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Levels of Mercury in Feathers of Clapper Rails (*Rallus crepitans*) over 45 Years in Coastal Salt Marshes of New Hanover County, North Carolina

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Abstract We sampled clapper rail (*Rallus crepitans*) feathers from museum specimens collected between 1965 and 2010 to investigate changes in mercury (Hg) availability in coastal marshes of New Hanover County, North Carolina. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was conducted to control for dietary shifts that may have influenced Hg exposure. Hg concentrations ranged from 0.96 to 9.22 µg/g (ppm), but showed no significant trend over time; diet ($\delta^{15}N$) or foraging habitat ($\delta^{13}C$) also provided little to no explanatory power to the variation in Hg concentrations among clapper rails. Our findings suggest the bioavailability of Hg to clapper rails in coastal North Carolina salt marshes has remained consistent over time, despite the global trend of increasing mercury in many other bird species, providing an excellent baseline for any future assessment of Hg availability to salt marsh birds in coastal North Carolina.

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Wetlands, fresh and estuarine, play an important role in the mobility and bioavailability of Hg, serving both as a sink for gaseous and inorganic forms of Hg (Hg^0 and Hg(II), respectively) as well as a net source for methylmercury (CH_3Hg or MeHg; Selvendiran et al. 2008). MeHg is the most bioavailable form of Hg that bioaccumulates and biomagnifies in aquatic and terrestrial food webs and can have significant impacts on wildlife – from reproductive impairment to decreased survival, and ultimately population-level impacts (Brasso and Cristol 2008). Owing to their hydrological properties and sediment characteristics and microbiota (including abundant sulfate-reducing bacteria) salt marshes typically show higher rates of methylation and subsequently higher concentrations of MeHg than open water habitats (Williams et al. 1994; Benoit et al. 2013; Heyes et al. 2006). Songbirds breeding and overwintering in freshwater wetlands and salt marshes across the country accumulate potentially harmful concentrations of Hg, even in remote areas (Shriver et al. 2006; Jackson et al. 2011; Winder and Emslie 2012; Winder et al. 2012).

Clapper rails (*Rallus crepitans*) are marsh birds that live in salt and brackish water marshes along North America's coastlines. As a resident species, clapper rails show a high degree of site fidelity and have a well-documented diet (Novak and Gaines 2006). In North Carolina, the diet is comprised primarily of the Atlantic marsh fiddler crab (*Uca pugnax*), a prey species of many other marsh birds, and part of the filtering and cycling of MeHg (Heard 1982; Koga et al. 1998; Cumbee et al. 2008). With strong site fidelity in salt marsh habitat, resident clapper rails can serve as excellent biomonitors of environmental

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contaminants, such as mercury (Hg) in salt marsh ecosystems (Odom 1971; Cumbee et al. 2008).

The purpose of the present study was to document the trend of Hg concentrations in clapper rails in coastal North Carolina by sampling museum specimens collected over a 45 year time period. The use of museum specimens allowed an assessment of historical Hg exposure within New Hanover County. Concurrent changes in trophic position were also investigated through the use of stable isotope analysis ($\delta^{15}N$, $\delta^{13}C$). Nitrogen isotopic values ($\delta^{15}N$) in avian tissues are commonly used to infer trophic level and diet, while carbon isotopic values ($\delta^{13}C$) help trace trends in habitat use (Peterson and Fry 1987; Jardine et al. 2006). The $\delta^{13}C$ values can aid in identifying the foraging habitat of an individual, whether terrestrial, aquatic, or marine through isotopic changes that occur during carbon fixation by the primary producer in a given food web (Peterson and Fry 1987; Jardine et al. 2006). The concurrent analysis of Hg concentrations and stable isotopes ($\delta^{15}N$, $\delta^{13}C$) in clapper rail feathers could provide explanatory power to any temporal variation in Hg concentrations that may have resulted from trophic shifts or changes in foraging habitat in this population over time (see Brasso and Polito 2013).

