

1 2015.2.14

2 Short Note to J Ornithology: 2nd revised version

3

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

6

7 Yutaka Watanuki¹, Takashi Yamamoto^{1,2}, Ai Yamashita¹, Chihiro Ishii³, Yoshinori
8 Ikenaka³, Shouta M.M. Nakayama³, Mayumi Ishizuka³, Yuya Suzuki¹, Yasuaki
9 Niizuma⁴, C.E. Meathrel⁵, R.A. Phillips⁶

10

11 ¹Graduate School of Fisheries Sciences, Hokkaido University, Minato-cho 3-1-1,
12 Hakodate 041-8611, Japan ywata@fish.hokudai.ac.jp, ²Arctic Environment
13 Research Center, National Institute of Polar Research, Midori-cho 10-3,
14 Tachikawa, 190-8518, Japan, ³Graduate School of Veterinary Medicine, Hokkaido
15 University, Kita-18 Nishi-9, Sapporo 060-0818, Japan, ⁴Faculty of Agriculture,
16 Meijyo University, Shiogamaguchi 1-501, Tenpaku-ku, Nagoya 468-8502,
17 ⁵LaTrobe University, Wodonga, Victoria 3086, Australia, ⁶British Antarctic Survey,
18 Natural Environment Research Council, High Cross, Madingley Road, Cambridge
19 CB3 0ET, UK

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°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
91 DOLT-4), and the detection limit was 0.2 ng/g (dry weight). For $\delta^{15}\text{N}$ analysis,
92 feathers were cleaned using 0.25M sodium hydroxide, rinsed in Milli-Q water and
93 dried at 60°C for 24 hrs. Dried samples were ground in an auto-mill after freezing
94 using liquid nitrogen. The nitrogen stable isotope ratio (in ‰) was measured
95 using a gas-source isotope ratio mass spectrometer (ANCA-GSL and Hydra
96 20-20, Sercon Ltd, UK), and is presented as deviations from atmospheric N_2 ,
97 where $\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N} \text{ sample} / {}^{15}\text{N}/{}^{14}\text{N} \text{ standard}) - 1] \times 1,000$. All samples were
98 measured in triplicate and average values used in all statistical tests. If the
99 coefficient of variation on triplicate measurements was over 0.3, the value with
100 the largest 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

ACKNOWLEDGEMENTS

174 We thank R. Bruce, B. Nishizawa, and A. Ito for field assistance, L. Hardy for
175 recovering geolocator from a beached bird. Research was carried out under La

176 Trobe University Animal Ethics Project AEC 10/32 (W) and Tasmanian
177 Department of Primary Industries Permits to Take Wildlife for Scientific Purpose
178 FA10161 and FA11212 held by CE Meathrel. The study was supported by JSPS
179 (#20241001, #22405007) and GRENE project.

180

181

REFERENCES

- 182 Bearhop S, Phillips RA, Thompson DR, Waldron S, Furness RW (2000)
183 Variability in mercury concentrations of great skuas *Catharacta skua*: the
184 influence of colony, diet and trophic status inferred from stable isotope
185 signatures. *Mar Ecol Prog Ser* 195:261-268.
- 186 Bond AL (2010) Relationships between stable isotopes and metal contaminants in
187 feathers are spurious and biologically uninformative. *Environ Pollut*
188 158:1182–1184
- 189 Bourgeois K, Dromzee D (2014) Moulting strategies of the Yelkouan Shearwater
190 *Puffinus yelkouan* during the breeding season. *J Ornithol* 155:265–271
- 191 Burger J, Gochfeld M (1997) Risk, mercury levels, and birds: relating adverse
192 laboratory effects to field biomonitoring. *Environ Res* 75:160-172
- 193 Carey MJ (2011) Sexual size dimorphism, within-pair comparisons and assortative
194 mating in the short-tailed shearwater (*Puffinus tenuirostris*). *Notornis* 58: 8-16
- 195 Carey MJ, Phillips RA, Silk JRD, Shaffer SA (2014) Trans-equatorial migration of
196 Short-tailed Shearwaters revealed by geolocators. *Emu* 114:352–359
- 197 González-Solís J, Smyrli M, Militão T, Gremillet D, Tveraa T, Phillips RA,
198 Boulinier T (2011) Combining stable isotope analyses and geolocation to
199 reveal kittiwake migration. *Mar Ecol Prog Ser* 435, 251-261

