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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.

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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,

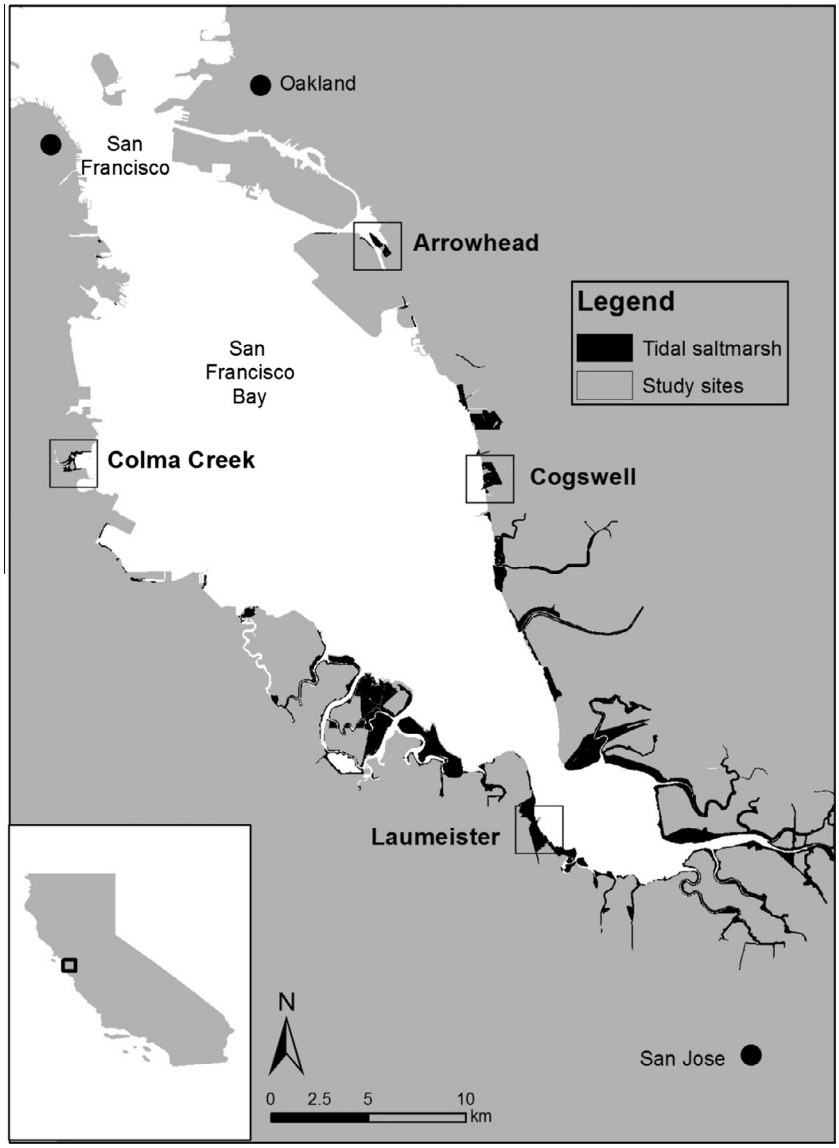

Fig. 1. Tidal marshes within San Francisco Bay, California sampled to assess dietary Hg exposure to California Clapper Rails during December – March of 2008 – 2009 and 2009 – 2010.

Table 1
Number of macro-invertebrate and fish samples collected and analyzed to assess mercury exposure to California Clapper Rails in San Francisco Bay, California from December – March of 2008 – 2009 and 2009 – 2010.

