Mercury Bioaccumulation in Estuarine Fishes: Novel Insights from Sulfur Stable Isotopes

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S Supporting Information

ABSTRACT: Estuaries are transitional habitats characterized by complex biogeochemical and ecological gradients that result in substantial variation in fish total mercury concentrations (THg). We leveraged these gradients and used carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) stable isotopes to examine the ecological and biogeochemical processes underlying THg bioaccumulation in fishes from the San Francisco Bay Estuary. We employed a tiered approach that first examined processes influencing variation in fish THg among wetlands, and subsequently examined the roles of habitat and within-wetland processes in generating larger-scale patterns in fish THg. We found that $\delta^{34}S$, an indicator of sulfate reduction and habitat specific-foraging, was correlated with fish THg at all three spatial scales. Over the observed ranges of $\delta^{34}S$, THg concentrations in fish increased by up to 860% within wetlands, 560% among wetlands, and 291% within specific impounded wetland habitats. In contrast, $\delta^{13}C$ and $\delta^{15}N$ were not correlated with THg among wetlands and were only important in low salinity impounded wetlands, possibly reflecting more diverse food webs in this habitat. Together, our results highlight the key roles of sulfur biogeochemistry and ecology in influencing estuarine fish THg, as well as the importance of fish ecology and habitat in modulating the relationships between biogeochemical processes and Hg bioaccumulation.

INTRODUCTION

Mercury (Hg) is a pervasive environmental pollutant.[1] In its organic form—methylmercury (MeHg)—it is a potent neuro-toxicant to fish, wildlife, and humans[2,3] that readily biomagnifies though food webs.[4] The bioaccumulation of mercury is closely tied to the biogeochemical processes that control MeHg production and availability,[5] as well as the ecological processes that govern biomagnification.[6] However, these processes are complex and difficult to measure in situ, thus hindering efforts to resolve their respective roles in influencing Hg concentrations in biological communities. Chemical tracers, such as stable isotopes, can be useful tools for disentangling some of the processes associated with MeHg cycling.[6–9]

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes are extensively utilized ecological tracers that provide spatially and temporally integrated insights into the dietary sources (e.g., benthic versus pelagic food web pathways) and trophic positions of consumers.[6,9] These isotopes have been widely applied in understanding the ecological processes that regulate MeHg bioaccumulation because MeHg biomagnifies up food chains[4] and because MeHg transfer largely follows energetic pathways that may differ among habitats with different prey communities or MeHg concentrations in prey.[10–12] In contrast to $\delta^{13}C$ and $\delta^{15}N$, sulfur ($\delta^{34}S$) stable isotopes are less

frequently utilized indicators of processes associated with Hg cycling (but see refs 7,8,13,14), despite the fact that they fractionate in response to sulfate reduction,[15,16] a key process associated with MeHg production.[5,17,18] In many waterbodies, fractionation associated with sulfate reduction results in aqueous sulfates that are isotopically enriched (i.e., have higher $\delta^{34}S$ values) relative to sedimentary sulfides, and the magnitude of this enrichment is positively correlated with sulfate reduction rates.[7,15,19] As with $\delta^{13}C$, $\delta^{34}S$ is generally conserved in consumers with little trophic enrichment.[20] Thus, the distinct $\delta^{34}S$ values of aqueous and sedimentary sulfur pools are often reflected in pelagic and benthic foraging consumers, respectively.[13,19,21,22] Further, $\delta^{34}S$ varies along a gradient from freshwater to marine habitats due to the large, highly enriched pool of marine sulfate, and $\delta^{34}S$ has been used to assess residency of estuarine fishes.[23,24] Thus, in some cases, $\delta^{34}S$ could provide unique insights into ecological and biogeochemical processes that are not apparent using $\delta^{13}C$ or $\delta^{15}N$.

