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Mercury concentrations in primary feathers reflect pollutant exposure in discrete non-breeding grounds used by Short-tailed Shearwaters

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Abstract We measured mercury concentrations ([Hg]) and nitrogen stable isotope ratios ($\delta^{15}N$) in the primary feathers of Short-tailed Shearwaters (*Puffinus tenuirostris*) that were tracked year-round. [Hg] were highest in 14 birds that used the Okhotsk and northern Japan Seas during the non-breeding period (2.5±1.4 μg/g), lowest in 9 birds that used the eastern Bering Sea (0.8±0.2 μg/g), and intermediate in 5 birds that used both regions (1.0±0.5 μg/g), with no effects of $\delta^{15}N$. The results illustrate that samples from seabirds can provide a useful means of monitoring pollution at large spatial scale.

Key word: geolocator; trophic level; northern North Pacific; monitoring

INTRODUCTION

Mercury (Hg) is diffused globally through atmospheric and oceanic transport (Laurier et al. 2004), methylated and biomagnified in the marine ecosystem (Jaeger et al. 2009) and has adverse physiological effects on consumers. Although reliable approaches to monitoring spatial pattern of marine Hg pollution are needed, intensive and repeated sampling of seawater in offshore regions is financially and logistically challenging. As an alternative, seabird feathers may offer a viable method for monitoring Hg pollution including over large spatial scales (Bearhop et al. 2000).

Studies of seabirds tracked using geolocators show that individuals from the same breeding colony may use various discrete regions in pelagic waters during the non-breeding period (Phillips et al. 2008). Recent study showed that individuals of Great Skuas (*Catharacta skua*) carried rather different levels of contaminants depending on their wintering area (Leat et al. 2013). If molt patterns are known or inferred, Hg concentrations of feathers grown at a specific time of the year may therefore provide valuable information on environmental exposure within particular regions (Ramos et al. 2009).

Short-tailed Shearwaters (*Puffinus tenuirostris*) are trans-equatorial migrants that breed in southern Australia and spend the non-breeding period (May–Sep) in the northern North Pacific. To assess Hg exposure in these non-breeding grounds, we tracked Short-tailed Shearwaters using geolocators, and sampled outer most primary feathers (P10) when the birds returned to the colony for later analysis of

Hg concentrations ([Hg]) and $\delta^{15}N$ (a proxy of trophic level; Jaeger et al. 2009).

STUDY AREA AND METHODS

Field work The study was conducted at Great Dog, Flinders Islands, Tasmania (40°15´S, 148°15´E). Geolocators (Mk15; British Antarctic Survey, Cambridge) were attached to 50 and 46 incubating birds in early December in 2009 and 2010, respectively. Geolocators weighed 2.5g (< 1% of mean body mass of study birds), and were attached to aluminum leg bands with plastic ties (Carey et al. 2014). Fifteen and 27 birds in the burrows were recaptured in early December 2010 and 2011, respectively (including 3 birds recaptured 2 years after deployment), and one recovered from a beached bird in 2010. Tracking data were obtained from 40 birds (3 loggers could not be downloaded). At recapture, 1 cm from the tip of P10 was collected and stored at -20 °C. Based on body measurements (Carey 2011), the sample of tracked birds was male-biased (32 males, 4 females, and 7 unknown) since males usually take the first incubation spell in early December when we conducted the fieldwork.

Track analysis The geolocators measured light intensity, and immersion and temperature in seawater. We estimated sunset and sunrise times from light curves, then derived latitudes on the basis of day length, and longitudes from the time of local midday and midnight. Day and night locations were averaged to give a single location per day. During the period around the equinoxes, when latitude cannot be estimated from the day length, we used the water-temperature data and the light-based longitudes to estimate the daily latitude from maps of remotely-sensed sea surface temperature (8-day composite, resolution 9 km, measured by Aqua-MODIS). Location data that were unreliable because of obvious interruptions around sunset and sunrise, or unrealistic flight speeds (>70 km/h) were replaced with those estimated by linear interpolation.

