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METALS AND POLYCYCLIC AROMATIC HYDROCARBONS IN COLONIAL WATERBIRD EGGS FROM LAKE ATHABASCA AND THE PEACE–ATHABASCA DELTA, CANADA

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Abstract—In 2009, aquatic bird eggs from a variety of species were collected from three sites in northern Alberta, Canada. Two sites were located in receiving waters of the Athabasca River, which drains the oil sands industrial region north of Fort McMurray, Alberta. The third site, located on the Peace River, was remote from the influence of the Athabasca River. Levels of mercury, arsenic, and polycyclic aromatic hydrocarbons (PAHs) were measured in the eggs along with nitrogen stable isotopes ($\delta^{15}N$) as an indicator of bird trophic position. Levels of As and PAHs in eggs were low, whereas Hg was measureable in all samples. Egg Hg levels increased with $\delta^{15}N$ values (a proxy of food web trophic position); however, some eggs exhibited Hg levels greater than expected based on trophic position. These eggs were from sites in receiving waters of the Athabasca River, namely, Mamawi Lake and Egg Island. Levels of Hg in egg pools were correlated with naphthalene levels, perhaps indicating a common source of contamination. Temporal comparison of Hg levels in California gull (*Larus californicus*) eggs from the Lake Athabasca colony indicated that egg Hg burdens increased 40% from 1977 to 2009. More research is required to evaluate temporal trends in levels of environmental contaminants and to identify sources. Environ. Toxicol. Chem. 2011;30:1178–1183. © 2011 SETAC

Keywords—Oil sands Bird eggs PAHs Mercury Athabasca River

INTRODUCTION

The Peace–Athabasca Delta is the largest boreal delta in the world and, in 1982, was designated a wetland of international significance under the Ramsar Convention [1]. It provides important nesting and staging habitat for millions of birds annually. As an example, endangered whooping cranes (*Grus americana*) migrate through the delta in the spring on their way to nesting grounds in Wood Buffalo National Park [2].

Adjacent regions also provide important habitat for nesting birds. Egg Island in western Lake Athabasca supports the largest breeding colony of Caspian terns (*Hydroprogne caspia*) in Alberta as well as harboring other waterbirds [3]. It was designated a provincial ecological reserve in 1992.

Concern is increasing with regard to the environmental impacts of industrial development in this region. Much of this concern stems from the production of synthetic crude oil derived from bituminous sands in the Athabasca oil sands [4,5]. Although commercial oil sands operations began in 1967, the pace of development has increased greatly since the 1990s and is expected to increase further in coming decades [6,7]. Oil sands development is currently centered in the region north of Fort McMurray. A major river, the Athabasca River, flows northward through Fort McMurray and the area where many of the oil sands operations are located. The river then discharges into the Peace–Athabasca Delta (via the Embarras River and Mamawi Creek) and western Lake Athabasca approximately 200 km downstream from Fort McMurray (Fig. 1).

Recent research has led to conflicting views regarding the impacts of oil sands development on contaminant levels in the Athabasca River [4,5,8]. Kelly et al. [4,5] concluded that contaminants associated with oil sands development included metals (e.g. Hg, As) and polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, fluorene, phenanthrene, and anthracene. Here, we measure levels of these compounds in waterbird eggs collected from three sites in northern Alberta (two located in receiving waters of the Athabasca River and one on the Peace River). We investigate how egg contaminant levels may be influenced by site of collection. In addition, we measure stable nitrogen isotopes ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) in eggs to examine how trophic position might influence egg contaminant levels. During trophic transfer, the heavy ^{15}N isotope is enriched relative to the lighter ^{14}N isotope, resulting in a stepwise increase in ^{15}N with trophic level [9]. Stable nitrogen isotope ($\delta^{15}N$) values generally increase by 3 to 4‰ from one trophic level to the next [10]. This is also true for avian eggs, in which protein $\delta^{15}N$ levels were 3.4‰ greater than those in the diet of laying females [11]. Gull and tern eggs are a useful matrix for gaining insights into local environmental conditions because they are generally formed using exogenous resources, that is, local food sources [12]. Hence, the chemical composition of the egg will reflect the chemical characteristics of the region around the breeding colony (for examples of gull eggs as indicators of regional contaminant sources, see Hebert et al. [13]).