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As transitional zones between freshwater and marine habitats, estuaries are characterized by diverse hydrologic, biogeochemical, and ecological gradients, which can result in large spatial and temporal variability in biota MeHg concentrations.[11,14,25,26] We exploited this diversity in the San Francisco Bay Estuary, CA, to better understand the ecological and biogeochemical processes influencing Hg bioaccumulation in estuarine fishes. Specifically, we coupled total Hg (THg) concentrations with stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotope ratios of fishes from 31 wetlands representing a range of habitats. We employed a tiered statistical approach that examined the relationships between fish THg concentrations and stable isotope ratios at multiple spatial and ecological scales. First, we examined large spatial scale processes associated with the substantial variation in fish THg concentrations observed among wetlands within the estuary.[26] In the second- and third-tiered analyses, we compared the relationships between THg and stable isotopes both among wetland habitats and within individual wetlands to better understand the roles of habitat-specific processes and local (within wetland) variation in shaping these larger scale patterns.

■ EXPERIMENTAL SECTION

Sample Collection, Processing, and Chemical Analyses. Between 2005 and 2008, we sampled fishes from 31 wetland sites representing five habitats (low salinity (20−40 ppt), moderate salinity (40−60 ppt), and high salinity (60−80 ppt) impounded wetlands, seasonal impounded wetlands, and tidal habitats (i.e., habitats that experience daily tidal action including: open bay, tidal sloughs, and breached saltponds that have direct tidal influence)) in the San Francisco Bay Estuary (Supporting Information (SI) Figure S1). These habitats represent a wide range of the biogeochemical and ecological variability within the San Francisco Bay Estuary. We sampled nine species of small, "forage" fishes (SI Table S1) with diverse foraging ecologies including generalists and both benthic and pelagic specialists. Fish were sampled using beach seines, gill nets, and minnow traps as described in Eagles-Smith and Ackerman.[26] Each fish was placed in a labeled polyethylene bag and stored on wet ice in the field for up to 6 h, then stored frozen at −20 °C until processing for THg and stable isotope analyses.

Prior to analysis, we rinsed each fish with deionized water, dried them with lint-free wipes, then measured standard length to the nearest millimeter using a fish board and wet mass to the nearest 0.01 g on an analytical balance. We oven-dried fish at 50 °C until they reached constant mass and then reweighed dried fish to determine moisture content. We homogenized dried fish to a fine powder in a ceramic mortar and pestle, and then stored them in a desiccator until analysis.

We measured THg as a proxy for MeHg because approximately 94% of all Hg in forage fishes from the San Francisco Bay Estuary is MeHg.[27,28] Total Hg was determined on a dry weight basis via Environmental Protection Agency method 7473.[29] Dry weight concentrations can be converted to wet weight concentrations using the species specific moisture contents provided in Eagles-Smith and Ackerman.[26] We analyzed stable isotope ratios in fish tissues using isotope ratio mass spectrometry and present ratios in standard δ-notation. See SI for additional details on chemical analyses including quality assurance measures.

Statistical Analyses. Total Hg concentrations in several species of fishes increased with fish length.[26] Therefore, in the species displaying significant THg-length relationships, we standardized each fish's THg concentration to the value predicted at the mean standard length for each species (SI Table S1). This was accomplished using analysis of covariance (ANCOVA) as described in Eagles-Smith and Ackerman[26] accounting for spatial and temporal variation in the relationship between THg and fish length. These size-standardized THg concentrations were natural log-transformed and utilized in all subsequent statistical analyses. We selected this approach rather than simply including fish length as a covariate in models because the relationships between THg and fish length varied among species and sites, and the inclusion of these interactions hindered model interpretation.

We employed a tiered statistical approach that examined the relationships between fish THg concentrations and stable isotope ratios (1) across the entire estuary, regardless of site or habitat; (2) within each of five habitats; and (3) within individual sites. The first tier allowed us to identify landscape-scale processes driving the substantial variation in fish THg concentrations observed among San Francisco Bay wetlands,[26] whereas the second and third tier analyses provided insights into the roles of habitat-specific processes and local (within wetland) variation in shaping these larger scale patterns.

