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Ecological factors differentially affect mercury levels in two species of sympatric marine birds of the North Pacific

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ABSTRACT

In 2003 and 2004, we measured mercury concentrations and $\delta^{15}N$ and $\delta^{13}C$ values in the whole blood of adults of two species of seabirds, Cassin's auklet (*Ptychoramphus aleuticus*) and rhinoceros auklet (*Cerorhinca monocerata*), during their prelaying, incubation, and provisioning periods. We also collected whole blood from the offspring of both seabirds. Among prey items, $\delta^{15}N$ values were higher in fish than in crustaceans, while $\delta^{13}C$ did not vary systematically between prey types. Mercury concentrations in prey showed little relationship with either stable isotope. In the zooplanktivorous Cassin's auklet, year, reproductive stage, and $\delta^{15}N$ and $\delta^{13}C$ stable isotope values explained only 14% of the variation in mercury concentrations in adult blood, and none of these variables had a statistically significant effect. In contrast, these same variables explained 41% of the variation in mercury levels in the more piscivorous rhinoceros auklet, and all but $\delta^{15}N$ values had statistically significant effects. Mercury concentrations in adult rhinoceros auklets were higher in 2003 than in 2004; higher prior to laying than during the incubation or provisioning periods; and increased with $\delta^{13}C$ values — but in just one of two years. In both species, mercury concentrations were substantially higher in adults than in nestlings. Our results accord with previous studies in showing that mercury concentrations can vary among years, species and age classes, while the marked variation with reproductive stage is noteworthy because it is so rarely considered. Our results may help to explain the disparate conclusions of previous studies: while many factors influence mercury concentrations in marine predators, they apparently do so in a manner that defies easy characterization. We believe that there is a need for more studies that consider a range of physiological, ecological and behavioral factors that might affect mercury burdens in marine predators.

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1. Introduction

Concentrations of some contaminants, such as certain heavy metals, have increased in a wide range of coastal marine biota over recent decades (Doney, 2010). While most trace metals in the marine environment are derived from natural sources (Gustin et al., 2000; Chen et al., 2008), there are a variety of additional anthropogenic inputs from agricultural runoff, mining operations, urban waste disposal, and atmospheric deposition from the burning of fossil fuels and the generation of electrical power (Wang et al., 2004). Mercury is one of the better-studied trace-metal contaminants in marine environments, especially in the form of methylmercury, largely because of concerns about its effects on the health of humans that consume seafood (Fitzgerald et al., 2007).

Colonial seabirds are frequently used as bioindicators in studies investigating temporal and spatial variation in the concentrations of mercury in the ocean (Elliott et al., 1992; Elliott and Scheuhammer, 1997; Thompson et al., 1998; Goodale et al., 2008). Seabirds are especially useful in this role because they are easy to sample through time at their large, spatially fixed breeding sites, and because some species accumulate mercury to high concentrations (Burger et al., 1993; Kim et al., 1996; Savinov et al., 2003; Elliott, 2005). The relative sensitivity of seabird species to the toxicological effects of mercury is a complex topic, possibly related to variation in the pattern and duration of molt and the capability to demethylate mercury (Thompson and Furness, 1989; Stewart et al., 1999). Nonetheless, there is the potential for sub-lethal concentrations of mercury to impair certain critical physiological and behavioral functions of seabirds (Scheuhammer et al., 2007; Wayland et al., 2010).

While there is a good general understanding of the sources and biological effects of mercury contamination in marine ecosystems, apparent inconsistencies in the information and important knowledge gaps persist (Fitzgerald et al., 2007). For example, it is well established that mercury tends to biomagnify within marine food webs. In recent decades, this has often been inferred from positive correlations between mercury concentrations and stable isotopes of

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nitrogen ($\delta^{15}N$) in tissues sampled from a wide range of food web components (Jarman et al., 1996; Braune et al., 2005). A positive correlation between tissue $\delta^{15}N$ values and mercury concentrations is often found within seabird communities (Campbell et al., 2005; Elliott, 2005; Anderson et al., 2009), although some studies report no such relationship in both intraspecific (Jaeger et al., 2009) and interspecific (Lavoie et al., 2010) comparisons. Others find that the strength of the correlation depends on the tissue sampled (Bond and Diamond, 2009).

In addition, because stable-carbon isotope measurements ($\delta^{13}C$) are typically higher in benthically linked rather than pelagic biota in marine foodwebs (Hobson and Welch 1992), and because benthic habitats are reducing environments where the contaminant accumulates in sediments (Fitzgerald et al., 2007), positive correlations between mercury load and $\delta^{13}C$ in seabird tissues may also occur. This has been reported in some seabird communities (Campbell et al., 2005), while in others the relationship between mercury concentrations and $\delta^{13}C$ was neutral (Lavoie et al., 2010). In fact, even negative relationships have been reported in both intraspecific (Bearhop et al., 2000a) and interspecific (Anderson et al., 2009) comparisons. The inconsistency may reflect that both isotopic and contaminant concentrations can be affected by several independent and related processes.

