Mercury bioaccumulation and risk to three waterbird foraging guilds is influenced by foraging ecology and breeding stage

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The influence of foraging habitat, trophic position, and exposure timing on mercury bioaccumulation and risk to reproduction is evaluated in three waterbird guilds.

1. Introduction

Mercury (Hg) exposure and bioaccumulation can have deleterious effects on wildlife populations (Evers et al., 2008). Methylmercury (MeHg), the most toxicologically significant Hg form, is produced in aquatic habitats by microbial activity (Gilmour et al., 1992), and biomagnifies through the food web (Kidd et al., 1995). Waterbirds in particular are at elevated risk to MeHg exposure because they forage in aquatic habitats and are highly sensitive to toxic effects of MeHg exposure (Evers et al., 2008; Heinz et al., 2009). These toxic effects can manifest through several mechanisms, including altered foraging efficiency (Adams and Frederick, 2008), behavior (Bouton et al., 1999; Evers et al., 2008), and tissue biochemistry (Hoffman et al., 2005), as well as reduced hatching success (Albers et al., 2007), and chick survival (Ackerman et al., 2008a). Ultimately, MeHg toxicity is thought to influence waterbird populations primarily through impaired productivity (Burgess and Meyer, 2008).

Because MeHg concentrations increase with each successive step in the food web (Wiener et al., 2003), trophic position is a primary consideration for evaluating toxicological risk of Hg exposure to waterbirds. Fish-eating birds generally have higher MeHg levels than other foraging guilds of waterbirds (Evers et al., 2005). However, foraging habitat is another important factor in determining MeHg bioaccumulation through the food web (Cristol et al., 2008; Eagles-Smith et al., 2008a; Evers et al., 2005). MeHg production is often higher in wetland habitats, such as marshes and floodplains, than in open-water bays and other coastal locations because wetland environmental conditions enhance microbial conversion of inorganic Hg to MeHg (Marvin-DiPasquale et al., 2003). Thus, lower trophic level birds that forage in wetland habitats may face greater risk to Hg exposure than would be estimated by trophic position alone. For example, wetland-dependent birds in San Francisco Bay that primarily consume invertebrates, such as black-necked stilts (Himantopus mexicanus) and California clapper rails (Rallus longirostris obsoletus) (Robinson et al., 1999; Eddleman and Conway, 1998), may be at higher risk to MeHg than some obligate piscivorous waterbirds, such as double-crested cormorants (Phalacrocorax auritus), because they forage in wetlands along the Bay's margin (Ackerman et al., 2007a) instead of the open-bay environment typically used by cormorants (Davis et al., 2006). Current levels of MeHg exposure to clapper rails and stilts in San Francisco Bay may impair egg hatchability (Schwarzbach et al., 2006) and chick survival (Ackerman et al., 2008a), whereas MeHg...
concentrations in cormorant eggs are well below effects-thresholds (Davis et al., 2006).

The timing of MeHg exposure also is an important determinant of risk to waterbirds. Since dietary MeHg is rapidly absorbed into the bloodstream (Bearhop et al., 2000) and distributed throughout the body (Eagles-Smith et al., 2008b), MeHg risk is correlated with recent MeHg uptake. If exposure to elevated MeHg occurs during or just prior to feather molt, dietary and tissue-stored MeHg may be sequestered into growing feathers and become toxicologically inert, diminishing long-term MeHg exposure and risk (Bearhop et al., 2000). Conversely, high Hg intake that occurs just prior to breeding can increase MeHg risk to reproduction because females deposit recently ingested MeHg into eggs (Heinz and Hoffman, 2004), which can impair egg hatchability and chick survival (Albers et al., 2007). Continued exposure to MeHg during incubation also can impair nesting behavior and incubation (Evers et al., 2008). Thus, birds that forage in habitats with elevated MeHg production just before breeding are likely to be at increased risk to MeHg toxicity.

