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Feather mercury concentration in streaked shearwaters wintering in separate areas of southeast Asia

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ABSTRACT: We measured mercury concentration ([Hg]) and nitrogen stable isotope values ($\delta^{15}N$) in tail feathers that were replaced during the non-breeding period of streaked shearwaters *Calonectris leucomelas* that bred on 3 islands in Japan. The birds' year-round movements were tracked and their breeding status was monitored. [Hg] was greater in males than in females, and was greatest in those birds spending their non-breeding period in the South China Sea (3.1 ± 1.5 µg g^{-1} dry weight), moderate in birds in the Arafura Sea (1.5 ± 0.7 µg g^{-1}), and lowest in birds in the Pacific Ocean north of New Guinea (0.8 ± 0.4 µg g^{-1}). Adverse effects of feather [Hg] on breeding status were not observed. This regional variation in feather [Hg] might partly reflect differences in the intake of Hg between these non-breeding areas in addition to accumulation during the late breeding period and the southward migration period.

KEY WORDS: Geolocator · Migration · $\delta^{15}N$ · Breeding

INTRODUCTION

Individual seabirds from the same breeding colony sometimes use different pelagic areas during their non-breeding periods (Phillips et al. 2007, Kopp et al. 2011), and can accumulate different levels of pollutants; for example, great skuas *Stercorarius skua* (Leat et al. 2013) and short-tailed shearwaters *Puffinus tenuirostris* (Watanuki et al. 2015). The usefulness and limitations of seabirds as indicators of regional variation in marine pollution can be explored by tracking individuals to determine the relationships between non-breeding areas and the accumulation of pollutants. Further, to use seabirds as sentinels, knowledge is needed regarding the effects of pollutants on their physiology, behavior, and breeding success.

The emission and pollution of mercury (Hg) in Southeast Asia and surrounding waters has become an important public concern (Pacyna et al. 2010). Streaked shearwaters *Calonectris leucomelas* spend their non-breeding period in one of 4 separate areas in the offshore waters between Southeast Asia and New Guinea (Fig. 1 below, see also Yamamoto et al. 2010). Their tail feathers, which we inferred are replaced during the non-breeding period, may be suitable indicators of Hg pollution in these areas. We tracked individuals and measured the mercury concentration ([Hg]) and the nitrogen stable isotope value ($\delta^{15}N$ as a proxy of trophic level) in tail feathers

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to identify bio-magnification in birds that bred at 3 colonies in Japan. We also monitored the breeding status (eggs or chicks) of most of the tracked birds.

MATERIALS AND METHODS

Fieldwork

Fieldwork was carried out on Sangan (39° 18′ N, 141° 58′ E), Mikura (33° 52′ N, 139° 14′ E), and Awa (38° 27′ N, 139° 13′ E) Islands (Fig. 1) during the breeding periods between 2006 and 2010 under permits from the Japanese Ministry of Environment and the Agency of Cultural Affairs. We hand-captured 269 birds returning to feed chicks in their burrows, sexed each, and attached geolocators (Mk 4 or Mk 5, British Antarctic Survey) (Yamamoto et al. 2010). Birds giving high- and low-pitched calls were identified as males and females, respectively, as the pitch is a reliable indicator of genetically determined sex in this species (Arima et al. 2014). We recaptured 214 birds a year later.

