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Biotransport of Metallic Trace Elements from Marine to Terrestrial Ecosystems by Seabirds

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Abstract: Physical systems, such as currents and winds, have traditionally been considered responsible for transporting contaminants. Although evidence is mounting that animals play a role in this process through their movements, we still know little about how such contaminant biotransport occurs and the extent of effects at deposition sites. In the present study, we address this question by studying how rhinoceros auklets (*Cerorhinca monocerata*), a seabird that occurs in immense colonies (~300 000 pairs at our study site, Teuri Island), affect contaminant levels at their colony and at nearby sites. More specifically, we hypothesize that contaminants are transported and deposited by seabirds at their colony and that these contaminants are passed on locally to the terrestrial ecosystem. To test this hypothesis, we analyzed the concentration of 9 heavy metal and metalloids, as well as $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, in bird tissues, plants, and soil, both within and outside of the colony. The results show that rhinoceros auklets transport marine-derived mercury (Hg), possibly from their wintering location, and deposit Hg via their feces at their breeding site, thereby contaminating plants and soils within the breeding colony. The present study confirms not only that animals can transport contaminants from marine to terrestrial ecosystems, potentially over unexpectedly long distances, but also that bird tissues contribute locally to plant contamination. *Environ Toxicol Chem* 2019;38:106–114.

Keywords: Biological transport; Rhinoceros auklet; Mercury; Bioaccumulation; Machine learning

INTRODUCTION

Migratory species, such as marine apex predators, can transport significant quantities of nutrients across ecosystem boundaries into recipient food webs (Michelutti et al. 2009). These animals often carry high contaminant loads as a result of both bioaccumulation and biomagnification through marine food webs (Fisk et al. 2003; Mallory and Braune 2012). Among top predators, recent studies have highlighted a major role played by seabirds in transferring contaminants from marine to terrestrial ecosystems (Sun et al. 2004; Evenset et al. 2007; Choy et al. 2010a, 2010b). Because seabirds nest in immense colonies that can be at the same location for centuries, they can create breeding sites with high concentrations of contaminants through the accumulation of feces, feathers, and carcasses (Blais et al. 2005; Ellis et al. 2006; Mulder et al. 2011; Bauer and Hoye 2014).

Among these contaminants, mercury (Hg) is particularly problematic because this trace metal is a neurotoxin occurring in ecosystems throughout the world. In contrast to some organic contaminants, Hg pollution is difficult to remediate (Elliott and Elliott 2016) because 1) Hg does not naturally degrade and 2) it is continually released by common industrial activities (Selin 2009; Vo et al. 2011; Mason et al. 2012; Driscoll et al. 2013). As such, Hg levels in surface oceans have tripled since the Industrial Revolution (Driscoll et al. 2013; Lamborg et al. 2014; Poulain et al. 2015). Contaminants such as Hg are being added to recipient sites at concentrations far beyond those individually transported by physical processes (Blais et al. 2007). Yet, among these physical drivers, the relative roles of anthropogenic versus

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biological vectors are difficult to tease apart (Lamborg et al. 2014) and, to date, have received little attention. Although the role of seabirds in transferring marine-derived nutrients (e.g., Farina et al. 2003; Ellis et al. 2006) and contaminants (e.g., Blais et al. 2007; Michelutti et al. 2010) across ecosystem boundaries is recognized, both the origin and the pathway from sea to land taken by these biotransported contaminants remain unclear.

We assessed the origin and pathway of allochthonous Hg transported and deposited by seabirds at their breeding colony and determined the impact of such biotransport on terrestrial ecosystems. To evaluate the role of seabirds as biovectors, we measured Hg concentrations in rhinoceros auklets (*Cerorhinca monocerata*) sampled at Teuri Island, in the Sea of Japan—one of the largest colonies of this species in the world, where approximately 300 000 pairs breed annually (Watanuki and Ito 2012). As already advocated (Mallory et al. 2010), we simultaneously sampled multiple proxies of seabird health (tissues, feces) as well as soil and plants within and outside the seabird colony to measure the impact of these sea-to-land biovectors. This allowed us to study metal biotransport in which auklets bioaccumulate Hg at sea through foraging, carry it in their tissues to their breeding site, and deposit it mainly in guano (auklets do not molt at breeding stage; Gaston and Dechesne 1996), which degrades and is eventually transferred to plants. To characterize this biotransport process, we measured Hg concentrations in soil, plant roots, and plant leaves at both auklet-affected sites and control sites, where no seabirds have nested at least within the past 30 yr (Y. Watanuki, unpublished data). Because Hg burden is often correlated with levels of other elements (Michelutti et al. 2010), we also measured levels of 8 other metals and metalloids in the same samples of roots to evaluate the effects of auklets on the root metal profiles.

MATERIALS AND METHODS

Ethical note

All work was conducted under the permits of the Ministry of the Environment, the Agency of Cultural Affairs, and Hokkaido University's Ethical Review Board. To avoid bird disturbance, handling of auklets was kept to a minimum.