Chemical analysis Each feather was split into two at the rachis for Hg and stable isotope analyses. For Hg analysis, the feathers were washed using 99.5% acetone and Milli-Q water and dried in an oven at 50°C for 24 hrs. We measured [Hg] using CV-AAS (Cold Vapor-Atomic Absorption Spectroscopy) and a Mercury Analyzer MA-3000 (Nippon Instruments Corporation, Japan). Hg recoveries were between 90% and 105% for the laboratory standards (fish; DORM-3 and DOLT-4), and the detection limit was 0.2 ng/g (dry weight). For $\delta^{15}N$ analysis, feathers were cleaned using 0.25M sodium hydroxide, rinsed in Milli-Q water and dried at 60°C for 24 hrs. Dried samples were ground in an auto-mill after freezing using liquid nitrogen. The nitrogen stable isotope ratio (in ‰) was measured using a gas-source isotope ratio mass spectrometer (ANCA-GSL and Hydra 20-20, Sercon Ltd, UK), and is presented as deviations from atmospheric N_2, where $\delta^{15}N = [(^{15}N/^{14}N \text{ sample} / ^{15}N/^{14}N \text{ standard}) - 1] \times 1{,}000$. All samples were measured in triplicate and average values used in all statistical tests. If the coefficient of variation on triplicate measurements was over 0.3, the value with the largest deviation was excluded from calculation of the mean.

Statistical analyses The effect of year and non-breeding grounds (fixed effects) and $\delta^{15}N$ (covariate) on [Hg], and the effects of year and non-breeding grounds (fixed) on $\delta^{15}N$ were examined by GLMs using SPSS statistics ver. 22. Sex was not included as a factor because of the male bias. No interaction terms were included. Means are presented ± SD.

RESULTS

Non-breeding grounds Feather samples of Short-tailed Shearwaters were not collected in every case, or too small for analysis, so [Hg] and $\delta^{15}N$ data were available for 28 tracked birds in this study. During the non-breeding period, 14 birds stayed in the southern Okhotsk and northern Japan Seas (WEST), 9 birds (including three tracked for two years) migrated to the eastern Bering Sea and around the Aleutian Islands (EAST), and 5 birds initially used the western region but later moved to the eastern region (MIX) (Fig. 1).

Mercury and $\delta^{15}N$ There was no significant effect of year ($F_{(1,23)}=1.863$, $P=0.185$) or $\delta^{15}N$ ($F_{(1,23)}=0.053$, $P=0.820$) on [Hg], but the effect of non-breeding grounds was significant ($F_{(2,23)}=6.789$, $P=0.002$). Mean [Hg] was higher in WEST than EAST birds (Fig. 2; $P<0.05$, Bonferroni post-hoc test), and did not differ significantly between MIX and WEST or EAST birds ($P>0.05$). The effect of non-breeding grounds on $\delta^{15}N$ was marginally significant ($F_{(2,24)}=3.336$, $P=0.053$) with $\delta^{15}N$ tended to be higher in EAST (15.2±2.9‰) than WEST (13.4±0.8‰) and MIX birds (13.0±1.7‰). Year effect was not significant ($F_{(1,24)}=0.229$, $P=0.637$).

DISCUSSION

Primary molt of Short-tailed Shearwaters has been recorded during late June and July (non-breeding period) and is completed before return to the breeding site (Marchant and Higgins 1990). Based on stable isotope and other data, the Sooty Shearwaters *Puffinus griseus* also start to molt primaries on arrival at the non-breeding grounds in the north-western Atlantic (Hedd et al. 2012). Although there was substantial variation in molting patterns among populations and individuals in Yelkouan Shearwaters *Puffinus yelkouan*, they rarely molted wing feathers during breeding (Bourgeois and Dromzee 2014). Thus, the Hg concentrations in P10 of the short-tailed shearwaters in our study presumably reflect exposure to pollutants in the non-breeding grounds. Further information on molt sequence in this species, including birds of different status and from different colonies, would be valuable, however, as chemical analysis of different feather types may provide details on pollutant exposure during the non-breeding period at a finer temporal and spatial scale (González-Solís et al. 2011).

