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Stable isotope values delineate the non-breeding distributions of sooty shearwaters *Puffinus griseus* in the North Pacific Ocean

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ABSTRACT: Following breeding, sooty shearwaters *Puffinus griseus* leave New Zealand waters and migrate to 1 of 3 distinct areas in the North Pacific Ocean, effectively exploiting environmental resources across a large proportion of this northern ocean basin. In this study, we combined electronic tracking technology with stable isotope analyses ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of feathers grown during the non-breeding period in order to evaluate whether isotope signatures can be used to identify specific non-breeding areas used by sooty shearwaters. A region to the east of Japan was utilised by the majority of tracked birds, whereas others used areas off the west coast of North America. Stable isotope values of feathers allowed the discrimination of individuals that used each of the 3 different non-breeding areas, and suggested that birds off Japan can be further separated into 'coastal' and 'offshore' groups. Our results confirm the utility of using stable isotope analysis, validated by tracking devices, as a tool to determine distribution and habitat use of a long-range oceanic migrant, the sooty shearwater. These results also highlight the resource connectivity between the northern and southern Pacific Ocean basin.

KEY WORDS: Isoscape · Migratory connectivity · New Zealand · Seabird · Spatial distribution

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INTRODUCTION

The sooty shearwater *Puffinus griseus* is an abundant seabird that breeds on islands in the southwest Pacific Ocean and around South America. In New Zealand, censuses in 1994–2004 suggest an estimated total breeding population of 4.2–4.7 million pairs, with an overall population of approximately 20 million individuals (Newman et al. 2009). However,

over recent decades, there is growing evidence to indicate marked declines in sooty shearwater populations (Veit et al. 1996, 1997, Scofield & Christie 2002, Scott et al. 2009), and the conservation status of sooty shearwater in New Zealand is defined as 'at risk—declining' (Robertson et al. 2013).

At-sea observations (Spear & Ainley 1999) and tracking using electronic archival devices (Shaffer et al. 2006) have delineated the transequatorial pan-

Pacific migrations of sooty shearwaters following breeding. Further, Shaffer et al. (2006) reported that sooty shearwaters utilised 1 of 3 discrete zones in the North Pacific Ocean (off Japan, Alaska or California), and remained within one of these zones for the duration of the non-breeding period. This has potentially important life-history implications for sooty shearwaters. Migratory connectivity, the relationship between population distributions during the breeding and non-breeding periods, affects the linkages between spatial variation in environmental conditions and reproductive performance and survival (e.g. see Webster et al. 2002) and is increasingly important in a world where the rate of oceanographic and climatic change is unprecedented (Veit et al. 1996, 1997, Ainley & Hyrenbach 2010, Clucas 2011).

Tissues synthesised at different times during an animal's annual cycle reflect local isotopic signatures, allowing movement patterns to be determined (Hobson 2007). Because seabirds generally moult during the non-breeding period, isotope values of feathers reflect the birds' diet, and hence the local environment, at this time (e.g. Cherel et al. 2000, 2013, Phillips et al. 2009, Ramos et al. 2009). In the absence of comprehensive baseline 'isoscape' information to describe geographic variation in isotope ratios, the stable isotope approach to identifying non-breeding destinations of seabirds is especially powerful when validated by simultaneous electronic tracking of a sample of the population (Phillips et al. 2007, Jaeger et al. 2010, González-Solís et al. 2011, Rayner et al. 2011, Hedd et al. 2012, Militão et al. 2013).

Here we combine stable isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) from sooty shearwaters that were simultaneously tracked, using global location sensing (GLS) archival loggers, on their trans-Pacific migrations from New Zealand to test the utility of delineating non-breeding regions in the northern Pacific Ocean using isotopic markers.