Study site and field collections

Teuri Island (44°24′N, 141°17′E) lies 38 km off Haborocho harbor along the northwestern coast of Hokkaido, in the northern Sea of Japan. In 2016, adult rhinoceros auklets were caught by hand during incubation (May) and chick-provisioning (June–July) periods. Blood (1 mL) was drawn from the brachial vein using 25-G syringes. On collection, blood samples were immediately centrifuged to separate plasma from red blood cells and stored in a freezer at –20 °C. To collect fecal samples, birds were placed in a box before blood collection. We released the birds typically within 10 min, regardless of whether fecal samples were successfully collected. No individual was sampled more than once. Whole plants were collected during incubation and chick-provisioning periods from both the auklet-affected (i.e., center of the colony) and unaffected (i.e., outside of colony; Figure 1) sites. We set up study plots at 2 auklet-affected sites (approximately 100 m apart) and at 3 control sites to examine the effect of auklets on the entire island. We focused vegetation collection on the Scandinavian small-reed (*Calamagrostis purpurea* (Trin.) Trin. subsp. *langsdorfii* (Link) Tzvelev; *Iwanogariyasu* [イワノガリヤス] in Japanese), the dominant plant species at both the auklet-affected and control sites. Surface soil samples were simultaneously taken near the plant sample sites using a trowel. In addition, soil core samples were taken near the plant and soil sample sites using a 4.4-cm internal diameter custom-made corer that was pushed directly into the soil to identify the longitudinal effect of auklet deposition on soil profiles. The core tubes were pushed until they met strong resistance (~9–12 cm), indicating that the entire sedimentary record was retrieved. The cores were sectioned at 1-cm intervals on site using a vertical extruder. We finally note that although the area occupied by the colony has expanded or contracted since the early 1970s, the sites that we selected remained well within or well outside of the colony (respectively) over the past approximately 50 yr (Y. Watanuki, personal observation).

Laboratory analyses

All samples were shipped frozen to the Department of Environmental Veterinary Sciences, Hokkaido University. We then followed standard laboratory procedures for each analysis. More specifically, for plant samples, whole plants were rinsed in distilled water and the roots and leaves extracted. Feces and the extracted plant samples were air-dried, weighed, dried at 50 °C in an oven for approximately 48 h, and manually homogenized prior to analysis of each sample. For animal tissues, red blood cells were analyzed as wet weight for Hg analysis but freeze-dried for stable isotope analysis prior to the analyses. Soil and plant samples were divided in half, with one-half used for measuring metal and metalloid concentrations and the rest used for measuring stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), which are proxies for feeding habitat and trophic level,

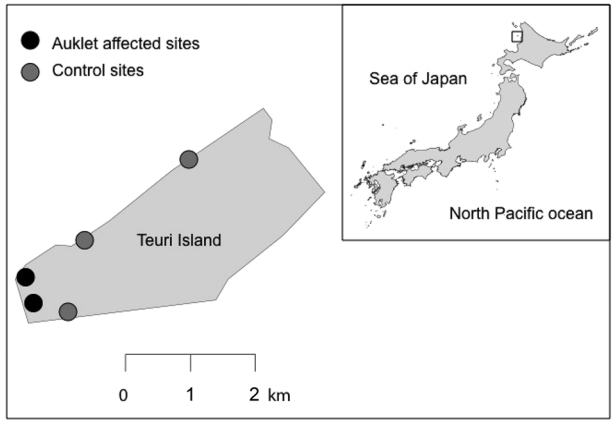

FIGURE 1: Map showing the location of Teuri Island in the North Pacific. An unfilled square symbol indicates the location of Teuri Island; black-filled circles indicate auklet-affected sites; gray-filled circles indicate control sites.

respectively, and to examine the impacts of those in soil and plant samples (Newsome et al. 2007). Blood and fecal samples were analyzed separately for measuring metal and metalloid concentrations and stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) because of the small number of samples. Sample sizes for each analysis are shown in Supplemental Data, Table S1.

Total Hg analyses

Concentrations of total Hg in red blood cells, feces, soil, root, and leaf samples were determined using a Direct Thermal Decomposition Mercury Analyzer (MA-3000; Nippon Instruments). After preparation of the calibration standards, the concentration of Hg was measured by thermal decomposition. Analytical accuracy for Hg was determined by analyzing one or 2 blank samples with each sample set, as well as standard reference materials: BCR-320, DOLT-4, and tomato leaves obtained from the Canadian National Research Council. Recoveries of all standard reference materials were within the certified range of values (BCR-320, $98 \pm 4\%$, $n = 2$; DOLT-4, $101 \pm 8\%$, $n = 2$; tomato leaves, $98 \pm 2\%$, $n = 12$; average $\pm$ standard deviation [SD]). The detection limit of the analyzer was at 0.001 ng. Concentrations of Hg are reported for blood in micrograms per gram wet weight and for the remaining matrices in micrograms per gram dry weight.