First, we assessed the relationships between fish THg concentrations and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ across all sites, independent of habitat using linear mixed-effect models within an information theoretic framework. Specifically, we ranked a set of a priori candidate models that included all combinations of species as a fixed effect; $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ as covariates; and two-way interactions between species and each isotope. We also included the combination of wetland site and year (wetland-year) as a random effect in order to nest individual fish within the wetland and year where they were captured, thus accounting for the influence of spatial-temporal variation in baseline $\delta^{15}N$ values on the relationship between THg and $\delta^{15}N$.[30] We evaluated models using Akaike's Information Criterion (AIC_c) adjusted for smaller sample sizes, the difference in AIC_c between the best model and each alternative model (ΔAIC_c), Akaike weights (w_i), and evidence ratios.[31] We defined models with $\Delta AIC_c \leq 2$ as plausible alternatives to the top ranked model except when alternative models differed only in the addition of uninformative variables (i.e., variables that do not improve model fit).[32] We evaluated the effect and relative importance of individual variables using model-averaged coefficients, 85% confidence intervals of the model-averaged coefficients,[32] and variable weights (V; calculated as the sum of the Akaike's weights from each candidate model containing the variable).[31] Because the top ranked model from this analysis included species × $\delta^{13}C$, species × $\delta^{15}N$, and species × $\delta^{34}S$ interactions (SI Table S2), we also analyzed species-specific model sets for the five most common species (Long-Jawed Mudsucker (*Gillichthys mirabilis*), Mississippi Silversides (*Menidia audens*), Rainwater Killifish (*Lucania parva*), Top-smelt (*Atherinops affinis*), and Threespine Stickleback (*Gasterosteus aculeatus*)) that occurred in >10 wetland sites and comprised approximately 88% of all fish sampled. These species-specific model sets included all combinations of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ as covariates and each model included wetland-year as a random-effect. We also included a null model that contained only the intercept and the random effect of wetland-year (SI Table S3).

Table 1. Structure and Ranking Criteria of Candidate Models Describing Relationships among Size-Standardized Total Mercury Concentrations and Carbon (δ^{13}C), Nitrogen (δ^{15}N), and Sulfur (δ^{34}S) Stable Isotopes in Five Habitats of the San Francisco Bay Estuary, California[a]

