Environmental Pollution 243 (2018) 1750-1757

Contents lists available at ScienceDirect

**Environmental Pollution** 

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

# Seabird plastic ingestion differs among collection methods: Examples from the short-tailed shearwater $\stackrel{\star}{\times}$



POLLUTION

Airam Rodríguez<sup>a, b, c, \*</sup>, Francisco Ramírez<sup>d</sup>, M. Nazaret Carrasco<sup>c</sup>, André Chiaradia<sup>a</sup>

<sup>a</sup> Conservation Department, Phillip Island Nature Parks, P.O. Box 97, Cowes, 3922, Victoria, Australia

<sup>b</sup> Department of Evolutionary Ecology, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Avda. Américo Vespucio 26, 41092, Seville, Spain

<sup>c</sup> Canary Islands' Ornithology and Natural History Group (GOHNIC), La Malecita s/n, 38480 Buenavista del Norte, Tenerife, Canary Islands, Spain

<sup>d</sup> Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Facultat de Biologia, Avda. Diagonal 643, Barcelona, Spain

# A R T I C L E I N F O

Article history: Received 9 June 2018 Received in revised form 2 September 2018 Accepted 2 September 2018 Available online 5 September 2018

Keywords: Artificial light Light-induced mortality Marine debris Microplastics Monitoring program Plastic ingestion Stranded Wreck

# ABSTRACT

Despite the increase of literature on seabird plastic ingestion in recent years, few studies have assessed how plastic loads vary according to different sampling methods. Most studies use necropsies of seabirds with a natural cause of death, e.g. beached or predated, to determine plastic loads and monitor marine debris. Sampling naturally dead seabirds may be biased as they have perished because of their intrinsic factors, e.g. poor body condition, high parasite loads, sickness or predation, affecting estimates of plastic loads. However, seabirds killed accidentally may be more representative of the population. Here, we used the short-tailed shearwater Ardenna tenuirostris to test different sampling methods: naturally beached fledglings and accidentally road-killed fledglings after being attracted and grounded by artificial lights. We compared plastic load, body condition, and feeding strategies (through using feathers'  $\delta^{13}$ C and  $\delta^{15}$ N isotope niche) between beached and road-killed fledglings. Beached birds showed higher plastic loads, poorer body condition and reduced isotopic variability, suggesting that this group is not a representative subsample of the whole cohort of the fledgling population. Our results might have implications for longterm monitoring programs of seabird plastic ingestion. Monitoring plastic debris through beached birds could overestimate plastic ingestion by the entire population. We encourage the establishment of refined monitoring programs using fledglings grounded by light pollution if available. These samples focus on known cohorts from the same population. The fledgling plastic loads are transferred from parents during parental feeding, accumulating during the chick-rearing period. Thus, these fledglings provide a higher and valuable temporal resolution, which is more useful and informative than unknown life history of beached birds.

© 2018 Elsevier Ltd. All rights reserved.

# 1. Introduction

Humanity has benefited from plastics by improving health, safety, energy savings or material maintenance (Andrady, 2015). However, the advantages of plastic such as low-cost, light weight and durable are causing them to accumulate at alarming levels, producing a major global environmental problem (UNEP, 2016). Despite multiple international, national and regional agreements

E-mail address: airamrguez@ebd.csic.es (A. Rodríguez).

aimed at reducing marine plastic debris, plastic production continues to rise and 4.8–12.7 million metric tons of plastic are estimated to enter annually in the oceans (Jambeck et al., 2015).

The accumulation of plastic debris is a threat to marine biodiversity (Rochman et al., 2016). For marine vertebrates, ingestion, entanglement and contaminant release from plastics are the main threats (Kühn et al., 2015; Tanaka et al., 2015; Teuten et al., 2009). Over 200 seabird species are affected by plastic ingestion today (Gall and Thompson, 2015; Kühn et al., 2015). It is estimated that 99% of seabird species will ingest at least a plastic item by 2050 (Wilcox et al., 2015). Given the negative effects of plastic marine debris on wildlife, monitoring is crucial to assess their impact. Among different taxa, the exposure to plastic pollution has been extensively studied in seabirds (Gall and Thompson, 2015), and



<sup>\*</sup> This paper has been recommended for acceptance by Dr. Chen Da.

<sup>\*</sup> Corresponding author. Department of Evolutionary Ecology, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Avda. Américo Vespucio 26, 41092, Seville, Spain.

they have been proposed as a key group for marine plastic pollution monitoring purposes (Ryan, 2008). The usefulness of seabird as a monitoring tool has been recognized by the 1992 Oslo and Paris Conventions for the protection of the marine environment. They have developed an Ecological Quality Objective based on plastic mass ingested by Northern fulmars *Fulmarus glacialis* to assess plastic pollution at North Sea (van Franeker et al., 2011).