Extraction and analysis of heavy metals and metalloids

We measured concentrations of 8 elements (cadmium [Cd], chromium [Cr], cobalt [Co], nickel [Ni], copper [Cu], zinc [Zn], lead [Pb], and arsenic [As]) per Nakata et al. (2016), using an inductively coupled plasma-mass spectrometer (ICP-MS; 7700 series, Agilent Technologies). Briefly, all laboratory materials and instruments used in the heavy metal analysis were washed with 2% nitric acid (HNO_3) and rinsed at least twice with distilled water. Samples of approximately 1.0 g of plant roots were dried for 48 h in an oven at 50 °C. Dried samples were placed in prewashed digestion vessels, followed by acid digestion using nitric acid (atomic absorption spectrometry grade, 60%; Kanto Chemical) and hydrogen peroxide (Cica reagent, 30%; Kanto Chemical) in a microwave digestion system (Speed Wave MWS-2). After cooling, each mixture was transferred into a plastic tube, and various elements were determined using an ICP-MS. Analytical quality control was performed using the DORM-3 and DOLT-4 certified reference materials (National Research Council of Canada). Replicate analysis of these reference materials showed good recoveries (95–105%, $n = 5$, respectively). The instrument detection limits for Cd, Cr, Co, Ni, Cu, Zn, Pb, and As were 0.2, 0.5, 0.5, 0.5, 1.0, 0.1, 1.0, and 2.0 $\mu g\,kg^{-1}$, respectively.

Stable isotope analyses

All processed and homogenized samples were shipped to the Port and Airport Research Institute in Yokosuka, Japan. To remove inorganic carbon, all samples except for blood were acidified with 1N HCl and dried at 60 °C. Lipids were not removed from blood and plant samples because of expected low lipid levels. Isotopically fractionated metabolites, such as urea and ammonium, as well as inorganic carbon were removed from fecal samples using a 2 to 1 chloroform to methanol soak and rinse (Kuwae et al. 2008). Isotope fractionation in catabolism occurs when nitrogen in amino acid is deaminated to produce metabolites depleted in ^{15}N (Fry 2006). Thus, uric acid, which may be a major nitrogen metabolite in fecal samples, is not fractionated because it is not produced through deamination. Both $\delta^{13}C$ and $\delta^{15}N$ were measured with an isotope ratio mass spectrometer (Delta Plus Advantage; Thermo Electron) coupled with an elemental analyzer (Flash EA 1112; Thermo Electron). Results were reported in delta notation in parts per thousand relative to Vienna PeeDee Belemnite (VPDB; $\delta^{13}C$) and air ($\delta^{15}N$). L-Histidine ($\delta^{13}C$–PDB $= -10.18‰$, $\delta^{15}N$–air $= -7.81‰$; Shoko) was included as an internal standard every fifth sample to check analytical accuracy. Based on within-run replicate measurements of multiple standards (L-histidine, L-alanine [$\delta^{13}C$–VPDB $= -19.6‰$, $\delta^{15}N$–air $= 10.1‰$; Shoko]; L-alanine [$\delta^{13}C$–VPDB $= -19.6‰$, $\delta^{15}N$–air $= 26.1‰$; Shoko]), measurement precision for both $\delta^{13}C$ and $\delta^{15}N$ values was estimated to be always at or below 0.3‰.

Statistical analyses

Elemental compositions were $\log_{10}$-transformed prior to analysis. We examined the effect of site (auklet-affected vs control), stage (incubation vs chick provisioning), $\delta^{15}N$ and $\delta^{13}C$ in roots, and an interaction term of stage $\times$ $\delta^{15}N$ on Hg in roots when using linear models. Model selection was based on Akaike's information criterion (AIC). The model that received the lowest AIC was designated as the best model. Models within 2.0 units of the best model were considered indistinguishable from the best model (Burnham and Anderson 2002). To account for uncertainty in the model selection process, a model averaging approach was used, in which the parameter estimates of factors included in the adequate models were weighed with the corresponding Akaike weights and averaged. To visualize if the auklet-affected site could be teased apart from control sites, a principal component analysis (PCA) was performed with 9 heavy metal (Cd, Pb, Hg, Cu, Zn, Cr, Co, Ni) and metalloid (As) concentrations in roots. To assess the predictive power of metal and metalloid concentrations on determining the presence of auklets, we employed a supervised machine-learning algorithm based on adaptive boosting (Freund and Schapire 1996). The classifier's accuracy was determined by 10-fold cross-validation (CV_{10}), where the algorithm is trained on nine-tenths of the data and the last decile is used to compute a confusion matrix; this process was repeated 100 times. Finally, significance between the 2 types of sites for the 9 element concentrations was assessed based on the Dunn test, and general linear models were fitted to the data. All statistical analyses were performed in R 3.4.2 (R Development Core Team 2017). For comparison purposes in *Discussion*, we assumed a moisture of 65% in red blood cells (Tartu et al. 2014; Bond and Robertson 2015) and 79% in whole blood (Eagles-Smith et al. 2008) and that