	Number of samples				
Taxa	Arrowhead	Colma Creek	Cogswell	Laumeister	Total
Eastern mudsnail	11	9	18	9	47
Baltic clam	2	10	10	6	28
Soft-shell clam	1	6	7	5	19
Ribbed horse mussel	8	6	31	10	55
Mud crab	14	7	14	3	38
Ragworm	4	5	10	3	22
Staghorn sculpin	2	2	14	6	24
Total	42	45	104	42	233

yet statistically non-significant, spatial pattern. Hg concentrations differed more among sites for Baltic clams and ragworms, with Arrowhead having the highest Hg concentrations followed by Laumeister, Cogswell, and Colma Creek. The overall pattern of relatively higher Hg in diet items from Arrowhead, Cogswell, and Laumeister compared to lower Hg in diet items from Colma Creek matched the spatial pattern described previously for Hg in Clapper Rail blood (Ackerman et al., 2012) (Fig. 2).

Across all marshes and size classes, Hg concentrations were highest in eastern mudsnails, followed by Baltic clams, soft-shell clams, ragworms, ribbed horse mussels, staghorn sculpin, and mud crabs (Fig. 3). Eastern mudsnails posed a moderate Hg risk relative to the Shore et al. (2011) dietary threshold for total Hg effects on avian reproduction, with 26% of samples exceeding 0.25 μg/g (ww) compared to 11% for Baltic clam, 9% for ragworms, 2% for ribbed horse mussels, and 0% for mud crabs and soft-shell clams.

