingestion by shearwaters using fatty acid analysis. Conserv. Physiol. 2019, 7, (1).
55. Coffin, S.; Huang, G.-Y.; Lee, I.; Schlenk, D., Fish and seabird gut conditions enhance desorption of estrogenic chemicals from commonly-ingested plastic items. Environ. Sci. Technol. 2019, 53, (8), 4588-4599.
56. Laganà, P.; Caruso, G.; Corsi, I.; Bergami, E.; Venuti, V.; Majolino, D.; La Ferla, R.; Azzaro, M.; Cappello, S., Do plastics serve as a possible vector for the spread of antibiotic resistance? First insights from bacteria associated to a polystyrene piece from King George Island (Antarctica). Int. J. Hyg. Environ. Health 2018, 222, (1), 89-100.
57. Li, J.; Zhang, K.; Zhang, H., Adsorption of antibiotics on microplastics. Environ. Pollut. 2018, 237, 460-467.
58. Provencher, J. F.; Vermaire, J. C.; Avery-Gomm, S.; Braune, B. M.; Mallory, M. L., Garbage in guano? Microplastic debris found in faecal precursors of seabirds known to ingest plastics. Sci. Total Environ. 2018, 644, 1477-1484.
59. Mattsson, K.; Johnson, E. V.; Malmendal, A.; Linse, S.; Hansson, L.-A.; Cedervall, T., Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 2017, 7, (1), 11452.
60. Martínez-Gómez, C.; León, V. M.; Calles, S.; Gomáriz-Olxima, M.; Vethaak, A. D., The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. Mar. Environ. Res. 2017, 130, 69-76.
61. Avery-Gomm, S.; Borrelle, S. B.; Provencher, J. F., Linking plastic ingestion research with marine wildlife conservation. Sci. Total Environ. 2018, 637–638, 1492-1495.
62. Croxall, J. P.; Butchart, S. H. M.; Lascelles, B.; Stattersfield, A. J. J.; Sullivan, B. J. J.; Symes, A.; Taylor, P., Seabird conservation status, threats and priority actions: a global assessment. Bird Cons. Int. 2012, 22, 1-34.
63. Hamer, K. C.; Schreiber, E. A.; Burger, J., Biology of marine birds. CRC Press: Boca Raton, Florida, USA, 2002.
64. Phillips, R. A.; Gales, R.; Baker, G. B.; Double, M. C.; Favero, M.; Quintana, F.; Tasker, M. L.; Weimerskirch, H.; Uhart, M.; Wolfaardt, A., The conservation status and priorities for albatrosses and large petrels. Biol. Conserv. 2016, 201, 169-183.
65. Dagleish, M. P.; Ryan, P. G.; Girling, S.; Bond, A. L., Clinical pathology of the critically endangered Gough Bunting (Rowettia goughensis). J. Comp. Pathol. 2017, 156, (2), 264-274.
66. O'Brien, A. L.; Dafforn, K. A.; Chariton, A. A.; Johnston, E. L.; Mayer-Pinto, M., After decades of stressor research in urban estuarine ecosystems the focus is still on single stressors: A systematic literature review and meta-analysis. Sci. Total Environ. 2019, 684, 753-764.

67. Quillfeldt, P.; Masello, J. F.; Möstl, E., Blood chemistry in relation to nutrition and ectoparasite load in Wilson's Storm-petrels Oceanites oceanicus. Polar Biol. 2004, 27, (3), 168-176.