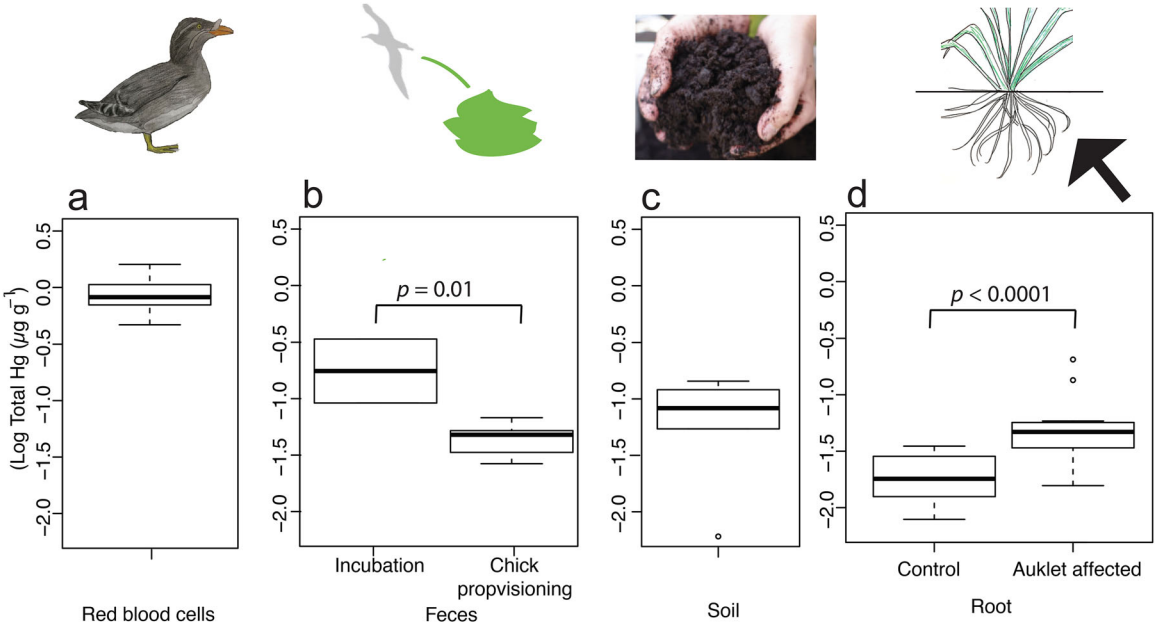

FIGURE 2: Schematic simplified mercury (Hg) transfer pathway via seabirds. Mercury concentration (**a**) in red blood cells (wet wt, $n = 21$), (**b**) in auklet feces (dry wt) during incubation ($n = 2$) and chick-provisioning periods ($n = 7$), (**c**) in soil ($n = 10$), and (**d**) in roots at control ($n = 15$) and Auklet-affected ($n = 11$) sites collected from Teuri Island during the auklet breeding season in 2016. Boxplots show the first quartile, median, third quartile, and range of $\log_{10}$-transformed concentrations.

red blood cells consist of 45% of the whole blood, and that most Hg is accumulated in red blood cells (Bond and Robertson 2015).

RESULTS

Hg and stable isotope ratios in red blood cells and feces

We detected quantifiable concentrations of Hg in blood and feces in all individuals. Mean [Hg] in auklet red blood cells during the present study period (±1SD [range]) was 0.86 ± 0.27 (0.47–1.6) μg g^{-1} wet weight (Figure 2a) and was similar between incubation and chick-provisioning periods (Table 1; $P = 0.06$). Mean [Hg] in auklet feces during the entire study period was 0.08 ± 0.10 μg g^{-1} dry weight and decreased from incubation to chick rearing (Table 1; $t = 2.25$, $P = 0.04$, $df = 8$). Stable isotope ratios, $\delta^{15}N$ ($\Delta AIC = 1.71$) or $\delta^{13}C$ ($\Delta AIC = 1.99$), varied independently of Hg in red blood cells but were stage-dependent (Supplemental Data, Figure S1) through time (from incubation to chick rearing); $\delta^{15}N$ increased, whereas $\delta^{13}C$ decreased (Table 1; Supplemental Data, Figure S1).

Hg and stable isotope results in plants and soil

Mean [Hg] in roots at the auklet-affected site was higher than that at the control site ($p < 0.0001$; Figure 2d), but mean concentrations in leaves were marginally detectable and similar between the 2 sites ($P = 0.08$; Table 2). Thus, only roots were considered for further statistical analyses. Mean [Hg], $\delta^{15}N$, and $\delta^{13}C$ in soil were similar between auklet-affected and control sites (Table 2 and Figure 2c). We did not find any auklet effects on [Hg], $\delta^{15}N$, and $\delta^{13}C$ in soil core samples at any given depth (Supplemental Data, Table S2). Although we did not compare identical depths, because values did not vary with depth at either site, we concluded that levels were likely similar among sites. Among root samples, the fitting of linear models explaining root [Hg] showed that site (auklet affected vs control), $\delta^{15}N$ (positive),

TABLE 1: Total mercury concentrations (in micrograms per gram), stable isotope ratios ($\delta^{15}N$, $\delta^{13}C$ in parts per thousand) and sample sizes (*n*) in red blood cells (in wet wt) and fecal samples (feces, in dry wt) during incubation and chick-provisioning periods[a]

	Sample	Incubation	Converted in dry wt	*n*	Chick provisioning	Converted in dry wt	*n*
Hg	RBC	0.99 ± 0.32	≈2.83 ± 0.32	9	0.77 ± 0.18	≈2.20 ± 0.18	12
	Feces	0.22 ± 0.17		2	0.05 ± 0.01		7
$\delta^{15}N$	RBC	10.90 ± 0.30		24	11.9 ± 0.40		28
	Feces	11.60 ± 1.60		4	12.7 ± 0.60		12
$\delta^{13}C$	RBC	–19.00 ± 0.30		24	–20.00 ± 0.40		28
	Feces	–21.60 ± 1.10		4	–23.80 ± 0.60		12

[a]Assuming moisture content of 65% in red blood cells, converted values in red blood cells in dry wt are provided for comparable purpose.