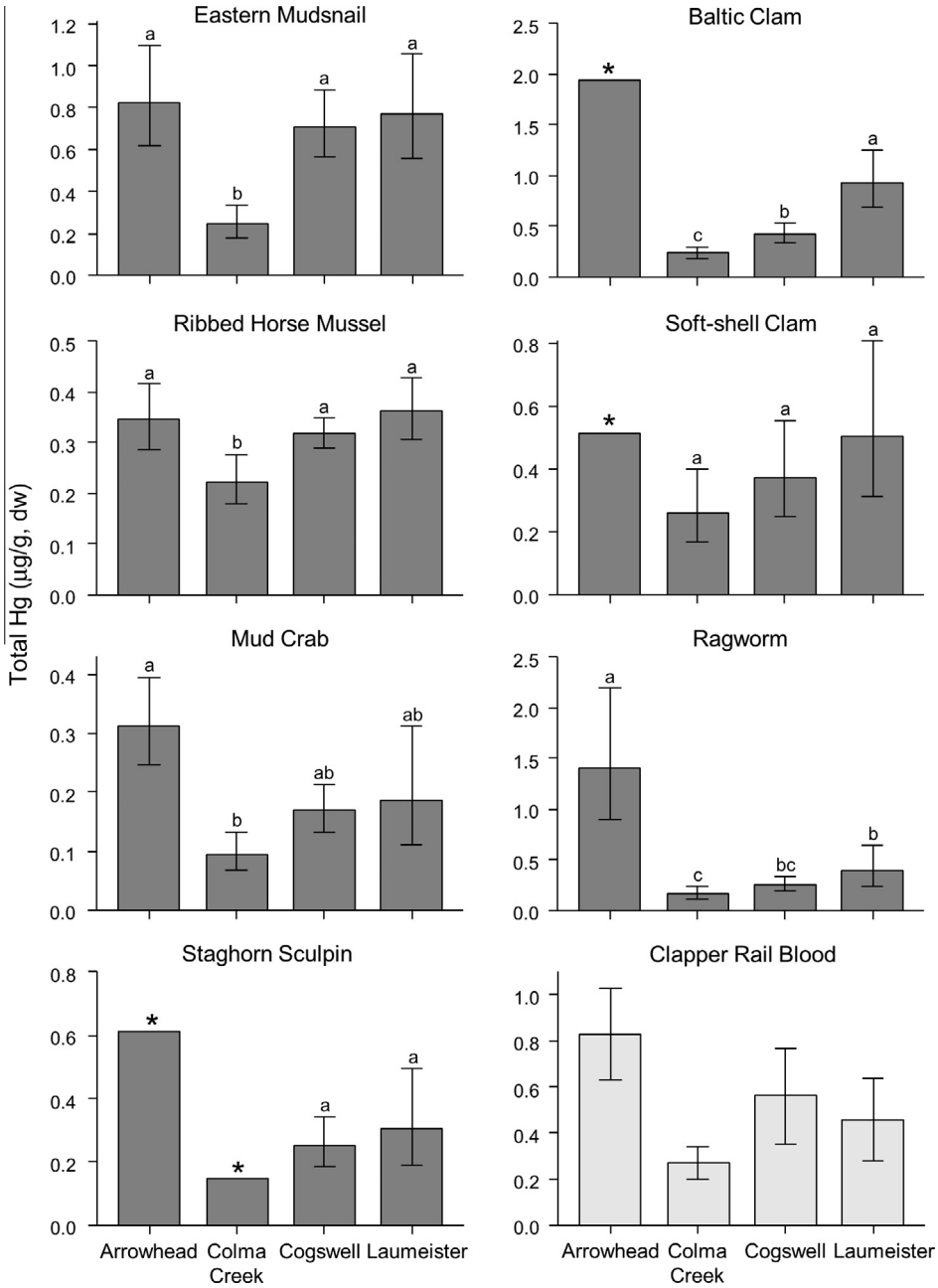

Fig. 2. Least-squares means (dw, ±95% CI) for concentrations of total Hg in macro-invertebrate and fish taxa (grey bars) consumed by California Clapper Rails within four tidal marshes in San Francisco Bay, California, December – March 2008 – 2009 and 2009 – 2010. Means sharing the same letter within the same dietary taxonomic group do not differ significantly. Model averaged whole blood Hg concentrations (ww, +95%CI) in Clapper Rails at these same sites in 2006–2010 (white bars, Ackerman et al., 2012) are displayed for visual comparison. Asterisks denote that the geometric mean was used for visual comparison because sample sizes were insufficient to allow statistical comparisons.

Staghorn sculpin also posed moderate Hg risk to birds depending on the threshold used for evaluation, whereby 25% of samples exceeded the Depew et al. (2012) 0.10 µg/g (ww) threshold for behavioral impairment, but no samples exceeded the Burgess and Meyer (2008) 0.30 µg/g (ww) threshold for reproductive impairment (Fig. 3).

4. Discussion

4.1. Spatial patterns

The consistent pattern of higher Hg in diet taxa from Arrowhead, Laumeister, and Cogswell compared to lower Hg in diet taxa from Colma Creek matched the spatial pattern in Clapper Rail blood described by Ackerman et al. (2012). These congruent results indicate that Clapper Rails are exposed to Hg from a geographically limited area. This pattern was expected because radio-marked birds have strong site fidelity to a single marsh and adults display limited dispersal movements (Casazza et al., 2008). For example, only 4 individual Clapper Rails out of 139 radio-marked in the San Francisco Bay emigrated away from the marsh where they were captured (USGS unpublished data, Rohmer, 2010).

Without a *priori* knowledge of spatial patterns in Clapper Rail blood (Ackerman et al., 2012), we might expect Hg to decrease with increasing distance from the Guadalupe River flowing into the southern head of the Bay. The New Almaden mercury mine