68. Work, T. M.; Rameyer, R. A., Mass stranding of Wedge-tailed Shearwater chicks in Hawaii. J. Wildl. Dis. 1999, 35, (3), 487-495.
Table 1
Flesh-footed Shearwater fledgling blood components, morphometrics, and ingested plastic on Lord Howe Island during 2014-2017.

| Parameter               | Units  | Mean  | SD   | Min  | Max   | n  | Log-transformed |
|-------------------------|--------|-------|------|------|-------|----|----------------|
| **Blood composition**   |        |       |      |      |       |    |                |
| Packed cell volume      | L/L    | 0.45  | 0.50 | 0.31 | 0.56  | 33 | No             |
| White blood cells       | 10^9/L | 5.41  | 4.82 | 0.20 | 21.5  | 41 | Yes            |
| % Heterophils           | %      | 53    | 21   | 16   | 88    | 41 | NA             |
| Heterophils             | 10^9/L | 2.7   | 2.8  | 0.1  | 14.7  | 41 | Yes            |
| % Lymphocytes           | %      | 39    | 22   | 6    | 82    | 41 | NA             |
| Lymphocytes             | 10^9/L | 2.2   | 2.9  | 0.1  | 13.1  | 41 | Yes            |
| % Monocytes             | %      | 7     | 6    | 0    | 32    | 41 | NA             |
| Monocytes               | 10^9/L | 0.4   | 0.5  | 0.0  | 2.3   | 41 | No             |
| % Eosinophils           | %      | 1     | 2    | 0    | 8     | 41 | NA             |
| Eosinophils             | 10^9/L | <0.1  | <0.1 | 0.0  | 0.2   | 41 | No             |
| % Basophils             | %      | 1     | 2    | 0    | 10    | 41 | NA             |
| Basophils               | 10^9/L | 0.1   | 0.1  | 0.0  | 0.6   | 41 | No             |
| **Liver function**      |        |       |      |      |       |    |                |
| Aspartate aminotransferase | IU/L   | 439   | 854  | 78   | 6176  | 53 | Yes            |
| Glutamate dehydrogenase | IU/L   | 10    | 8    | 1    | 46    | 53 | Yes            |
| Bile acids, fasting     | mmol/L | 13    | 11   | 0    | 41    | 52 | No             |
| **Kidney function**     |        |       |      |      |       |    |                |
|                          | mmol/L  | 1.7 | 1.6 | 0.1 | 11.1 | 53  | Yes |
|--------------------------|---------|-----|-----|-----|------|-----|-----|
| Urea                     |         |     |     |     |      |     |     |
| Uric acid                | mmol/L  | 0.4 | 0.7 | 0.0 | 4.5  | 53  | Yes |
| Blood proteins           |         |     |     |     |      |     |     |
| Total protein            | g/L     | 33  | 10  | 15  | 64   | 49  | Yes |
| Albumen                  | g/L     | 13  | 5   | 5   | 26   | 50  | Yes |
| Globulin                 | g/L     | 20  | 6   | 10  | 43   | 49  | Yes |
| Albumen:Globulin         | ratio   | 0.6 | 0.2 | 0.1 | 1.0  | 49  | NA  |
| Other blood parameters   |         |     |     |     |      |     |     |
| Calcium                  | mmol/L  | 2.3 | 0.2 | 1.9 | 2.7  | 50  | No  |
| Creatine kinase          | IU/L    | 4602| 13374| 521| 94466| 52  | Yes |
| Cholesterol              | mmol/L  | 12.0| 7.0 | 3.7 | 42.1 | 53  | Yes |
| Amylase                  | IU/L    | 305 | 121 | 63  | 596  | 53  | No  |
| Glucose                  | mmol/L  | 14.0| 4.8 | 1.2 | 34.5 | 53  | Yes |
| Bird morphometrics       |         |     |     |     |      |     |     |
| Bird mass                | g       | 562 | 175 | 180 | 805  | 53  | No  |
| Wing chord               | mm      | 289 | 30  | 223 | 323  | 51  | No  |
| Culmen                   | mm      | 40.7| 2.1 | 35.9| 47.0 | 51  | No  |
| Head+bill                | mm      | 92.6| 3.8 | 84.6| 102.6| 51  | No  |
| Ingested plastic         |         |     |     |     |      |     |     |
| Plastic mass             | g       | 7   | 0   | 0   | 37.3267| 53  | No  |
| Plastic count            | items   | 21  | 45  | 0   | 263  | 53  | NA  |
Table 2