TABLE 2: Total mercury concentration, stable isotope ratio ($\delta^{15}N$, $\delta^{13}C$), and sample size (*n*) in roots and leaves of Scandinavian small-reed and soil at auklet-affected (affected) and control (control) sites

		Incubation				Chick provisioning			
	Sample	Affected	*n*	Control	*n*	Affected	*n*	Control	*n*
Hg	Roots	0.04 ± 0.01	6	0.02 ± 0.01	9	0.07 ± 0.07	7	0.02 ± 0.01	9
	Leaves	0.0077 ± 0.0023	6	0.0051 ± 0.0022	9	0.0097 ± 0.0048	6	0.0099 ± 0.0049	17
	Soil	0.06 ± 0.01	2	0.09 ± 0.07	3	0.06 ± 0.05	9	0.09 ± 0.05	7
$\delta^{15}N$	Roots	6.0 ± 1.5	8	6.15 ± 2.8	7	7.6 ± 2.3	10	7.1 ± 2.4	7
	Leaves	4.7 ± 1.9	8	4.7 ± 1.9	9	8.4 ± 3.5	6	6.7 ± 2.8	18
	Soil	10.0 ± 2.4	2	9.2 ± 2.3	3	10.5 ± 1.9	10	9.4 ± 2.0	9
$\delta^{13}C$	Roots	–27.4 ± 1.1	8	–28.0 ± 0.5	7	–27.2 ± 1.1	10	–27.3 ± 1.1	7
	Leaves	–27.5 ± 1.3	8	–28.3 ± 0.3	9	–28.3 ± 1.2	6	–28.1 ± 1.1	18
	Soil	–25.6 ± 0.2	2	–27.3 ± 0.7	3	–25.5 ± 0.3	10	–26.4 ± 1.1	9

TABLE 3: Model selection results by change in Akaike's information criterion based on linear models explaining $\log_{10}$-transformed Hg in roots of Scandinavian small-reed at Teuri in 2016 ($\log_{10}[Hg] \sim \sum$ variables)[a]

	Model ($\sum$ variables)	*df*	ΔAIC	w_i
Hg Roots	**Site + $\delta^{15}N$**	**22**	**0.00**	**0.39**
	Site + stage + $\delta^{15}N$	21	1.07	0.23
	Site + stage + $\delta^{13}C$	21	2.89	0.09
	Site + stage + $\delta^{15}N$ + $\delta^{13}C$ + stage × $\delta^{15}N$	19	3.02	0.09
	Site + stage	27	3.03	0.09
	Site + stage + $\delta^{15}N$ + $\delta^{13}C$	20	3.06	0.09
	Site	28	5.38	0.03
	$\delta^{15}N$	23	13.82	0.00
	Stage × $\delta^{15}N$	21	14.14	0.00
	$\delta^{13}C$	23	15.95	0.00
	Stage	28	21.06	0.00

[a]The best model is in boldface. The number of parameters (*df*) and Akaike weight are also shown. Potential factors included are site (auklet-affected vs control), stage (incubation vs chick provisioning), $\delta^{15}N$ and $\delta^{13}C$ in the roots, and an interaction term of stage × $\delta^{15}N$. Models within 2 Akaike's information criterion units of the best model are shaded in light gray.
AIC = Akaike's information criterion; w_i = Akaike weight.

and breeding stage (incubation vs chick provisioning) were variables included in the best models (with ΔAIC <2; Table 3).

Concentration of metals and metalloids in roots

Based on a PCA, the concentrations of the 9 elements (Cd, Pb, Hg, Cu, Zn, Cr, Co, Ni, and As) allow discrimination of the 2 types of sites, with some overlap (Figure 3). To differentiate affected from nonaffected sites based on these 9 concentrations, the trained adaptive boosting classifier showed that only 3 of them (As, Cd, and Co) are critical (importance values &gt CV_{10} classification success rate = 80%; Supplemental Data, Figure S2) and hence provide a distinct signature for auklet presence. Indeed, all 3 have significantly different concentrations among sites (at the 0.5% level), with, however, [Cd] being higher at the control site (Figure 4). Mercury is not directly a critical feature for classification for 3 reasons: 1) [Hg] covaries with [As] and [Co], with all 3 elements explaining most of the concentration variance among sites (50%; Figure 3); 2) [As] and [Co] are both orthogonal to (independent of) [Cd]; and 3) all 3 concentrations, [As,] [Co], and [Cd], are an order of magnitude

