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No relationship between gastrointestinal parasite intensities or mercury burdens on fluctuating asymmetry in Common Eider (*Somateria mollissima*) mandibles

Corey A. Scobie[1,*], Jesser G. Panopi[2], Erin M. Bayne[2], Jennifer F. Provencher[3], and H. Grant Gilchrist[4]

[1]Royal Alberta Museum, 9810–103a Avenue NW, Edmonton, Alberta T5J 0G2 Canada

[2]Department of Biological Sciences, University of Alberta, CW405 Biological Sciences Building, Edmonton, Alberta T6G 2E9 Canada

[3]Department of Biology, Carleton University, 1125 Colonel By Drive, Raven Road, Ottawa, Ontario K1A 0H3 Canada

[4]National Wildlife Research Centre, Environment and Climate Change Canada, 1125 Colonel By Drive, Raven Road, Ottawa, Ontario K1A 0H3 Canada

*Corresponding author: Corey.Scobie@gov.ab.ca

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Abstract
Mercury (Hg) emissions have increased since 1950 and biomagnification in Arctic ecosystems can affect animals, particularly at higher trophic levels. Exposure to Hg can negatively affect young developing animals, resulting in altered morphology and ultimately, lower fitness. We examined the relationship of mandible fluctuating asymmetry (FA) with gastrointestinal helminth intensity and breast muscle Hg concentration in Common Eider (*Somateria mollissima borealis*). Procrustes analysis of variance indicated significant FA but relatively high measurement error. Based on multiple linear regression modelling, there was no significant relationship between FA and Hg concentration or parasite burden. There may be a mismatch in trying to relate amount of Hg and parasite intensity in adults to FA that would have occurred early in life during skeletal development.

Key words: Mercury; parasite; Common Eider; fluctuating asymmetry; skeletal asymmetry; *Somateria mollissima*

Introduction
Mercury (Hg) emissions have increased since 1950, primarily due to coal combustion (Streets *et al.* 2011). In the atmosphere, emitted Hg may undergo reactions that result in the deposition of Hg onto the land and oceans (Krabbenhoft and Sunderland 2013). Deposited Hg is then methylated and converted into methylmercury (MeHg), which may accumulate across trophic levels, particularly in aquatic food webs (AMAP 2011; Krabbenhoft and Sunderland 2013). Methylmercury is the most toxic form of mercury to animals. In the Arctic, it is estimated that 74.2–94.4% of Hg in animals originates from an anthropogenic source (Dietz *et al.* 2009).

Like other contaminants, Hg can affect biological processes such as function of the central nervous system, hormonal regulation, and reproduction in animals. Mercury toxicity in birds often results in lower reproductive output, detrimental nesting behaviour, reduction in feeding rates, and thereby reduced juvenile survival (Scheuhammer *et al.* 2007). Compiled experimental and correlational studies also reveal Hg's adverse impacts on avian reproduction, behaviour, endocrine system, and immunocompetence (Whitney and Cristol 2018).

In general, reduced immune function caused by contaminants may increase host susceptibility to parasites (Sures 2006) and several studies have linked increased parasite load with higher Hg exposure. For instance, Glaucous Gulls (*Larus hyperboreus*) show higher acanthocephalan parasite intensities with higher Hg levels (Sagerup *et al.* 2009). Zebra Finches (*Taeniopygia guttata*) provided with MeHg in their diet showed greater coccidian parasite intensities instead of lower parasite intensities during the anticipated parasite expulsion timeframe (Smith *et al.* 2018).

Fluctuating asymmetry (FA; Klingenberg 2015) is a biological assessment that has been used to determine contaminant and parasite impacts on animals (Møller 1992; Jenssen *et al.* 2010; Rodríguez-González *et al*. 2020). FA refers to the structural discrepancies in left-right sides of a structure and its divergence from the expected ideal phenotype during an organism's development (Klingenberg 2003; Nijhout and Davidowitz 2003). FA studies focus on the idea that environmental stress lowers the individual's ability to mitigate the developmental variations on each side of the organism, resulting in higher asymmetry between the two sides of an organism's structure (Klingenberg 2015). For instance, studies on small mammals reported higher levels of skull FA with increased exposure to environmental contaminants (Oleksyk *et al.* 2004; Sánchez-Chardi *et al.* 2013; Yalkovskaya *et al.* 2016).