Aquatic birds examined in the present study represent apex predators in this ecosystem. As such, they act as effective integrators of environmental change. For example, fish-eating birds have been shown to reflect temporal changes in food web dynamics [14] and alterations in environmental contaminant levels [15]. Here we present the first temporal comparison

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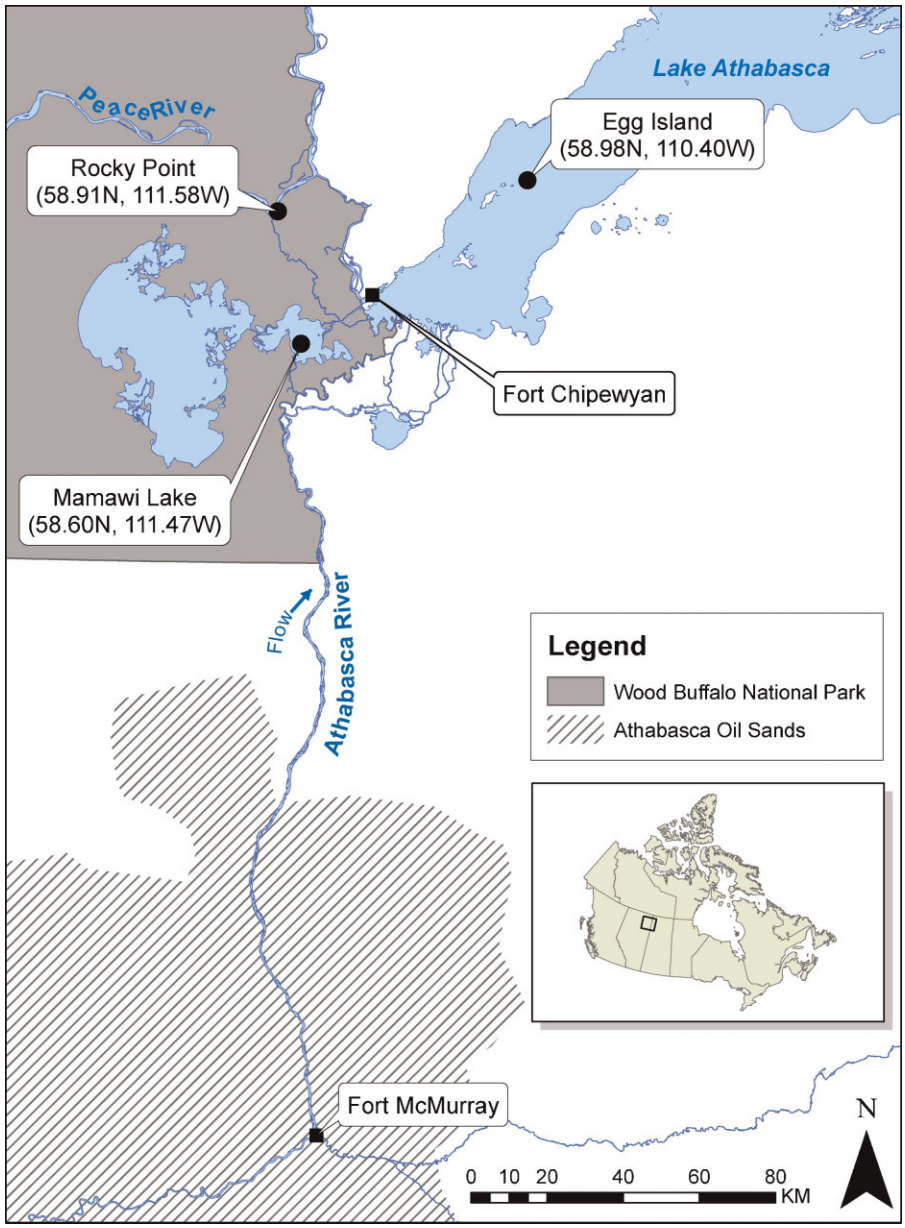

Fig. 1. Waterbird egg collection locations (circles) in northern Alberta, Canada. Eggs were collected from two sites (Mamawi Lake and Rocky Point) in Wood Buffalo National Park in 2009. Eggs were also collected from Egg Island, Lake Athabasca in 1977 and 2009. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

of contaminant levels in aquatic bird eggs from Lake Athabasca. These samples were collected before and after the large-scale expansion of oil sands operations. In conjunction with information regarding avian populations and ecology [3], the data reported here provide a baseline against which to evaluate future environmental change and possible impacts on wildlife.