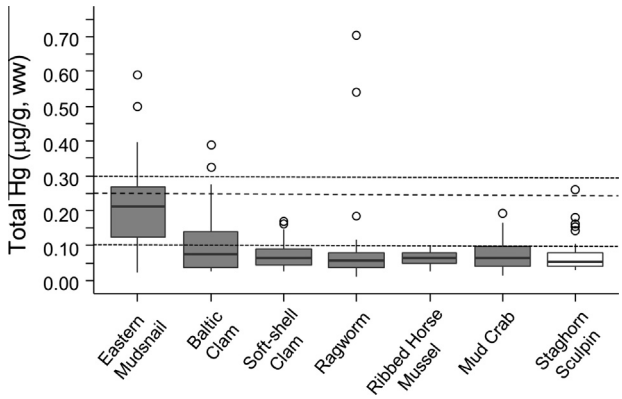

Fig. 3. Boxplots describing Hg concentrations (µg/g, ww) in macro-invertebrate (grey bars) and fish (white bars) taxa consumed by California Clapper Rails across four tidal marshes in San Francisco Bay, California. The small long-dashed line indicates a 0.25 µg/g (ww) threshold of total Hg in invertebrates broadly associated with adverse effects on avian reproduction (Shore et al., 2011). The large short-dashed lines indicate of 0.10 and 0.30 µg/g (ww) thresholds of MeHg in fish associated with behavioral (Depew et al., 2012) and reproductive (Burgess and Meyer, 2008) impairment, respectively, in piscivorous birds.

in the Guadalupe River watershed is a strong point source of Hg entering the Bay, and studies of MeHg in water (Davis et al., 2012), sediment and fish (Greenfield and Jahn, 2010; Greenfield et al., 2013a), and birds (Ackerman et al., 2007, 2008; Eagles-Smith et al., 2009) have described high Hg concentrations in the South San Francisco Bay, yet relative hotspots of Hg in fish near Arrowhead and Colma Creek have also been documented (Greenfield et al., 2013b). Moreover, consistently low Hg in diet items and Clapper Rail blood at Colma Creek relative to the other three marshes is particularly intriguing. Marsh elevation does not ostensibly explain spatial patterns of Hg in our study given that Colma Creek and Arrowhead are both low elevation tidal marshes that experience more frequent tidal inundation compared to mid- to high-elevation marshes at Cogswell and Laumeister. However, marsh age may be a contributing factor towards this pattern, whereby younger marshes have had less time to accumulate organic matter that can enhance Hg methylation and entry into Clapper Rail food webs (Lambertsson and Nilsson, 2006). Colma Creek was a mudflat until it was invaded by hybrid *Spartina* in the mid-1980s, while Cogswell (which typically had the second lowest Hg concentrations in Clapper Rail diet items) was restored from a salt production pond to tidal marsh beginning in 1980 (Overton et al., 2014). In contrast, Arrowhead and Laumeister are >100 years old, and hence have had substantially more time to accumulate organic matter from marsh vegetation litter. Thus, older tidal marshes may present greater Hg risk than younger marshes if they provide more labile organic matter for microbes (Yee et al., 2008). Furthermore, these patterns combined further illustrate that inorganic Hg is not limiting in the Estuary (Davis et al., 2012), and marsh-specific biotic and abiotic factors are likely the strongest drivers of MeHg exposure to Clapper Rails.

4.2. Relative Hg risk of diet items to Clapper Rails

Comparison of total Hg wet-weight concentrations in macro-invertebrate prey to levels of potential impairment for bird reproduction (Shore et al., 2011) indicated non-uniform risk across taxa. Clapper Rails are generalist foragers (Rush et al., 2012), so individual birds that consume more eastern mudsnails may incur greater Hg risk because over one-quarter of samples from this taxa exceeded 0.25 µg/g (ww). Eastern mudsnails are highly invasive in the San Francisco Bay, and have a wide diet breadth that spans periphyton, small invertebrates, and detritus from plants and animals (Cohen and Carlton, 1995). Invertebrates that ingest Hg from multiple routes of exposure can accumulate Hg at a faster rate compared to invertebrates primarily exposed through the dissolved fraction of Hg in water (Cardoso et al., 2012). This process may explain why Hg concentrations were higher for mudsnails than for filter-feeding bivalves, but it does not explain lower Hg concentrations in ragworms and mud crabs that also forage through multiple routes. Elevated Hg concentrations in ragworms from Arrowhead were similar to those reported by Diaz-Jaramillo et al. (2013) from a contaminated estuary in Chile, whereas low Hg concentrations in mud crabs from all four tidal marshes in our study were similar to those from a relatively uncontaminated estuary in the Diaz-Jaramillo et al. (2013) study. Although sample sizes for Baltic clams from Arrowhead were too small to permit statistical comparisons, both samples from that site exceeded the 0.25 µg/g (ww) risk level. Regardless of the cause, Hg concentrations in eastern mudsnails represent a relatively consistent risk of Hg exposure to Clapper Rails across tidal marshes, whereas ragworms and Baltic clams represent more variable risk compared to the other invertebrate taxa sampled in our study.