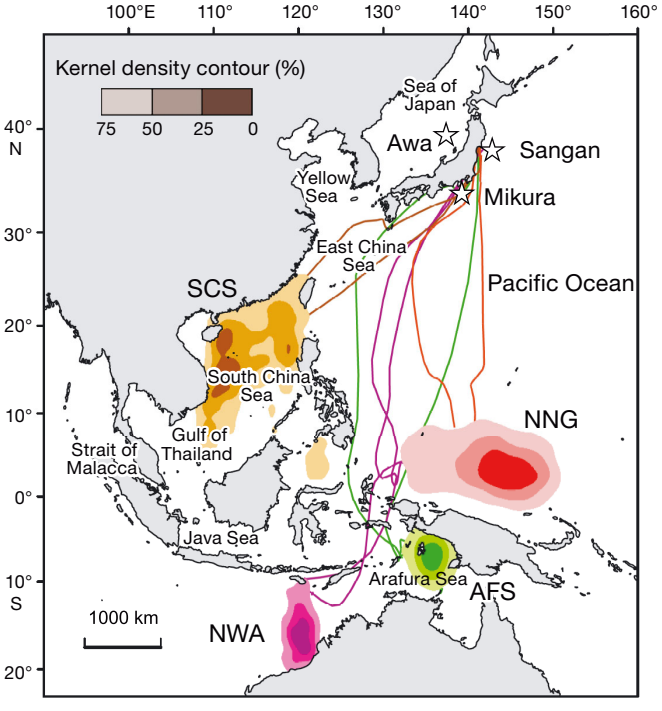

Fig. 1. (☆) Study colonies on the Mikura, Sangan, and Awa Islands and non-breeding areas (color shaded) of 186 tracked streaked shearwaters. Non-breeding areas were the South China Sea (SCS, n = 18, brown), the Pacific Ocean north of New Guinea (NNG, n = 129, red), the Arafura Sea (AFS, n = 37, green), and the Indian Ocean northwest of Australia (NWA, n = 2, purple). The kernel density contours represent the proportions of the overall kernel density surface from the highly utilized core area (dense color, 25%), through intermediate areas (50%), to the periphery of the winter distribution (pale color, 75%). Percentages indicate probability of a bird's position occurring within each range. See Yamamoto et al. (2010) for details. Marginal seas in the tropical to temperate western North Pacific are also shown. Typical migration routes for each non-breeding area are shown

Geolocators recorded the maximum light intensity during each 10 min period and the water temperature every 10 min after continuous immersion for 20 min. Sunset and sunrise times were estimated from the light curves, and latitude and longitude were derived from day length and the time of local midday and midnight, respectively (Yamamoto et al. 2010). During the 2 wk before and after the equinoxes, the latitudes could not be obtained from day length, so they were estimated from the recorded water temperature, the remotely sensed sea surface temperature (8 d composite data, resolution 9 km, measured by Aqua-MODIS), and the light-based longitude. Location data that were not validated because of interruptions around sunset and sunrise or unrealistic flight speed (e.g. >35 km h^{-1} sustained over 48 h) were corrected by using linear interpolation of neighboring locations. On the basis of our mapped data, we assumed that birds had reached or left their non-breeding areas after crossing predetermined boundaries (see Yamamoto et al. 2010). The key non-breeding areas for birds were identified separately by generating kernel density maps using the ArcGIS Spatial Analyst Density tool (ESRI, Redlands, CA, USA) with a cell size of 50 km and a search radius of 200 km. The non-breeding areas identified were South China Sea (SCS), the Pacific Ocean north of New Guinea (NNG), the Arafura Sea (AFS), and the Indian Ocean northwest of Australia (NWA) (Fig. 1).

The total mass of the geolocator with the plastic leg ring was 7 g (Mk 4) or 6 g (Mk 5), which represents about 1.2% of the average body mass of captured birds (576 ± 73 g, average ± SD, n = 100), and is smaller than the smallest proportional mass (i.e. <3%) that affects behavior in albatrosses and shearwaters (Phillips et al. 2003). Body mass did not differ between birds with or without devices at recapture (Yamamoto et al. 2008). Upon recovery, we removed the logger and leg ring; we did not observe any injuries to the birds' legs. The recovery rate of the loggers was high (80%, or 214 of 269 birds) in comparison with other studies of shearwaters (40% in González-Solís et al. 2007, 60% in Navarro et al. 2007).

We sampled the outermost tailfeathers (R6) that we inferred were replaced during the non-breeding

period, as in Cory's shearwaters *Calonectris diomedea* (Ramos et al. 2009a). To corroborate this, we confirmed that none of 30 streaked shearwaters rearing chicks on Nakanokami Island (Okinawa) and Sangan Island in 2014 began molting tail-feathers. The breeding status of the birds in the following season was determined either by direct observation of eggs or chicks or by recording the visits to burrow nests using geolocator data (Yamamoto et al. 2012) during the incubation and chick rearing periods.

