



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

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20

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

29

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

31

## INTRODUCTION

32

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

41

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

50

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

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

57

58

## STUDY AREA AND METHODS

59 **Field work** The study was conducted at Great Dog, Flinders Islands, Tasmania  
60 ( $40^{\circ}15'S$ ,  $148^{\circ}15'E$ ). Geolocators (Mk15; British Antarctic Survey, Cambridge)  
61 were attached to 50 and 46 incubating birds in early December in 2009 and 2010,  
62 respectively. Geolocators weighed 2.5g ( $< 1\%$  of mean body mass of study birds),  
63 and were attached to aluminum leg bands with plastic ties (Carey et al. 2014).  
64 Fifteen and 27 birds in the burrows were recaptured in early December 2010 and  
65 2011, respectively (including 3 birds recaptured 2 years after deployment), and  
66 one recovered from a beached bird in 2010. Tracking data were obtained from 40  
67 birds (3 loggers could not be downloaded). At recapture, 1 cm from the tip of P10  
68 was collected and stored at  $-20^{\circ}\text{C}$ . Based on body measurements (Carey 2011),  
69 the sample of tracked birds was male-biased (32 males, 4 females, and 7  
70 unknown) since males usually take the first incubation spell in early December  
71 when we conducted the fieldwork.

72

73 **Track analysis** The geolocators measured light intensity, and immersion and  
74 temperature in seawater. We estimated sunset and sunrise times from light curves,  
75 then derived latitudes on the basis of day length, and longitudes from the time of  
76 local midday and midnight. Day and night locations were averaged to give a  
77 single location per day. During the period around the equinoxes, when latitude  
78 cannot be estimated from the day length, we used the water-temperature data and  
79 the light-based longitudes to estimate the daily latitude from maps of

80 remotely-sensed sea surface temperature (8-day composite, resolution 9 km,  
81 measured by Aqua-MODIS). Location data that were unreliable because of  
82 obvious interruptions around sunset and sunrise, or unrealistic flight speeds (>70  
83 km/h) were replaced with those estimated by linear interpolation.

84

85 **Chemical analysis** Each feather was split into two at the rachis for Hg and stable  
86 isotope analyses. For Hg analysis, the feathers were washed using 99.5% acetone  
87 and Milli-Q water and dried in an oven at 50°C for 24 hrs. We measured [Hg]  
88 using CV-AAS (Cold Vapor-Atomic Absorption Spectroscopy) and a Mercury  
89 Analyzer MA-3000 (Nippon Instruments Corporation, Japan). Hg recoveries were  
90 between 90% and 105% for the laboratory standards (fish; DORM-3 and DOLT-4),  
91 and the detection limit was 0.2 ng/g (dry weight). For  $\delta^{15}\text{N}$  analysis, feathers were  
92 cleaned using 0.25M sodium hydroxide, rinsed in Milli-Q water and dried at 60°C  
93 for 24 hrs. Dried samples were ground in an auto-mill after freezing using liquid  
94 nitrogen. The nitrogen stable isotope ratio (in ‰) was measured using a  
95 gas-source isotope ratio mass spectrometer (ANCA-GSL and Hydra 20-20,  
96 Sercon Ltd, UK), and is presented as deviations from atmospheric  $\text{N}_2$ , where  $\delta^{15}\text{N}$   
97 =  $[(^{15}\text{N}/^{14}\text{N} \text{ sample} / ^{15}\text{N}/^{14}\text{N} \text{ standard}) - 1] \times 1,000$ . All samples were measured in  
98 triplicate and average values used in all statistical tests. If the coefficient of  
99 variation on triplicate measurements was over 0.3, the value with the largest  
100 deviation was excluded from calculation of the mean.

101

102 **Statistical analyses** The effect of year and non-breeding grounds (fixed effects)  
103 and  $\delta^{15}\text{N}$  (covariate) on [Hg], and the effects of year and non-breeding grounds

104 (fixed) on  $\delta^{15}\text{N}$  were examined by GLMs using SPSS statistics ver. 22. Sex was  
105 not included as a factor because of the male bias. No interaction terms were  
106 included. Means are presented  $\pm$  SD.

107

108

## RESULTS

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

116

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

126

127

## DISCUSSION

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

142

143 [Hg] in P10 of our Short-tailed Shearwaters (0.8 – 2.5 µg/g, Fig. 2) were lower  
144 than those in the flight feathers that were replaced during the non-breeding period  
145 by other species (1.2 – 3.9 µg/g in Cory's Shearwater *Calonectris diomedea*, Ramos  
146 et al. 2009; 4.3 – 6.0 µg/g in Great Skua, Bearhop et al. 2000), and lower than the  
147 levels sometimes associated with impaired reproduction (>5.0µg/g, Burger and  
148 Gochfeld 1997). We further found that feather [Hg] were higher for the  
149 Short-tailed Shearwaters that spent the non-breeding period in the southern  
150 Okhotsk and northern Japan Seas (WEST), than those in the eastern Bering Sea  
151 and around the Aleutian Islands (EAST). The influence of  $\delta^{15}\text{N}$  on [Hg] in



152 feathers was not significant in our study birds, in comparison with results from  
153 other seabird species (Bond 2010). In addition, the  $\delta^{15}\text{N}$  of WEST birds tended to  
154 be lower than that of EAST birds, and so the differences in trophic level would not  
155 explain the higher [Hg] in the WEST birds unless we postulate a substantial  
156 disparity in isotopic baselines in the two regions.

157

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

166

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

172

173

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228

229

230

## FIGURE CAPTIONS

231

232 Fig. 1 Kernel density map of Short-tailed Shearwaters with different distributions  
233 during the non-breeding period: (a) WEST (14 birds), (b) MIX (5 birds), and  
234 (c) EAST (9 birds). The kernel density contours represent the proportions of  
235 the overall kernel density surface from the highly utilized, core area (black:  
236 25%) to the periphery of the winter distribution (light grey: 95%).