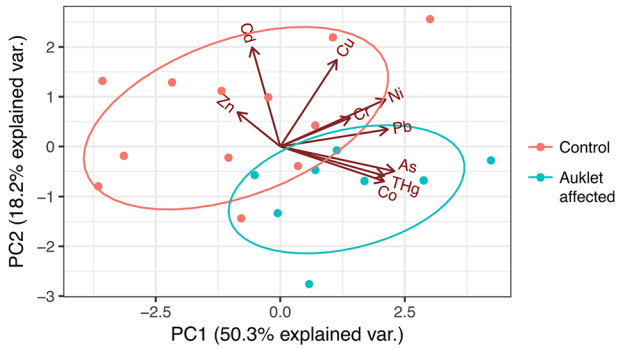

FIGURE 3: A principal components analysis of 9 metals (As, Cd, Pb, Hg, Cu, Zn, Cr, Co, Ni) in roots between auklet-affected and control sites. Ellipses are shown in 95% concentration of points. PC = principal component; var. = variance.

higher than [Hg], whose signal is hence overwhelmed (Figure 4). Most remarkably, although Hg is not critical in distinguishing auklet presence at a given site, Hg is the element that is the most affected by auklets ($p < 10^{-3}$) at their breeding site.

DISCUSSION

Seabirds are critical biovectors in ecosystems because of their frequent commutes between sea and land during the breeding season (Blais et al. 2007; Mallory et al. 2015). Based on a quasi-experimental approach, we evaluated the possible role of seabirds as biovectors of allochthonous Hg from oceanic into terrestrial ecosystems, by focusing on a highly mobile marine top predator, the rhinoceros auklet. We show that seabirds deposited allochthonous Hg from sea to land via their feces, at their breeding colony, and that allochthonous Hg was then transferred to terrestrial plants, thereby crossing ecosystem boundaries.

Hg fluxes in seabirds through their breeding season

We detected Hg in both red blood cells and feces of all individuals assayed, confirming that auklets carry detectable amounts of Hg not only in their tissues such as liver and kidney (Ishii et al. 2014) but also in their fecal matter. Average blood