[Hg] in P10 of our Short-tailed Shearwaters (0.8 – 2.5 μg/g, Fig. 2) were lower than those in the flight feathers that were replaced during the non-breeding period by other species (1.2 – 3.9 μg/g in Cory's Shearwater *Calonectris diomedea*, Ramos et al. 2009; 4.3 – 6.0 μg/g in Great Skua, Bearhop et al. 2000), and lower than the levels sometimes associated with impaired reproduction (>5.0μg/g, Burger and Gochfeld 1997). We further found that feather [Hg] were higher for the Short-tailed Shearwaters that spent the non-breeding period in the southern Okhotsk and northern Japan Seas (WEST), than those in the eastern Bering Sea and around the Aleutian Islands (EAST). The influence of $\delta^{15}N$ on [Hg] in feathers was not significant in our study birds, in comparison with results from other seabird species (Bond 2010). In addition, the $\delta^{15}N$ of WEST birds tended to be lower than that of EAST birds, and so the differences in trophic level would not explain the higher [Hg] in the WEST birds unless we postulate a substantial disparity in isotopic baselines in the two regions.

The spatial pattern of Hg pollution in offshore waters of the northern North Pacific Ocean has been examined by sampling seabird tissues and seawater during research cruises. [Hg] in the liver of Glaucous-winged Gulls (*Larus glaucescens*) increased towards the west along the Aleutian Island chain (Ricca et al. 2008). [Hg] in seawater was higher in the western North Pacific shelf area off Japan than in the basin and central North Pacific (Laurier et al. 2004). These findings provide general support for the spatial pattern in Hg exposure found in the Short-tailed Shearwaters tracked in our study.

Although the method we applied provided information on pollutant levels only in the areas visited by the tracked birds, our study nevertheless demonstrates the utility of this technique for monitoring the spatial pattern of Hg pollution in large offshore regions, which are beyond the ranges of breeding birds and where ship-based sampling is expensive and logistically challenging.

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FIGURE CAPTIONS

Fig. 1 Kernel density map of Short-tailed Shearwaters with different distributions during the non-breeding period: (a) WEST (14 birds), (b) MIX (5 birds), and (c) EAST (9 birds). The kernel density contours represent the proportions of the overall kernel density surface from the highly utilized, core area (black: 25%) to the periphery of the winter distribution (light grey: 95%).

Fig. 2 Mercury (Hg) concentration (in μg/g dry weight) of the outermost primary feather of Short-tailed Shearwaters that spent the non-breeding period in the southern Okhotsk Sea and northern Japan Sea (WEST, 14 birds), in the eastern Bering Sea and around the Aleutian Islands (EAST, 9 birds), or that moved from WEST to EAST (MIX, 5 birds).