Materials and Methods

Feathers were sampled from museum study skins of resident clapper rails collected in New Hanover County, North Carolina, during summer months from 1965 to 2010 (n = 63). We used birds collected only in New Hanover County to control for natural variation in isotope composition and Hg availability that can occur at broader geographic scales. Forty-six of the specimens sampled are housed at the University of North Carolina Wilmington (UNCW) Natural History Collection and 14 are housed at the North Carolina Museum of Natural Sciences in Raleigh. Three additional rails were collected from local hunters in New Hanover County in 2010. No mercuric preservatives were applied to any museum specimens used in this study. Independent of collection, each specimen had three body contour feathers plucked from the upper half of the breast to control for molt on each resident bird during the same season (Pyle 2008). The vane of three feathers per individual were cut into small (~3 mm) segments using stainless steel scissors to create a single sample from each bird. A random sub-sample of feather segments was removed for Hg analysis (~0.01 g) and a second random sub-sample was removed for stable isotope analysis to have paired stable isotope and Hg values from a mixture of the same three feathers per individual.

Feather segments for Hg analysis were rinsed in three cycles of acetone and deionized water to remove any confounding external deposits, and then air dried under a fume hood for 24 h (Hobson 1999). Samples were analyzed by cold-vapor atomic absorption spectroscopy for total Hg using a Milestone DMA-80 (Milestone Inc, Sheldon CT, USA). Because nearly all Hg present in feathers is in the form of methylmercury, a measurement of total Hg concentration was used as a proxy for the highly bioavailable form (Bloom 1992; Bond and Diamond 2009; Payne and Taylor 2010). Hg concentrations were only analyzed in a sub-set of individuals for which stable isotope values were available owing to budgetary and time constraints (n = 30, stratified across decades).

Each set of 20 samples analyzed was preceded and followed by two method blanks, a sample blank, and two samples each of standard reference material (DORM-4, DOLT-5; fish protein, and dogfish liver certified reference materials, respectively, provided by National Research Council Canada). All feather Hg concentrations are reported as parts per million (ppm), fresh weight. Mean percent recoveries for standard reference materials were 92.3 % ± 5.0 % (DORM-4) and 83.8 % ± 7.9 % (DOLT-5). Percent difference between duplicates of standard reference materials were 5.5 % and 9.4 %, respectively. The detection limit of the assay was 0.005 ng Hg.

To remove lipids which could affect the isotope readings, the feather segments were soaked in a 2:1 (chloroform: methanol) solution overnight, then drained. After draining, the feathers were rinsed again in a fresh methanol solution and allowed to dry completely overnight. A total of 400–500 µg from each feather sample was then loaded into tin cups and flash-combusted to analyze for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes through an interfaced Delta V Plus continuous-flow stable isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Raw δ values were normalized on a two-point scale using depleted and enriched glutamic acid reference materials USGS-40 and USGS-41 (National Institute of Standards and Technology, Gaithersburg, MD, USA). Sample precision was 0.1 and 0.2 for ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), respectively. Stable isotope ratios are expressed in δ notation in per mil units (‰), according to the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \cdot 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The $R_{standard}$ values were based on the Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

Log-transformed Hg concentrations were used in all statistical comparisons. Linear regression analyses were used to examine the relationships between log Hg concentrations and $\delta^{13}C$ and $\delta^{15}N$. Linear regression analysis also was used to independently examine the relationship between log Hg concentrations, $\delta^{13}C$, and $\delta^{15}N$ as well as each metric over time (time treated as a continuous variable). All means are provided ± SD; untransformed Hg concentrations were used in figure axes for clarity. All analysis were completed in R, Version 3.3.0 (R Core Team 2016).

Results and Discussion

Total Hg concentrations in feathers from clapper rails collected in coastal North Carolina from 1965 to 2010 ranged from 0.96 to 9.22 ppm. Hg concentrations ($X^2 = 1.65$, $df = 4$, $p = 0.80$), $\delta^{15}N$ values ($X^2 = 5.00$, $df = 4$, $p = 0.29$), and $\delta^{13}C$ values ($X^2 = 1.38$, $df = 4$, $p = 0.85$) did not differ significantly among decades with the Kruskal-Wallace test and we found no relationships between Hg, $\delta^{15}N$, or $\delta^{13}C$ over time using linear regression (Fig. 1). While there appeared to be a significant, positive relationship between Hg concentration and $\delta^{15}N$, the model fit was weak ($R^2 = 0.13$, $p = 0.054$). No relationship was found between Hg and $\delta^{13}C$, nor $\delta^{15}N$ and $\delta^{13}C$ (Fig. 2).