A variety of ecological and behavioral factors other than trophic level and feeding habitat, such as seasonal movements between environments varying in mercury exposure and foraging range (Braune et al., 2005; Campbell et al., 2005), can also cause mercury concentrations to vary among avian species and individuals. Within species, mercury concentrations have been shown to vary with reproductive stage, possibly due to changes in the organ mass and body composition of breeding birds (Wayland et al., 2005), and to be higher in adults than in their offspring (Evers et al., 2005; Kojadinovic et al., 2007; Goodale et al., 2008). Many of these factors are poorly studied however, especially the causes of variation within species; marked inter-individual differences often remain largely unexplained (Bearhop et al., 2000a). Given the range and obvious complexity of the issues, there is need for more studies that concurrently assess a wide range of ecological factors that might cause concentrations of contaminants such as mercury to vary in marine biota.

We collected blood samples from breeding adults and the single nestlings of two seabirds, Cassin's auklet (*Ptychoramphus aleuticus*) and rhinoceros auklet (*Cerorhinca monocerata*), at Triangle Island, British Columbia, Canada, to examine how a suite of factors influence their mercury concentrations even though we have no reason to believe that the contaminant is currently impacting these populations. We also collected a suite of prey items. Our sampling protocol was designed to enable us to test for differences due to (1) year: our study spanned two years, one (2003) an El Niño year in which neither of our study species bred successfully, the other (2004) a more typical year in terms of oceanography and seabird breeding success (Hipfner et al., 2008; Wolf et al., 2009); (2) species: Cassin's auklets feed primarily on zooplankton in offshore habitats while breeding, while rhinoceros auklets are more generalist feeders (zooplankton, squid and fish) that undergo an upward trophic shift over the course of their breeding season (Davies et al., 2009; Ito et al., 2009). The two species differ in other relevant traits including body mass and annual adult survival rate (at our study site, ~180 g and 81% in Cassin's auklet and ~475 g and 88% in rhinoceros auklet; Morrison et al., submitted for publication); (3) reproductive stage: we sampled adults during the three main phases of their breeding cycle: prior to laying eggs, while incubating eggs, and while provisioning nestlings; (4) age: we sampled nestlings at the same time as we sampled provisioning adults; (5) $\delta^{15}N$, which measures relative trophic level (with elevated $\delta^{15}N$ values signifying higher trophic level feeding); and (6) $\delta^{13}C$, which gauges foraging habitat type (with elevated $\delta^{13}C$ values signifying a more benthic-nearshore feeding habit).

Of note, whole blood offers important advantages compared to other widely sampled tissues in a study such as ours: first, it provides a direct, accurate and non-lethal assessment of local exposure to mercury (Evers et al., 2005; Bearhop et al., 2000b; French et al., 2010); and second, there is likely to be reasonable overlap in the timeframe for integration into the blood system of mercury (half-life of 30–65 days in a large seabird, the 1218 g Great Skua, *Catharcta skua*; Bearhop et al., 2000b) and $\delta^{15}N$ and $\delta^{13}C$ (half-lives of approximately 14 days in Cassin's auklet and 20 days in rhinoceros auklet based on the allometric relationship in Carleton and Martinez del Rio, 2005; see also Bearhop et al., 2002).

2. Methods

2.1. Study site and field collections

Triangle Island (50°52′ N, 129°05′W) lies 46 km off Cape Scott at the northwestern tip of Vancouver Island, in relatively shallow waters (<200 m) some 25 km from the edge of the continental shelf.

In 2003, whole specimens of two important prey items in Cassin's auklet diets, the copepod *Neocalanus cristatus* and the euphausiid *Euphausia pacifica* (Hipfner, 2009), were collected on shipboard plankton tows along the continental shelf margin near Triangle Island. These crustaceans were immediately fresh-frozen (Mackas et al., 2007). We also collected a suite of fish prey directly from provisioning rhinoceros auklets trapped at night using hand-held nets at Triangle Island: Pacific saury *Cololabis saira*, juvenile rockfish *Sebastes* sp., and adult Pacific sandlance *Ammodytes hexapterus*. These fish samples were frozen at −10 °C in the field.

We caught adult Cassin's and rhinoceros auklets in pheasant nets erected between PVC poles during each of the prelaying, incubation, and provisioning periods. Full details can be found elsewhere (Morrison et al., in review). Blood (0.5–1.0 ml) was drawn from the brachial vein using 27 g syringes, after which the birds were banded, measured and released. No individual was sampled more than once. For each species, consecutive sampling dates were separated by >30 days, to allow for adequate turnover of elements in whole blood (Fig. 1). We also collected blood samples (0.5 ml) from nestlings pulled from burrows at approximately four weeks of age, during the same time period when we were sampling adults. All blood samples were stored in microcentrifuge tubes and frozen in the field at −10 °C.