Results from linear models examining the relationships between plastic ingestion and clinical pathology or body condition in Flesh-footed Shearwaters. Test statistics from multivariate tests are Wilk’s $\lambda$, and those from univariate tests are F-statistics. Significant results ($p < 0.05$) include parameter estimates ($\beta \pm SE$) and are in bold.

| Parameter                      | Plastic presence | Number of pieces | Plastic mass |
|-------------------------------|------------------|------------------|--------------|
|                               | Test statistic   | p-value          | $\beta \pm SE$ | Test statistic | p-value | $\beta \pm SE$ | Test statistic | p-value | $\beta \pm SE$ |
| Blood composition             |                  |                  |              |                |         |              |                |         |              |
| Packed cell volume            | 1.64             | 0.21             | 1.67         | 0.21           | 2.90    | 0.10           |                |         |              |
| White blood cell count        | 1.35             | 0.25             | 0.37         | 0.55           | 0.63    | 0.43           |                |         |              |
| WBC composition               | 0.88             | 0.47             | 0.89         | 0.52           | 0.96    | 0.91           |                |         |              |
| Liver function (AST, GLDH, bile acids) | 0.95             | 0.49             | 0.97         | 0.73           | 0.95    | 0.46           |                |         |              |
| Kidney function (urea, uric acid) | 0.99             | 0.91             | 0.87         | 0.05           | 0.90    | 0.07           |                |         |              |
| Urea                          | 0.20             | 0.66             |              |                |         |              |                |         |              |
| Parameter                        | Value 1 | Value 2 | Value 3 |
|---------------------------------|---------|---------|---------|
| Uric acid                       | 4.05    | 0.05    | 0.003   |
| Blood proteins (albumen, globulin) | 0.98  | 0.64    | 0.99  | 0.87    | 0.98  | 0.66    |
| Other blood parameters          | -0.001 ±| -0.007 ±|
| Calcium                         | 5.62    | 0.022   | -0.133 ± 0.056 | 5.06  | 0.029   | 0.001  | 4.096  | 0.048  | 0.004  |
| Creatine kinase                 | 3.56    | 0.07    | 2.68   | 0.11    | 1.72   | 0.20    |
| Cholesterol                     | 4.66    | 0.036   | 0.276 ± 0.128 | 0.33  | 0.57    | 0.13   | 0.72    | 5.190 ± |
| Amylase                         | 0.25    | 0.62    | 1.68   | 0.20    | 6.037  | 0.018   | 2.112  |
| Glucose                         | 1.97    | 0.17    | 0.14   | 0.71    | 0.05   | 0.83    |
| Bird morphometrics              | -101.061 ± |
| Bird mass                       | 4.45    | 0.040   | 47.933 | 0.03   | 0.86   | 0.28   | 0.60    |
|          | Value 1 | Value 2 | Value 3    | Value 4 | Value 5 | Value 6 | Value 7 |
|----------|---------|---------|------------|---------|---------|---------|---------|
| Wing chord | 7.71    | 0.008   | -22.131 ± 8.973 | 0.08    | 0.79    | 0.30    | 0.59    |
| Culmen    | 10.22   | 0.002   | -1.757 ± 0.550  | 0.01    | 0.97    | 0.06    | 0.81    |
| Head+bill | 13.28   | <0.001  | -3.528 ± 0.968  | 0.03    | 0.96    | 0.01    | 0.92    |

390
391
Table 3

Blood chemistry parameters (mean ± SD, range in parentheses) for juvenile seabirds from the literature.