MATERIALS AND METHODS

In June, 2009, 62 freshly laid eggs (i.e., no embryonic development) were collected from three sites in northern Alberta, Canada (Fig. 1). Two of the sites were located in Wood Buffalo National Park (Mamawi Lake and Rocky Point), and the third site was located in western Lake Athabasca (Egg Island). The Mamawi Lake and Egg Island sites receive waters from the Athabasca River and are downstream of the region encompassing oil sands operations north of Fort McMurray (Fig. 1). The site closest to the Athabasca River was the Mamawi Lake location, approximately 25 km linear distance from the river. Mamawi Lake receives approximately 6% of the total flow of the Athabasca River via the Embarras River and Mamawi Creek [16]. At that site, 10 eggs were collected from each of two species: common tern (*Sterna hirundo*) and ring-billed gull (*Larus delawarensis*). The Egg Island site, in western Lake Athabasca, was located farther from the Athabasca River, approximately 45 km linear distance from the river mouth. At Egg Island, eggs were collected from three species: Caspian tern (*Hydroprogne caspia,* $n = 10$), California gull (*Larus californicus* $n = 10$), and herring gull (*Larus argentatus*, $n = 2$). Herring gull samples were limited by the number of nests present on the island. The third site at Rocky Point was located in the Peace River watershed. This site was removed from the influence of the Athabasca River. At Rocky Point, eggs were collected from common tern ($n = 10$) and ring-billed gull ($n = 10$) nests. At all sites, eggs were collected from different nests.

After collection, eggs were transported to the National Wildlife Research Centre (NWRC; Ottawa, ON, Canada). The eggs were opened and the contents (yolk and albumen) homogenized using chemically clean techniques. Individual homogenized samples ($n = 62$) were stored frozen (−40°C) in the National Wildlife Specimen Bank [17]. Individual egg samples collected in 2009 were used for total Hg, total As, and stable nitrogen (N) isotope ($^{15}N/^{14}N$) analyses. In addition, subsamples from each egg were used to create an equal-weight pooled sample for each species at each collection site ($n = 7$). These pooled samples were used for PAH analysis.

Total Hg analyses were completed at NWRC using the following methods. Egg samples were weighed into plastic, acid-washed vials, then freeze dried, and dry mass was recorded. Hg was quantified as follows: 10 to 25 mg of dried egg material was thermally and chemically decomposed within the decomposition furnace of an advanced Hg analyzer (AMA-254; LECO Corporation). The AMA-254 software calculated mercury concentrations. Quality control was maintained by using certified reference materials (Dolt-3 and Tort-2 from the National Research Council [NRC] of Canada, Oyster Tissue 1566b from the National Institute of Standards and Technology [NIST]), sample replicates, and blanks. Limit of quantification for Hg was 0.006 μg/g (dry wt). Results are reported in micrograms per gram dry weight.

Analyses for As were also completed at the NWRC. Briefly, 0.05 g (dry wt) of egg material was weighed into plastic, acid-washed tubes and digested using 0.25 ml of reverse-osmosis–purified water and 1.0 ml of 70% nitric acid. Samples were stored at room temperature overnight and on the following day were loosely capped and heated for 4 h at 100°C. Cool, digested samples were diluted tenfold with reverse-osmosis–purified water to 2 ml, and an internal standard was added. Arsenic concentrations were measured with an inductively coupled plasma mass spectrometer (ICP-MS; ELAN 9000; Perkin Elmer). The performance of the ICP-MS was evaluated by using certified reference materials (Dolt-3 and Tort-2 from NRC, CRM 8415 Whole Egg Powder from NIST). Quality assurance/quality control protocols also involved the analysis of duplicates and blank samples. Theoretical detection limit (TDL; calculated as 3× standard deviation of 10 blanks) for As was 0.028 μg/g (dry wt). Limit of quantification (5× TDL) for As was 0.142 μg/g (dry wt). Results are reported in micrograms per gram dry weight.