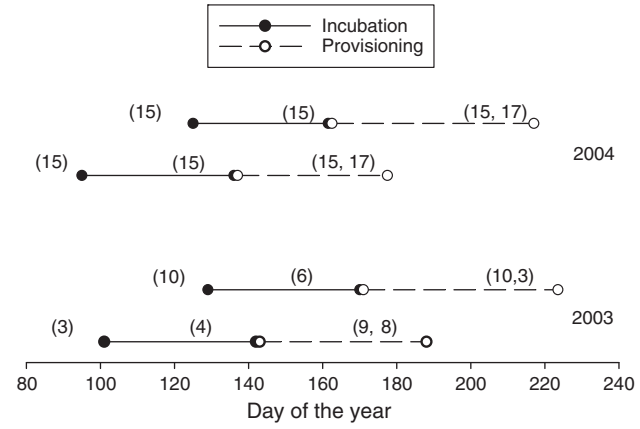

Fig. 1. Sampling scheme relative to the timing of seabird breeding stages. Dots represent median laying, hatching and fledging dates for Cassin's auklets (bottom of each pair) and rhinoceros auklets (top of each pair) in 2003 (bottom pair) and 2004 (top pair). Numbers represent the numbers of individuals sampled on that date for prelaying adults (far left), incubating adults (middle), and provisioning adults and nestlings (far right pair). Note that consecutive sampling dates were always separated by at least 30 days.

2.2. Laboratory analyses

All blood and prey samples were shipped frozen to the Environment Canada laboratory at the National Wildlife Research Centre (NWRC) in Ottawa.

For each prey item, copepod, euphausiid, saury, rockfish and sandlance, the samples were prepared as three equal subpools for analysis. Those subpools were weighed, and then homogenized using a Retsch ball mill. Blood samples were thawed, weighed to determine their moisture content, and then freeze-dried and homogenized. The freeze-dried samples were divided in half, with one half remaining at NWRC for measurement of total mercury concentrations, the other sent to the Environment Canada lab of KAH for measurement of stable isotope ratios of carbon and nitrogen (see below).

Concentrations of mercury in freeze-dried blood and prey samples were determined using an AMA-254 advancer mercury analyser. Results are reported in $\mu g\, g^{-1}$ dry weight. Quality assurance procedures included use of certified reference materials (CRM — Daily Calibration Check Standards from Institute for Reference Materials and Measurements: ERM®-CE278 Mussel Tissue and BCR®-463 Tuna Fish). The accuracy of the method was confirmed by analyzing the concentration of certified reference materials Dolt-3 and Tort-2 from NRC and Oyster Tissue 1566b from NIST. To check for the homogeneity of mercury in the samples, 16 samples were analyzed in duplicate. Replicates of the certified reference materials were also analyzed to check the calibration of the instrument, the within-run precision, and the reproducibility of the method. The practical detection limit of the instrument is 0.12 ng Hg, which corresponds to 0.006 $\mu g\, g^{-1}$ in the average 0.020 g dry mass sample.

Recoveries of total mercury for the daily calibration check standards ranged from 98.7 to 105.3% — within acceptable limits. Based on 16 replicate measurements, measurement precision for mercury was estimated to be 1.84% ± 2.60 SE for blood samples. For two prey samples, one fish and one crustacean, the measurement precision was estimated to be 1.1% and 0.3%, respectively.

Stable-carbon and nitrogen isotope assays of whole dried blood and homogenized prey items were performed on 1 mg subsamples of powdered material at the stable isotope facility of the Department of Soil Science, University of Saskatchewan. Lipids were not removed from blood samples due to expected low lipid levels. However, we used a 2:1 chloroform:methanol soak and rinse to remove lipids from prey samples to avoid effects of differential concentrations of isotopically ($\delta^{13}C$) light lipids. Samples were first loaded into tin cups and combusted in a Robo-Prep elemental analyzer at 1200 °C. The resultant CO_2 and N_2 gases were separated and analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer, with every fifth sample separated by two (albumin) laboratory standards. Results were reported in delta notation in parts per thousand (‰) relative to Air ($\delta^{15}N$) and VPDB ($\delta^{13}C$). Based on within-run replicate measurements of albumen standards, measurement precision for $\delta^{13}C$ and $\delta^{15}N$ values was estimated to be ±0.1‰ and ±0.3‰, respectively.

2.3. Statistical analyses

All statistical analyses were performed using the Fit Model function in JMP v. 8.0.2 (SAS Institute, 2010), with Generalized Linear Model personality, normal error structure and an identity link function. For all tests, diagnostic plots in the Fit Model function were inspected to ensure that residuals were approximately normally distributed. First, we used a multivariate analysis of variance (MANOVA) on $\delta^{15}N$ and $\delta^{13}C$ values in the blood of Cassin's auklets and rhinoceros auklets in relation to year, species and reproductive stage, the latter including both the three breeding phases for adults (prelaying, incubation and provisioning) as well as nestlings. Next, we examined mercury levels in the blood of breeding adults in relation to year, species, reproductive stage, as well as $\delta^{15}N$ and $\delta^{13}C$ and their interaction with species. Lastly, we examined the mercury levels in the blood of provisioning adults compared to the nestlings in relation to year, species, $\delta^{15}N$, $\delta^{13}C$, and the species x age class interaction term.

3. Results

3.1. Mercury and stable isotopes in prey species

Mercury levels in relation to $\delta^{15}N$ and $\delta^{13}C$ values in homogenized prey items are shown in Fig. 2. As expected, $\delta^{15}N$ values were higher in the three species of fish (saury, rockfish and sandlance) than in the two crustaceans (*N. cristatus* and *Euphausia pacifica*), while, as reported previously in this system (Davies et al., 2009), $\delta^{13}C$ values did not vary systematically between fish and crustaceans. Mercury levels in prey did not increase systematically with trophic level based on $\delta^{15}N$ values, in that mercury levels in sandlance were below those in euphausiids — and showed no clear systematic variation with habitat type based on $\delta^{13}C$ values.