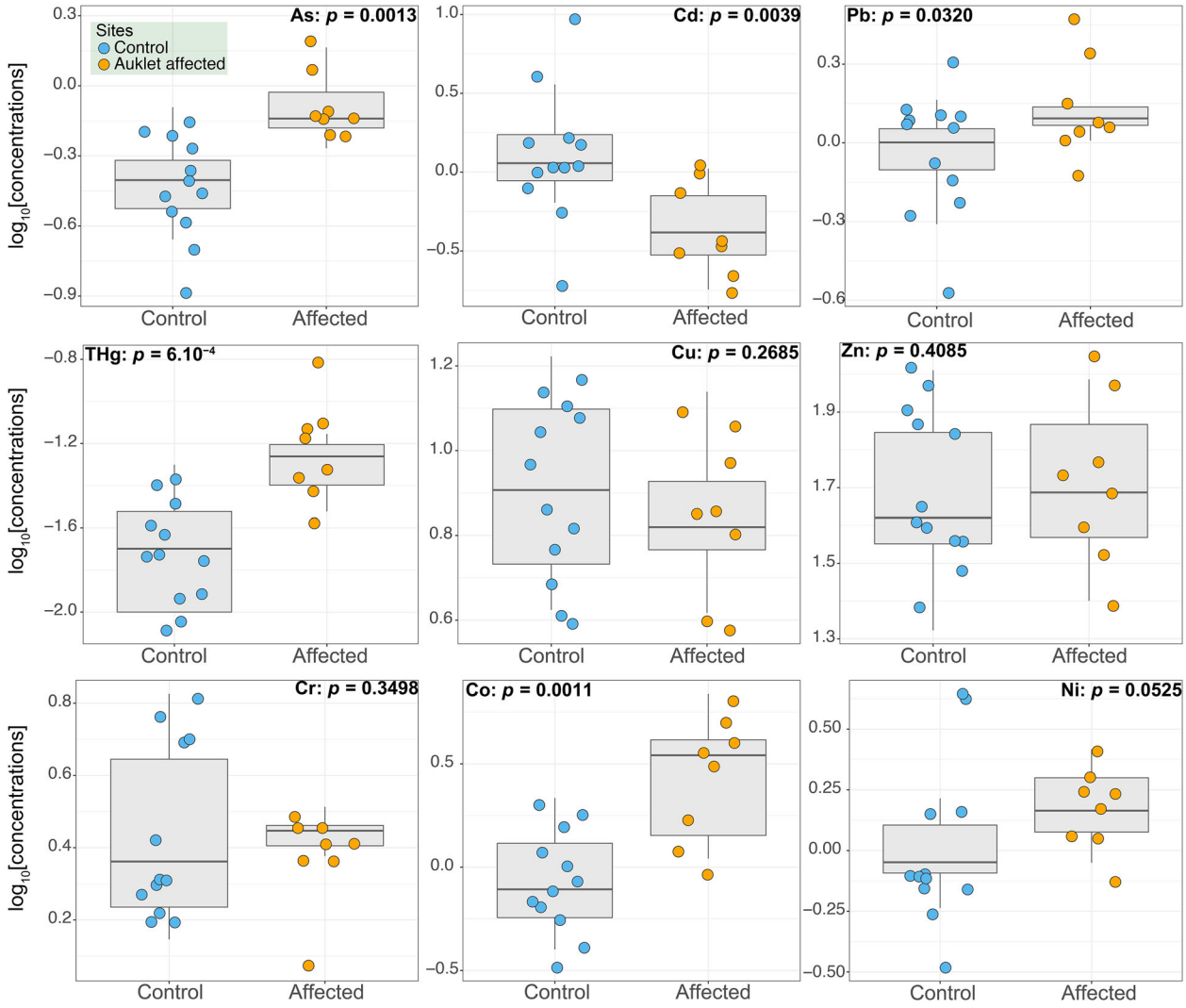

FIGURE 4: Metal concentrations in roots (dry wt) at auklet-affected and control sites. The response variables are each analyzed element that was $\log_{10}$-transformed. "Control" indicates samples collected at the control site ($n = 12$) and "Auklet affected" indicates samples collected at the auklet-affected sites ($n = 8$). Individual values are shown as dots. Boxplots show the first quartile, median, third quartile, and range of $\log_{10}$-transformed concentrations. THg = total Hg.

[Hg] measured at Teuri (2.46 ± 0.76 1SD $\mu g\, g^{-1}$ dry wt; Table 1) was higher than that reported at 1.75 $\mu g\, g^{-1}$ dry weight on average (note: this is measured in whole blood [Hipfner et al. 2011]) in a Canadian auklet population in the eastern Pacific or in other piscivorous species such as the Antarctic petrel (*Thalassoica antarctica*, at 0.84 $\mu g\, g^{-1}$ dry wt [Carravieri et al. 2018]). Although other work reported higher concentrations in seabirds (at 8.22 $\mu g\, g^{-1}$ dry wt on average in brown skuas, *Stercorarius antarcticus* [Goutte et al. 2014]; 2.7 $\mu g\, g^{-1}$ dry wt in snow petrels, *Pagodroma nivea* [Tartu et al. 2014]; see also review in Ackerman et al. 2016), the lowest [Hg] that we detected in red blood cells (1.34 $\mu g\, g^{-1}$ dry wt, converted from wet wt) still exceeds the avian affect threshold (1.20 $\mu g\, g^{-1}$ dry wt in double-crested cormorant, *Phalacrocorax auratus* [Gibson et al. 2014]; 1.20 $\mu g\, g^{-1}$ dry wt in black-legged kittiwake, *Rissa tridactyla* [Tartu et al. 2014]). It is, however, unclear whether or to what extent such elevated Hg contamination levels in Teuri auklets are possibly detrimental to their health.

Such detrimental effects potentially occur throughout the breeding season of auklets because their mean blood Hg concentrations were essentially identical between the 2 breeding stages. The time frames for integration of Hg into the blood system (half-life of 30–65 d reported in great skuas [Bearshop et al. 2000]) and of $\delta^{15}N/\delta^{13}C$ (half-life of $\sim$ 20 d in this system [Carleton and Martinez del Rio 2005]) are approximately the same as the length of their breeding season, thus ruling out the possibility that high tissue [Hg] and isotopic ratio changes were attributable to proximal (i.e., at colony) causes and suggesting that most of the Hg measured in auklets comes from their wintering grounds. Although it is likely that foraging habitats and/or prey of auklets changed as breeding progressed (i.e., average $\delta^{13}C$ values and $\delta^{15}N$ changed over the course of breeding; Supplemental Data, Figure S1), blood [Hg] was constant throughout the breeding season, further suggesting that either no Hg intake occurred during breeding or [Hg] in auklets was at a dynamic equilibrium. However, that blood [Hg]

was stage-independent whereas blood $\delta^{13}C$ and $\delta^{15}N$ were stage-dependent is unexpected. Indeed, previous work on rhinoceros auklets at Teuri showed that, as these birds shift from smaller crustaceans such as copepods (for self-feeding during incubation) to larger fish (for chick provisioning), their $\delta^{15}N$ values increase (Ito et al. 2010). Because Hg levels in copepods are lower than those in fish, this dietary shift should lead to a concomitant increase in blood Hg (Davoren and Burger 1999), which we did not observe in the present study. Although unexpected, the present results are, however, not unique because Hg levels do not always systematically increase with trophic levels (Hipfner et al. 2011), and other studies also failed to show clear patterns between $\delta^{15}N$ and Hg within single seabird populations (Elliott et al. 1992; Hipfner et al. 2011; Tartu et al. 2014; Elliott and Elliott 2016). These conflicting results suggest that blood [Hg] during breeding results from a dynamic equilibrium, between Hg intake and offloading, and that a lack of increase in blood [Hg] concomitant to an increase in $\delta^{15}N$ might be attributable to Hg contents being either higher than expected in copepods or lower than expected in larger fish, possibly attributable to a shift in the availability of preys that are usually harvested by auklets during chick provisioning.