Chemical analyses

Feathers were stored in a freezer (–20°C). Vanes of a feather were removed from the rachis, and one side was used for [Hg] analysis and the other side for $\delta^{15}N$ analyses. For [Hg] analysis, the vane was washed in 99.5% acetone and Milli-Q water and dried at 50°C in an oven for 24 h. Approximately 10 barbs were sampled randomly from each of the tip, middle, and base of a feather, mixed, and then weighed on an electronic balance. Total Hg in this mixture was measured with a Direct thermal decomposition mercury analyzer (MA-3000, Nippon Instruments). After preparation of calibration standards, the concentration of total mercury was measured by thermal decomposition. Recovery rates of Hg for the certified reference material, DOLT-4 (dogfish liver, National Research Council, Canada), ranged from 92 to 103% (94.3 ± 4.2%). The detection limit was 2.0 pg of total Hg.

For $\delta^{15}N$ analyses, the vane was washed in distilled water, stored in methanol/chloroform (2:1 v/v) overnight, dried at room temperature, freeze-dried for 36 to 48 h, cut as small as possible, and then homogenized with a mortar and pestle. $\delta^{15}N$ was measured using a Delta Plus advantage isotope ratio mass spectrometer (IR-MS) coupled with a Flash EA 1112 elemental analyzer (Thermo Electron). $\delta^{15}N$ values are expressed in δ notation as the deviation from standard (atmospheric nitrogen) in parts per thousand (‰):

$$\delta^{15}N = [(^{15}N/^{14}N_{sample} / {}^{15}N/^{14}N_{standard}) - 1)] \quad (1)$$

We used L-histidine as secondary isotopic reference material. The average and SD of $\delta^{15}N$ of L-histidine given by the provider (SI Science) are –7.74 ± 0.04‰. We measured the L-histidine value every 3 to 6 samples. The average values of $\delta^{15}N$ of L-histidine within runs were between –7.85‰ and –7.50‰ and the overall average was –7.71 ± 0.24‰ (n = 145).

Statistical analyses

We examined the effects of colony, non-breeding area, sex, $\delta^{15}N$, and non-breeding area × $\delta^{15}N$ interaction on the tail feather [Hg] (log-transformed for normality) using a generalized linear mixed model (GLMM) in which the year was treated as a random factor. We similarly examined the effects of colony, non-breeding area, and [Hg] on the presence or absence (1/0) of eggs or chicks (assuming a binomial distribution). Model selection was based on Akaike's information criteria (AIC). Models with ΔAIC < 2.00 were considered as adequate (Burnham & Anderson 2010). When only a single adequate model was selected, it was designated the best model. When multiple adequate models were selected, a model-averaging approach was used where the parameter estimates of factors included in the adequate models with ΔAIC < 2 (see Table S3 in the Supplement) were weighted with the corresponding Akaike weight and averaged. Differences in $\delta^{15}N$ between groups were examined by Kruskal-Wallis test. Statistical analyses were carried out in R v. 3.1.1 (R Development Core Team 2014) and SPSS Statistics 22 (IBM) software.

RESULTS

Among 214 birds recaptured, 186 were tracked successfully. Tail feather samples from some birds were unavailable for chemical analyses, and the sex of a few birds was not determined. This left 126 birds for the analysis of factors explaining [Hg] (Table 1). Among these birds, the presence of eggs or chicks was not determined for some birds, giving 122 and 94 birds in the final analyses of factors explaining the presence of eggs and chicks, respectively.

Sex and non-breeding area were included as factors in the best model explaining the tail-feather [Hg] (Table 2, Table S1 in the Supplement at www.int-res.