Stable nitrogen isotope analyses were conducted at the University of Ottawa's G.G. Hatch Stable Isotope Laboratory using 1 mg (±0.2 mg) of dried egg tissue encapsulated in tin. Isotope analysis was completed with a CE 1110 Elemental Analyser (CE Instruments), followed by gas chromatographic separation and online analysis by continuous-flow with a DeltaPlus Advantage isotope ratio mass spectrometer (Thermo Scientific) coupled with a ConFlo III (G.G. Hatch Isotope Laboratories). Data were normalized using international standards for calibration, and quality control was maintained through sample duplicates. Stable isotope values were reported in delta notation in parts per thousand (‰, per mil) relative to atmospheric air standard. Analytical precision, based on repeat measures of a standard (C-55), was ±0.2‰.

Pooled samples were used to measure 16 PAHs; PAH analysis was completed at the University of Windsor's Great Lakes Institute for Environmental Research using the following methods. Ten-gram (wet wt) aliquots of egg pools were mixed with anhydrous sodium sulfate and Soxhlet extracted using acetone:hexane. Extracts were concentrated and extracted three times with hexane. The three hexane extracts were collected, concentrated, and added to a chromatographic column (glass wool stopper and anhydrous sodium sulfate), in which they were eluted with hexane. The extract was rotaevaporated to a final volume of 2 ml and transferred into a florisil/sodium sulfate column. Samples were eluted successively with hexane (fraction 1), 15% dichloromethane/hexane (fraction 2), and 60% DCM/hexane (fraction 3). Fractions were collected separately, and each was concentrated (after adding 5 ml isooctane) to approximately 2 ml. For PAH analysis, fractions 2 and 3 were combined, rotoevaporated to 2 ml, and injected (splitless) into an HP model 6890/5970 GC-MSD with a 60-m DB-5 column (0.25 mm inside diameter [i.d.] × 0.10 μm DB-5 film thickness; J&W Scientific). Quality assurance/quality control was ensured using standard reference materials (NIST SRM 1944) and method blanks. Polycyclic aromatic hydrocarbons were identified based on retention times of quantification ions and the ratio of abundances of the confirmation ions that were comparable with those of calibration standards. Polycyclic aromatic hydrocarbon concentration calculations were performed in Windows NT HP Chem Station3. Limits of detection for 16 PAHs ranged from 0.03 ng/g to 1.11 ng/g (wet wt). Concentrations for PAHs were reported as nanograms per gram wet weight.

In addition to the samples collected in 2009, historical samples were retrieved from the National Wildlife Specimen Bank (NWRC), in which they had been kept frozen at −40°C since collection. These samples comprised 10 California gull eggs collected in June, 1977, from the Egg Island colony. Upon arrival at NWRC in 1977, the eggs were opened and the contents placed in chemically clean glass jars. At the time of processing, it was noted that there were signs of embryonic development in all eggs (i.e., they were not freshly laid). As incubation progresses, water and lipid content of eggs declines but not contaminant content [18]; hence, with development, chemical concentration data are biased high. To ensure the validity of temporal comparisons using data from freshly laid eggs (2009) and developed eggs (1977), egg Hg burdens were calculated (mass of egg contents × concentration). Methods for chemical analysis of these samples were identical to those described for the samples collected in 2009. Individual eggs were used for all analyses except for PAH analysis, in which one pooled sample was analyzed.

Intersite and species differences in Hg concentrations were examined using analysis of variance (ANOVA) followed by Tukey's HSD test for unequal sample sizes. Student's *t* test was used to examine differences in Hg concentrations/burdens in California gull eggs collected in 1977 and 2009. Prior to these analyses, Hg concentration/burden data were $\log_{10}$ transformed to meet assumptions underlying parametric statistics. Arsenic data did not meet parametric assumptions, so nonparametric statistics (Mann-Whitney *U* test) were used for temporal comparisons. Untransformed $\delta^{15}N$ data met the requirements for parametric analysis. Pearson product-moment correlations were used to examine relationships between variables.