To evaluate the roles of foraging habitat and exposure timing, as well as trophic position on Hg bioaccumulation, we assessed Hg concentrations in tissues of five waterbird species comprising three distinct foraging guilds: fish-eating seabirds (Forster’s terns; Sterna forsteri, and Caspian terns; Hydroprogne caspia), invertebrate-foraging shorebirds (black-necked stilts, hereafter stilts, and American avocets; Recurvirostra americana, hereafter avocets), and open-water, benthic-foraging sea ducks (surf scoters; Melanitta perspicillata, hereafter scoters) in the San Francisco Bay (SFB) estuary, CA. The guild approach is valuable because estuarine waterbirds form well-documented, distinct guilds distinguished by their feeding methods and habitat use (Takekawa et al., 2001). Thus, Hg exposure can be evaluated based on large-scale foraging strategies (such as trophic position) occurring among guilds, and small-scale strategies (such as foraging micro-habitat) that occur within guilds. Four of the five species studied (Forster’s terns, Caspian terns, stilts, and avocets) breed locally, whereas scoters breed in Alaska and the Northwest Territories of Canada, and overwinter in SFB.

The SFB estuary has a legacy of Hg contamination from historic mining in the Sierra Nevada and Coast Range Mountains (Conaway et al., 2008), as well as the New Almaden Mercury Mine in the Guadalupe River watershed. Large amounts of inorganic Hg have been transported to the estuary where it resides in the sediments and can be converted to MeHg through microbial processes (Martin-DiPasquale et al., 2003). Due to the high level of contamination, and the extensive wetland restoration program within SFB, there is concern Hg may be impairing reproduction of wildlife in the estuary (Davis et al., 2003). We predicted that among guilds, Hg would be highest in higher trophic level species, and yet that within guilds, similar trophic level species would have different Hg concentrations depending on their foraging habitat and timing of exposure.

2. Methods

2.1. Study location

We conducted our study at several locations throughout the greater SFB Region (37.8° N, 122.3° W) where waterbirds overwinter, stage during migration to their breeding grounds, and breed in large numbers (Page et al., 1999; Rintoul et al., 2003; Strong et al., 2004). Sampling occurred within four major regions in SFB: Lower South Bay, Central South Bay and Central Bay, North Bay, and Suisun Bay (Fig. 1). More detail on sampling locations is provided in Ackerman et al. (2007a, 2008b).

2.2. Sample collection

Briefly described are our sampling methods, but see related studies on other topics (Ackerman et al., 2007a, 2008b; Eagles-Smith et al., 2008b) for more details. We captured and collected adult scoters overwintering in SFB and pre-breeding and breeding avocets, stilts, and Forster’s tern adults from several locations throughout the SFB in 2004–2006. Wintering scoters were captured between October and March, pre-breeding avocets and stilts were captured and collected between February and April, and pre-breeding Caspian and Forster’s terns were captured and collected between April and May. We captured and collected breeding avocets, stilts, and Forster’s and Caspian terns between April and July. Tissues were collected and stored as described in Eagles-Smith et al. (2008b).

2.3. Sample processing and chemical determination

We processed and analyzed all samples for total mercury (THg) as described in Eagles-Smith et al. (2008b). Recoveries of certified reference materials averaged 102.1 ± 0.9% (N = 329), and matrix spike recoveries averaged 97.7 ± 1.3% (N = 154). Blood, feathers, and muscle tissue were only analyzed for THg because previous studies have shown that >95% of Hg in these tissues is MeHg (Evers et al., 2005). Conversely, the percent MeHg in organs can be variable (Eagles-Smith et al., 2009), thus a subset of livers were analyzed for MeHg. Absolute relative percent difference for duplicates in all tissues averaged 7.8 ± 1.9%. Livers were analyzed for MeHg at Battelle Marine Sciences Laboratory (Sequim, WA, USA) following EPA method 1630 (www.epa.gov/waterscience/methods/method/mercury/1630.pdf), cold vapor atomic fluorescence. MeHg recoveries averaged 106.2 ± 1.83% (N = 57) and 96.8 ± 1.83% (N = 175) for certified reference materials and matrix spikes, respectively. Absolute relative percent difference for duplicates averaged 7.8 ± 1.5% (N = 42).

2.4. Statistical analyses

All Hg concentrations are reported in μg/g (parts per million) dry weight (dw), except for blood which is reported in wet weight (ww). All Hg values were natural-log transformed prior to statistical analyses to normalize residuals and meet the assumptions of general linear models, and we used JMP V5.0.3 (SAS Institute, 2005) for all statistical models. Unless otherwise specified, we report all results as back-transformed least squares means ± standard error from model outputs, and standard errors were estimated using the delta method (Williams et al., 2002). We conducted three separate evaluations of Hg levels in waterbirds based on the timing of occurrence in the estuary for each species.