Table 1. Number of sampled streaked shearwaters (males, females) breeding at each colony and spending their non-breeding period in the South China Sea (SCS), Arafura Sea (AFS), Pacific Ocean north of New Guinea (NNG) and Indian Ocean northwest of Australia (NWA)

Colony	SCS	AFS	NNG	NWA
Awa Island	4, 4	3, 9	13, 7	0, 0
Mikura Island	0, 0	0, 0	11, 4	0, 0
Sangan Island	5, 0	2, 8	29, 25	0, 2

Table 2. Parameter estimates for factors included in the best model explaining tail feather [Hg] of all birds (Table S1 in the Supplement) and those of males and females (Table S2). For models explaining the presence/absence of eggs or chicks, the averaged parameter estimates of factors included in the adequate models with ΔAIC < 2 (Table S3) are shown. Potential factors included non-breeding areas (NB) in the South China Sea (SCS), Pacific Ocean north of New Guinea (NNG), Arafura Sea (AFS), and Indian Ocean northwest of Australia (NWA); colonies (CO) on the islands of Sangan (SAN), Mikura (MIK) and Awa (AWA); sex (SX), and log-transformed [Hg] (HG)

Independent variable	Factor	Parameter estimate ± SE	*t*	p
[Hg] all birds	Intercept	0.04972 ± 0.06878	0.723	0.52
	NB(NNG)	−0.31242 ± 0.04649	−6.719	<0.001
	NB(SCS)	0.23450 ± 0.06746	3.476	<0.001
	NB(NWA)	−0.18604 ± 0.13814	−1.347	0.181
	SX(male)	0.19970 ± 0.03490	5.722	<0.001
[Hg] male	Intercept	0.30200 ± 0.08423	3.586	<0.001
	NB(NNG)	−0.35445 ± 0.08811	−4.023	<0.001
	NB(SCS)	0.21689 ± 0.10505	2.065	0.043
[Hg] female	Intercept	0.18585 ± 0.04880	3.808	<0.001
	CO(MIK)	−0.21534 ± 0.10019	−2.149	0.036
	CO(SAN)	−0.23243 ± 0.05483	−4.239	<0.001
	NB(NNG)	−0.24051 ± 0.05401	−4.453	<0.001
	NB(SCS)	0.08915 ± 0.09836	0.906	0.369
	NB(NWA)	−0.07342 ± 0.13093	−0.561	0.577
Independent variable	Factor	Averaged parameter estimate ± SE	*Z*	p
Presence of egg	Intercept	2.0409 ± 0.6423	3.177	0.001
	CO(MIK)	15.6443 ± 2437.6022	0.006	0.995
	CO(SAN)	−1.5341 ± 0.6254	2.453	0.014
	NB(NNG)	0.7591 ± 0.6230	1.218	0.223
	NB(SCS)	0.1696 ± 0.8977	0.189	0.85
	NB(NWA)	−18.8403 ± 7670.5022	0.002	0.998
	HG	−0.4677 ± 1.0217	0.458	0.647
Presence of chick	Intercept	1.0015 ± 0.5093	1.967	0.049
	CO(MIK)	−1.4151 ± 0.7723	1.832	0.067
	CO(SAN)	−1.1622 ± 0.5320	2.184	0.029
	HG	−1.0123 ± 0.8855	1.143	0.253

com/articles/suppl/m546p263_supp.pdf). [Hg] was greater in males (1.4 ± 1.1 µg g^{-1} dry weight, n = 67) than in females (0.9 ± 0.6 µg g^{-1}, n = 59). [Hg] was greater in birds spending the non-breeding period in SCS (3.1 ± 1.5 µg g^{-1}) than in AFS (1.5 ± 0.7 µg g^{-1}), which was greater than in NNG (0.8 ± 0.4 µg g^{-1}) (Fig. 2, Table 2). [Hg] in birds spending the non-breeding period in NWA (0.8 µg g^{-1}) did not differ from that in AFS (Table 2).