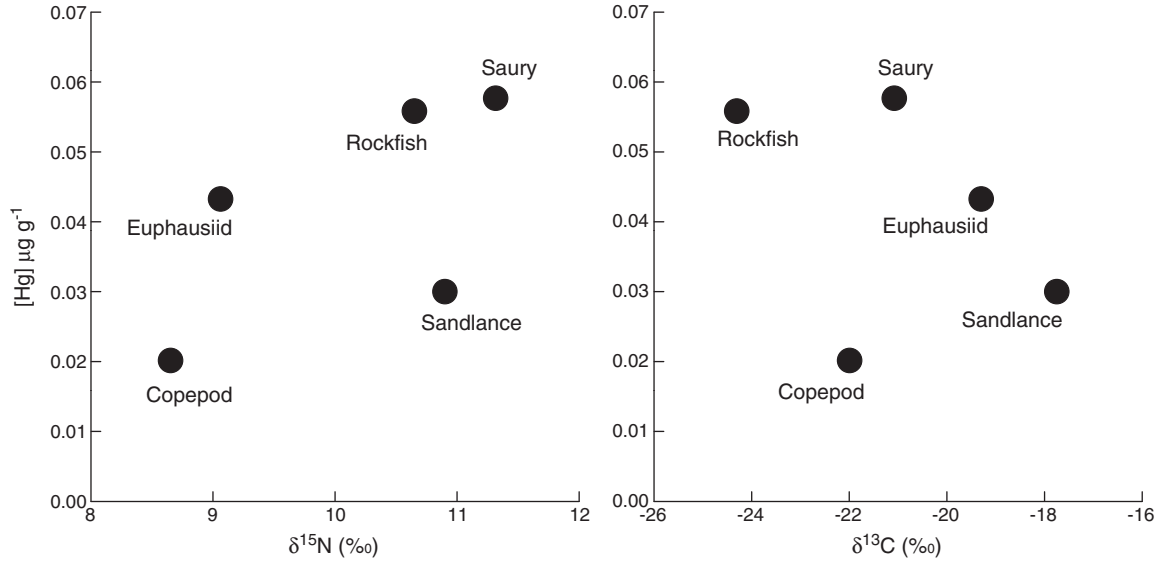

Fig. 2. Mercury levels in homogenates of the various prey types collected in 2004 in relation to their $\delta^{15}N$ and $\delta^{13}C$ values.

Table 1
Results for the full ANOVA model for explaining variation in $\delta^{15}N$ and $\delta^{13}C$ values in the blood of adult (all reproductive stages) and nestling Cassin's auklets and rhinoceros auklets at Triangle Island in 2003 and 2004. For full models, $R^2 = 0.70$ ($\delta^{15}N$) and 0.43 ($\delta^{13}C$).

Variable	Source	df	SS	*F*-ratio	*p*-value
$\delta^{15}N$	Year	1	19.115	38.842	<0.001
	Species	1	150.450	305.701	<0.001
	Stage	3	24.761	16.771	<0.001
$\delta^{13}C$	Year	1	1.308	4.537	0.035
	Species	1	2.967	10.289	0.002
	Stage	3	30.668	35.449	<0.001

3.2. Stable isotopes in seabird blood

In the full MANOVA model, each of year, species and reproductive stage (all three adult phases plus nestlings) had highly statistically significant effects on $\delta^{15}N$ and $\delta^{13}C$ values (all $p<0.001$). Therefore, we ran separate models for each isotope. The full model including year, species and reproductive stage explained most of the variation in $\delta^{15}N$ values ($R^2 = 0.70$, $F_{5,170} = 79.82$, $p<0.001$), and all three independent variables had statistically significant effects (Table 1). Based on parameter estimates and their standard errors (Table 2), $\delta^{15}N$ values were higher in 2003 than in 2004, higher in rhinoceros auklets than in Cassin's auklets, and lowest in incubating adults but highest in provisioning adults and nestlings (Fig. 3). For $\delta^{13}C$, the full model explained much of the variation ($R^2 = 0.43$, $F_{5,170} = 25.89$, $p<0.001$), and all three independent variables were statistically significant (Table 1). Based on parameter estimates and their standard errors (Table 2), $\delta^{13}C$ values were higher in 2004 than in 2003, higher in rhinoceros auklets than in Cassin's auklets, and lowest in provisioning adults and nestlings but highest prior to laying and during incubation (Fig. 3).