2.4.1. Tri-guild analysis

The first analysis compared liver THg levels among all 5 species, bounded by the dates and regions for which sampling times overlapped among species. Scoters overwinter in the estuary and are the only species we studied that solely breeds elsewhere (Savard et al., 1998). Forster’s terns and Caspian terns breed in the estuary and primarily overwinter elsewhere (Cuthbert and Wires, 1999; McNicholl et al., 2001), and avocets and stilts are resident in the estuary year-round (Robinson et al., 1997, 1999). Thus, all 5 species co-occur in the estuary for a short time period in the early spring. Therefore, to compare Hg levels among all 5 species, we initially compared only those birds sampled between 1 March and 23 April, since this represents the best approximation of the pre-breeding season when all 5 species were sampled and simultaneously present in SFB. Since scoters were sampled in different habitats than the other species (open-bay vs. bay margins), we categorized sites based on general geographic regions for this first level of analysis (Central South Bay/Central Bay, and North Bay). We used several linear models (GLMs) to simultaneously test for differences in liver THg levels among species, regions, sexes, and years.

2.4.2. Surf scoters

Next, we evaluated spatial and temporal variation in liver THg concentrations for scoter separately from the other species because they breed outside the estuary, and we therefore were not able to add a breeding stage effect like we did for the other 4 species. For this analysis, we used the entire scoter data set (from 28 October to 21 April, 2004–2005), sampled at all regions. We included three regions (Central Bay, North Bay, and Suisun Bay), whereas we did not sample the other 4 species in Suisun Bay. We also only analyzed THg in scoter livers, whereas we analyzed multiple tissues for the other species. We used analysis of covariance (ANCOVA) with region, year, and sex as factors, and Julian date as the covariate. We also included the interaction term Julian date × site in our initial model, and removed it if P > 0.10.

2.4.3. Locally breeding birds

Finally, we evaluated Hg bioaccumulation in several tissues (blood, liver [THg and MeHg], muscle, kidney, and breast and head feathers) of the four species that breed locally in SFB (avocets, stilts, Caspian terns, and Forster’s terns). In our global model we tested the main effects of species, breeding stage (pre-breeding vs. breeding), region (Lower South Bay, Central South Bay, and North Bay), sex, and year. Our global model structure also included a species × breeding stage interaction to test whether time-influenced Hg levels similarly among species. We found significant interactions between species and breeding stage (see Results), therefore we conducted separate GLMs for each species and breeding stage to test for differences among species, and separate ANOVA models for each species to test differences between breeding stages.

3. Results

We sampled 174 Forster’s terns, 50 Caspian terns, 217 stilts, 439 avocets, and 158 scoters. Across all regions, sexes, years, and dates,
the geometric mean THg concentrations (µg/g) in two commonly measured tissues, blood (ww) and liver (dw), respectively, were 1.37 ± 0.10 and 9.58 ± 0.89 in Forster’s terns, 1.41 ± 0.14 and 8.58 ± 0.89 in Caspian terns, 1.20 ± 0.06 and 7.57 ± 0.62 in stilts, and 0.24 ± 0.01 and 2.60 ± 0.25 in avocets. THg concentrations were 3.11 ± 0.17 in scoter livers.

3.1. Tri-guild analysis

Total Hg concentrations in livers of all five species sampled only during the time period when they co-occurred in the estuary differed among species ($F_{4,151} = 35.12, P < 0.0001$) and years ($F_{2,151} = 7.17, P = 0.001$), but not between sexes ($F_{1,151} = 2.71, P = 0.10$) or regions ($F_{1,151} = 0.89, P = 0.35$). When controlling for the other main effects, liver THg concentrations were highest in Caspian terns, followed by Forster’s terns, stilts, scoters, and avocets (Fig. 1).