habitat	structure	k	LogLik	ΔAIC$_c$	w_i	ER
Low Salinity Impounded Wetlands						
	δ^{13}C + δ^{15}N + δ^{34}S	7	−352.88	0.00	0.98	1.00
	δ^{15}N + δ^{34}S	6	−357.61	7.42	0.02	40.90
	δ^{13}C + δ^{34}S	6	−363.05	18.30	0.00	9.40×10^3
	δ^{34}S	5	−364.59	19.35	0.00	1.59×10^4
	δ^{13}C + δ^{15}N	6	−392.22	76.63	0.00	4.37×10^{16}
	δ^{15}N	5	−395.55	81.27	0.00	4.44×10^{17}
	NULL	4	−412.90	113.95	0.00	5.53×10^{24}
	δ^{13}C	5	−412.80	115.77	0.00	1.37×10^{25}
Moderate Salinity Impounded Wetlands						
	δ^{34}S	5	−46.49	0.00	0.55	1.00
	δ^{13}C + δ^{34}S	6	−46.48	2.07	0.19	2.82
	δ^{15}N + δ^{34}S	6	−46.49	2.10	0.19	2.85
	δ^{13}C + δ^{15}N + δ^{34}S	7	−46.48	4.19	0.07	8.12
	NULL	4	−57.68	20.30	0.00	2.56×10^4
	δ^{13}C	5	−57.08	21.19	0.00	4.00×10^4
	δ^{15}N	5	−57.48	21.99	0.00	5.97×10^4
	δ^{13}C + δ^{15}N	6	−57.02	23.16	0.00	1.07×10^5
High Salinity Impounded Wetlands						
	δ^{34}S	5	7.85	0.00	0.51	1.00
	δ^{13}C + δ^{34}S	6	8.26	1.58	0.23	2.20
	δ^{15}N + δ^{34}S	6	7.89	2.32	0.16	3.18
	δ^{13}C + δ^{15}N + δ^{34}S	7	8.45	3.67	0.08	6.27
	NULL	4	2.19	9.00	0.01	90.13
	δ^{13}C	5	2.26	11.18	0.00	267.43
	δ^{15}N	5	2.19	11.32	0.00	287.77
	δ^{13}C + δ^{15}N	6	2.26	13.57	0.00	886.00
Seasonal Impounded Wetlands						
	δ^{34}S	5	−22.88	0.00	0.44	1.00
	δ^{13}C + δ^{34}S	6	−22.36	1.34	0.23	1.95
	δ^{15}N + δ^{34}S	6	−22.65	1.90	0.17	2.59
	δ^{13}C + δ^{15}N + δ^{34}S	7	−21.52	2.08	0.16	2.83
	δ^{13}C	5	−33.89	22.03	0.00	6.09×10^4
	NULL	4	−35.66	23.28	0.00	1.13×10^5
	δ^{13}C + δ^{15}N	6	−33.85	24.30	0.00	1.89×10^5
	δ^{15}N	5	−35.59	25.43	0.00	3.33×10^5
Tidal Habitats						
	NULL	4	−69.41	0.00	0.19	1.00
	δ^{15}N	5	−68.51	0.40	0.15	1.22
	δ^{13}C + δ^{15}N	6	−67.41	0.41	0.15	1.23
	δ^{13}C	5	−68.56	0.49	0.15	1.28
	δ^{13}C + δ^{15}N + δ^{34}S	7	−66.66	1.18	0.10	1.81
	δ^{13}C + δ^{34}S	6	−67.89	1.37	0.09	1.98
	δ^{34}S	5	−69.06	1.50	0.09	2.11
	δ^{15}N + δ^{34}S	6	−68.16	1.91	0.07	2.60

[a]All models include wetland-year and species within wetland-year as random effects. k: number of estimated parameters in each model; −logLik: log-likelihood of the model; ΔAIC$_c$: difference in the AIC$_c$ value of the current model and the top model in the candidate set; ER: evidence ratio between the current model and the top model in the candidate set; w_i: Akaike weight of the current model, indicating the likelihood of the current model being the top model in the candidate set.

Because fish THg concentrations vary substantially among wetland habitats in the San Francisco Bay Estuary,[26] our second tier analysis assessed whether the relationships between fish THg concentrations and stable isotope ratios differed among habitats. This analysis allowed us to identify habitat-specific processes that may contribute to the large-scale variation observed across the larger estuary system. To do so, we utilized a quantitative model selection framework similar to that described above, to assess whether relationships between fish THg concentrations and δ^{13}C, δ^{15}N, and δ^{34}S differed among these habitats while accounting for the effects of species, wetland site, and year. Our global model included habitat as a fixed-effect; δ^{13}C, δ^{15}N, and δ^{34}S as covariates; and fish species and wetland-year as random-effects. We also included each two-way interaction between habitat and δ^{13}C, δ^{15}N, and δ^{34}S. Because the top-ranking model included interactions between

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habitat and both δ^{15}N and δ^{34}S (SI Table S5), we also developed sets of habitat-specific models that included each isotope as covariates, as well as species and wetland-year as random-effects (Table 1). We then ranked the resulting eight a priori candidate model sets for each habitat separately and assessed variable importance using model-averaged coefficients and variable weights as described above.