3.3. Mercury in adult seabird blood

The full model including year, species, reproductive stage, as well as $\delta^{15}N$ and $\delta^{13}C$ and their interactions with species, explained most of the variation in mercury concentrations in adult birds ($R^2 = 0.66$, $F_{8,112} = 29.90$, $p<0.001$). Year, species and reproductive stage had statistically significant effects, but $\delta^{15}N$ and $\delta^{13}C$ did not (Table 3). Based on parameter estimates and their standard errors (Table 4), mercury concentrations were higher in 2003 than in 2004, higher in rhinoceros auklets than in Cassin's auklets, and higher during the prelaying period than during incubation or while provisioning nestlings, but with no significant difference between the latter two stages. However, an interpretation of the direct effects of $\delta^{15}N$ and $\delta^{13}C$ on mercury concentrations is confounded by the strong interactions these variables had with species (Table 3). Therefore, we reassessed the effects of $\delta^{15}N$ and $\delta^{13}C$ separately in the two species, and including year and reproductive stage, both of which were statistically significant in the full model (Table 3).

The full model including year, reproductive stage, $\delta^{15}N$ and $\delta^{13}C$ did not strongly predict variation in mercury concentrations in Cassin's auklets ($R^2 = 0.14$, $F_{5,54} = 1.87$, $p = 0.11$), and no single independent variable had a statistically significant effect (all

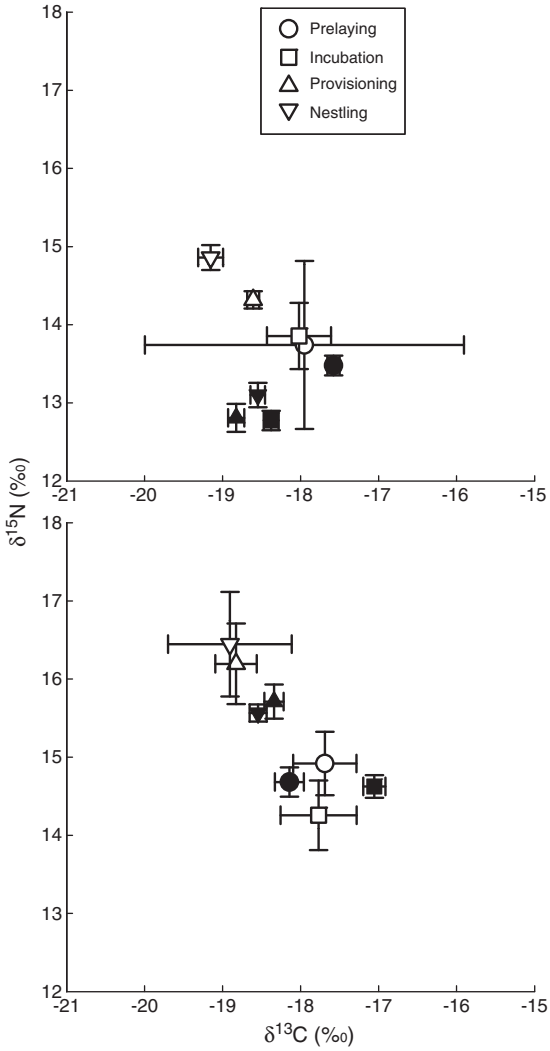

Fig. 3. Bivariate plot showing the mean ± SE values for $\delta^{15}N$ in relation to $\delta^{13}C$ in Cassin's auklet (top) and rhinoceros auklet (bottom) during the 3 breeding stage plus nestlings in 2003 (open symbols) and 2004 (closed symbols).

Table 2
Parameter estimates for terms in the full model for explaining variation in $\delta^{15}N$ and $\delta^{13}C$ values in the blood of adult (all reproductive stages) and nestling Cassin's auklets and rhinoceros auklets at Triangle Island in 2003 and 2004.

Variable	Term	Estimate	SE
$\delta^{15}N$	Intercept	14.452	0.058
	Year (2003)	0.365	0.059
	Species (Cassin's auklet)	−0.932	0.537
	Stage (prelaying)	−0.210	0.093
	Stage (incubation)	−0.513	0.095
	Stage (provisioning)	0.317	0.089
	Stage (nestling)	0.410	0.091
$\delta^{13}C$	Intercept	−18.269	0.045
	Year (2003)	−0.095	0.045
	Species (Cassin's auklet)	−0.131	0.072
	Stage (prelaying)	0.380	0.093
	Stage (incubation)	0.461	0.073
	Stage (provisioning)	−0.398	0.068
	Stage (nestling)	−0.442	0.070

Table 3
Results for the full ANCOVA model for explaining variation in mercury levels in the blood of adult Cassin's auklets and Rhinoceros auklets at Triangle Island during 3 reproductive stages in 2003 and 2004. For the full model, $R^2 = 0.66$.

Source	df	SS	*F*-ratio	*p*-value
Year	1	6.027	19.114	<0.001
Species	1	24.754	78.506	<0.001
Reproductive stage	2	11.005	17.451	<0.001
$\delta^{15}N$	1	2.006	1.873	0.174
$\delta^{13}C$	1	1.379	0.493	0.484
Species*$\delta^{15}N$	1	2.347	7.436	0.007
Species*$\delta^{13}C$	1	2.938	9.316	0.003

Table 4
Parameter estimates for terms in the full model for explaining variation in mercury levels in the blood of adult Cassin's auklets and rhinoceros auklets at Triangle Island during 3 reproductive stages in 2003 and 2004.