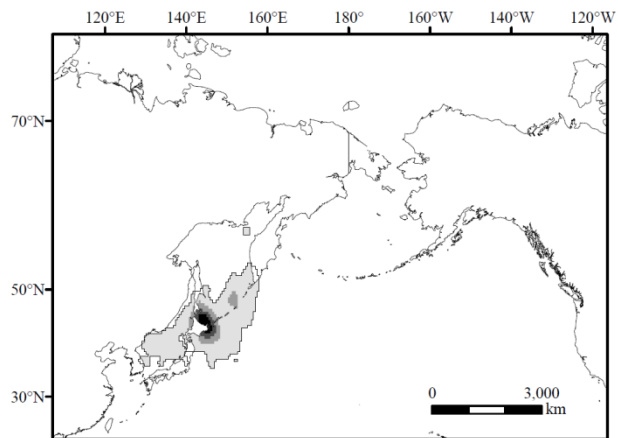
237

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

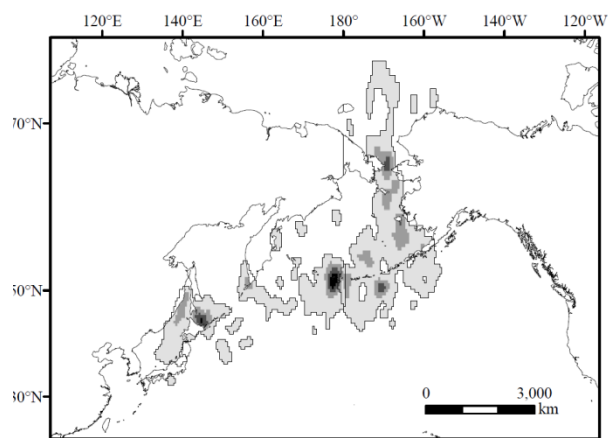
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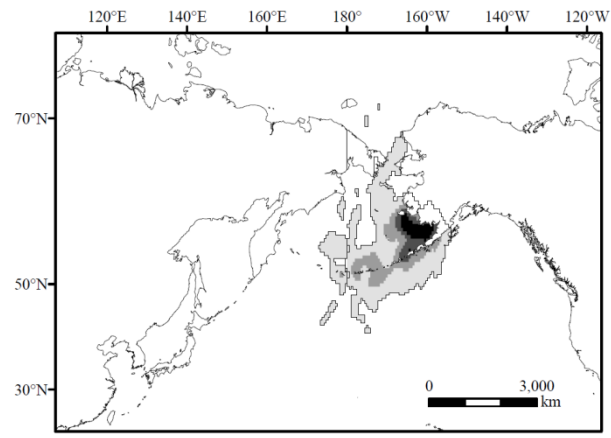
(a) WEST



(b) MIX



(c) EAST



Kernel density contour (%)

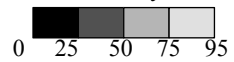


Fig. 1

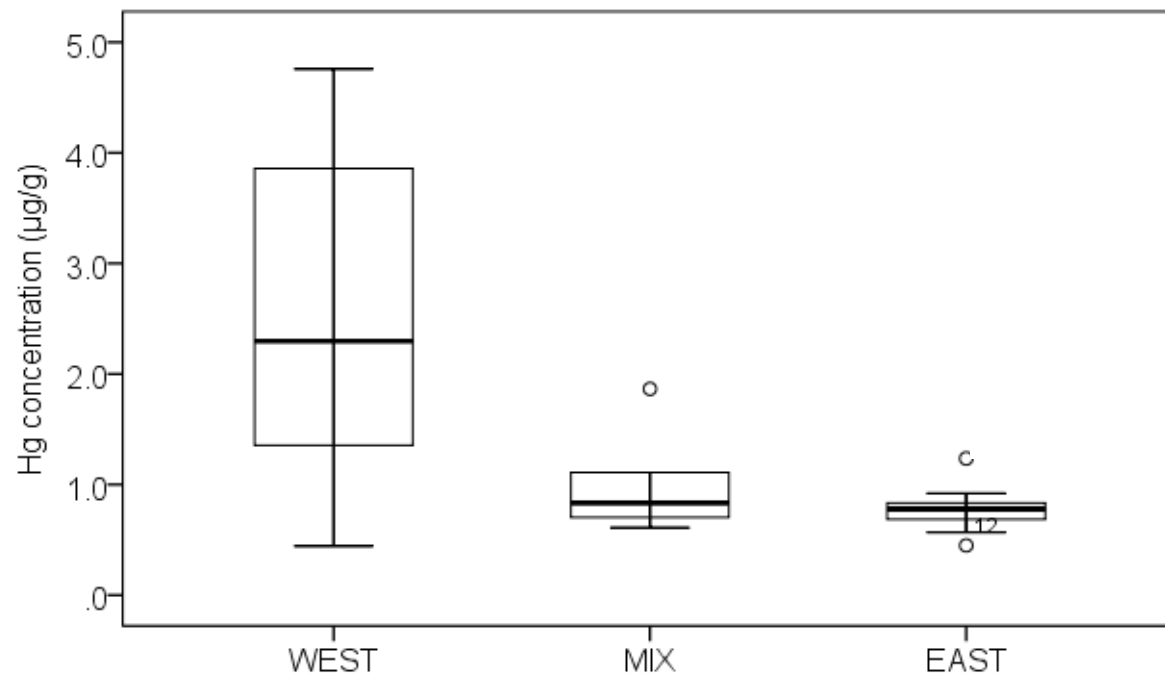


Fig. 2