3.2. Surf scoter spatial and temporal trends

Total Hg concentrations in scoter livers from all sites and dates increased with collection date ($F_{1,150} = 74.20, P < 0.0001$; Fig. 2), indicating that scoters accumulated Hg in their livers as they spent more time in the estuary. The global model also indicated differences among sites ($F_{2,150} = 3.94, P = 0.02$). THg concentrations tended to be higher in scoters from Suisun Bay (3.53 ± 0.44) and North Bay (3.43 ± 0.34) than in scoters from the Central Bay (2.73 ± 0.27), although Tukey–Kramer pairwise tests (all $P > 0.05$) suggested that the effect of site was relatively small. We initially included an interaction term (site × date) in our ANCOVA model to evaluate if slopes of THg concentrations with date differed among locations; however, there was no evidence of differential accumulation rates among regions ($F_{2,148} = 2.20, P = 0.12$). THg concentrations in scoter livers also differed between sexes ($F_{1,150} = 4.61$,
always lowest in avocets (Table 1, Fig. 3). Mercury concentrations in blood were higher for males (all $F_{1,150} = 3.63, P = 0.06$) than females which increased between 2.1 and 2.3 times (Table 1, Fig. 5). However, North Bay breeders also had higher concentrations than Central South Bay birds (Table 1, Fig. 4), whereas regions did not differ during the pre-breeding time period. In breeding birds, Hg concentrations in all internal tissues differed between sexes (all $F > 5.38, P < 0.02$), with males higher than females (Tukey–Kramer, all $P < 0.05$). We again found no differences in Hg concentrations between years for blood THg or muscle THg (all $F \leq 1.94, P \geq 0.13$), whereas years differed in liver THg and liver MeHg (all $F \geq 4.35, P < 0.04$) of breeders.

### 3.4. Breeding stages

#### 3.4.1. Pre-breeding birds

Total Hg or MeHg (liver only) concentrations in all internal tissues of pre-breeding birds differed among species (all $F \geq 26.85, P < 0.0001$) and regions (all $F \geq 15.96, P < 0.0001$), but not between years (all $F \leq 0.42, P > 0.52$). When controlling for other main effects, THg in blood of pre-breeders was highest in Caspian terns ($1.62 \pm 0.28$) and stilts ($1.11 \pm 0.09$), Forster’s tern blood concentrations ($0.88 \pm 0.09$) did not differ from stilts, but were lower than Caspian terns, and all three species had more elevated THg concentrations in their blood than avocets ($0.19 \pm 0.01$). For all other internal tissues of pre-breeders, THg concentrations did not differ among Caspian terns, Forster’s terns, or stilts, and were always lowest in avocets (Table 1, Fig. 3). Mercury concentrations in blood were higher for males than females ($F_{1,170} = 24.90, P < 0.0001$), but there were no sex differences in the other internal tissues (all $F \leq 1.70, P \geq 0.15$). THg and MeHg (liver only) concentrations of all internal tissues were highest in Lower South Bay birds (Tukey–Kramer, $P < 0.05$), and did not differ between North Bay and Central South Bay birds (Tukey–Kramer, all $P > 0.05$; Fig. 4).

#### 3.4.2. Breeding birds

Although we again found differences in Hg concentrations among species (all $F \geq 26.95, P < 0.0001$) and regions (all $F \geq 25.80, P < 0.0001$) in breeders, the pairwise comparisons indicated that the relative rank of species and regions differed from those of pre-breeders. Breeding Forster’s terns had the highest Hg concentrations of all species for all internal tissues, concentrations did not differ between Caspian terns and stilts, and avocets again had the lowest concentrations (Table 1, Fig. 3). Among regions, Lower South Bay breeders again had the highest Hg concentrations (Table 1, Fig. 4). Additionally, Hg concentrations did not differ between sexes (all $F \leq 2.93, P \geq 0.08$; Fig. 5) or years (all $F \leq 2.34, P \geq 0.13$), except in blood (blood THg: $F_{1,210} = 6.71, P = 0.01$).

### 3.5. Species

#### 3.5.1. Forster’s terns

Breeding stage was an important driver of Hg concentrations in Forster’s tern tissues (all $F \geq 15.48, P < 0.0001$), with breeding terns between 2.5 and 2.9 times higher than pre-breeders (Table 1, Fig. 3). Concentrations also differed between sexes (all $F \geq 5.41, P < 0.02$) and among regions (all $F \geq 4.61, P \leq 0.1$). Mercury in male terns was approximately 1.4 times higher than in female terns (Table 1, Fig. 5). Moreover, the increase in Hg concentrations from pre-breeding to breeding was greater for males, which increased 3.3–4.2 times, than females which increased between 2.1 and 2.3 times (Table 1). Regionally, Lower South Bay birds had higher concentrations than North Bay or Central South Bay (Fig. 4) birds, which did not differ from each other.