Hg concentrations in the feathers of clapper rails collected in New Hanover County, North Carolina, between 1965 and 2010 varied significantly among individuals and no temporal trend was detected. Feather Hg concentrations averaged 3.12 ± 1.94 ppm over the 45 year study period. Of the 30 birds collected, 53 % (16/30) had Hg concentrations that exceeded the lowest observed adverse effects level (LOAEL) correlated with a 10 % reduction in

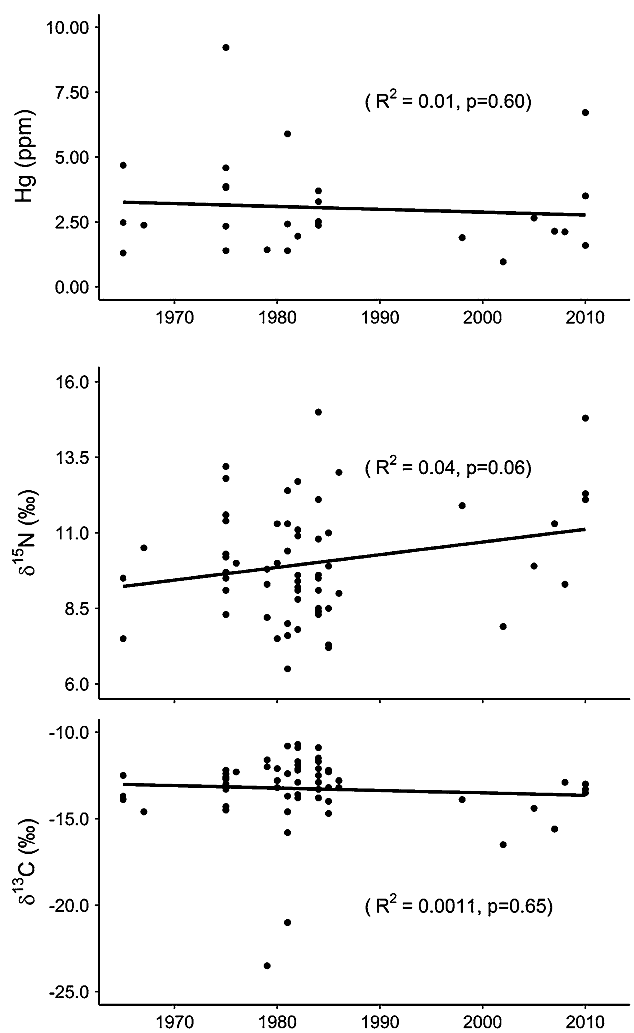

Fig. 1 Trends in Hg concentrations (*top*), diet ($\delta^{15}N$, *middle*), and foraging habitat ($\delta^{13}C$, *bottom*) of clapper rails in New Hanover County, North Carolina, over the past 45 years