| Parameter | Units | Pterodroma sandwichensis | Diomedea immutabilis | Oceanites oceanicus | Stranded Ardenna pacifica | Healthy Ardenna pacifica |
|-----------|-------|--------------------------|----------------------|---------------------|---------------------------|-------------------------|
| Blood composition | (n = 56; 1994) | (n = 40; 1993) | (n = 17; 2000) | (n = 45; 1994) | (n = 69; 1994) |
| Packed cell volume | L/L | | | | 0.39 ± 0.47 (0.3-0.4) |
| White blood cells | $10^9$/L | 18.1±11.1 (5.2-65.1) | 55.3 | 14.3±8.4 (3.0-54.4) | 29.8±13.9 (9.5-76.6) |
| Heterophils | $10^9$/L | 2.3±1.3 (0.2-6.0) | 6.7±2.1 (3.8-9.9) | 8.4±5.4 (2.1-32.9) | 2.5±0.9 (0.9-5.0) |
| Lymphocytes | $10^9$/L | 13.9±10.6 (1.3-58.6) | 25.5±9.8 (14.9-42.9) | 5.4±4.3 (0.4-21.2) | 26.0±13.5 (7.6-70.0) |
| Monocytes | $10^9$/L | 0.09±0.16 (0.00-0.77) | 0.00 | 0.36±0.61 (0.00-3.36) | 0.2±0.2 (0.0-0.77) |
| Eosinophils | $10^9$/L | 0.68±0.58 (0.09-2.73) | 4.49 | 0.04±0.09 (0.00-0.58) | 0.74±2.21 (0.00-14.81) |
| Basophils | $10^9$/L | 1.16±0.68 (0.18-3.26) | 1.74 | 0.15±0.14 (0.0-0.48) | 0.34±0.44 (0-2.68) |

Liver function
| **Aspartate** | IU/L | 106±28 (71-169) | 98±21 (57-133) | 366±231 (169-1740) | 153±41 (95-274) |
|----------------|------|-----------------|----------------|--------------------|-----------------|
| **Kidney function** | | | | | |
| **mmol/** | | | | | |
| Uric acid | L | 2.2±0.6 (1.2-3.5) | 3.5±1.7 (1.4-7.6) | 7.3±5.5 (1.4-24.7) | 6.2±4.3 (1.2-15.8) |
| **Blood proteins** | | | | | |
| **Total protein** | g/L | 2.7±0.9 (1.7-6.5) | 3.9±0.8 (2.7-5.0) | 2.6±0.1 (1.7-3.6) | 3.2±0.6 (2.1-4.4) | 3.2±0.4 (2.6-4.2) |
| Albumen | g/L | 1.3±0.4 (1.0-3.3) | 1.5±0.3 (1.1-1.9) | 1.5±0.2 (1.1-2.0) | 1.7±0.2 (1.4-2.2) |
| Globulin | g/L | 1.3±0.5 (0.7-3.2) | 2.4±0.5 (1.5-3.2) | 1.6±0.4 (1.0-2.4) | 1.5±0.2 (1.2-2.0) |
| **Other blood parameters** | | | | | |
| **mmol/** | | | | | |
| Calcium | L | 10.1±0.8 (8.5-12.2) | 12.2±0.7 (11.3-13.5) | 11.0±1.9 (5.1-14.0) | 11.8±1.2 (7.8-13.8) |
| Creatine kinase | IU/L | 114±90 (23-344) | 534±266 (194-1146) | 1091±1142 (65-6000) | 899±899 (147-4473) |
| Glucose | L | 316±35 (222-375) | 136±23 (83-175) | 133±44 (22-248) | 202±23 (145-255) |
| **Source** | | | | | |
| Work | | | | | |
| Work | | | | | |
| Quillfeldt, et al. | | | | | |
| Work and Rameyer | | | | | |
| Work and Rameyer | | | | | |
Figure captions

Figure 1. Blood chemistry parameters and measures of body condition that were significantly related to plastic ingestion in Flesh-footed Shearwaters (see also Table 2). In boxplots, solid lines are medians, boxes are the inter-quartile range (25\textsuperscript{th} & 75\textsuperscript{th} percentile), whiskers are the 5\textsuperscript{th} and 95\textsuperscript{th} percentile, and dots are final outliers. In other plots, solid lines are regressions with the model confidence interval shaded in grey.