#### 3.5.2. Caspian terns

As with Forster’s terns, Hg concentrations differed between pre-breeding and breeding Caspian terns for all tissues (all $F \geq 5.18, P \leq 0.03$) except blood THg ($F_{1,44} = 0.62, P = 0.43$). We again found that Hg concentrations in breeders were substantially elevated from those of pre-breeders (Fig. 3), though the relative increase during time periods was lower in Caspian terns (Table 1) than in Forster’s terns. Mercury concentrations in Caspian tern tissue also differed among regions (all $F \geq 5.23, P < 0.01$), but not between years (all $F \leq 3.60, P \geq 0.06$), or sexes (all $F = 3.54, P > 0.07$; Fig. 5). For all tissues, Hg concentrations were highest in Lower South Bay and North Bay birds, and lowest in Central South Bay birds (Fig. 4).

#### 3.5.3. Black-necked stilts

Unlike the two tern species, there were generally no differences in Hg concentrations between pre-breeding and breeding stilts (all $F \leq 3.63, P \geq 0.06$; Fig. 3), except in muscle tissue ($F_{1,104} = 6.80, P = 0.01$) where breeders had higher concentrations ($3.09 \pm 0.39$) than pre-breeders ($2.28 \pm 0.22$). There were similar regional effects (all $F \geq 17.62, P < 0.0001$) where stilts from the Lower South Bay had higher Hg concentrations than either North Bay or Central South Bay birds (Fig. 4), which did not differ from one another. Additionally, Hg concentrations did not differ between sexes (all $F \leq 3.02, P \geq 0.08$; Fig. 5) or years (all $F \leq 2.34, P \geq 0.13$), except in blood (blood THg: $F_{1,210} = 6.71, P = 0.01$).

#### 3.5.4. American avocets

Consistent with our results from the other locally breeding species, there were no differences in avocet tissue Hg...
concentrations between years (all F ≤ 2.46, P ≥ 0.12). Mercury concentrations differed among regions (all F ≥ 12.87, P < 0.0001), with avocets from the Lower South Bay having higher Hg concentrations than avocets from either North Central or Central South Bay regions (Fig. 4), which did not differ from one another. Mercury concentrations were higher in breeding than pre-breeding birds for all species (all F ≤ 4.66, P ≤ 0.03; Fig. 3), except liver (F<sub>1,119</sub> = 2.43, P = 0.12). Finally, we found sex differences in THg concentrations in blood (F<sub>1,428</sub> = 21.38, P < 0.001; Fig. 5), but not the other tissues (all F ≤ 2.03, P ≥ 0.16; Fig. 5).

### Table 1

Total mercury (THg; μg/g dw [ww for blood]) concentrations (geometric mean ± 1 SE) in tissues of waterbirds representing three foraging guilds in San Francisco Bay, CA, USA. Sample sizes in parentheses NS – Not sampled.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pre-breeding</th>
<th>Breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td><strong>Lower South Bay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood THg</td>
<td>1.04 ± 0.17 (38)</td>
<td>1.32 ± 0.17 (38)</td>
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<tr>
<td>Liver THg</td>
<td>7.17 ± 3.04 (9)</td>
<td>7.80 ± 1.73 (11)</td>
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<tr>
<td>Liver MeHg</td>
<td>6.77 ± 3.55 (9)</td>
<td>9.21 ± 1.72 (6)</td>
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<tr>
<td>Muscle THg</td>
<td>2.30 ± 1.00 (9)</td>
<td>2.40 ± 0.49 (9)</td>
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<tr>
<td>Kidney THg</td>
<td>8.58 ± 3.40 (9)</td>
<td>9.58 ± 1.79 (11)</td>
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<tr>
<td>Breast feather THg</td>
<td>6.56 ± 0.87 (40)</td>
<td>12.15 ± 1.73 (18)</td>
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<tr>
<td>Head feather THg</td>
<td>4.51 ± 0.64 (38)</td>
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<td><strong>Central South Bay</strong></td>
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<tr>
<td>Blood THg</td>
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<td>0.86 ± 0.15 (13)</td>
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<td>Liver THg</td>
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<td>4.74 ± 0.60 (9)</td>
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<tr>
<td>Liver MeHg</td>
<td>0.78 (1)</td>
<td>4.23 ± 0.29 (5)</td>
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<tr>
<td>Muscle THg</td>
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<td>1.55 ± 0.18 (9)</td>
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<td>Liver MeHg</td>
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<td>Head feather THg</td>
<td>8.75 ± 2.78 (13)</td>
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### Table 3.6