RESULTS AND DISCUSSION

Differences in egg contaminant levels, 2009

For the 2009 samples, Hg levels in all eggs were well above the limit of quantification. Conversely, egg As concentrations were low and close to detection limits. Concentrations of As in 15 of the egg samples were below the TDL, and the

Table 1. Mean Hg and As concentrations (μg/g dry wt) and $\delta^{15}N$ values (‰) in waterbird eggs[a]

Species	Site	N	Mean Hg	95% Confidence interval		Mean As	SD	Mean $\delta^{15}N$	SD
				Lower	Upper				
COTE	Mamawi Lake	10	1.50 AB*	1.06	2.11	0.08	0.03	11.41 A	1.75
RBGU	Mamawi Lake	10	0.33 E*	0.24	0.46	0.02	0.01	8.64 C	2.02
CATE	Egg Island	10	1.65 A	1.44	1.89	0.04	0.03	10.76 AB	0.89
HERG	Egg Island	2	1.56 ABC	1.41	1.72	0.07	0.01	10.06 ABC	0.68
CAGU	Egg Island	10	0.56 CD	0.41	0.76	0.06	0.03	9.44 BC	1.11
COTE	Rocky Point	10	0.93 B	0.83	1.03	0.09	0.03	10.75 AB	0.80
RBGU	Rocky Point	10	0.45 DE	0.36	0.57	0.04	0.02	8.58 C	1.64

[a] Geometric mean Hg concentrations (μg/g dry wt; ±95% confidence interval) and arithmetic mean As and $\delta^{15}N$ values (‰; ± 1 SD) in waterbird eggs collected in 2009 from Wood Buffalo National Park (Mamawi Lake and Rocky Point) and Egg Island, Lake Athabasca. CATE = Caspian tern; COTE = common tern; HERG = herring gull; CAGU = California gull; RBGU = ring-billed gull. Means sharing the same letter are not significantly different (Tukey's HSD test for unequal sample sizes). N = number of eggs; SD = standard deviation.

* *t* test intraspecific comparisons of Hg between Mamawi Lake and Rocky Point, COTE $p = 0.007$, RBGU $p = 0.099$.

remaining 47 samples contained As at concentrations below the limit of quantification. Although most (47) samples contained detectable As, the uncertainty associated with these measurements precluded detailed interpretation of the 2009 data.

There were statistically significant differences among species and locations for Hg (ANOVA, $p < 0.0001$; Table 1). Geometric mean Hg levels were greatest in tern eggs from Egg Island and Mamawi Lake and in herring gull eggs from Egg Island (Table 1). For As, arithmetic mean values (±1 standard deviation [SD]) are reported in Table 1, but no further statistical analysis was conducted given the low As concentrations in eggs. We saw interspecific differences in arithmetic mean egg $\delta^{15}N$ values (ANOVA, $p < 0.0001$; Table 1), along with a positive correlation between egg $\log_{10}$-transformed Hg levels and stable N isotope ($\delta^{15}N$) values ($r = 0.45$, $p = 0.001$; Fig. 2) when data from all locations and species were used. Mercury in eggs is comprised primarily of methylmercury [19], which biomagnifies [20]. Organisms occupying higher trophic positions are expected to have greater Hg levels. Therefore, the positive relationship between $\delta^{15}N$ values and Hg levels was expected. However, most of the Caspian tern (9/10) and herring gull (2/2) eggs from Egg Island and half the common tern eggs from Mamawi Lake (5/10) contained greater levels of Hg than were expected based on trophic position alone (Fig. 2).

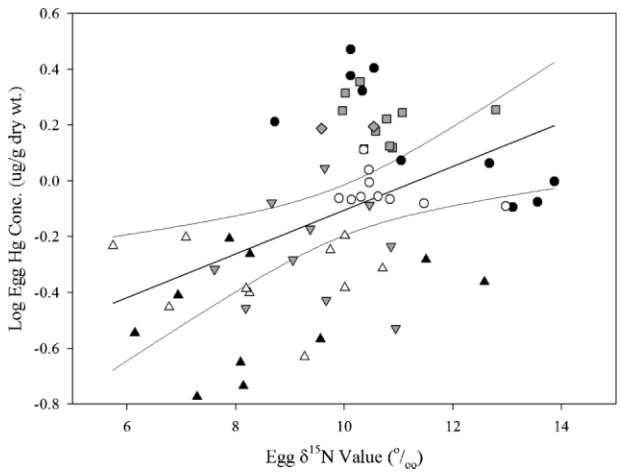

Fig. 2. Correlation (with 99% CI) between individual egg $\delta^{15}N$ value and $\log_{10}$ Hg concentration (μg/g dry wt) for eggs collected from all species and sites in northern Alberta, Canada, 2009. Squares = Caspian tern; circles = common tern; diamonds = herring gull; triangles = ring-billed gull; inverted triangles = California gull. Sites are coded by color: black, Mamawi Lake; gray, Egg Island; white, Rocky Point.

