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Dietary mercury exposure to endangered California Clapper Rails in San Francisco Bay

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ABSTRACT

California Clapper Rails (*Rallus longirostris obsoletus*) are an endangered waterbird that forage in tidal-marsh habitats that pose risks from mercury exposure. We analyzed total mercury (Hg) in six macro-invertebrate and one fish species representing Clapper Rail diets from four tidal-marshes in San Francisco Bay, California. Mercury concentrations among individual taxa ranged from lowest at Colma Creek (mean range: 0.09–0.2 μg/g dw) to highest at Cogswell (0.2–0.7), Laumeister (0.2–0.9) and Arrowhead Marshes (0.3–1.9). These spatial patterns for Hg matched patterns reported previously in Clapper Rail blood from the same four marshes. Over 25% of eastern mudsnails (*Ilyanassa obsolete*) and staghorn sculpin (*Leptocottus armatus*) exceeded dietary Hg concentrations (ww) often associated with avian reproductive impairment. Our results indicate that Hg concentrations vary considerably among tidal-marshes and diet taxa, and Hg concentrations of prey may provide an appropriate proxy for relative exposure risk for Clapper Rails.

1. Introduction

The California Clapper Rail (*Rallus longirostris obsoletus*, hereafter 'Clapper Rail') is a state and federally listed endangered species restricted to tidal marsh habitats in the San Francisco Bay in California (USFWS, 1973). Population declines have been attributed to an array of factors including market hunting in the late 19th and early 20th centuries, predation by introduced mammals, habitat loss, and exposure to environmental contaminants (Albertson and Evens, 2000; Takekawa et al., 2011; USFWS, 2013). Indirect linkages between habitat loss and environmental contaminants pose contemporary trade-offs for Clapper Rail population recovery. Widespread loss of tidal marsh habitat to salt production ponds and drainage for urban in-fill, coupled with habitat alterations caused by invasive plants and impending sea level rise, represent salient threats to the recovery of this tidal-marsh obligate species (USFWS, 2013). At the same time, sediments in the San Francisco Bay are highly polluted with mercury (Hg) derived from historic mercury and gold mining in the Bay's expansive watershed (Davis et al., 2012; Donovan et al., 2013). Importantly, the many channels and sloughs bisecting tidal marsh habitats are not only areas where Clapper Rails forage, but they also are conducive for converting inorganic Hg to highly toxic and bioaccumulative methyl Hg (MeHg) because they experience frequent wetting and drying from tidal cycles (Marvin-DiPasquale et al., 2003) and have high inputs of organic matter derived from high rates of primary production (Lambertsson and Nilsson, 2006). Hence, tidal marsh habitats required by Clapper Rails also present inherent risks to birds by exposure to Hg. High levels of Hg in Clapper Rail eggs were first reported by Lonzarich et al. (1992), and Hg exposure has since been implicated as a potential cause of reduced reproductive output (Schwarzbach et al., 2006) and impaired body condition (Ackerman et al., 2012). Clapper Rail embryos also have relatively lower survival rates than other waterbird species when exposed to *in-ovo* MeHg dosing (Heinz et al., 2009), which further highlights the potential risk that Clapper Rails have to Hg exposure in tidal marsh habitats.

Elucidating sources of Hg exposure to Clapper Rails is important for developing conservation strategies to promote species recovery. San Francisco Bay is one the most intensively studied natural laboratories for Hg ecotoxicology in the world (Davis et al., 2012), yet few studies have quantified variation in Hg among tidal-marsh macro-invertebrates and fish that are integral components of Clapper Rail diet. Determining variation in Hg concentrations associated with specific dietary taxa is necessary to explain

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trophic routes of Hg exposure and relative Hg risk to Clapper Rails. Rates of bioaccumulation of Hg in biota vary spatially in San Francisco Bay due to variation among *in-situ* biogeochemical factors that facilitate Hg methylation, and to a lesser degree, proximity to sources of inorganic Hg entering the Bay (Conaway et al., 2008; Davis et al., 2012; Eagles-Smith and Ackerman, 2009; Greenfield and Jahn, 2010; Greenfield et al., 2013a; Marvin-DiPasquale et al., 2003). Further, Clapper Rails are spatially constrained to small home ranges that rarely include more than one marsh (Casazza et al., 2008; Overton et al., 2014; Rohmer, 2010; Takekawa et al., 2011), and Clapper Rail Hg concentrations vary substantially among tidal marshes (Ackerman et al., 2012). Identifying whether Hg concentrations in Clapper Rail diet items also differ among tidal marshes would further illustrate the localized exposure of Hg contamination.

Our objectives were to: (1) describe variation in Hg concentrations among a wide range of Clapper Rail diet taxa across four tidal-marshes in South San Francisco Bay; (2) compare these patterns to Hg in adult Clapper Rails from the same marshes (Ackerman et al., 2012); and (3) assess the relative Hg risk to Clapper Rails associated with each diet taxa.

2. Methods

2.1. Study area and sample collection

We collected invertebrate and fish taxa representing dietary items consumed by Clapper Rails (Albertson and Evens, 2000; Rush et al., 2012) at the four tidal marsh in South San Francisco Bay described by Ackerman et al. (2012) and Overton et al. (2014). Briefly, the 4 marshes from north to south comprised Arrowhead (10 ha), Colma Creek (25 ha), Cogswell (60 ha), and Laumeister (36 ha) (Fig. 1). Invasive hybrid cordgrass (*Spartina alterniflora* × *foliosa*) dominated Arrowhead, Colma, and Cogswell, whereas as native pickleweed (*Sarcocornia pacifica*), gumplant (*Grindelia* sp.), and Pacific cordgrass (*Spartina foliosa*) characterized marsh vegetation at Laumeister.