**Feather mercury**

THg concentrations differed among species for both feather types (all F ≥ 24.46, P < 0.0001). Breast feather THg concentrations in Caspian terns (10.31 ± 1.28), Forster’s terns (10.05 ± 0.68), and stilts (9.46 ± 0.82) were higher than in avocets (2.59 ± 0.23), but did not differ from one another. Alternatively Hg concentrations in head feathers were highest in stilts (13.24 ± 1.30), did not differ between Caspian terns (8.42 ± 1.13) and Forster’s terns (7.62 ± 0.56), and were lowest in avocets (4.67 ± 0.45). Total Hg concentrations differed between years for breast feathers (F<sub>1,446</sub> = 5.20, P = 0.02), but not

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head feathers \((F_{1,441} = 0.13, P = 0.72)\). Conversely, Hg concentrations differed among regions for head feathers \((F_{2,441} = 10.30, P < 0.0001)\), but not breast feathers \((F_{2,446} = 2.37, P = 0.09)\) suggesting that head feather growth may be more closely tied to the timing and locations of tissue sampling than breast feathers. Indeed, as with internal tissues, head feather Hg concentrations were higher in the Lower South Bay \((9.99 \pm 0.76)\) than the Central South Bay \((6.22 \pm 0.56)\), whereas concentration in North Bay birds \((8.05 \pm 0.70)\) did not differ from those of the other two regions. However, unlike our results from internal tissues, we found no effect of breeding stage on head feather or breast feather \((all F < 1.46, P > 0.12)\) Hg concentrations. Mercury concentrations for both feather types differed between sexes \((all F \geq 6.74, P \leq 0.01)\) with males having higher levels than females (Fig. 5).

4. Discussion

Mercury concentrations in fish-eating birds (Forster’s terns and Caspian terns) were generally the highest of all five species, a result consistent with other studies that have shown elevated Hg concentrations in upper trophic waterbirds (Borga et al., 2006; Braune, 1987; Evers et al., 2005). Yet, we also found relatively high Hg levels in stilts, especially in comparison to avocets, which often co-occur and forage on similar foods (Robinson et al., 1997, 1999). Notably, Hg concentrations in tissues of all species showed substantial spatial and temporal variation, illustrating the importance of these factors in determining Hg concentrations in waterbirds across foraging guilds. Mercury levels in birds from the Lower South Bay, North Bay, and Suisun Bay (scoters only) were generally higher than in the Central South Bay or Central Bay, likely a result of either upstream Hg sources or greater marsh area and in situ
production of MeHg. Mercury concentrations also increased with time spent in the estuary. Scoter liver THg concentrations roughly tripled from their fall arrival in SFB to just before their spring departure to breed. This is particularly notable because it suggests that scoters may carry heavy contaminant loads accumulated while overwintering in SFB to their boreal breeding grounds, where Hg exposure would otherwise likely be relatively low. In SFB, breeding birds generally had higher Hg concentrations than pre-breeding birds, which may indicate elevated risk of reproductive impairment. This was particularly evident in Forster’s terns, where Hg concentrations were 2–4 times higher in breeding than pre-breeding terns, and for which a high proportion of breeding birds exceeded toxicity thresholds developed for other waterbird species (Evers et al., 2008).

4.1. Differences within guilds

4.1.1. Forster’s tern and Caspian terns

The differences in Hg concentrations of Forster’s tern tissues compared to those in Caspian terns are striking. Upon arrival in SFB, Caspian terns had Hg concentrations exceeding all other species. Forster’s terns had relatively low pre-breeding Hg concentrations in comparison with Caspian terns, similar to what we measured in stilts and scoters, which both forage on invertebrates. However, within approximately 2 months, Hg concentrations in Forster’s terns nearly tripled, exceeding those in breeding Caspian terns or any of the other species we examined. It is unclear why these two species had such different Hg levels upon entering the estuary, but it may be due to differential wintering distributions, diet, or molt cycles. Little is known about wintering locations of Forster’s terns or Caspian terns, but most of the Pacific Coast populations overwinter in Mexico and Guatemala and follow coastal migration routes (McNicholl et al., 2001; Cuthbert and Wires, 1999). It is also possible that some individuals overwinter in the estuary or along the coast of California. Future satellite telemetry studies would be valuable for determining wintering locations.