Non-breeding area was included as a factor in the best model for each sex (Table S2); [Hg] was greater in SCS than in AFS for males, and it was greater in AFS than in NNG for females and males (Fig. 2, Table 2). Colony was also included in the best model for females (Table S2): [Hg] was greater in females breeding on Awa Island than on Sangan and Mikura Islands (Table 2).

The $\delta^{15}N$ of feathers did not differ significantly among the non-breeding areas (p = 0.835) or between males and females (p = 0.126). $\delta^{15}N$ and $\delta^{15}N$ × non-breeding area were not included in the best models explaining feather [Hg] (Tables S1 & S2). Regressions were significant for AFS (r^2 = 0.334, $F_{1,20}$ = 10.013, p = 0.005) and for SCS (r^2 = 0.331, $F_{1,11}$ = 5.454, p = 0.039) but not for NNG (r^2 = 0.00, $F_{1,87}$ = 0.028, p = 0.867) (Fig. 3).

Four adequate models were selected for explaining the presence of eggs, and 3 for chicks (Table S3). Model averaging showed that colony was more important (relative importance of 1.00) than non-breeding area (0.52) and [Hg] (0.30) in explaining the presence of eggs, and that colony was more important (0.83) than [Hg] (0.34) in explaining the presence of chicks. Averaged parameter estimates indicated that the probability of having eggs or chicks was smaller on Sangan Island than on Awa Island (Table 2).

DISCUSSION

Factors affecting feather [Hg]

Kinetics of Hg in birds and timing of molt can influence [Hg] of each feather. The effect of feather $\delta^{15}N$ on [Hg] was not significant overall or in birds that used NNG, though birds in AFS and in SCS showed weak positive correlations. In other species of seabirds, similar inconsistent results have been observed (Nisbet et al. 2002, Ramos et al. 2009b). These inconsistencies may arise because the feather $\delta^{15}N$ reflects the current food intake when feathers are growing during 2 to 3 wk, while the feather [Hg] reflects not only the current intake but also the previously accumulated Hg (Bond 2010). In a dose–response experiment in adults of Cory's shearwater, the half-life of [Hg] in blood was 38 to 65 d (Monteiro & Furness 2001). Feather [Hg] in great skuas reflects blood [Hg] when the feathers are growing (Bearhop et al. 2000). Thus the tail feather [Hg] possibly reflects Hg intake during at least 1 mo before the molt.

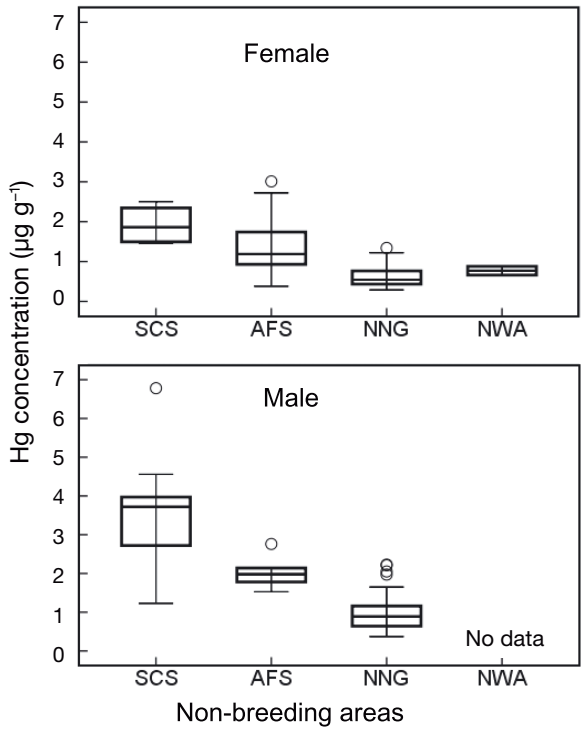

Fig. 2. Box plots of the tail-feather [Hg] (μg g^{-1} dry weight) of streaked shearwaters spending their non-breeding period in SCS (9 males, 4 females), AFS (5 males, 17 females), NNG (53 males, 36 females), and NWA (2 females). The bottom and top of each box are the 1st and 3rd quartiles, and the band in the middle is the median (2nd quartile). The bottom and top whiskers are the minimum and maximum values, respectively, excluding outlier values shown as open circles (ouside 1.5 × interquartile range). See Fig. 1 legend for abbreviations of non-breeding areas