In contrast, one common tern egg from Rocky Point had Hg levels greater than were expected based on trophic position. The Mamawi Lake and Egg Island sites were in receiving waters of the Athabasca River and point to the possibility of an upstream Hg source in that river. Current egg Hg concentrations (dry wt range 0.17–2.96 μg/g, wet wt range 0.04–0.66 μg/g) were generally below levels associated with avian reproductive impairment (0.5–2.0 μg/g wet wt) [21]. Only four eggs (three common tern eggs from Mamawi Lake and one Caspian tern egg from Egg Island) contained concentrations greater than 0.5 μg/g wet weight. Furthermore, seabirds such as those examined here may be much less sensitive to Hg than other avian groups [21]. Studies of embryo survival after Hg injection into eggs indicated that tern and gull species showed low to medium sensitivity to Hg exposure [22]. Therefore, it is unlikely that current Hg levels pose a threat to these birds.

Only limited interpretation of the egg PAH data was possible because of the analysis of pooled samples. Polycyclic aromatic hydrocarbon concentrations were low, which is typical for eggs [23]. However, PAHs were above detection limits in some egg samples (Table 2) and were similar to values reported elsewhere [23]. Polycyclic aromatic hydrocarbons were detected most frequently in Caspian terns from Egg Island, followed by common terns and ring-billed gulls from Mamawi Lake and herring gulls from Egg Island. These analyses indicate that levels of Hg and PAHs were greatest at sites in receiving waters of the Athabasca River (Tables 1 and 2). Furthermore, there was a significant positive relationship between egg Hg concentrations (species/site geometric means) and levels of naphthalene in the pooled egg samples ($r = 0.79$, $p = 0.03$; Fig. 3), providing evidence of a possible common source of Hg and PAH contamination associated with the Athabasca River.

Temporal trends in egg contaminant levels, 1977 versus 2009

For all of the California gull eggs collected in 1977, Hg and As levels were above limits of quantification. Arithmetic mean (±1 SD) As concentrations (1977, 0.24 ± 0.02 μg/g dry wt; 2009, 0.06 ± 0.03 μg/g dry wt) were greater in the eggs collected in 1977 than 2009 (Mann–Whitney *U* test, $p < 0.001$). For PAHs, only naphthalene was detected in either the 1977 or the 2009 California gull egg pools. However, only in 1977 was the naphthalene concentration (12 ng/g wet wt) above detection limits. The higher concentrations in the 1977 samples could, in part, have reflected their more advanced stage of development (and concomitant loss of moisture and lipid) compared with the eggs from 2009. Low As and PAH levels in California gulls from 2009 can be explained by these gulls' relatively low

Table 2. PAH concentrations (ng/g wet wt) in pooled egg samples from all species and sites in northern Alberta, Canada, 2009[a]

Species	Site	NA	PHE	AN	B*k*F	B*a*P	D*ah*A	B*ghi*P
COTE	Mamawi Lake	1.1	ND	ND	ND	ND	ND	0.4
RBGU	Mamawi Lake	<0.33[b]	0.8	0.9	ND	ND	ND	ND
CATE	Egg Island	0.5	0.6	0.8	0.1	0.1	ND	ND
HERG	Egg Island	0.6	ND	ND	0.1	ND	ND	ND
CAGU	Egg Island	<0.33[b]	ND	ND	ND	ND	ND	ND
COTE	Rocky Point	<0.33[b]	ND	ND	ND	ND	0.1	ND
RBGU	Rocky Point	ND	ND	ND	0.1	ND	ND	ND
	DL	0.33	0.16	0.15	0.05	0.07	0.08	0.24