MATERIALS AND METHODS

Geolocation data and analysis

Tracking data were obtained from a total of 23 sooty shearwaters breeding at 3 sites in New Zealand. Ten birds from Whenua Hou (Codfish Island; 46° 48' S, 167° 42' E) and 4 birds from Mana Island (41° 6' S, 174° 48' E) were tracked between January to December in 2005 or 2006, and correspond to a subset of the birds included in Shaffer et al. (2006). Sooty

shearwaters from Whenua Hou and Mana were equipped with Lotek LTD2400 (Lotek Wireless) GLS archival loggers. Further details of device deployments are given in Shaffer et al. (2006). A further 9 breeding birds were tracked from Kauwahaia Island (36° 54' S, 174° 24' E) between December 2011 and December 2012. These sooty shearwaters were captured, and subsequently re-captured, by hand from marked burrows and equipped with a British Antarctic Survey Mk15 GLS logger. The Mk15 GLS loggers were attached to a metal leg band using a cable tie threaded inside the metal band and around the device. Additionally, the logger was glued to a curved section of plastic band, which in turn was glued to the outside of the metal leg band, preventing the logger rotating around the metal band.

Location data downloaded from retrieved loggers were processed following established methods as detailed by Phillips et al. (2004) and Shaffer et al. (2005, 2006). Phillips et al. (2004) noted that on average, the error in locations derived using GLS loggers was 186 ± 114 km. Spatial analysis was limited to GLS locations during the core non-breeding period (May to October), i.e. when birds were in the North Pacific Ocean and excluding the short periods of rapid northerly and southerly migration. All data were mapped using a Mollweide projection. Utilisation distributions were generated from GLS locations using the Kernel Density function in ArcGIS (ESRI, v. 10.2; search radius = 200 km; cell size = 50 km). The probability contours (90, 75 and 50%) were created for each density raster using the isopleth function in Geospatial Modelling Environment (Spatial Ecology).

Stable isotope analysis

Four to 6 small, fresh-looking body feathers (i.e. dark, sooty-grey feathers as opposed to faded brown and abraded feathers) were sampled either from the dorsal area or from the flanks of each sooty shearwater when the birds were recaptured to retrieve GLS loggers. Fresh feathers were sampled to ensure collection of material that had been re-grown during the preceding non-breeding period. Feathers were stored in sealed plastic bags until analysis. In the laboratory, feathers were cleaned of any surface contamination by washing in distilled water and then ethanol, rinsed again with distilled water, dried in an oven at 50°C for 24 h and then allowed to equilibrate to ambient laboratory temperature (approximately 20°C). Cleaned and dried feathers were cut into very

small pieces using stainless-steel scissors and approximately 0.5 mg weighed accurately into a tin capsule. Stable carbon and nitrogen isotope ratios were determined using a DeltaPlus (Thermo-Fisher Scientific) continuous flow, isotope ratio mass spectrometer linked to an NA 1500 elemental analyser (Fisons Instruments) at the National Institute of Water and Atmospheric Research (NIWA) stable isotope laboratory in Wellington. ISODAT (Thermo-Fisher Scientific) software calculated $\delta^{13}\text{C}$ values against the international standard Carrara Marble NSB-19 (National Institute of Standards and Technology [NIST]) correcting for ^{17}O , with $\delta^{15}\text{N}$ values calculated against the international air standard. Isotopic values were further corrected against NIST standards (NIST 8573 USGS40 L-glutamic acid, NIST 8548 IAEA-N2 ammonium sulphate, and NIST 8542 IAEA-CH-6 sucrose) using a 3-point normalisation process. Results are presented using standard δ notation in units of parts per thousand (‰). Repeat analysis of NIST standards produced data accurate to within 0.19‰ for $\delta^{15}\text{N}$ and 0.36‰ for $\delta^{13}\text{C}$ with replicates of an internal DL-leucine (DL-2-amino-4-methylpentanoic acid, $\text{C}_6\text{H}_{13}\text{NO}_2$; Lot 127H1084, Sigma) laboratory standard giving a precision of better than 0.26‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Variation in stable isotope values among sooty shearwaters that spent the non-breeding period in different regions was assessed using 1-way ANOVA for each element separately, followed by Tukey HSD tests to identify significantly different mean values.

RESULTS

All tracked sooty shearwaters spent the non-breeding period at 1 of 3 regions in the North Pacific Ocean: an area to the east of Japan used by the majority of birds (16 of the 23 tracked; 70%), an area to the south of Alaska and west of northwestern Canada in the Gulf of Alaska (3 individuals; 13%), or an area along the western coasts of North America and the Baja Peninsula, Mexico (4 individuals; 17%) (Fig. 1). Individuals did not move between these 3 regions during the non-breeding period. For those birds in waters off Japan, there was a further distinction between those that remained relatively close to the eastern coasts of Japan in an area bounded by 30–50° N and 140–160° E (e.g. Bird 53089; Fig. 2a), and individuals that also spent time at 30–50° N, but further offshore, between 140–180° E (e.g. Bird 26752; Fig. 2b).