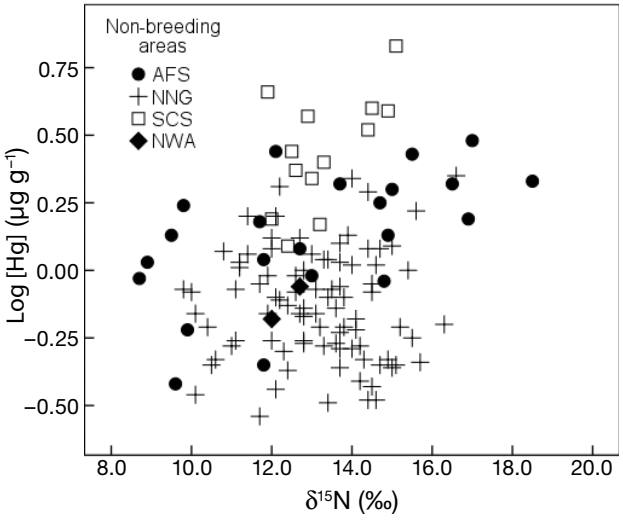

Fig. 3. Effects of $\delta^{15}N$ on tail feather [Hg] (log-transformed) of streaked shearwaters spending their non-breeding period in AFS, NNG, SCS, and NWA. See Fig. 1 legend for abbreviations of non-breeding areas

Cory's shearwater (Ramos et al. 2009a) and the Yelkouan shearwater *Puffinus yelkouan* (Bourgeois & Dromzee 2014) replace the outermost tail feathers (R6) last after they leave their colonies. As the streaked shearwaters did not replace their tail feathers during the chick-rearing period, we assumed that they did so during the non-breeding period, though we could not determine the actual timing of molt. In Yelkouan shearwaters (Bourgeois & Dromzee 2014), the timing of feather molt does not change with sex or breeding success, but in Cory's shearwaters (Alonso et al. 2009), it varies across colonies that have different migration strategies and between individuals of different breeding status.

Two factors might contribute to the 4-fold differences in the tail feather [Hg] of streaked shearwaters using different non-breeding areas (Fig. 2). First, R6 [Hg] might reflect the intake of Hg in the non-breeding areas in addition to Hg accumulated during the latter breeding period and the southward migration period. The effect of colony on R6 [Hg] was not significant when males and females were combined, but the significant effect in females (Table 2) indicates the influence of Hg accumulated during the breeding period also. Birds using SCS during the non-breeding period started migration later than the others and spent 20 d in the East China Sea (ECS), where they possibly fed during their southward migration (Yamamoto et al. 2010; Fig. 1). Thus, the higher R6 [Hg] of SCS birds might reflect Hg intake during the latter breeding period and the southward migration through the ECS. The timing of migration did not differ between birds in in NNG and AFS (Yamamoto et al. 2010).

Second, the greater average feather [Hg] in birds staying in SCS might be associated with the average trophic level of their prey. Streaked shearwaters feed on epipelagic fish and squid during the breeding season (Matsumoto et al. 2012), but their diet during the non-breeding period remains unknown. The $\delta^{15}N$ of the R6 of streaked shearwaters using SCS did not differ significantly from that of the others. Stable isotope values of a series of feathers (R1 to R6, for example) could show dietary consistency of individuals throughout the non-breeding period, though we did not collect such series of feathers in this study. The $\delta^{15}N$ of marine top predators reflects not only their trophic level, but also the baseline $\delta^{15}N$ in their habitat (Graham et al. 2010). We do not have concurrent data of baseline $\delta^{15}N$ in the study areas. Published data show that $\delta^{15}N$ of seawater, surface sediment, and zooplankton ranges widely among samples collected within the same areas (Table S4).