In a recent review on ingested debris in marine megafauna. approximately 70% of seabird studies involved necropsies of complete specimens (Provencher et al., 2017). Most of the 85 studies compiled in the review used at least a potentially biased sampling method, i.e. beached birds following wrecking events (24 studies), found dead birds by predation on colony (16 studies) or admitted to rehabilitation centres (2 studies) (Provencher et al., 2017). Seabirds involved in wrecks, predation events or rehabilitation centres might not be a representative sample of populations, as they constitute the naturally perishing low-quality individuals of the population. For example, beach washed seabirds might be unable to discriminate real food from non-food items such as plastics prior to stranding or they may show higher mortality because of high plastic loads (Ryan, 1987). The same bias may apply to birds that were predated or admitted into rehabilitation centres. Thus, while beached birds could help to monitor spatiotemporal trends in plastic marine debris, they may be less useful or representative to quantify plastic ingestion at the population level. The rate and frequency of plastic ingestion can be used to estimate the quantity of plastic ingested by seabird populations, providing information on changes over time. Therefore, a comparison of sampling methods is required to assess whether plastic loads are influenced by the method of sampling as highlighted in several recent studies (Avery-Gomm et al., 2016; Provencher et al., 2017). Despite the increasing number of seabird plastic ingestion studies in recent years, just eight studies compare between sampling methods (Provencher et al., 2017; See Discussion below for details). Here, we compared seabird plastic ingestion, body condition and stable isotopes ( $\delta^{13}$ C and  $\delta^{15}$ N) from two sources of dead birds: 1) beached birds perished under natural conditions; and 2) road-killed birds after they were grounded by street lights, accidentally killed by anthropogenic threats. To our best knowledge, neither the probability of a petrel being grounded by artificial lights nor the probability of being consequently killed after grounding is related to intrinsic factors (e.g. nutritional; but see Rodríguez et al., 2012b, 2017b). Road-killed birds could be therefore a better representation of the population than beached birds, likely poor-quality individuals. Two dimensions ( $\delta^{13}$ C and  $\delta^{15}$ N)  $\delta$ -spaces (formally named isotopic niches) are frequently used to investigate the trophic structure of populations; and isotopic variability is widely considered a proxy to the trophic niche width (Newsome et al., 2007; Swanson et al., 2015). Thus, stable isotope spaces could shed light to elucidate if sampled individuals are representative of the whole population. As beached birds likely represent the poor-quality fraction of the population, we expected this group would show poorer body condition and reduced isotopic variability than road-killed individuals. Further, we expected that beached birds would have higher plastic loads than road-killed birds as low-quality individuals would likely be more prone to be fed with plastics ingested by their parents (note we sampled fledglings).

We used the short-tailed shearwater *Ardenna tenuirostris* from Phillip Island, Australia in this study, an abundant long-distance migratory seabird breeding in southern Australia and Tasmania. This species is a good study case as it ingests and accumulates abundant plastic items, feeding only at sea (Acampora et al., 2014; Carey, 2011; Cousin et al., 2015; Vlietstra and Parga, 2002). The population size at Phillip Island is estimated of 450,000 breeding pairs (BirdLife International, 2017), producing a high number of fledglings. The inevitably fraction of dead fledglings provides an exceptional opportunity to compare among sampling methods while helping to shed light on the global problem of seabird plastic ingestion. The fledglings' cause of death are wrecks at sea and roadkill after being disorientated and grounded by artificial lights (Peter and Dooley, 2014; Rodríguez et al., 2017b). By focusing on fledglings from Phillip Island, we have avoided potential confounding effects and sources of variation, providing a homogeneous sample of fledglings belonging to the same cohort, from the same colony and in the same year of collection.

#### 2. Methods

#### 2.1. Model species and study area

Our study was conducted on Philip Island (38°29'; 145°15'), Victoria, Australia, an "Important Bird Area" supporting more than 1% of the global population of the short-tailed shearwater (BirdLife International, 2017). During the two fledging seasons of 2015 and 2016, we collected dead short-tailed shearwater fledglings from 17 April to 5 May. They were beached by natural causes or road-killed as a consequence of attraction and disorientation caused by artificial lights (Rodríguez et al., 2017b).

Many fledglings are unable to take off once they sit on the sea surface, leading to wrecks. Winds and waves wash-out these birds onto beaches. We visited beaches at sunrise and looked for freshly dead beach-washed fledglings. We conducted early morning visits to collect birds before they were predated by scavengers (Carey, 2011), mainly Pacific gulls *Larus pacificus*. Fresh corpses with intact gastrointestinal tracts were collected and frozen until dissection.

To mitigate light-induced mortality of fledglings grounded by artificial lights, Phillip Island Nature Parks rangers conduct night rescue patrol along the main road of the island, collecting grounded birds off the road and releasing them in the safety of the colony (Rodríguez et al., 2017b). Unfortunately, some birds die before being rescued as a consequence of collisions with fence and power wires, buildings, cars or even crashing to the ground (Rodríguez et al., 2014). These birds were collected by the Rangers and kept frozen until dissection.