Term	Estimate	SE
Intercept	1.339	2.478
Year (2003)	0.264	0.060
Species (Cassin's auklet)	−0.739	0.083
Stage (prelaying)	0.491	0.085
Stage (incubation)	−0.244	0.083
Stage (provisioning)	−0.248	0.100
$\delta^{15}N$	−0.107	0.078
$\delta^{13}C$	−0.072	0.103
Species*$\delta^{15}N$	0.184	0.067
Species*$\delta^{13}C$	0.275	0.090

$F \leq 1.19$, all $p > 0.28$). Further, standard errors around parameter estimates overlapped zero. Thus, there was no indication that any of our suite of ecological predictor variables appreciably influenced mercury concentrations in Cassin's auklets (Fig. 4).

For rhinoceros auklets, in contrast, the full model including year, stage, $\delta^{15}N$ and $\delta^{13}C$ values had good explanatory power ($R^2 = 0.41$, $F_{5,65} = 9.10$, $p < 0.001$), and all variables had statistically significant effects ($p \leq 0.02$) with the exception of $\delta^{15}N$ ($p = 0.998$). Based on parameter estimates and their standard errors (Table 5), mercury concentrations in adult rhinoceros auklets were higher in 2003 than in 2004, peaked prior to laying (Fig. 4), and increased with $\delta^{13}C$ values. It is clear from Fig. 5 however that there was a positive relationship between mercury concentrations and $\delta^{13}C$ values only in 2003, but as we had no *a priori* reason to expect this yearly difference we did not include the year × $\delta^{13}C$ term in our model. When we added that interaction term, it markedly improved the explanatory power of the model ($R^2 = 0.56$) and the interaction term was highly statistically significant ($p < 0.001$).

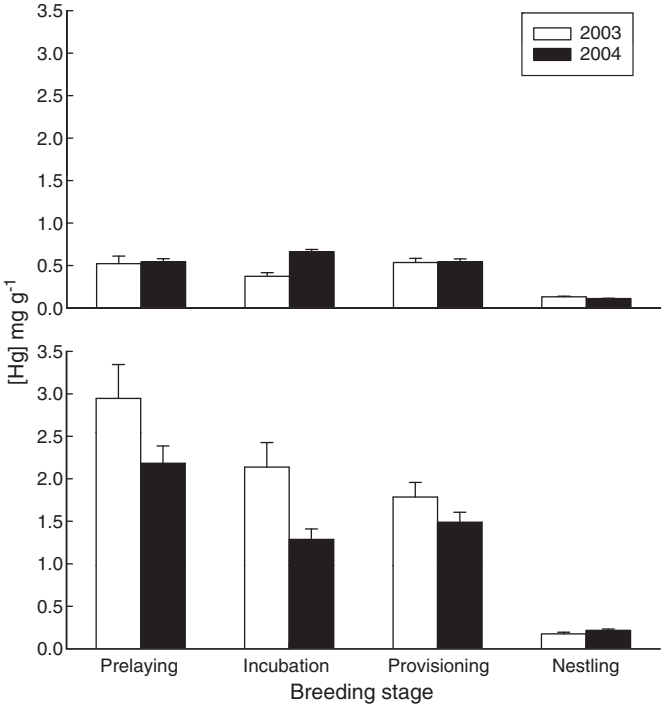

Fig. 4. Mercury levels (mean ± SE, in μg/g dry mass) in blood samples from Cassin's auklet (top) and rhinoceros auklet (bottom) during three reproductive stages in 2003 (open bars) and 2004 (closed bars): prior to laying (PL), during incubation (INC) and while provisioning nestlings (PROV). Also shown are values in nestlings.

Table 5
Parameter estimates for terms in the full model for explaining variation in mercury levels in the blood of adult rhinoceros auklets at Triangle Island during 3 reproductive stages in 2003 and 2004.

Term	Estimate	SE
Intercept	8.020	3.576
Year (2003)	0.342	0.091
Stage (prelaying)	0.600	0.124
Stage (incubation)	−0.517	0.179
Stage (provisioning)	−0.083	0.198
$\delta^{15}N$	0.000	0.132
$\delta^{13}C$	0.337	0.145

3.4. Mercury in seabird blood in relation to age

The full model including year, species, age class, $\delta^{15}N$, $\delta^{13}C$, and species × age class accounted for most of the variation in mercury concentrations in provisioning adults and nestlings ($R^2 = 0.86$, $F_{6,87} = 88.612$, $p < 0.001$). All independent variables except $\delta^{15}N$ had statistically significant effects (Table 6). Based on parameter estimates and their standard errors (Table 7), mercury concentrations were higher in 2003 than in 2004, higher in rhinoceros auklets than in Cassin's auklets, higher in adults than in nestlings, and increased with $\delta^{13}C$ values but had no relationship with $\delta^{15}N$ values. In addition, the species × age class term was significant, reflecting that the difference in mercury concentrations of Cassin's auklet provisioners (least squares mean of 0.634 μg g^{-1} ± 0.069 SE) and their offspring (0.230 μg g^{-1} ± 0.067 SE) was considerably less than that between