The average values of $\delta^{15}N$ of seawater given in each study also ranged widely within SCS (2.8 to 5.5‰) and ECS (3.3 to 6.7‰), presumably in relation to season, location, or depth (Table S4, Fig. S1). This range of the average $\delta^{15}N$ of seawater collected in SCS includes the range of the average $\delta^{15}N$ of seawater in the northwestern Pacific (4.4 to 5.2‰), while the average $\delta^{15}N$ of sediments and zooplankton in SCS lie within the ranges of average $\delta^{15}N$ of these materials collected in the surrounding areas (Table S4, Fig. S1). The only exception is a high $\delta^{15}N$ value reported in the sediments collected in NNG (see Fig. 1 in Tesdal et al. 2013). Thus, using published data we could not find strong evidence indicating large directional deviations of baseline $\delta^{15}N$ in SCS from those in surrounding areas. The greater feather [Hg] of SCS birds, therefore, could not be explained only by the trophic level unless we postulate a substantial disparity in isotopic baselines in these areas. To understand the effects of bio-magnification, however, the measurements of spatial and temporal variations in baseline $\delta^{15}N$ all over the seas off Southeast Asia and surrounding waters have to be carried out.

The tail-feather [Hg] was greater in males than in females. Sex differences in feather [Hg] have been reported in various bird species and can be attributed to sex differences in diet and in parental roles (Robinson et al. 2012). We did not find a significant sex difference in feather $\delta^{15}N$. Excretion of Hg through egg production might decrease the Hg burden of females at egg laying and might thus influence the sex difference in the tail feather [Hg].

Regional variation

Reported [Hg] in the air and fish collected in SCS and in the air, sediment, tuna, whales, and dolphins in ECS seem to be higher than those reported in other marginal seas in southeast Asia and North Pacific (Table S5, Fig. S2). This trend does not contradict the regional pattern of feather [Hg] that we found in streaked shearwaters. The greatest total gaseous [Hg] in the air in ECS and the average atmospheric gaseous elemental [Hg] in SCS are greater than those in the North Pacific and the Sea of Japan (Table S5, Fig. S2). Total [Hg] in the sea bottom surface sediment is greater in SCS and the ECS inner shelf than in the Yellow Sea, the ECS middle shelf, and the coast of northern Australia (Table S5). Although geographical patterns of [Hg] in the muscle of various marine fish species collected in fish markets are not apparent, [Hg] might be slightly greater in SCS and adjacent areas (Gulf of Thailand and Java Sea) than in ECS and the Straits of Malacca (Table S5, Fig. S2). Muscle [Hg] from different tuna species and red meat [Hg] from different whale or dolphin species are greater in ECS and Taiwan than in the northwestern Pacific, the central Pacific, and the sea around Australia (Table S5, Fig. S2). Marine pollution in the Arafura Sea has been suspected (Morrison & Delaney 1996), and our result indicates levels of Hg pollution in this area might be lower than SCS but higher than NNG.

Laboratory and field studies of birds indicate that [Hg] of 5 to 40 µg g^{-1} dry weight in the feathers is associated with impaired reproduction (Burger & Gochfeld 1997). In our study, the tail feather [Hg] exceeded 5 µg g^{-1} in just 1 out of 126 birds. Although the feather [Hg] was included in some models explaining breeding status, colony was a more important factor than [Hg]. Thus, Hg pollution in the streaked shearwaters' non-breeding areas might not be as high as the level associated with apparent reproductive failure. However, [Hg] burden can be greater during the breeding period because of the accumulation of Hg in soft tissues between molts (Furness 1993). Thus, the effect of feather [Hg] during the period of maximum burden, possibly during the latter breeding period, has to be examined.

Further studies of the kinetics of Hg in seabirds and the timing of molt of each feather need to be carried out to verify that seabirds are reliable and effective indicators. Nevertheless, our results indicate that the feather [Hg] of seabirds together with their year-round movements can improve our understanding of the spatial pattern of Hg pollution, especially in offshore areas where direct water and sediment sampling is financially and logistically challenging.

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