Summary stable isotope results are presented in Table 1. There were significant differences in both mean $\delta^{13}\text{C}$ ($F_{2,20} = 31.1$, $p < 0.01$; Table 1) and $\delta^{15}\text{N}$ ($F_{2,20} = 68.6$, $p < 0.01$; Table 1) in feathers of sooty shearwaters that spent the non-breeding period in the 3 regions. Feather stable isotope values were relatively depleted in both ^{15}N and ^{13}C in birds off Japan, enriched in birds from the western seaboard of the USA and Mexico, and had intermediate values in birds from the Gulf of Alaska (Table 1). Among those birds from the east of Japan, those that spent the non-breeding period relatively close to the Japan-

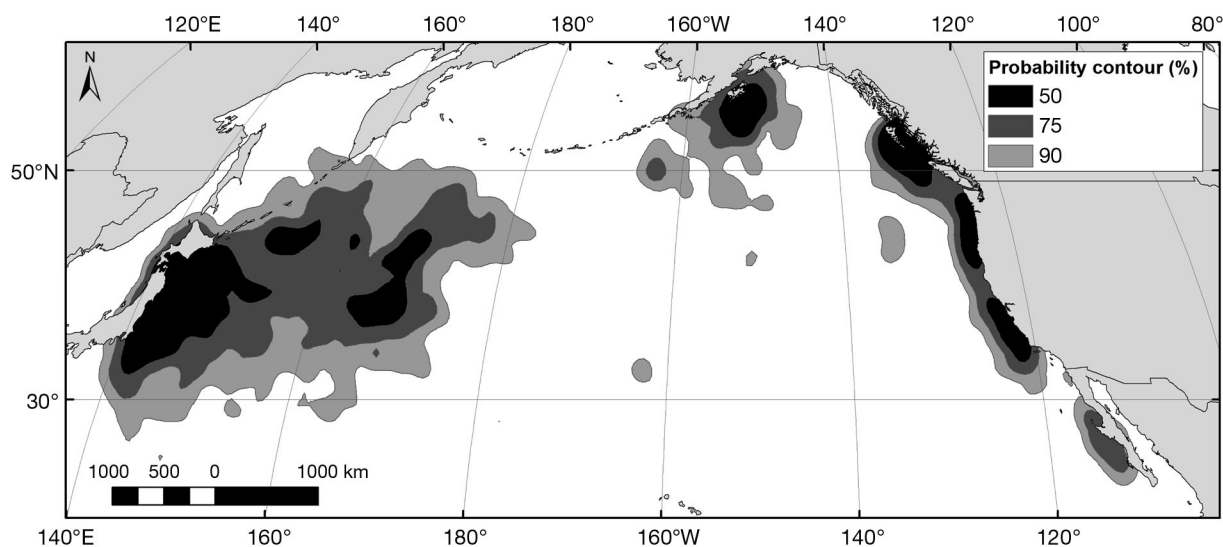


Fig. 1. Kernel density plot showing the 90, 75 and 50% probability contours for the non-breeding period for 23 *Puffinus griseus* tracked from 3 breeding sites in New Zealand