#### 2.2. Morphological measurements of birds

Data recorded for each individual bird were: date, location (beach or road), body mass, wing length, culmen (from the base of forehead feathers in the centre of their nasal tube to distal extent of the hooked bill), bill depth (from the base of forehead feathers to ventral surface of lower mandible) and tarsus (Rodríguez et al., 2017b). We have not measured six culmen and seven bill depth measures due to the heads were eaten (n = 1) or destroyed by vehicle collisions (n = 6). For these birds, we used the mean of each measurement in the analysis. The biometrics were taken using a digital balance (nearest 1 g), a ruler (nearest 1 mm) and an electronic calliper (nearest 0.01 mm). To minimise biases during weighing, water logged at the plumage of beached birds was removed after freezing the corpses, as water in a solid state is easier to remove from plumage. For the stable isotope analyses, we collected several ventral feathers, stored in paper envelopes at room temperature (see below). Given that we sampled fledglings, ventral feathers were grown during the nestling period, and therefore, their stable isotopes analyses provide diet information during such period (February–April).

#### 2.3. Dissection and plastic processing

We dissected the birds to extract the ventriculus and proventriculus, where plastic items are accumulated. The gut content was transferred to a petri dish and rinsed in water under a binocular microscope. We searched visually for plastic items, discarding particles shorter than 1 mm. Against indications of Provencher et al. (2017), we discarded the use a 1 mm sieve as some plastic threads were narrower than 1 mm in width but longer in length, which could have passed through the sieve undetected. Quantifying plastics shorter than 1 mm was beyond the scope of our study because potential cross-contamination of small fibres and they contribute little to plastic mass (van Franeker et al., 2011). Each plastic item was broadly categorized as industrial or user plastics and whether it was accumulated in the ventriculus or proventriculus, following Provencher et al. (2017). Plastics were measured (±0.01 mm) using an optic stereo-microscope (x8, Zeiss, SteREO Discovery.V8). We used a digital balance  $(\pm 0.0001 \text{ g})$  to weight the plastic load per individual. Given that our aims are not to provide a benchmark for plastic ingestion in short-tailed shearwaters, we do not show at the manuscript all the standardized metrics recommended by Provencher et al. (2017). However, we provide raw data in the Supplementary material to facilitate any future comparisons.

#### 2.4. Stable isotope analyses

We used  $\delta^{13}$ C and  $\delta^{15}$ N values, which are frequently used to study trophic level and inshore-offshore habitat use, as they integrate dietary information that reflects specific periods of time, i.e. when analysed tissues were synthesized (Kowalczyk et al., 2014; Newsome et al., 2007). The analyses of two or more stable isotope signatures can be used to define quantifiable ecological niche space (Newsome et al., 2007; Swanson et al., 2015). The overlap, width, and position of such niche spaces can inform about the trophic interactions of individuals or populations (Newsome et al., 2007; Swanson et al., 2015). Prior to stable isotope analyses, ventral feathers were cleaned with successive rinses of chloroformmethanol (2:1) solution. Feather samples were then freeze-dried and powdered, transferring 0.3–0.4 mg of each sample into tin capsules. Isotopic analyses were performed at the Stable Isotope Laboratory of the Estación Biológica de Doñana, CSIC, Seville, Spain (www.ebd.csic.es/lie/index.html). Samples were combusted at 1020 °C using a continuous flow isotope-ratio mass spectrometer by means of a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer. Stable isotope ratios were expressed in the standard  $\delta$ -notation (‰) relative to Vienna Pee Dee Belemnite ( $\delta^{13}$ C) and atmospheric N<sub>2</sub> ( $\delta^{15}$ N). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement error of ±0.1‰ and ±0.2‰ for  $\delta^{13}$ C and  $\delta^{15}$ N, respectively. The standards used were EBD-23 (cow horn, internal standard), LIE-BB (whale baleen, internal standard) and LIE-PA (razorbill feathers, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency.

# 2.5. Statistical analyses

We calculated a body size index (BSI) and a body condition index (BCI) for each individual following Rodríguez et al. (2017b). We conducted a principal component analysis (PCA) to the centred and scaled four morphometric variables and the first principal component was used as a BSI. The four morphometric variables were positively correlated to BSI (Pearson correlation coefficients: 0.77, 0.58, 0.74 and 0.70 for wing length, culmen, bill depth, and tarsus, respectively), and therefore, the first principal component (BSI) was a more reliable measure of bird size than any single morphometric variable. The BCI was calculated as the standardized residuals of an ordinary least squares regression between body mass on BSI, where positive and negative values indicate that birds are heavier or lighter than the average in the population, respectively (Rodríguez et al., 2012b, 2017b).