We collected diet samples at all marshes prior to the breeding season during the winter months (December – March) of 2008 – 2009 and 2009 – 2010. We targeted macro-invertebrates and fish representative of the broad dietary breath of Clapper Rails (Albertson and Evens, 2000; Rush et al., 2012) that included eastern mudsnail (*Ilyanassa obsolete*), Baltic clam (*Macoma balthica*), soft-shell clam (*Mya arenaria*), ribbed horse mussel (*Geukensia demissa*), polychaete ragworms, (family Neridae), mud crab (*Hemigrapsus* sp.), and staghorn sculpin (*Leptocottus armatus*). Of these diet items, mudsnails, soft-shell clams, and ribbed horse mussels are non-indigenous in San Francisco Bay (Cohen and Carlton, 1995). Mudsnails, clams, mussels, and ragworms were collected by either sieving surficial sediments (0–10 cm) in marsh channels through a 5.0 mm mesh or hand-picking, and minnow traps were used to collect sculpin and mud crabs. Diet samples were collected at 3–5 tidal channels spaced >100 m apart that spanned the interior, mid, and outer regions of each marsh. Samples collected at each channel were placed in separate Whirl-Pac™ or clean Ziploc™ bags, stored on ice, and frozen at −20 °C within 12 h of collection. All sampling occurred during low-tide.

2.2. Sample preparation and Hg analysis

Prior to Hg determination, all samples were scrubbed free of external mud and debris with deionized water, measured to nearest cm in length, and patted dry with lint-free wipes. Shells and carapaces were removed from mollusks and soft-tissues analyzed for Hg. Staghorn sculpin were analyzed for Hg on a whole body basis following the methods of Eagles-Smith and Ackerman (2009). Ribbed horse mussel and staghorn sculpin were large enough to analyze for Hg on an individual basis. Multiple (3–6) individuals for eastern mudsnail, Baltic clams, soft-shell clams, and ragworms required compositing to achieve adequate sample mass (>1.0 g, wet weight (ww)) for Hg determination. For these taxa, individuals were composited by size (±1–2 cm length) to minimize any size-oriented variation in Hg.

Samples were oven-dried at 40 °C for 48–72 h until a constant dry-mass was achieved, then ground to a fine powder with a mortar and pestle. Concentrations of total Hg were determined following U.S. Environmental Protection Agency Method 7473 using a Milestone DMA-80 Direct Mercury Analyzer (Milestone, Monroe, CT, USA). Protocols for quality control and assurances are described by Ackerman et al. (2012). In our study, recoveries of Hg from certified reference materials and calibration verifications averaged 98.7% (SD = 3.0, $n = 18$) and 98.2% (SD = 6.7, $n = 29$), respectively. Relative percent difference for duplicate samples averaged 7.9% (SD = 8.0, $n = 19$). All concentrations are reported as total Hg.

2.3. Statistical analysis

We tested for differences in Hg concentrations (dry weight, dw) among marshes within each taxonomic group using analysis of variance in Program R (R-Core-Team, 2012). We fit animal length (binned by size class) as a covariate to further account for differences in size that could be related to variation in Hg burden. All Hg concentrations were natural log transformed prior to analysis, and back-transformed least-squares means and 95% CIs are reported. Geometric mean Hg concentrations without estimates of variance for Baltic clams and soft-shell clams at Arrowhead Marsh, and staghorn sculpin at Arrowhead and Colma Creek are presented because of small sample sizes (Table 1). Accordingly, we did not fit a full model testing for the main effects or interaction between marsh and taxa. Marsh-specific patterns of Hg in diet taxa were then graphically compared to patterns described for Hg in Clapper Rail blood from the same marshes (Ackerman et al., 2012). Relative Hg risk to Clapper Rails associated with each macro-invertebrate taxa was assessed by comparing the measured wet-weight Hg concentrations to a lowest observable adverse effects level (LOAEL) of 0.25 μg/g (ww) broadly indicative of dietary concentrations of total Hg that may negatively affect avian reproduction (Shore et al., 2011). Because MeHg comprises 94% of total Hg in whole fish from San Francisco Bay (Ackerman et al., 2013), Hg risk from total Hg in staghorn sculpin (ww) was assessed in relation to thresholds of 0.10 μg/g (ww) associated with behavioral impairment (Depew et al., 2012) and 0.30 μg/g (ww) associated with reproductive impairment (Burgess and Meyer, 2008) in piscivorous birds.

3. Results

We collected and analyzed 233 prey samples across the 4 tidal marshes to estimate risk of Hg exposure to Clapper Rails (Table 1). Length classes ranged from 1 to 3 cm for eastern mudsnails, 1–3 cm for Baltic clams, 1–5 cm for soft-shell clams, 3–10 cm for ribbed horse mussels, 2–7 cm for ragworms, 2–5 cm for mud crabs, and 3–6 cm for staghorn sculpin.

Hg concentrations differed significantly ($F \geqslant 4.7$, $P \leqslant 0.005$) among marshes for all taxa except soft-shell clams and staghorn sculpin ($F \leqslant 1.8$, $P \geqslant 0.2$) (Fig. 2). Hg concentrations were consistently lower in diet items from Colma Creek compared to concentrations from the other three marshes. Hg concentrations at Arrowhead, Cogswell, and Laumeister were similar for eastern mudsnails, ribbed horse mussels, and mud crabs, and Hg concentrations in soft-shell clams and staghorn sculpin followed a similar,