200 Hedd A, Montevecchi WA, Otley H, Phillips RA, Fifield DA (2012)
201 Trans-equatorial migration and habitat use by sooty shearwaters *Puffinus*
202 *griseus* from the South Atlantic during the nonbreeding season. Mar Ecol
203 Prog Ser 449:277–290

204 Jaeger I, Hop H, Gabrielsen GW (2009) Biomagnification of mercury in selected
205 species from an Arctic marine food web in Svalbard. Sci Total Environ
206 407:4744–4751

207 Laurier FJG, Mason RP, Whalin GAG (2004) Mercury distribution in the North
208 Pacific ocean–20 years of observations. Mar Chem 90:3–19

209 Leat EHK, Bourgeon S, Magnúsdóttir E, Gabrielsen GW, Grecian WJ, Hanssen SA,
210 Ólafsdóttir K, Petersen A, Phillips RA, Strøm H, Ellis S, Fisk AT, Bustnes JO,
211 Furness RW, Borgå K (2013) Influence of wintering area on persistent organic
212 pollutants in a breeding migratory seabird. Mar Ecol Prog Ser 491, 277-293.

213 Marchant S, Higgins PJ (1990) Handbook of Australian, New Zealand and
214 Antarctic Birds. Volume 1: Ratites to ducks: Part A: Ratites to petrels.
215 Melbourne, Oxford University Press, pp 632–644

216 Phillips RA, Croxall JP, Silk JRD, Briggs DR (2008) Foraging ecology of
217 albatrosses and petrels from South Georgia: two decades of insights from
218 tracking technologies. Aquat Conserv Mar Freshw Ecosyst 17:6–21

219 Ramos R, González-Solís J, Forero MG, Moreno R, Gomez-Diaz E, Ruiz X,
220 Hobson KA (2009) The influence of breeding colony and sex on mercury,
221 selenium and lead levels and carbon and nitrogen stable isotope signatures in
222 summer and winter feathers of *Calonectris* shearwaters. Oecologia
223 159:345–354

224 Ricca MK, Miles AK, Anthony RG (2008) Sources of organochlorine
225 contaminants and mercury in seabirds from the Aleutian archipelago of
226 Alaska: inferences from spatial and trophic variation. *Sci Total Environ*
227 406:308–323
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%).

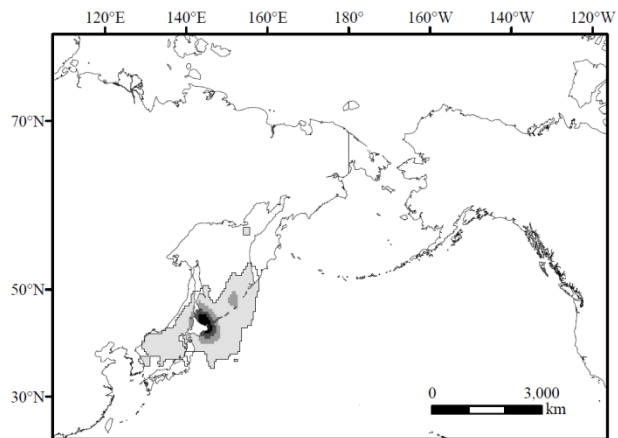
237

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).