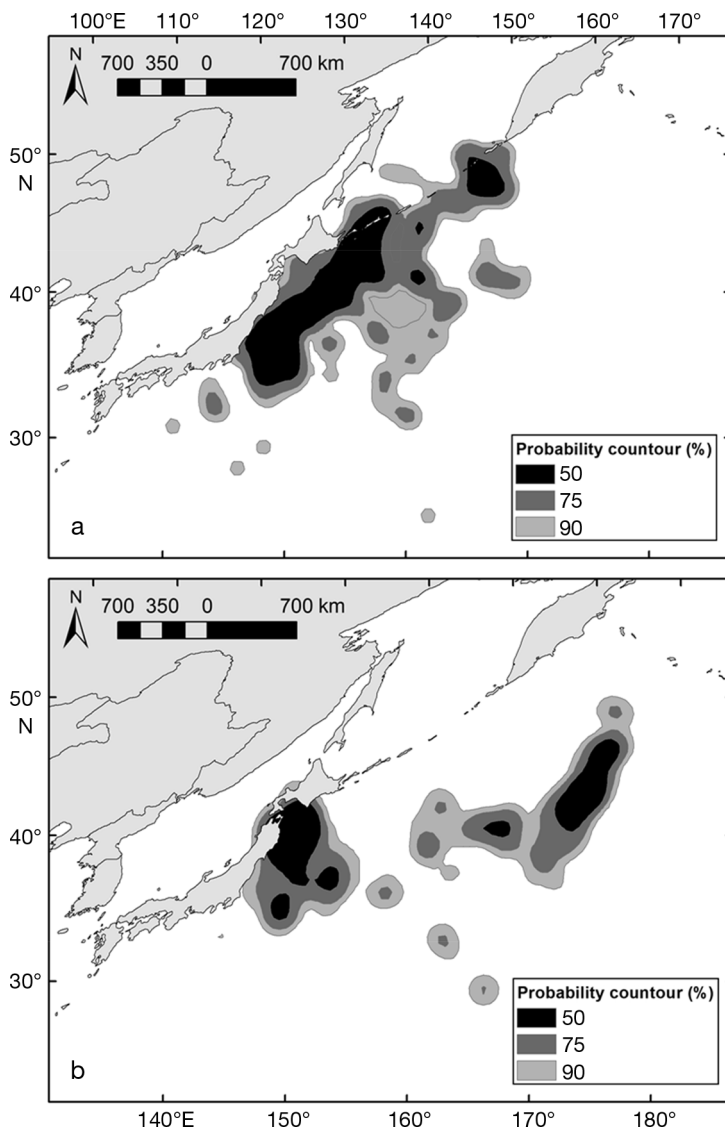


Fig. 2. Kernel density plot showing the 90, 75 and 50% probability contours for 2 *Puffinus griseus* that spent the non-breeding period off the east coast of Japan: (a) Bird 53089 remained relatively close to the coast, and (b) Bird 26752, which spent time further offshore

Table 1. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values (as ‰) in body feather samples from *Puffinus griseus* tracked using GLS loggers from breeding sites in New Zealand. Region 1: east of Japan; Region 2: Gulf of Alaska and west of Canada; Region 3: west of USA and Baja Peninsula. Mean values sharing a superscript letter are not significantly different (see 'Results')

Region	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean	1 SD	Mean	1 SD
1	16	-18.2 ^a	0.6	12.3 ^a	0.8
2	3	-16.7 ^b	1.3	15.3 ^b	0.6
3	4	-15.4 ^b	0.1	16.8 ^c	0.8

ese coast ($n = 5$) had significantly lower $\delta^{15}\text{N}$ values (mean $\pm$ SD: $11.5 \pm 0.8\text{‰}$) than those from further offshore ($n = 11$; mean $\pm$ SD: $12.6 \pm 0.4\text{‰}$; $t_{14} = 3.88$, $p < 0.01$), whereas the $\delta^{13}\text{C}$ values were less distinct and not significantly different (mean $\pm$ SD of $-18.0 \pm 0.8\text{‰}$ and $-18.4 \pm 0.5\text{‰}$ for inshore and offshore, respectively; $t_{14} = 1.06$, $p = 0.31$).

DISCUSSION

The tracking data for 9 sooty shearwaters reported here, combined with the 14 sooty shearwaters previously reported in Shaffer et al. (2006), highlight the importance of 3 distinct non-breeding destinations in the North Pacific Ocean (Fig. 1). Furthermore, there was no migratory connectivity between these regions. This pattern of widespread dispersal across the entire North Pacific Ocean to 1 of 3 discrete areas contrasts with tracking data reported for sooty shearwaters breeding in the South Atlantic Ocean, which migrate northwards to the North Atlantic Ocean during the austral winter (Hedd et al. 2012). These birds spent the non-breeding period in 2 distinct areas: (1) west of the mid-Atlantic ridge and (2) south of the Grand Bank, although 1 bird moved from the mid-Atlantic ridge to the northeast Atlantic Ocean (west of Ireland), before migrating south (Hedd et al. 2012). Despite utilising areas in relatively close proximity at the presumed outset of primary feather moult, these 2 groups of shearwaters exhibited distinct isotopic values for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Hedd et al. 2012).