We used Fisher-Pitman permutation tests to evaluate differences in frequency distributions of nine variables (collection date, number of plastics, plastic mass, maximum length of plastics, body mass, BSI, BCI,  $\delta^{13}$ C and  $\delta^{15}$ N) between locations (beach vs. road) and years (2015–2016). The null hypothesis is that the group distributions are identical in shape and variability (Neuhäuser and Manly, 2004). We show 99% confidence intervals of P-values based on null distributions obtained via 9999 Monte Carlo replicates. No differences in collection date were detected between beached and road-killed birds (Table 1) nor in any plastic load variables between years (99% confidence intervals of P-values = 0.30–0.33, 0.39–0.42 and 0.32–0.34 for number of plastic, mass of plastic and maximum length of plastics, respectively). Thus, each individual was treated as one record regardless date and year of collection.

We also employed multidimensional niche spaces to assess the overlap between beached and road-killed birds. A high overlap would indicate that both bird groups are similar, while a low overlap would indicate differences between bird groups. In addition, the overlap metric is directional (asymmetric), i.e. the probability that a beached individual will be found in the niche of road-killed birds can be different to the probability that a road-killed individual is found in the niche of beached birds (Swanson et al., 2015). Thus, even if no differences are detected in univariate analyses, a group could account for only a small fraction of the

Table 1

Summary of variables of plastic loads, condition and stable isotope composition of short-tailed shearwater *Ardenna tenuirostris* fledglings found dead on beaches and roads at Phillip Island, Australia (2015–2016). SD = standard deviation, c.i. = confidence intervals.

	Beach	SD	range	n	Road	SD	range	n	P-value	
	mean				mean				lower 99% c.i.	upper 99% c.i.
Collection date (Julian date; $1 = 1$ April) <i>Plastic loads</i>	27.46	4.93	22 April, 3 May	26	28.06	3.71	17 April, 5 May	114	0.497	0.522
Number of plastic items	8.23	6.00	1, 26	26	7.11	4.91	0, 22	114	0.042	0.053
Mass of plastics (g)	0.24	0.26	0.0056, 1.0472	26	0.13	0.10	0.000, 0.5421	114	0.001	0.003
Maximum length (mm)	13.18	12.75	4.731, 60.000	26	8.90	4.37	3.634, 31.000	111	0.005	0.009
Condition of birds										
Body mass (g)	344.76	83.76	204, 516	26	522.64	105.47	254, 784	114	0.000	0.001
Body size index	-1.26	1.65	-4.161, 2.585	26	0.29	1.17	-2.174, 2.939	114	0.000	0.001
Body condition index	-0.80	0.85	-2.707, 0.688	26	0.18	0.95	-1.976, 2.784	114	0.000	0.001
$\delta^{13}C$	-21.02	0.27	-21.410, -20.290	25	-20.81	0.39	-21.980, -19.780	105	0.010	0.015
$\delta^{15}$ N	12.50	0.42	11.660, 13.420	25	12.72	0.51	11.620, 13.900	104	0.044	0.055

variability of the other group. Although this method was originally designed for stable isotope data, it can be applied to any continuous ecological niche indicator in multiple dimensions (Giménez et al., 2018; Swanson et al., 2015). We used an ellipsoid Bayesian framework (nicheRover; Lysy et al., 2014) to estimate the niche region and overlap probability between beached and road-killed individuals in three different multidimensional spaces defined by plastic (number of plastics, plastic mass and maximum length of plastics), body condition (body mass, BSI, BCI) and stable isotopes (ventral feathers'  $\delta^{13}$ C and  $\delta^{15}$ N). Niche region was defined as a multidimensional region in which an individual of the group (beached or road-killed) has the 95% of probability of being found (Swanson et al., 2015), as in other ellipsoid methods (Jackson et al., 2011). Overlap metrics (mean and 95% credible intervals) were estimated through Monte Carlo-Markov Chain (MCMC) simulations based on 1000 draws from which overlap parameters were calculated. For each group (beached or road-killed) and every pair of plastic load variable (number, mass, and maximum length), we displayed 10 randomly chosen elliptical projections of the plastic niche to visualize the relationships among variables (Swanson et al., 2015).

Finally, we run Pearson correlation tests to assess the relationship between plastic loads (number of plastics, plastic mass and maximum length of plastics) and BCI. All statistical analyses were conducted in R (version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results and discussion

Beached birds had lower body condition than road-killed fledglings (Table 1). Overlap analyses for condition niche indicated a very low overlap between both groups (upper 95% credible interval limit of beached birds is higher than lower 95% credible interval of road-killed birds; 41.4 vs 39.6; Fig. 1). These findings confirm that beached fledglings are biased towards low-quality individuals with higher chances of being water-logged after being washed or wrecked at the beach (Rodríguez et al., 2017b). We went one step farther by assessing isotopic variability and demonstrating lower  $\delta^{13}$ C and  $\delta^{15}$ N values in beached than road-killed fledglings (Table 1). Overlap analyses for stable isotope niche indicated a high overlap of beached bird niche on road-killed birds (95% credible interval = [88.4, 99.6]), but a significantly lower overlap of roadkilled bird niche on beached birds (95% credible interval = [54.2, 85.6]; Fig. 1). The high isotopic variability for road-killed birds indicated a wide range of their parents feeding strategies, whereas the relatively low  $\delta^{15}$ N and  $\delta^{13}$ C values for beached individuals could indicate that food delivered by parents was preferentially based on low-level trophic pelagic prey (Hobson et al., 1994; Hussey et al., 2014). Beached birds accounted for just a small fraction of the total range of adult feeding strategies. Our results show clear isotopic and body condition differences between the two groups of fledglings, indicating that beached birds might be lowquality fledglings (Rodríguez et al., 2017b).