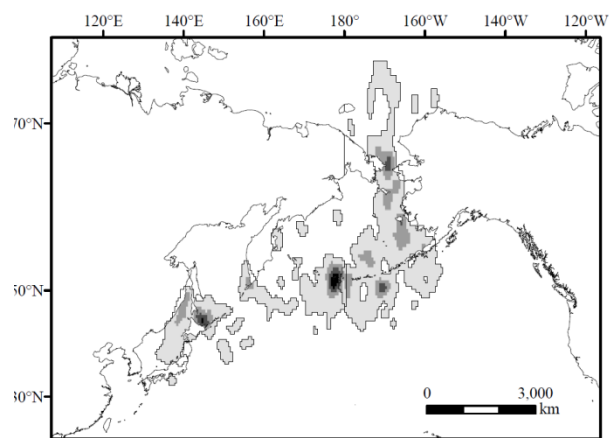
243

244

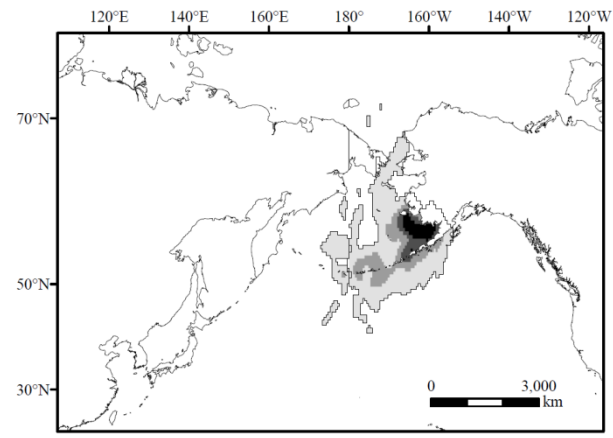
(a) WEST



(b) MIX



(c) EAST



Kernel density contour (%)

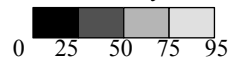


Fig. 1

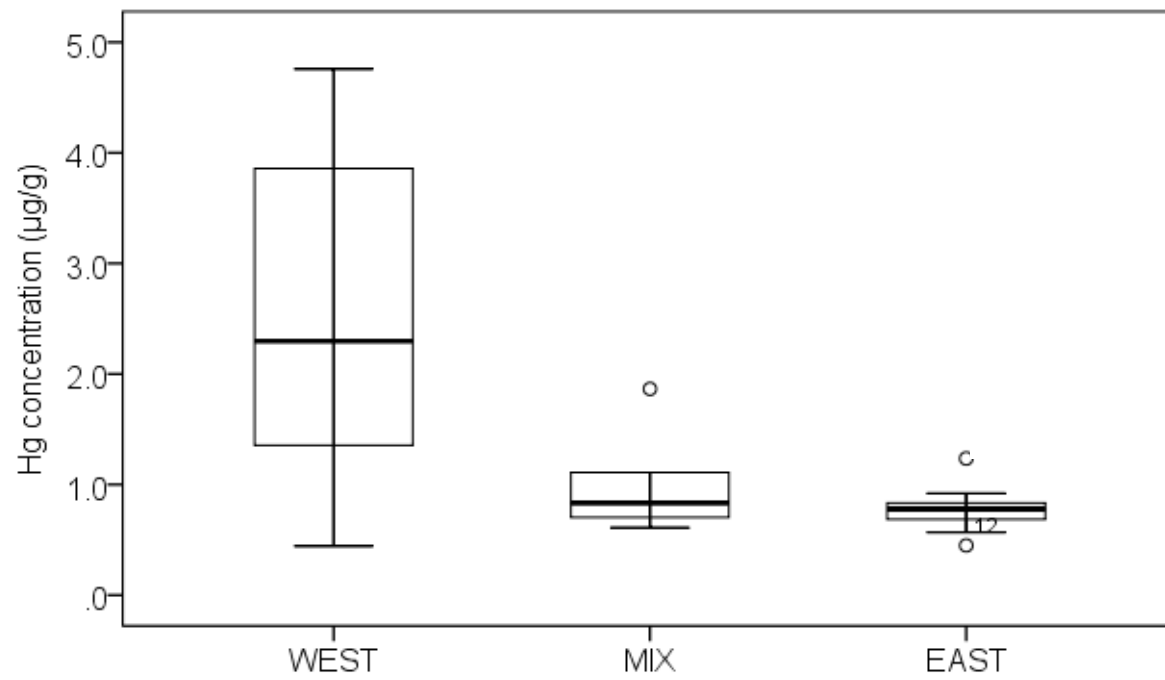


Fig. 2