In this study, we evaluated the relationship between individual FA values, Hg content, and parasite intensity in Common Eider (*Somateria mollissima borealis*). Common Eiders are sea ducks found in coastal regions in the Arctic and subarctic zones (Goudie *et al.* 2000). Bivalves, gastropods, and crustaceans comprise the majority of prey items that eiders consume (Waltho and Coulson 2015). Common Eiders often become infected with endoparasitic helminths such as acanthocephalans and cestodes through their consumption of intermediate crustacean hosts such as amphipods (Friend and Franson 1999; McLaughlin 2008; Nikolov *et al.* 2008). The endoparasites harboured by eiders include different species of digeneans, cestodes, acanthocephalans, and nematodes (Bishop and Threlfall 1971; Borgsteede *et al.* 2005). Wayland *et al.* (2001a) showed that in the Canadian Arctic, nematode numbers increase with higher Hg levels in Common Eiders. Parasite stress in Common Eider ducklings are also known to reduce nutrient availability and cause inflammation of the intestinal mucosa (Hollmén *et al.* 1999). Parasites may impede the intake of necessary nutrients by inducing mucosa layer damage in the gastrointestinal tract (Hollmén *et al.* 1999).

Overall, the stresses induced by Hg contamination and the resulting parasite intensity during development in young Common Eiders might reduce the ability of individuals to alleviate developmental variations on the skull, leading to higher observable FA in adults. Therefore, we predicted greater FA in the skulls of adult Common Eiders with higher levels of Hg and greater gastrointestinal parasite intensity. In this study, we assumed that the Hg concentration and parasite intensity in adults reflects Hg exposure and parasites during development.

Methods

Eiders were collected in Cape Dorset, Nunavut in May 2011 as part of the annual Indigenous hunt. We used the skulls from 39 adult male Common Eiders, along with the corresponding wing chord (cm), total Hg (dry weight) in pectoral muscle tissue, and genus level helminth parasite intensity for each individual (see Provencher *et al.* [2016] for additional details of methods used to determine amount of mercury and parasite intensity; the parasite intensity parameter Provencher *et al.* [2016] used included non-infected birds that both Margolis *et al.* [1982] and Rózsa *et al.* [2000] consider to be parasite abundance, not intensity). The skin was removed from the cranium and lower mandible of each specimen, which were then cleaned by Dermestid Beetles (*Dermestes maculatus*) and bleached with 3% hydrogen peroxide.

Landmarks (distinct locations for three dimensional measurements) chosen for FA analysis for vertebrate skulls often try to capture the whole shape of the skull (Oleksyk *et al.* 2004; Urošević *et al.* 2015; del Castillo *et al.* 2016). Mandibles were chosen for digitization rather than the whole skull because past FA studies show that the greatest potential effects of contaminants are on mandible FA (Sánchez-Chardi *et al.* 2013; Yalkovskaya *et al.* 2016). As mentioned in Klingenberg (2015), structures that have object symmetry, like skulls, should possess single landmarks on the midline of the structure and paired landmarks on the left and right side of the structure to ensure the required data are gathered for the analyses. Based on these criteria, we chose dorsal, lateral, and ventral landmarks that reflected the overall mandible shape. Measurement errors that can affect FA analysis are often associated with difficulty in finding and distinguishing the landmarks on the structure (Klingenberg 2015). To reduce the likelihood of measurement errors, the locations of the landmarks in this study were chosen because they were easily distinguished and past studies have found them to be repeatable.

In total, 20 landmarks were digitized on 39 mandibles (Figures 1 and 2; Table 1) using a MicroScribe 3D Digitizer (Solution Technologies Inc., Oella, Maryland, USA). Ten mandibles (26%) were digitized twice to calculate measurement error. Mandibles were secured in modelling clay on a raised wooden platform (11.0 cm tall × 4.5 cm wide × 9.7 cm long) clamped to a steady table. An elastic further secured the mandible by holding it between landmarks 5 and 9, 15 and the platform. All landmark digitization occurred from October 2017 to August 2018 and was performed by J.G.P.

Procrustes fit and Procrustes ANOVA (analysis of variance) in program MorphoJ (Klingenberg 2011) was used to acquire FA scores. The Procrustes fit removes configurational size, position and orientation differences, and determines shape differences among individuals (Klingenberg 2015). A Procrustes

FIGURE 1. Landmarks on the dorsal surface of a Common Eider (*Somateria mollissima*) mandible. Photo: C.A. Scobie.