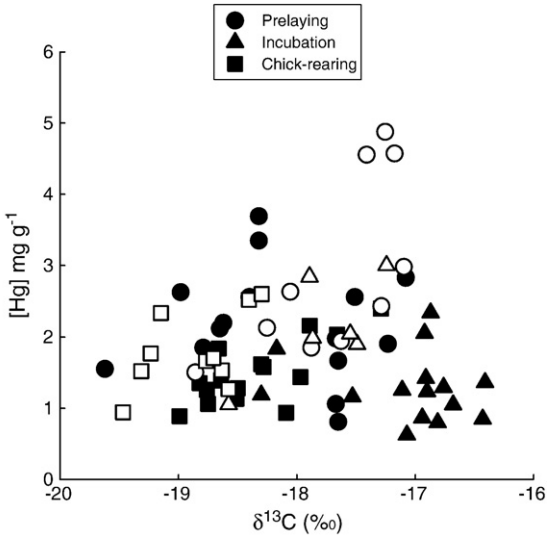

Fig. 5. Mercury levels (in μg/g dry mass) in blood collected from adult rhinoceros auklets in relation to their $\delta^{13}C$ values in 2003 (open symbols) and 2004 (closed symbols). The regression line is for 2003 only.

Table 6
Results for the full ANCOVA model for explaining variation in mercury levels in the blood of adult (provisioning) and nestling Cassin's auklets and rhinoceros auklets at Triangle Island in 2003 and 2004. For the full model, $R^2 = 0.86$.

Source	df	SS	*F*-ratio	*p*-value
Year	1	0.351	5.341	0.023
Species	1	1.914	29.127	<0.001
Age	2	16.787	255.467	<0.001
$\delta^{15}N$	1	0.046	0.704	0.404
$\delta^{13}C$	1	0.583	8.879	0.004
Species*Age	1	5.035	76.629	<0.001

Table 7
Parameter estimates for terms in the full model for explaining variation in mercury levels in the blood of adult (provisioning) and nestling Cassin's auklets and rhinoceros auklets at Triangle Island in 2003 and 2004.

Term	Estimate	SE
Intercept	4.852	1.456
Year (2003)	0.098	0.042
Species (Cassin's auklet)	−0.324	0.060
Age (nestling)	−0.438	0.027
$\delta^{15}N$	−0.038	0.045
$\delta^{13}C$	0.195	0.066
Species*age	−0.236	0.027

rhinoceros auklet provisioners (1.754 μg g^{-1} ± 0.113 SE) and theirs (0.406 μg g^{-1} ± 0.123 SE). However, the relative difference was similar, in that adult values were approximately 3–4 times higher than those of nestlings in both species.

4. Discussion

In accord with previous studies conducted elsewhere (Braune et al., 2005; Campbell et al., 2005; Anderson et al., 2009), we found that a variety of factors influenced mercury concentrations in two species of seabirds breeding at Triangle Island. Overall, mercury concentrations in adult seabird blood were higher (by ~0.3 μg g^{-1} on average) in 2003 than in 2004. Year-to-year differences in mercury concentrations have been observed in some seabird communities (Wayland et al. 2005), but not others (Bearhop et al., 2000a), while in our study mercury concentrations varied between years in rhinoceros auklets but not in Cassin's auklets.

Yearly differences in mercury loads could be a function of dietary differences, and in rhinoceros auklets, the higher mercury concentrations in 2003 compared to 2004 accord with the elevated $\delta^{15}N$ values in 2003. More specifically, rhinoceros auklet nestling diets in 2003 compared to 2004 included less Pacific sandlance (32% vs 39% by biomass) and more Pacific saury (54% vs 21%; Hipfner et al., 2008); and in comparing between these two prey types, saury had higher $\delta^{15}N$ values and higher mercury concentrations. However, it is striking that there was no corresponding difference in the mercury loads of rhinoceros auklet nestlings in the two years. And further, while Cassin's auklet nestling diets in 2003 compared to 2004 likewise included more of a high trophic-level, high-mercury prey type (euphausiids, 34% vs 56% by biomass) and less of a low trophic-level, low-mercury prey type (copepods, 45% vs 26%; Hipfner, 2009), blood mercury concentrations did not vary between years in Cassin's auklet adults and nestlings. Rather than diet, large-scale environmental processes that affect the total atmospheric deposition of mercury to coastal ocean regions (Fitzgerald et al., 2007) may largely underlie interannual variation in mercury loads in this marine system. For example, there was almost twice as much precipitation in the spring and summer of 2003, an El Niño year, compared to 2004, based on weather records collected at Sartine Island, 15 km east of Triangle Island (Hipfner et al. 2010).

As found in other seabird communities (Braune et al., 2005; Goodale et al., 2008), mercury concentrations varied between species after controlling for other factors: concentrations were higher (by ~0.9 μg g^{-1} on average) in rhinoceros auklets than in Cassin's auklets, consistent with a previous report (Elliott and Scheuhammer, 1997). This interspecific difference accords with the combined tendency of rhinoceros auklets to feed both at a higher trophic level and in more benthic or nearshore environments (Davies et al., 2009). Nonetheless, it seems likely that other species-specific life-history (such as longevity and gender), behavioral and physiological traits that we did not consider also play important roles (Anderson et al., 2009). Note too that the strong interactions between species and $\delta^{15}N$ and $\delta^{13}C$ blood values indicate that trophic and habitat effects did not act consistently on the two species.