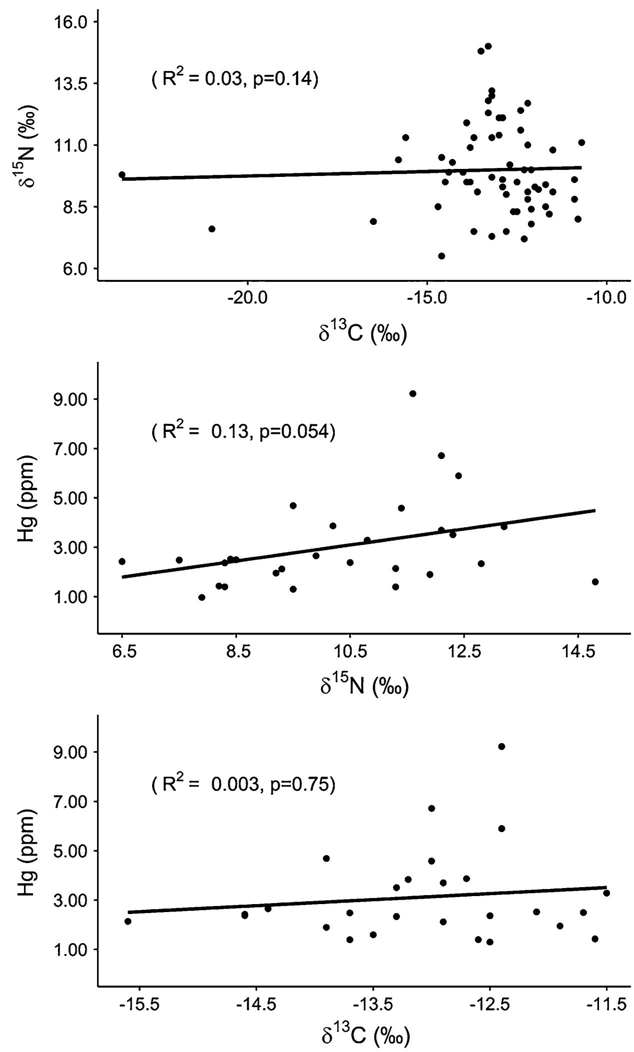

Fig. 2 Relationships between diet ($\delta^{15}N$) and foraging habitat ($\delta^{13}C$), diet ($\delta^{15}N$) and Hg concentrations, and foraging habitat ($\delta^{13}C$) and Hg concentrations in clapper rails from New Hanover County, North Carolina, for all years combined

reproductive success in songbirds (2.4 ppm; Jackson et al. 2011). There were individual birds that exceeded 2.4 ppm in all decades exclusive of the single individual collected in 1990. However, it should be noted that no clear LOAEL for birds currently exists in the literature; reported values range dramatically, between 5.0 and 40.0 ppm, depending on species (Wolfe et al. 1998; Burger and Gochfeld 2000; Evers et al. 2008) in which case only 13 % (4/30) individuals in the present study would be considered at risk for adverse effects.

Despite a range of $\delta^{15}N$ values within and among years, individuals with higher $\delta^{15}N$ values did not necessarily have higher Hg concentrations as would be predicted based on the principle of biomagnification. It appears that the diet and foraging habits of clapper rails have remained constant over past 45 years. Individual variation in Hg accumulation, to the degree found in the present study, is fairly common within a population at any point in time (Brasso and Cristol 2008; Jackson et al. 2011; Hartman et al. 2013). However, the lack of a positive or negative temporal trend in Hg over the past 45 years in New Hanover County along with a consistent diet suggests that the environmental conditions related to the bioavailability of Hg have remained fairly stable in this region.

A recent study of *Ammodramus* sparrows wintering in coastal North Carolina salt marshes found 16 %–55 % of individuals (*A. nelson*, 16 %; *A. caudacutus*, 25 %; and *A. maritimus*, 55 %) to have Hg concentrations in their body feathers that fell above the lowest adverse effects level linked to impaired reproduction (Winder and Emslie 2011, 2012). This result raised concern for clapper rails as they occupy similar habitats as sparrows in this region, though they do so year-round. While no previous work has been conducted to assess the risk of exposure to Hg in this region, several studies have investigated Hg accumulation and its effects in clapper rails across their range. Clapper rail embryos were ranked as showing medium sensitivity to MeHg (on a scale of low, medium, high) with LC_{50}s (lethal concentration required to kill 50 % of the population) reached at concentrations between 0.25 and 1 ppm of MeHg injected into wild-collected eggs (Heinz et al. 2009). Lonzarich et al. (1992) found clapper rail eggs in North Carolina had mean mercury levels of 0.15 ppm (0.12–0.33). Further, clapper rails in an estuarine marsh in Georgia showed evidence of a positive relationship between Hg concentration (mean muscle Hg: 1.40 ppm, wet weight) and the degree of double DNA strand breakage in birds from a contaminated site (Novak and Gaines 2006). Ackerman et al. (2012) found a negative relationship with mercury concentration and body condition in California clapper rails in which body mass losses of 5 %–7 % were found across the range of Hg concentrations in breast feathers (3.68–20.2 ppm).

A common challenge when investigating Hg exposure in a population is the lack of baseline data, especially over long time spans. Global trends in mercury over similar and longer time frames than ours in other bird species, including albatrosses, gulls and petrels have shown an increasing trend (Thompson et al. 1993, Burgess et al. 2013, Bond et al. 2015). Museum specimens are excellent resources for providing a direct means for assessing historic conditions to address temporal gaps in the literature. In this case, breast feathers from a resident species, the clapper rail, have proven to be a valuable source of long-term data for assessing changes in Hg exposure in coastal North Carolina salt marshes. While the Hg concentrations and limited dietary fluctuation of non-migratory resident birds suggest stability in this ecosystem, the elevated Hg concentrations in just over half of the individuals sampled suggests that portions of the population, as well as other more sensitive or higher trophic level species, may be at risk of adverse effects.

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