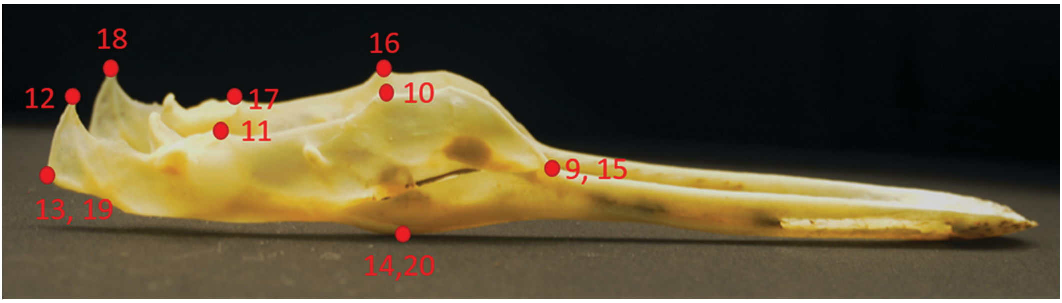

FIGURE 2. Landmarks on the lateral surface of a Common Eider (*Somateria mollissima*) mandible. Landmarks with two numbers associated with it are replicated in the same area on the opposite lateral surface of the mandible. Photo: C.A. Scobie.

TABLE 1. Landmark definitions on the lateral and dorsal surfaces of the Common Eider (*Somateria mollissima*) mandible.

No.	Definition
Dorsal	
1	Tip of dentary (adjacent to the very anterior point where dentary splits into two)
2, 3	Supra-angular
3, 7	Very posterior tip of the protruding process towards the inside of the mandible in the angular/articular region
4, 6	Most anterior tip of the protruding process towards the inside of the mandible in the angular/articular region
5	Most posterior point where the dentary splits into two (adjacent with dentary tip)
Lateral	
9, 15	Point where the dentary articulates with the other bones. Point directly adjacent to the vacuity, occurs at the bottom of the curved bone
10, 16	Uppermost point of the protruding process before the supra-angular
11, 17	Pointed edge in the articular/angular region the lies just before articular surface
12, 18	Uppermost pointed tip of the articular
13, 19	Most posterior point of the curve at the bottom of the articular
14, 20	Most posterior point in the articulation between dentary and the latter half of the mandible (or the vacuity)

fit was selected with alignment with the principal axes. A Procrustes ANOVA was then used to acquire FA values for each individual, with the assumption that isotropic variation at all landmarks was identical. Procrustes ANOVA uses the total variation derived from the differences between each individual configuration and the average configuration and allocates it into individual, reflection (comparison of sides of symmetrical object), and the interaction between individuals and reflection variation, as well as measurement error (Klingenberg *et al.* 2002). MorphoJ provides individual FA values as Procrustes FA values or Mahalanobis distances (Klingenberg and Monteiro 2005; Klingenberg 2011).

Total Hg was measured from muscle tissue from the left pectoral muscle of each eider. Most of the total Hg found in aquatic birds is comprised of the toxic form (MeHg; Houserova *et al.* 2007), so we used total Hg in our analyses with the assumption that most was likely MeHg. The intestines of each eider were examined thoroughly and all helminths found were identified to genus. Initial dataset exploration showed right-skewed counts for *Lateriporus* (cestode or tape worm), *Microsomacanthus* (cestode), and *Profilicollis* (acanthocephalan or spiny-headed worm) data. These data were log-transformed to remove non-normality. Program STATA 11 (StataCorp 2009) was used to perform a multiple linear regression analysis involving Hg concentration (Hg/g of dry weight) and parasite intensity for log-*Lateriporus*, log-*Microsomacanthus*, and log-*Profilicollis* and categorical variables *Fimbriarioides* (a cestode), unidentified cestode, in relation to Mahalanobis distances measuring FA, while controlling for wing length.

Results

The analysis indicated highly significant variation in symmetry among individuals ($F_{1026,988} = 6.72$, $P < 0.0001$; Table 2). Similarly, directional asymmetry or the variation among reflections was significant ($F_{26,988} = 47.80$, $P < 0.0001$; Table 2), which means there was variation among averages of the two sides of the left and right side of the mandibles. The analysis also showed significant FA or reflection variations among individuals ($F_{988,477} = 2.43$, $P < 0.0001$; Table 2), indicating a difference between the average of all left sides and all right sides of the mandibles. The F-value for the interaction between individual and reflection (Table 2) indicates the magnitude of FA relative to the measurement error (Klingenberg 2015). Our F-ratio indicates that measurement error was relatively high, but twice as much variation was explained by FA than measurement error (Table 2).