Within species, we found that neither $\delta^{15}N$ nor $\delta^{13}C$ values had particularly strong effects on mercury concentrations; in fact, only $\delta^{13}C$ values had any statistically discernable effect, and that in just one of two years (2003) and in one of two species (rhinoceros auklet). The generally weak relationships could reflect that there was no strong systematic covariation between mercury concentrations and isotopic values within the prey base around Triangle Island. But it is also possible that the contaminant and isotopic variables decouple because they reflect dietary inputs over slightly different time periods (Bearhop et al., 2000a). The balance of factors underlying interspecific variation in mercury concentrations may differ from that for intraspecific variation (Bearhop et al., 2000a; Anderson et al., 2009).

Mercury concentrations in breeding adults varied among three reproductive stages, being higher during the prelaying period than during the incubation or provisioning periods. Again, however, this was true only for rhinoceros auklets — mercury concentrations in Cassin's auklets varied little over the course of their breeding seasons. The fact that levels peaked prior to laying suggests that neither trophic-level effects, nor foraging habitat effects, were key factors, because $\delta^{15}N$ values in rhinoceros auklets were low then, the normal pattern in this species (Davies et al., 2009), and similar to values during incubation, by which time mercury concentrations had dropped substantially. Further, while high mercury concentrations would be expected from the high $\delta^{13}C$ values prior to laying, the prelaying $\delta^{13}C$ values were similar to those in incubating birds. In common eiders *Somateria mollissima*, by contrast, mercury concentrations in blood were lowest prior to laying, when adults gorge and gain mass, and were higher late in the nesting period, when adult birds fast and lose substantial amounts of mass from body stores and internal organs. This observation led Wayland et al. (2005) to suggest that blood mercury concentrations were affected by body condition.

Like other auks (Elliott et al., 2008), rhinoceros auklets undergo a loss of mass between incubation and chick rearing, although in contrast to common eiders this loss of mass is primarily a consequence of reducing lipid stores rather than organ mass or breast muscle mass (Niizuma et al., 2002). However, in our study, mercury concentrations did not differ between the incubation and chick rearing periods, suggesting that body condition alone does not adequately explain the differences in mercury concentrations with breeding stage in rhinoceros auklets.

We considered two alternative explanations for the elevated mercury concentrations in prelaying rhinoceros auklets. First, they might reflect spatial variation in exposure to mercury. Given the relatively long half-life of mercury in seabird blood (Bearhop et al., 2000b), concentrations measured early in the breeding season probably include contaminants acquired prior to or during passage back to the breeding colony, whereas mercury values measured during the incubation and provisioning periods (which on average take 45 and 54 days respectively in rhinoceros auklets) likely gauge contaminant levels in the local environment. Rhinoceros auklets spend the early spring period in continental shelf waters (Kenyon et al., 2009), which may be more contaminated as a result of accumulation from long-term riverine discharge of mercury accumulated annually in snowpack (Kirk and St. Louis, 2009; Leitch et al. 2007). Second, we would expect seasonal reductions if females transfer large amounts of mercury from body stores to eggs (Bond and Diamond, 2009). However, samples collected on the colony prior to laying are more likely to be male biased, based on sex-specific colony attendance patterns in other auks (Wanless and Harris, 1988).

Finally, we found that mercury concentrations were 3–4 times higher in adult blood than in nestling blood collected during the same period in both Cassin's and rhinoceros auklets. While it is normal for adults to have higher contaminant levels than juveniles (Elliott et al., 1992; Kojadinovic et al., 2007), the adult-to-juvenile ratio was

considerably higher in two auks, Atlantic Puffin *Fratercula arctica* (6.3:1) and Razorbill *Alca torda* (7.6:1) in the Gulf of Maine (Goodale et al., 2008) than in our study species in the more offshore environment at Triangle Island. The ratios at Triangle Island, however, were similar to or lower than those found in several piscivorous freshwater birds (Evers et al., 2005). Unlike provisioning adults, growing nestlings have an outlet for mercury through depuration into growing feathers (Spalding et al., 2000), and we suggest that this likely explains much of the age-related difference, given our own and previous results suggesting that adult and nestling diets are similar in Cassin's and rhinoceros auklets (Davies et al., 2009).

To conclude, we found that a variety of factors influenced mercury concentrations in the blood of two species within a northeast Pacific seabird community, but in a complex and in some instances unequal manner that defied easy characterization. In particular, while trophic level and foraging habitats consistently appear to play important roles in determining interspecific differences in mercury levels, their effects appear to be weak (trophic level) or inconsistent (foraging habitat) when considered intraspecifically (Bearhop et al., 2000a). While previous studies (Goodale et al., 2008), like ours, clearly indicate that adults are likely to be better indicators of mercury exposure than nestlings, our results point to the need to be mindful that mercury concentrations can vary appreciably from year to year, and also within a single breeding season — the latter a fact that has very rarely been considered (Wayland et al., 2005). Certainly in the case of the rhinoceros auklets studied at Triangle Island, mercury monitoring would need to target a specific stage in breeding, such as prelaying adults or eggs.

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