Both Caspian terns and Forster’s terns are primarily fish-eaters, but Caspian terns tend to take larger prey than Forster’s terns (Cuthbert and Wires, 1999; McNicholl et al., 2001), and Hg concentrations in fish often increase with fish length (Wiener et al., 2003). Thus, Hg concentrations in pre-breeding Caspian terns may have been elevated relative to Forster’s terns because of differences in prey size and associated Hg levels. During the breeding time period Caspian terns presumably still preyed on larger fish, yet their Hg concentrations were substantially lower than Forster’s terns’. This is likely due to the fact that Forster’s terns foraged along the Bay’s margins in salt ponds and marshes (Ackerman et al., 2008a,b) where microbial MeHg production is elevated relative to the open-bay (Marvin-DiPasquale et al., 2003). Conversely, Caspian terns forage more in the open-bay than Forster’s terns, and Hg concentrations in fish from the open-bay are generally lower than in salt ponds and marshes along the Bay’s margins. For example, size-
corrected THg concentrations in Northern anchovy (*Engraulis mordax*) and Pacific staghorn sculpin (*Leptocottus armatus*) collected during the same time periods were $0.37 \pm 0.03$ and $0.27 \pm 0.02 \mu g/g dw$, respectively, in the open-bay, whereas they were $1.01 \pm 0.13$ and $0.51 \pm 0.03 \mu g/g dw$, respectively, from wetlands along the Bay’s margins (Authors, unpublished data).

4.1.2. American avocets and black-necked stilts

Foraging habitat also may explain the distinctive species differences in Hg concentrations we found between invertebrate-foraging shorebirds. Mercury levels in stilt tissues were nearly 5 times higher than those in avocets. In fact, Hg concentrations in stilts approached those in fish-eating Caspian terns. These differences between stilts and avocets are particularly striking because both species co-occur in wetlands along the estuary’s margins (Rintoul et al., 2003) and they both forage on crustaceans and other small invertebrates. However, their relative use of micro-habitats within wetlands may explain these differences. Pre-breeding avocets used a much higher proportion of tidal mudflats and tidal marsh than stilts, whereas stilts utilized managed and muted-tidal pickleweed (*Sarcocornia pacifica*) marshes more so than avocets (Ackerman et al., 2007). This differential habitat use also has been supported by stable isotope data showing a C3 (pickleweed; *Spartina* spp.) carbon signature in stilt tissues and a C4 (cordgrass; *Spartina* spp.) carbon signature in avocet tissues (Ackerman et al., 2007), indicating that each species obtained their prey from different micro-habitats. Wetlands have been shown to be zones of enhanced MeHg production (Hall et al., 2008), and there is evidence that marshes and areas with higher vegetation root density also show greater MeHg production than mudflats and un-vegetated sediments (Canario et al., 2007). Moreover, recent work in the SFB showed that sediment MeHg concentrations in pickleweed habitats were 2.9–3.8 times higher than in sediments from mudflat or cordgrass habitats along transects in the same marsh (L. Windham-Meyers, Pers. Comm).

Avocets and stilts also showed contrasting temporal patterns in Hg levels from pre-breeding to breeding time periods. There were no differences in Hg concentrations between time periods for stilts, whereas avocet Hg concentrations increased 2–3-fold from pre-breeding to breeding time periods. Both species are year-round residents in the estuary, thus differential migration patterns and pre-breeding exposure are not likely explanations. During the pre-breeding season, blood THg concentrations did not change with date in either species (Ackerman et al., 2007), but pre-breeding Forster’s terns showed distinct increases in Hg levels over time once they arrived in SFB (Ackerman et al., 2008b). The increase in avocet Hg concentrations from pre-breeding to breeding may reflect increased foraging effort along land-water boundaries associated with establishing island nesting territories. In general, avocets forage in mudflats and open-water up to 25 cm deep