Mercury was detected in the muscle tissue of all eiders and they had an average concentration of 0.7 µg/g dry weight (Table 3). Parasite intensity and Hg concentration did not significantly predict variation in Mahalanobis distances ($R^2 = 0.17$, $F_{7,31} = 0.90$, $P = 0.51$; Table 3). An apparent positive relationship between mercury and FA (Figure 3; Table 3) was not significant ($t_{38} = 1.81$, $P = 0.08$).

Discussion

We did not find a significant relationship between

TABLE 2. Procrustes ANOVA results for adult male Common Eider (*Somateria mollissima*) mandibles assuming identical isotropic variation for all landmarks. Results include analyses of digitized left and right sides of 39 mandibles, each with nine paired landmarks and two median landmarks.

Variables	SS	MS	df	*F*	*P*
Individual	0.140	0.00014	1026	6.72	<0.0001
Reflection	0.025	0.00097	26	47.80	<0.0001
Ind × Reflection	0.020	0.00002	988	2.43	<0.0001
Error	0.004	0.00001	477		

TABLE 3. Results of multiple linear regression analysis looking at Mahalanobis fluctuating asymmetry values of 39 adult male Common Eider (*Somateria mollissima*) mandibles from Cape Dorset, Nunavut, Canada in relation to mercury content and parasite intensity. Descriptive statistics are also provided for variables included in the model: average (median for parasite intensity), range of variables, and prevalence of each type of parasite.

Variable	β	SE	*P*	Average (range)	Prevalence (%)
Wing	−0.14	0.10	0.18	29.5 cm (27.5–31.1)	—
Mercury	0.67	0.37	0.08	0.7 µg/g (0.3–1.2)	—
Lateriporus (cestode)	−0.07	0.07	0.36	9 (0–191)	90
Profilicollis (acanthocephalan)	0.01	0.06	0.87	4.5 (0–144)	69
Fimbriarioides (cestode)	−0.22	0.74	0.11	0 (0–1)	3
Unidentified cestode	−0.10	0.54	0.85	0 (0–2)	5
Microsomocanthus (cestode)	−0.02	0.05	0.75	100 (0–1000)	87
Intercept	7.07	3.02	0.03		

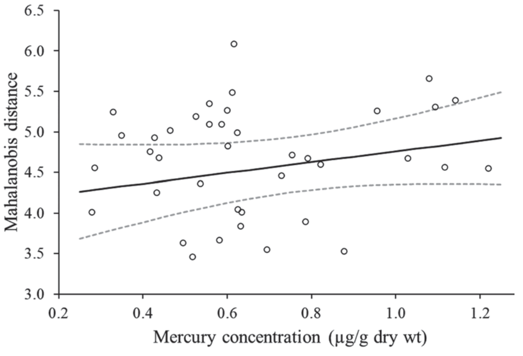

FIGURE 3. Predicted Mahalanobis fluctuating asymmetry values of 39 adult male Common Eider (*Somateria mollissima*) mandibles from Cape Dorset, Nunavut, Canada in relation to mercury concentration. The predictive model used median values of *Lateriporus*, *Profilicollis*, and *Microsomacanthus* and reference categorical values for *Fimbriarioides* and unidentified cestodes. Open circles are the raw data used in the model. Dashed lines represent 95% confidence interval.

FA in Common Eider mandibles and Hg concentration or parasite intensity. Our results do not support our initial hypothesis of higher FA with increasing muscle tissue Hg levels and parasite burden.

Unfortunately, Hg concentration in adult eider tissues does not necessarily reflect the amount of mercury birds are exposed to during skeletal development. Because birds have several ways of reducing their Hg load, we are unable to determine the amount of Hg to which eiders were exposed during development using Hg levels in the tissues of adult eiders. For instance, adult Cory's Shearwater (*Calonectris borealis*) deposit dietary Hg into their feathers (Monteiro and Furness 2001). Reduced brain, muscle, and liver Hg levels were also found after molting and Hg excretion in European Starling (*Sturnus vulgaris*; Whitney and Cristol 2017). The different Hg elimination processes throughout the avian body could have decreased the Hg load in the eiders in our study, making adult Hg levels a poor reflection of Hg levels experienced during development.