All sampled birds contained at least one plastic item in their guts, except three road-killed birds (beached fledglings: n = 26, 95% Jeffreys confidence limits = 0.909–1.000; road-killed fledglings: n = 114, 95% Jeffreys confidence limits = 0.925–0.991). This agrees with previous studies on beached short-tailed shearwater fledglings from the same population, where 100% of individuals included at least one plastic item (Carey, 2011). Beached fledglings contained higher plastic loads, measured as number of plastic items, mass of plastic loads, and maximum length of plastic item per individual than road-killed fledglings (Table 1 and Fig. 2). Body condition index did not correlate with plastic loads (all 95% confidence intervals of Pearson's correlation coefficients including zero; number of

plastics = [-0.261, 0.068]; plastic mass = [-0.295, 0.031], maximum length = [-0.286, 0.044]). The plastic niche of road-killed birds was almost completely overlapped by the plastic niche of beached birds (mean overlap = 98%; 95% credible interval = [94.5, 100.0]; Fig. 1). However, the probability that a beached individual will be found on the road was considerably lower (mean overlap = 28%: 95% credible interval = [18.6, 40.5]; Fig. 1). Thus, not all beached birds shared the plastic niche space of road-killed birds (Fig. 1). In summary, beached fledglings showed poor body condition, lower  $\delta^{13}$ C and  $\delta^{15}$ N values, and higher plastic loads than road-killed fledglings, indicating strong differences in fledgling traits from the two samples. However, if the higher plastic loads of beached birds are due to poor foraging strategies of parents, which may lead to lower body condition of fledglings, or a higher availability of plastics at adult foraging locations cannot be disentangled with our observational data set.

Considering whether the collection method for specimens may influence the estimates of plastic ingestion is important to obtain reliable estimates (Avery-Gomm et al., 2016; Provencher et al., 2017). Some studies suggest no differences in plastic loads among sampling methods. For example, eight Procellariiformes species showed no significant differences in frequency of occurrence or number of plastics between birds caught by longline fisheries and birds found dead on the beach, although statistical comparisons were not controlled by species and collection year (Colabuono et al., 2009). Similarly, van Franeker and Meijboom (2002) concluded that plastic loads of beached northern fulmars did not differ between carcasses identified as accidentally killed and killed by starvation. although the factor 'death cause' was included in six of the nine final models selected by stepwise regressions. On the other hand, several studies have suggested that birds found dead on colonies or beaches, predated or admitted to wildlife rehabilitation centres have larger plastic loads than healthy birds or killed by stochastic events (Hutton et al., 2008; Ryan, 1987). Non-conclusive results suggest that plastic loads of beached petrels and puffins are above the average of the adult population, but potential spatial biases (sampled birds cannot belong to the same population) preven any final conclusion (Harris and Wanless, 1994; Ryan, 1987). Also, stomach lavage of wedge-tailed shearwater (Ardenna pacifica) fledglings showed lower plastic loads (measured as frequency of occurrence, number of plastics and mass of plastic ingested) than necropsied fledglings, although no statistical analyses were reported (Lavers et al., 2018). To our best knowledge, only two studies on a single species, the Laysan albatross (Phoebastria immutabilis), have properly tested plastic loads among naturally dead birds (potentially biased sample) and birds killed for the study (or incidentally injured/killed by vehicles), finding significant differences in line with our results (Auman et al., 1997; Fry et al., 1987).

While using data from beached birds for the long-term monitoring of marine debris is highly valued (Acampora et al., 2016; Ryan, 2008; Ryan et al., 2009; van Franeker et al., 2011; van Franeker and Law, 2015), we suggest additional sampling strategies to be adopted in some locations where light-induced mortality of seabirds occurs, providing a less biased source of information. Seabird mortality induced by artificial lights is a worldwide phenomenon mainly affecting fledglings close to their breeding colonies (Rodríguez et al., 2017a). In contrast to beached birds used in plastic ingestion studies, fledglings grounded by lights are a more homogeneous sample. Grounded birds belong to the same cohort and their plastic loads are delivered by their parents during the chickrearing period (Carey, 2011; Rodríguez et al., 2012a). In addition, the probability that a fledgling is grounded by lights and consequently killed do not seem related to its physiological state (at least no clear links between the probability of being alive or dead have been published; but see Rodríguez et al., 2012b, 2017b). In fact,