![Graphs showing mercury levels in different tissues of avocets and stilts](image-url)
et al., 1997). However, over the course of 40-days prior to nesting, the average daily distance from nests declined from more than 3000 m to around 200 m at nest initiation (Demers et al., 2008), and the average daily distance from their future nest site was more than 3-times smaller during the breeding time period than the pre-breeding time period (Demers et al., 2008). By foraging close to their nest location during breeding, avocets likely foraged on prey in shallower waters near the land-water boundary where MeHg production rates often are elevated (Marvin-DiPasquale et al., 2003; Mitchell et al., 2008). Conversely, stilts tend to forage in shallower water year-round (Robinson et al., 1999), and likely are exposed to similar Hg levels during both pre-breeding and breeding time periods. Alternatively, the temporal differences may be due to avocets and stilts having differential wintering locations within SFB.

4.2. Seasonal patterns and reproductive risk

Hg concentrations increased substantially between the pre-breeding and breeding time periods in Forster's terns, Caspian terns, and avocets, but not stilts. Further, although they do not breed locally, scoter THg concentrations increased nearly 3-fold between late October, when they arrived in the estuary, to April when they began to leave the estuary for their breeding grounds in Alaska and the Northwest Territories of Canada (Savard et al., 1998; Takekawa et al., in press). Similarly, Forster's tern Hg concentrations approximately tripled from pre-breeding (February–April) to breeding (May–June). These results suggest that the SFB estuary is an important source of Hg exposure for both migratory and locally breeding waterbirds. The results also highlight the rapid rate of Hg accumulation in waterbird tissues when transitioning to a high Hg environment such as SFB.

These increased Hg concentrations over time are particularly notable because they occurred during a very sensitive timeframe in the avian reproductive cycle. Reproduction is generally considered to be the most sensitive endpoint for MeHg toxicity in wildlife (Wiener et al., 2003; Scheuhammer et al., 2007), and toxicological responses to elevated Hg concentrations can be manifested in adults through several mechanisms (Adams and Frederick, 2008; Evers et al., 2008). Data across multiple taxa are still lacking, but a threshold for high risk of deleterious effects has been developed for common loon (Gavia immer) blood at 3 µg/g (ww), above which 41% fewer young were produced than loons with <1 µg/g Hg in blood (Evers et al., 2008). During the pre-breeding time period, blood THg concentrations in 0% of avocets, 16% of stilts, 10% of Caspian terns, and 13% of Forster's terns exceeded this threshold value (Fig. 6a). By the onset of breeding, 5–6% of avocets, stilts, and Caspian terns exceeded this value. Importantly, 48% of breeding Forster's terns had blood THg concentrations exceeding this high-risk threshold (Fig. 6b). The proportion of breeding birds at high risk due to elevated blood Hg concentrations are similar to results found for eggs, where approximately 0.5% of avocet, 12% of stilt, 7% of Caspian tern, and 47% of Forster's tern eggs from SFB exceeded a high-risk thresholds for impairment of 1.3 µg/g fresh ww (Evers et al., 2003). This congruence in risk between matrices suggests a coupling between Hg exposure in breeding adults, and deposition of Hg into eggs. Maternally deposited Hg, which is reflective of recent maternal dietary exposure (Heinz and Hoffman, 2004), can reduce hatchability (Albers et al., 2007), and early chick survival (Ackerman et al., 2008a). Thus our results suggest that Forster's terns in particular may be at substantial risk to reproductive impairment (Fig. 7). Importantly, we recognize that there can be substantial inter-specific differences in sensitivity to Hg, and urge caution in interpreting risk. We selected common loons as our benchmark for comparison because they are among the few waterbird species for which reproductive impairment has been linked to adult Hg exposure in the wild. However, it is currently unclear how avocet, stilt, Caspian tern, or Forster's tern Hg sensitivity compares with that of common loons.

5. Conclusions

Our results indicate that SFB waterbirds accumulated Hg to concentrations that place them at considerable risk to deleterious reproductive effects. This exposure varied as a function of micro-habitat, region, trophic level, and time spent in the estuary. These results are concerning because the SFB estuary is among the most important sites for wintering, migrating, and breeding waterbird populations along the Pacific Flyway (Page et al., 1999), and is a hotspot for Hg exposure and bioaccumulation. Thus, Hg contamination in the SFB estuary likely is a significant issue for waterbird conservation, and further efforts should strive to evaluate population-level impacts of Hg exposure to waterbirds through studies on reproduction and population dynamics.

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