An antagonistic interaction between consumed Hg and Selenium (Se) during development may have also reduced any impact of Hg on mandible symmetry. Selenium is found in relatively high levels in Arctic waterfowl (Stout *et al.* 2002; Braune and Malone 2005), including Common Eiders (Wayland *et al.* 2001b). Methylmercury is converted to inorganic Hg in animal tissues, where it may bind to Se and prevent further damage in the animal's body (Eagles-Smith *et al.* 2009; Scheuhammer *et al.* 2015). In Japanese Quail (*Coturnix japonica*) chicks, continuous exposure to Hg and Se diets led to dramatic mortality declines while exposure to Hg-only diets led to high mortality (Stoewsand *et al.* 1974). Studies on Mallard (*Anas platyrhynchos*; Hoffman and Heinz 1998) and Shaoxing Duck (*Anas platyrhynchos domesticus*; Ji *et al.* 2006) also attributed Se exposure and diet to the increases in antioxidant enzymes such as glutathione peroxidase, glutathione, and superoxide dismutase which reduces tissue and neurological damage and promotes MeHg removal from the body. For our study, Hg and Se intake during development possibly reduced the negative impacts of MeHg in Common Eiders through increased protection from oxidative stress.

In comparison to other studies, Common Loon (*Gavia immer*) with higher Hg burdens (>40 µg/g) showed greater feather weight asymmetry compared to adults with smaller Hg loads (<10 µg/g; Evers *et al.* 2008). These Hg levels are much greater than the maximum found in our study (1.2 µg/g). Herring *et al.* (2017) found inconsistent relationships between overall FA and tissue Hg levels in various water birds. They found no relationships between the overall FA and total blood and feather Hg levels for American Avocet (*Recurvirostra americana*), Black-necked Stilt (*Himantopus mexicanus*), and Caspian Tern (*Hydroprogne caspia*), but overall FA increased with higher Hg levels in breast feathers and blood for Forster's Tern (*Sterna forsteri*; Herring *et al.* 2017). Herring *et al.* (2017) concluded that different avian structures may exhibit different relationships with Hg levels in different tissues and that some species may not show relationships with FA and Hg. Further comprehensive studies should determine which species exhibit Hg-related FA by examining multiple structures at once and pinpointing the most affected structure due to contaminant exposure. Although prevalence was high, the amount of mercury found in Common Eiders in our study was quite low compared to other studies (0.83 µg/g blood Hg; Meattey *et al.* 2014) that also did not find adverse effects related to mercury.

We also did not find any relationship between FA and any of the helminth intensities in our study. Camphuysen *et al.* (2002) compiled levels of infection with *Profilicollis botulus* in Common Eider and found prevalence ranged from 76.7 to 100% with mean abundance ranging from 30 to 271 worms with a maximum of 1270 parasites in a single bird. Kats *et al.* (2007) also found *P. botulus* infection levels in Common Eider (prevalence = 83.8%; mean number = 109, range = 1–2938) far above what we observed, but infection did not contribute significantly to negative effects on body condition, regardless of age. The natural infection rates found in healthy juvenile and adult eiders suggest that helminths may not severely impact juvenile growth and development.

The Procrustes ANOVA results indicate moderate measurement error during mandible digitization, which could have contributed to our non-significant results. Van Dongen (2015) showed that high measurement error in FA studies weakens the relationship between the true and estimated individual FA values and leads to biased FA estimates. It is possible that the measurement error in our study resulted in underestimated or overestimated individual FA values, which possibly prevented us from finding significant relationships. Variation among individuals for some of the landmarks potentially led to inconsistencies in landmark digitization for the whole group of individuals, which resulted in higher measurement error. Future studies should focus on increasing sample size, choosing better and more pronounced landmarks, and allocating time to practice digitization before each session to reduce measurement error. If feasible, an alternate method could be used where the mandibles are scanned three-dimensionally and digital techniques used to measure symmetry. Checking all the samples with a pilot study may also be beneficial for determining problematic or appropriate landmarks to use and whether replicates are needed for the main study (Klingenberg 2015).

Author Contributions

Writing – Original Draft: J.G.P.; Writing – Review & Editing: C.S. and E.B.; Conceptualization: C.S.; Investigation: J.F.P. and J.G.P.; Methodology: G.G., J.F.P., and C.S.; Resources: G.G., J.F.P., C.S., and E.B.; Formal Analysis: J.F.P., C.S., and E.B.; Funding Acquisition: G.G. and J.F.P.; Visualization: J.G.P.

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