**Fig. 1.** Overlap plots representing the posterior probability that an individual from the location labelled on the Y axis (row) will be found within the niche (alpha = 95%) of the location indicated by the column header. Vertical red lines represent the mean (continuous) and 95% (dashed) credible intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



Fig. 2. Ten random elliptical projections of plastic niche region for each pair of plastic load variables (above the diagonal). Also displayed are one-dimensional density plots (diagonal), and two-dimensional scatter plots for each pairwise combination of plastic load variables (Number of plastics, plastic mass –g, and maximum length of plastics –mm).

light-induced mortality is related to nest location, distribution of light sources and the sources of fatal hazardous (busy roads, sharp cutting structures, dense urban matrix) in the grounding locations. Despite the implementation of improvements in rescue campaigns (the main action to reduce mortality of seabirds grounded by artificial lights), light pollution will inevitably cause the death of many petrel species across the world at a predictable time during the fledging season (Rodríguez et al., 2017a). On the other hand, fledglings grounded by lights only represent one part of the population (fledglings), and therefore, other collection methods should be used to extrapolate plastic loads of adults. Birds killed at fisheries could potentially be the optimal unbiased source, although more research is needed as birds caught by fisheries might be younger, hungrier, bolder or inexperienced than individuals not bycatched in fishery activities (Collet et al., 2017a, 2017b).

#### 4. Conclusions

Studies comparing plastic ingestion among collection methods represent only a small number of seabird species, and a plea for more studies in this sense has been made, indicating the little we know about how the collection methods affect ingested plastic detection (Avery-Gomm et al., 2016; Provencher et al., 2017). Our study provides evidence on how plastic ingestion varies according to collection methods in one seabird species; providing warning signs of the potential biases for using stranded animals as bioindicators (for a discussion on sea turtles see Casale et al., 2016). Using road-killed seabird fledglings grounded by light pollution resulted in alternative samples to monitor debris ingestion in the marine system by a healthy seabird population. The advantages are that these birds are from the same cohort, same age, their origin is known and they are in similar health condition to successfully fledged birds. The use of such samples would improve the quality of seabird marine debris monitoring programs while providing a standard tool to assess the effect of plastics in the marine ecosystem (Mallory et al., 2010).

#### Funding

This work was supported by the European Research Agency [Marie Curie International Outgoing Fellowship, 330655 FP7-PEOPLE-2012-IOF]; and the Spanish Ministry of Economy, Industry and Competitiveness [Juan de la Cierva – Incorporación, IJCI-2015-23913 and IJCI-2015-24531].

#### Acknowledgments

We are indebted to Alona Charuvi and Rebecca R. McIntosh for their help during dissections, and to Phillip Island Nature Parks rangers for collecting dead birds from roads. Thanks to Jan van Franeker, Javier Lenzi, and four anonymous reviewers for their constructive comments on early drafts.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.09.007.