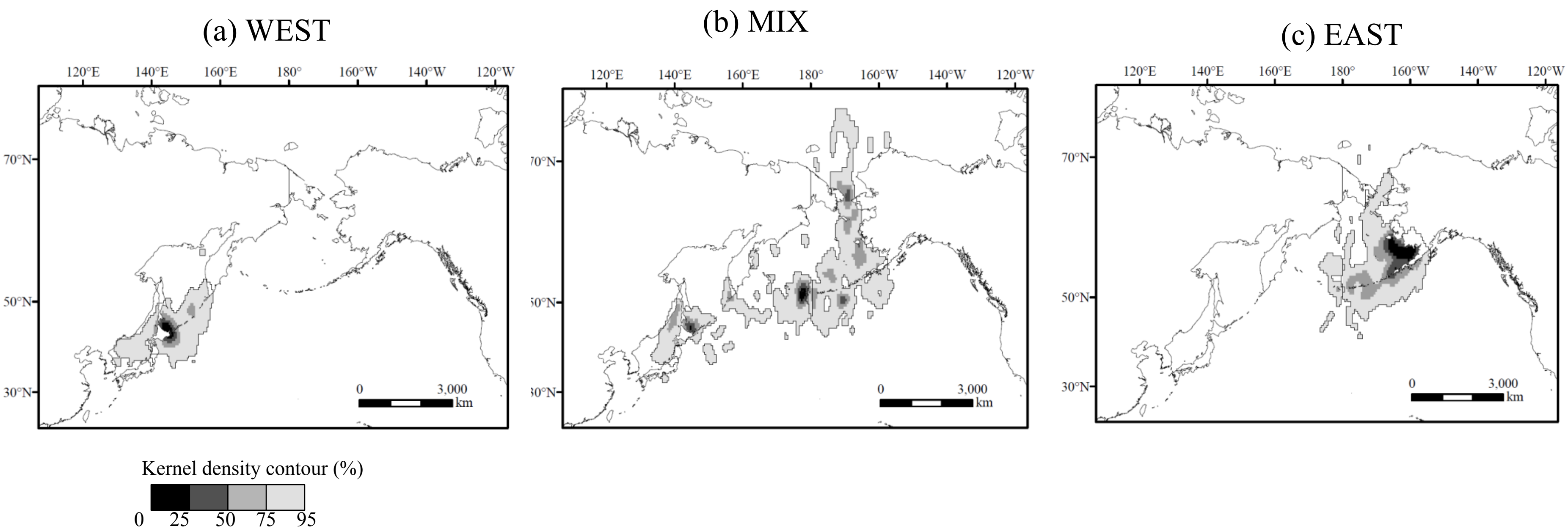
(a) WEST
(b) MIX
(c) EAST
120°E
140°E
160°E
180°
160°W
140°W
120°W
70°N
50°N
30°N
0
3,000
km
Kernel density contour (%)
0
25
50
75
95

Fig. 1

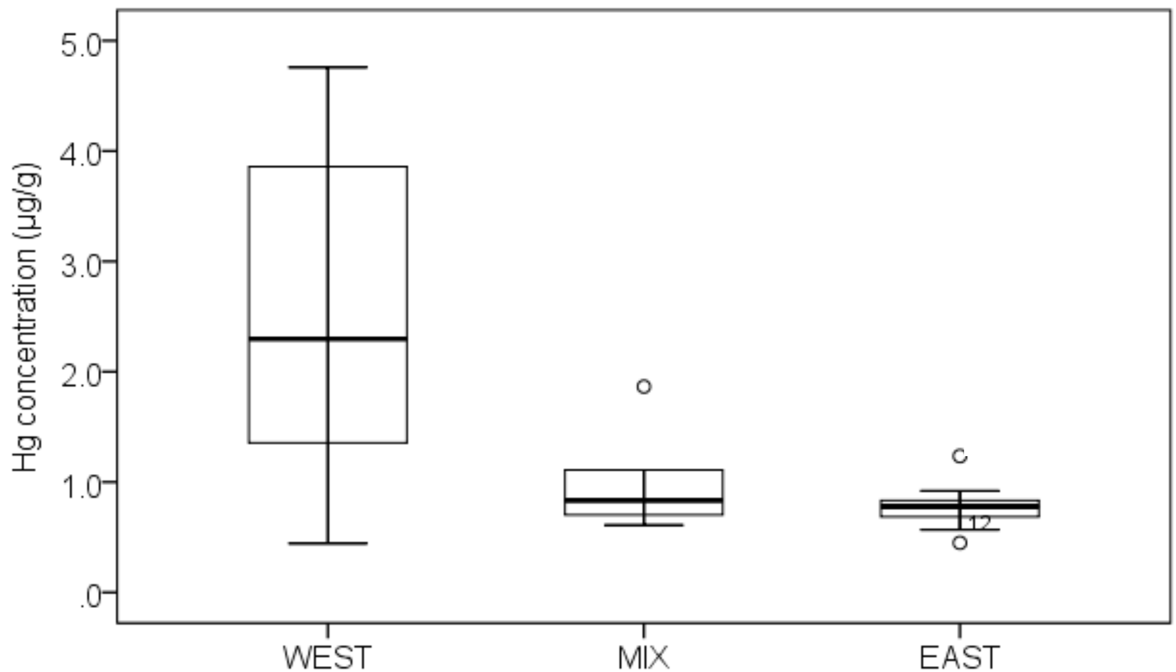

Fig. 2