Our analysis of Hg risk to Clapper Rails from macro-invertebrate prey involves two important caveats. First, the 0.25 µg/g wet-weight threshold in bird diets is meant to estimate risk of total Hg to avian reproduction across a wide range of species, and concentrations that exceed the threshold do not infer that adverse effects are occurring (Shore et al., 2011). Second, the percentage of total Hg comprised of MeHg in invertebrates varies dramatically within and among taxa, which makes estimation of Hg risk from measurements of total Hg less certain. For example, percentages of MeHg in macro-invertebrates from other estuaries similar to those sampled in our study can range from 6% to 100% of total Hg (Diaz-Jaramillo et al., 2013; Gagnon and Fisher, 1997; Kim et al., 2012; Riisgård et al., 1985; Sizmur et al., 2013), which largely relates to variation in depuration rates of inorganic Hg and foraging strategies (Gagnon and Fisher, 1997). Nevertheless, our assessments of site- and species-specific risks from dietary total Hg are likely robust given that body condition in Clapper Rails was negatively correlated with their blood Hg concentrations in birds from the same marshes sampled in our study (Ackerman et al., 2012), and depressed reproduction was reported by Schwarzbach et al. (2006).

In contrast to invertebrates, assessment of toxicity risk from fish is more straight-forward because MeHg generally comprises the majority of total Hg, as found for prey fish in San Francisco Bay (Ackerman et al., 2013). Consumption of staghorn sculpin by Clapper Rails appears to pose Hg risk comparable to that from mudsnails, whereby 25% of samples exceeded concentrations of 0.10 µg/g (ww) associated with behavioral impairment in piscivorous birds (Depew et al., 2012). However, no samples exceeded concentrations of 0.30 µg/g (ww) associated reproductive impairment. Among prey fish species, Hg concentrations in staghorn sculpin are intermediate among fishes inhabiting San Francisco Bay and its adjacent wetlands (Eagles-Smith and Ackerman, 2014) and nearshore channel habitats (Greenfield and Jahn, 2010). Our findings follow the overall pattern of elevated Hg concentrations in forage fish from San Francisco Bay compared to other worldwide estuaries (Eagles-Smith and Ackerman, 2014).

4.3. Relevance to management

The strong spatial correlation between Hg concentrations in Clapper Rail blood and their prey indicate that relative Hg risk to Clapper Rails can be assessed indirectly by measuring THg in potential prey items within specific marshes. This is an especially germane result given permitting restrictions associated with

capturing and handling an endangered species, or when evaluating marshes for potential reintroduction efforts into unoccupied habitats. However, assessment of risk would be made more precise by measurement of MeHg in prey.

Our results also indicate that Clapper Rail exposure to Hg is mediated most strongly by biogeochemical properties occurring at local rather than regional scales within San Francisco Bay. Hence, management designed to restore or enhance tidal marshes should also consider how planned actions (e.g. removal or addition of organic matter, alteration of wetting and drying frequency), might alter Hg risk to local Clapper Rail populations, particularly in marshes with high existing Hg risk. Efforts aimed at reducing the abundance of invasive eastern mudsnails might also ameliorate Hg risk to Clapper Rails, although the magnitude of the potential benefit of this action is uncertain given limited information regarding Clapper Rail diets. Hence, a more precise understanding of Clapper Rail forage selection is necessary to fully inform management actions that lower Hg risk for this highly endangered species.

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