Similarly, results presented here confirm that sooty shearwaters spending the non-breeding period at 1 of 3 North Pacific Ocean areas showed distinctive stable isotope values in their feathers (see 'Results' and Table 1). Most striking was the comparison between birds that spent the non-breeding period off Japan compared to those off the western seaboard of North America and Mexico. On average, there was a difference of 2.8‰ and 4.5‰ in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, in sooty shearwaters from the western and the eastern Pacific (Table 1). These results are in general agreement with modelled $\delta^{15}\text{N}$ values in the northern Pacific Ocean (Somes et al. 2010), and elevated $\delta^{15}\text{N}$ values in the eastern Pacific Ocean, which reflect the extensive local upwelling

system (see Graham et al. 2010). To our knowledge, a North Pacific Ocean-wide synthesis or modelling of $\delta^{13}\text{C}$ values at the base of food chains (an 'isoscape' for $\delta^{13}\text{C}$) has yet to be undertaken. Nevertheless, stable isotope data presented here for sooty shearwaters moulting in well-defined regions suggest that there is persistent, structured spatial variation in isotope ratios across the entire North Pacific Ocean basin. Jaeger et al. (2010) questioned the use of body feathers in this type of application of isotope data, but in the case of sooty shearwaters, body feathers appear to be replaced during long periods (ca. 150 d; Shaffer et al. 2006) of residency in one area during the non-breeding period, and the isotopic information integrated by body feathers accurately reflects local isotopic characteristics (see also Phillips et al. 2009). We have assumed that interannual variation in stable isotope values within regions is relatively small compared to the differences in stable isotope values between regions (see Graham et al. 2010). This assumption is supported by Ohman et al. (2012), who found that $\delta^{15}\text{N}$ values in zooplankton in the California Current system remained stable over a 54 yr time period, although there were annual and decadal fluctuations. Similarly, Chiba et al. (2012) reported interannual variation in $\delta^{15}\text{N}$ values of copepods across the North Pacific Ocean, but the shifts in $\delta^{15}\text{N}$ values from year to year were not as large as the different $\delta^{15}\text{N}$ values noted in this study in shearwaters tracked to Japan and those tracked to the western coasts of North America and the Baja Peninsula, Mexico (see Table 1).

Our study highlights the potential of using stable isotope signatures of body feathers taken from seabirds to identify non-breeding areas. For sooty shearwaters breeding in New Zealand, and perhaps other migratory seabird species that utilise distinct non-breeding areas, this technique represents a relatively non-invasive, straightforward approach that could be used to link non-breeding distribution, habitat characteristics or isotopic niche of large numbers of individuals with subsequent breeding performance, phenology and pollutant burdens (e.g. Sorensen et al. 2009, Leat et al. 2013). In particular, sampling body feathers for stable isotope analysis circumvents the need to deploy loggers, which are more expensive, impose an energetic cost during the long deployment period, and require the bird to be captured twice. The approach can also be used to assess hazards to human health. For example, the waters to the east of Japan, which are clearly of major importance for sooty shearwaters from New Zealand, have been affected by the discharge of radioactive material from the damaged Fukushima nuclear power plants

(Buesseler et al. 2011, Tanaka 2012). Stable isotope analysis of feathers taken from nesting birds could be used to identify not only those individuals that had spent the non-breeding period in that general region, but also potentially to discriminate those that were closer inshore (Fig. 2a) and possibly more contaminated. Subsequent monitoring for radionuclides could be focused on their chicks, particularly as this species is harvested for human consumption (Newman et al. 2009). Finally, analysis of stable isotopes in feathers provides a mechanism for conducting longitudinal studies of the same individuals across multiple years to explore not only non-breeding site fidelity, but also the consequences of spatial variation in natural and anthropogenic environmental change in non-breeding areas on subsequent breeding, in the case of sooty shearwaters at the other extreme of the world's largest ocean.

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