#### References

- Acampora, H., Lyashevska, O., Van Franeker, J.A., O'Connor, I., 2016. The use of beached bird surveys for marine plastic litter monitoring in Ireland. Mar. Environ. Res. 120, 122–129. https://doi.org/10.1016/j.marenvres.2016.08.002.
- Acampora, H., Schuyler, Q.A., Townsend, K.A., Hardesty, B.D., 2014. Comparing plastic ingestion in juvenile and adult stranded short-tailed shearwaters (*Puf-finus tenuirostris*) in eastern Australia. Mar. Pollut. Bull. 78, 63–68. https:// doi.org/10.1016/j.marpolbul.2013.11.009.
- Andrady, A.L., 2015. Plastics and Environmental Sustainability. John Wiley & Sons, Inc, Hoboken, NJ. https://doi.org/10.1002/9781119009405.
- Auman, H., Ludwig, J., Giesy, J., Colborn, T., 1997. Plastic ingestion by Laysan Albatross chicks on Sand Island, Midway Atoll, in 1994 and 1995. In: Albatross Biology and Conservation, pp. 239–244.
- Avery-Gomm, S., Valliant, M., Schacter, C.R., Robbins, K.F., Liboiron, M., Daoust, P.-Y., Rios, L.M., Jones, I.L., 2016. A study of wrecked Dovekies (*Alle alle*) in the western North Atlantic highlights the importance of using standardized methods to quantify plastic ingestion. Mar. Pollut. Bull. 113, 75–80. https:// doi.org/10.1016/j.marpolbul.2016.08.062.
- BirdLife International, 2017. Important Bird and Biodiversity Area Factsheet: Phillip Island [WWW Document]. URL. http://datazone.birdlife.org/site/factsheet/ phillip-island-iba-australia (accessed 6.12.17).
- Carey, M.J., 2011. Intergenerational transfer of plastic debris by Short-tailed Shearwaters (Ardenna tenuirostris). Emu 111, 229–234. https://doi.org/10.1071/ MU10085.
- Casale, P., Freggi, D., Paduano, V., Oliverio, M., 2016. Biases and best approaches for assessing debris ingestion in sea turtles, with a case study in the Mediterranean. Mar. Pollut. Bull. 110, 238–249. https://doi.org/10.1016/ j.marpolbul.2016.06.057.
- Colabuono, F.I., Barquete, V., Domingues, B.S., Montone, R.C., 2009. Plastic ingestion by Procellariiformes in southern Brazil. Mar. Pollut. Bull. 58, 93–96. https:// doi.org/10.1016/j.marpolbul.2008.08.020.
- Collet, J., Patrick, S.C., Weimerskirch, H., 2017a. Behavioral responses to encounter of fishing boats in wandering albatrosses. Ecol. Evol. 7, 3335–3347. https://doi.org/ 10.1002/ece3.2677.
- Collet, J., Patrick, S.C., Weimerskirch, H., 2017b. A comparative analysis of the behavioral response to fishing boats in two albatross species. Behav. Ecol. 28, 1337–1347. https://doi.org/10.1093/beheco/arx097.
- 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.
- Fry, D.M., Fefer, S.I., Sileo, L., 1987. Ingestion of plastic debris by laysan albatrosses and wedge-tailed shearwaters in the Hawaiian islands. Mar. Pollut. Bull. 18, 339–343. https://doi.org/10.1016/S0025-326X(87)80022-X.
- Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92, 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041.
- Giménez, J., Louis, M., Barón, E., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., Eljarrat, E., Barceló, D., Forero, M. G., de Stephanis, R., 2018. Towards the identification of ecological management units: a multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula. Aquat. Conserv. Mar. Freshw. Ecosyst. 28, 205–215. https://doi.org/ 10.1002/aqc.2814.
- Harris, M.P., Wanless, S., 1994. Ingested elastic and other artifacts found in puffins in Britain over a 24-year period. Mar. Pollut. Bull. 28, 54–55. https://doi.org/ 10.1016/0025-326X(94)90187-2.
- Hobson, K.A., Piattt, J.F., Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. J. Anim. Ecol. 63, 786–798.
  Hussey, N.E., MacNeil, M.A., McMeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G.,
- Hussey, N.E., MacNeil, M.A., McMeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T., Fisk, A.T., 2014. Rescaling the trophic structure of marine food webs. Ecol. Lett. 17, 239–250. https://doi.org/10.1111/ele.12226.
- Hutton, I., Carlile, N., Priddel, D., 2008. Plastic ingestion by flesh-footed shearwaters, *Puffinus carneipes*, and wedge-tailed shearwaters, *Puffinus pacificus*. Pap. Proc. R. Soc. Tasman. 141, 67–72.
- Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80, 595–602. https://doi.org/10.1111/j.1365-

2656.2011.01806.x.

- Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.) 347, 768–771. https://doi.org/10.1126/science.1260352.
- Kowalczyk, N.D., Chiaradia, A., Preston, T.J., Reina, R.D., 2014. Linking dietary shifts and reproductive failure in seabirds: a stable isotope approach. Funct. Ecol. 28, 755-765. https://doi.org/10.1111/1365-2435.12216.
- Kühn, S., Bravo Rebolledo, E.L., van Franeker, J.A., 2015. Deleterious effects of litter on marine life. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 75–116. https:// doi.org/10.1007/978-3-319-16510-3\_4.
- Lavers, J.L., Hutton, I., Bond, A.L., 2018. Ingestion of marine debris by wedge-tailed shearwaters (*Ardenna pacifica*) on lord howe island, Australia during 2005–2018. Mar. Pollut. Bull. 133, 616–621. https://doi.org/10.1016/ j.marpolbul.2018.06.023.
- Lysy, M., Stasko, A.D., Swanson, H.K., 2014. nicheROVER: (Niche) (R)egion and Niche (Over)lap Metrics for Multidimensional Ecological Niches. CRAN [WWW Document].
- Mallory, M.L., Robinson, S.A., Hebert, C.E., Forbes, M.R., 2010. Seabirds as indicators of aquatic ecosystem conditions: a case for gathering multiple proxies of seabird health. Mar. Pollut. Bull. 60, 7–12. https://doi.org/10.1016/ j.marpolbul.2009.08.024.
- Neuhäuser, M., Manly, B.F.J., 2004. The Fisher-pitman permutation test when testing for differences in mean and variance. Psychol. Rep. 94, 189–194. https://doi.org/ 10.2466/pr0.94.1.189-194.
- Newsome, S.D., Martinez del Rio, C., Bearhop, S., Phillips, D.L., 2007. A niche for isotopic ecology. Front. Ecol. Environ. 5, 429–436. https://doi.org/10.1890/ 060150.1.
- Peter, J., Dooley, S., 2014. The fatal shore: investigating one of the largest seabird wrecks in living memory. Aust. BirdLife 3, 24–27.
- Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal. Methods 9, 1454–1469. https://doi.org/ 10.1039/C6AY02419].
- Rochman, C.M., Browne, M.A., Underwood, A.J., van Franeker, J.A., Thompson, R.C., Amaral-Zettler, L.A., 2016. The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. Ecology 97, 302–312. https://doi.org/10.1890/14-2070.1.
- Rodríguez, A., Burgan, G., Dann, P., Jessop, R., Negro, J.J., Chiaradia, A., 2014. Fatal attraction of short-tailed shearwaters to artificial lights. PLoS One 9, e110114. https://doi.org/10.1371/journal.pone.0110114.
- Rodríguez, A., Holmes, N.D., Ryan, P.G., Wilson, K.-J., Faulquier, L., Murillo, Y., Raine, A.F., Penniman, J.F., Neves, V., Rodríguez, B., Negro, J.J., Chiaradia, A., Dann, P., Anderson, T., Metzger, B., Shirai, M., Deppe, L., Wheeler, J., Hodum, P., Gouveia, C., Carmo, V., Carreira, G.P., Delgado-Alburqueque, L., Guerra-Correa, C., Couzi, F.-X., Travers, M., Corre, M. Le, 2017a. Seabird mortality induced by land-based artificial lights. Conserv. Biol. 31, 986–1001. https:// doi.org/10.1111/cobi.12900.
- Rodríguez, A., Moffett, J., Revoltós, A., Wasiak, P., McIntosh, R.R., Sutherland, D.R., Renwick, L., Dann, P., Chiaradia, A., 2017b. Light pollution and seabird fledglings: targeting efforts in rescue programs. J. Wildl. Manag. 81, 734–741. https:// doi.org/10.1002/jwmg.21237.
- Rodríguez, A., Rodríguez, B., Carrasco, M.N., 2012a. High prevalence of parental delivery of plastic debris in Cory's shearwaters (*Calonectris diomedea*). Mar. Pollut. Bull. 64, 2219–2223. https://doi.org/10.1016/j.marpolbul.2012.06.011.
- Rodríguez, A., Rodríguez, B., Curbelo, Á.J., Pérez, A., Marrero, S., Negro, J.J., 2012b. Factors affecting mortality of shearwaters stranded by light pollution. Anim. Conserv. 15, 519–526. https://doi.org/10.1111/j.1469-1795.2012.00544.x.
- Ryan, P.G., 2008. Seabirds indicate changes in the composition of plastic litter in the Atlantic and south-western Indian Oceans. Mar. Pollut. Bull. 56, 1406–1409. https://doi.org/10.1016/j.marpolbul.2008.05.004.
- Ryan, P.G., 1987. The incidence and characteristics of plastic particles ingested by seabirds. Mar. Environ. Res. 23, 175–206. https://doi.org/10.1016/0141-1136(87) 90028-6.
- Ryan, P.G., Moore, C.J., van Franeker, J.A., Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Philos. Trans. R. Soc. B Biol. Sci. 364, 1999–2012. https://doi.org/10.1098/rstb.2008.0207.
- Swanson, H.K., Lysy, M., Power, M., Stasko, A.D., Johnson, J.D., Reist, J.D., 2015. A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. Ecology 96, 318–324. https://doi.org/10.1890/14-0235.1.
- Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M., Watanuki, Y., 2015. Facilitated leaching of additive-derived PBDEs from plastic by seabirds' stomach oil and accumulation in tissues. Environ. Sci. Technol. 49, 11799–11807.
- Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284.
- UNEP, 2016. Marine Plastic Debris and Microplastics Global Lessons and Research to Inspire Action and Guide Policy Chance. United Nations Environment Programme, Nairobi.
- van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N.,

Hansen, P.L., Heubeck, M., Jensen, J.K., Le Guillou, G., Olsen, B., Olsen, K.O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea. Environ. Pollut. 159,

 2609–2615. https://doi.org/10.1016/j.envpol.2011.06.008.
van Franeker, J.A., Law, K.L., 2015. Seabirds, gyres and global trends in plastic pollution. Environ. Pollut. 203, 89–96. https://doi.org/10.1016/ j.envpol.2015.02.034.

van Franeker, J.A., Meijboom, A., 2002. Marine Litter Monitoring by Northern

- Fulmars: a Pilot Study. Wageningen, the Netherlands. Vlietstra, L.S., Parga, J.A., 2002. Long-term changes in the type, but not amount, of ingested plastic particles in short-tailed shearwaters in the southeastern Bering Sea. Mar. Pollut. Bull. 44, 945–955. https://doi.org/10.1016/S0025-326X(02) 00130-3.
- Wilcox, C., Van Sebille, E., Hardesty, B.D., 2015. Threat of plastic pollution to seabirds is global, pervasive, and increasing. Proc. Natl. Acad. Sci. Unit. States Am. 112, 11899–11904. https://doi.org/10.1073/pnas.1502108112.