However, a constant blood [Hg] may not just be attributable to extrinsic factors but might also result from intrinsic features such as the long-term persistence of Hg in auklet tissues (Monteiro and Furness 1995). Age may also be a confounding variable because Hg may bioaccumulate in body tissues with age in marine vertebrates (Thompson 1990; but see Furness et al. 1990). Experimental studies showed that excretion of Hg via feces was approximately 22% of the intake in black-headed gull chicks (*Chroicocephalus ridibundus*; Lewis and Furness 1991). Although we could not control Hg intake, our recorded mean Hg level in feces was 3% of the recorded blood Hg when data were pooled. Auklet species do not molt during the breeding phase (Pyle 2009; Sorensen et al. 2010), and thus, biological transport by auklets occurs via feces and potentially carcasses and/or abandoned eggs rather than via feathers. However, fecal [Hg] decreased between the 2 breeding stages, that Hg in consumed prey most likely increases through breeding stages, an alternative explanation must be sought. Because sample sizes for feces analyses are quite small (see Table 2), the interpretation of these results requires caution.

Hg transfer from contaminated seabirds to plants and soil

The roots of the plants at the colony were found to have higher Hg levels than those at the control sites (Table 2), suggesting that the origin of this contamination was likely to be seabirds. Although Hg levels in roots are much lower than in auklet tissues and feces, the detected Hg is likely to be organic Hg as a result of biotransport and should not be detected under Japanese Environmental Guidelines (Ministry of Environment Japan 2008).

In the present study, we set up 3 control plots at Teuri where auklets or other seabirds are rarely observed flying over or perching (A. Shoji, personal observation). Although we did not experimentally manipulate auklet activity or even their contamination levels, there is growing evidence supporting a causal relationship between contaminated seabirds and contaminated plants because allochthonous inputs from seabirds significantly affect the chemical properties of both soil and flora in the proximity of their colonies (Breuning-Masden et al. 2008; Zwolicki et al. 2015, 2013; Ziolek and Melke 2014). Although seabirds can efficiently transfer heavy metals to both sediments and aquatic plants (Godzik 1991; Blais et al. 2005; Evenset et al. 2007), the present results are based on a novel approach that allowed us to show evidence for a direct transfer from a marine to a terrestrial ecosystem, by simultaneously collecting samples along the biotransport chain, from the biovectors to the terrestrial plants. Indeed, as the present study sites are separated by at most 2 km and are all located in similar soil, geologic, and atmospheric contexts, it is unlikely that variations in soil, geology, or atmospheric deposition rates explain the differences in [Hg] or in the concentrations of other metals that we measured. Therefore, the most likely source of Hg in roots is from contaminated seabirds, which most likely bring Hg from outside of the colony area, from their wintering grounds.

Mercury in roots does not seem to be transferred to the leaves of contaminated plants. Indeed, although Hg in leaves was similar between auklet-affected and control sites, it was remarkably lower than in roots (Table 2). Although few studies have examined Hg uptake by plant tissues in wild populations, Tomiyasu et al. (2005) reported that Hg in leaves is lower than in roots in goldenrod (*Solidago altissima*) and showed that Hg does not seem to move from roots to leaves. Although their results suggest that Hg in leaves may originate from the air and not from the roots, this may also explain why our Hg levels in leaves are lower than in roots—and are also site-independent—if the Hg intake pathways differ in leaves and roots. We suggest that Hg in roots results from plant uptake from the soil, where Hg was deposited by contaminated seabirds via their fecal matter.

Under this scenario, it could be expected that Hg in the soil of auklet-affected sites would be higher than at our control sites. Contrary to this expectation, however, we found that Hg in soil was not different between auklet-affected and control sites, yet still being significantly higher than in contaminated roots (Figure 2). Note that we measured Hg as total Hg concentration. A possible explanation of this counterintuitive result is that inorganic Hg contained in the soil may have overwhelmed the signal from biovectors (Lubick and Malakoff 2013). For instance, Asian dust storms containing Hg from China occur year-round but are more intense in the auklet's spring breeding season (Japan Meteorological Agency 2017). Indeed, our soil Hg concentrations exceeded soil quality guidelines (Ministry of Environment Japan 2008). We propose that organic Hg (methylmercury, the form of Hg that can pass through biological membranes [Mason et al. 1996]), which is carried and deposited by auklets, is more efficiently absorbed by the roots than inorganic Hg. Because no information on the efficiency of this process seems to be available, further quantification of organic Hg and tracing of Hg in soil with stable Hg isotope ratios ($\delta^{200}Hg$ [Lepak et al. 2015]) would help resolve some of these issues.

Although we found that biotransport significantly affected total Hg, other metals that we measured were not affected in the same way or extent. Indeed, although higher concentrations of Hg, As, Pb, and Co were found in roots at the auklet-affected site, the reverse was found for Cd. The reason the Cd concentration in roots at the control site was higher than at the auklet affected site is unclear but may not be biologically meaningful because of the large variance at each site.

We demonstrated that the concentration profile of metal and metalloid contaminants alone creates a signature for plants impacted by seabirds and that rhinoceros auklets act as a major biovector of such contaminants, transferring Hg and other metalloids from oceanic to terrestrial ecosystems. In theory, the source of this Hg can either be local, resulting from their foraging activity during the breeding season, or be from the distant wintering grounds. However, we provide some evidence downplaying the importance of local foraging sources of Hg transferred to local plants, suggesting instead that transferred Hg potentially resulted from their migratory behavior. The present study illustrates the importance of accounting for this source of contamination in terrestrial habitats when assessing the environmental risk of bioaccumulated contaminants.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4286.

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