[a] Polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g wet wt) in egg pools collected in 2009 from Wood Buffalo National Park (Mamawi Lake and Rocky Point) and Egg Island, Lake Athabasca, Alberta, Canada. Polycyclic aromatic hydrocarbons reported were measurable in at least one pooled sample. CATE = Caspian tern; COTE = common tern; HERG = herring gull; CAGU = California gull; RBGU = ring-billed gull. DL = detection limit; ND = not detected; NA = naphthalene; PHE = phenanthrene; AN = anthracene; B*k*F = benzo[*k*]fluoranthene; B*a*P = benzo[*a*]pyrene; D*ah*A = dibenzo[*a,h*]anthracene; B*ghi*P = benzo[*ghi*]perylene.
[b] Detected but below limit of quantification.

trophic position compared with other species examined here (e.g., terns, herring gull). This limits the usefulness of California gull eggs as indicators of As and PAH temporal trends. Unfortunately, historical samples are available only from that species. Based on the results reported here, no evidence exists of temporal increases in egg As or PAH levels.

Geometric mean Hg concentrations were greater in the 2009 eggs (0.56 μg/g dry wt) compared with the 1977 eggs (0.47 μg/g dry wt), but this difference was not statistically different (*t* test, $p = 0.28$). However, comparing contaminant concentration data between years might not be appropriate, because more advanced embryonic development in the 1977 eggs would have biased egg concentrations high. Hence, comparison of egg contaminant burdens is more appropriate. Geometric mean Hg burdens were significantly greater in the 2009 eggs (34.0 μg/egg dry wt) compared with those from 1977 (24.3 μg/egg dry wt; *t* test, $p = 0.043$). Geometric mean Hg burdens in eggs increased 40%; Hg concentrations in Athabasca River walleye (*Sander vitreus*) increased approximately 30% between 1976 and 2005 [24]. At present, it is unclear what Hg sources are most important in terms of contributing to increased egg Hg concentrations through time. Further monitoring is required to confirm or refute this apparent trend. However, based on the intersite differences reported above, local sources of Hg associated with the Athabasca River may be important.

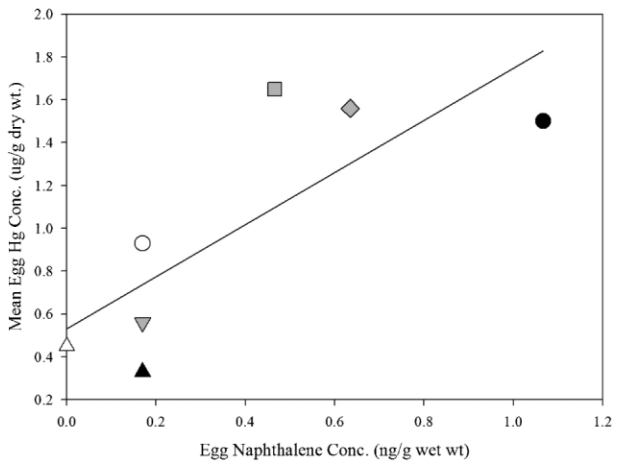

Fig. 3. Correlation between geometric mean Hg (μg/g dry wt) concentrations and naphthalene (ng/g wet wt) concentrations in pooled egg samples from all species and sites in northern Alberta, Canada, 2009. When naphthalene concentrations were lower than the limit of quantification, half the limit of detection was used. Squares = Caspian tern; circles = common tern; diamonds = herring gull; triangles = ring-billed gull; inverted triangles = California gull. Sites are coded by color: black, Mamawi Lake; gray, Egg Island; white, Rocky Point.

Future monitoring and research

The present study has provided baseline data regarding levels of contaminants in eggs of colonial waterbirds breeding in northern Alberta. Information was also generated regarding the feeding ecology of the study species ($\delta^{15}N$ values). Combined with population census information [3], these chemical and biological data begin to provide a foundation against which to evaluate future environmental change. Temporal analysis of contaminant trends indicated an increase in egg Hg burdens from 1977 to 2009 but no such increase for As or PAHs. Further monitoring is required to confirm or refute these trends, and research should focus on higher trophic level species (obligate fish-eating birds, such as terns) from a variety of sites, including reference areas (e.g., Peace River). Research is also required to identify sources contributing to increased Hg loadings in this area. Contamination from oil sands development is one possibility, but other external Hg sources must also be considered [25]. It is clear that these issues can only be addressed through increased biological and abiotic monitoring in this region. Conservation of bird populations will require regular monitoring to ensure that we have the capacity to detect environmental change and associated wildlife impacts.

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