Finally, in our third tier analysis we evaluated the relationships between fish THg and stable isotope ratios within individual wetland sites to better understand how local variation in biogeochemical and ecological conditions influence Hg bioaccumulation. To accomplish this, we used an approach similar to that outlined in our previous analyses. For each of 25 wetland sites with $n > 15$ individual fish, we developed a model set consisting of eight a priori candidate models with δ^{13}C, δ^{15}N, and δ^{34}S as covariates and, when multiple years were sampled, year as a random effect. For this analysis we did not include fish species as a factor because the ecological variation among species is reflected as differences in their stable isotope values and stable isotopes provide continuous quantitative variables for examining this ecological variation both within and among species.[6,9] As in our previous analyses, we used AIC$_c$, ΔAIC$_c$, model weights, and evidence ratios to rank a set of a priori candidate models that included all possible combinations arising from this global model, as well as a null model that only included an intercept and the random effect.

RESULTS

Factors Influencing Fish Mercury Across Wetland Sites. In our first tier of analysis, the most parsimonious model describing fish THg concentrations across wetland sites included species, all three isotopes (δ^{13}C, δ^{15}N, δ^{34}S), and the interactions between species and each isotope, as well as the wetland-year random effect ($w = 0.55$; SI Table S2). One other model was within ΔAIC$_c \leq 2.0$ and included all of the same parameters except the species $\times$ δ^{13}C interaction was excluded ($w = 0.45$; SI Table S2). Using evidence ratios, we estimated that the top model was 97.8, 8.3×10^3, and 3.3×10^{39} times more likely than the same models without the main and interaction effects of δ^{13}C, δ^{15}N, and δ^{34}S, respectively. Although variable weights strongly supported the importance of species, δ^{13}C, δ^{15}N, and δ^{34}S (all $V = 1.0$), the interactions between species and δ^{15}N, δ^{34}S, and δ^{13}C suggested that the relationships between THg concentrations and each isotope varied substantially among species.

Because interactions between species and stable isotope ratios were important in our global model, we also conducted separate analyses for each of the five most common species (mudsucker, killifish, silverside, Topsmelt, and stickleback). The top model for all five species included δ^{34}S, but the inclusion of other parameters varied among species (SI Table S3). The top models explaining THg concentrations in mudsuckers and killifish across all sites included only δ^{34}S and had Akaike weights of 0.39 and 0.36, respectively. Several other models were within ΔAIC$_c \leq 2.0$ of the top model for both species, but all consisted of δ^{34}S and various uninformative parameters (SI Table S3).[32] The top model for silversides included δ^{13}C and δ^{34}S ($w = 0.58$). The next best model included δ^{13}C, δ^{34}S, and δ^{15}N (ΔAIC$_c = 1.06$; $w = 0.34$), though δ^{15}N was considered uninformative (SI Table S3). Using evidence ratios, the top model for silversides was estimated to be 10.3 and 341.6 times more likely than similar models without δ^{13}C and δ^{34}S, respectively. The most

parsimonious model for Topsmelt included δ^{15}N and δ^{34}S ($w = 0.34$). The model including δ^{15}N, δ^{34}S, and δ^{13}C ($w = 0.27$) and a model including only δ^{34}S ($w = 0.17$) were also plausible, but δ^{13}C was again considered uninformative (SI Table S3). The top model was only 2.3 times more likely than the model with only δ^{34}S, illustrating the over-riding importance of δ^{34}S in the top model. In stickleback, the top model included all three isotopes ($w = 0.30$), although the simplified model including only δ^{34}S also had very strong support (ΔAIC$_c = 0.11$; $w = 0.29$; SI Table S3). In fact, the evidence ratio of 1.1 between these top two models again indicated that δ^{34}S had the strongest relationship with THg concentrations in fish. Other models with ΔAIC$_c \leq 2.0$ included δ^{34}S with either δ^{13}C or δ^{15}N as uninformative parameters (SI Table S3).

In all five species, variable weights indicated that δ^{34}S was by far the most important variable influencing THg concentrations in fish (SI Table S4). Model-averaged coefficients estimated that THg concentrations in fish increased by 26−155% for every 5‰ increase in δ^{34}S and 68−562% (median 122%) over the range of observed δ^{34}S values in each species (Figure 1).
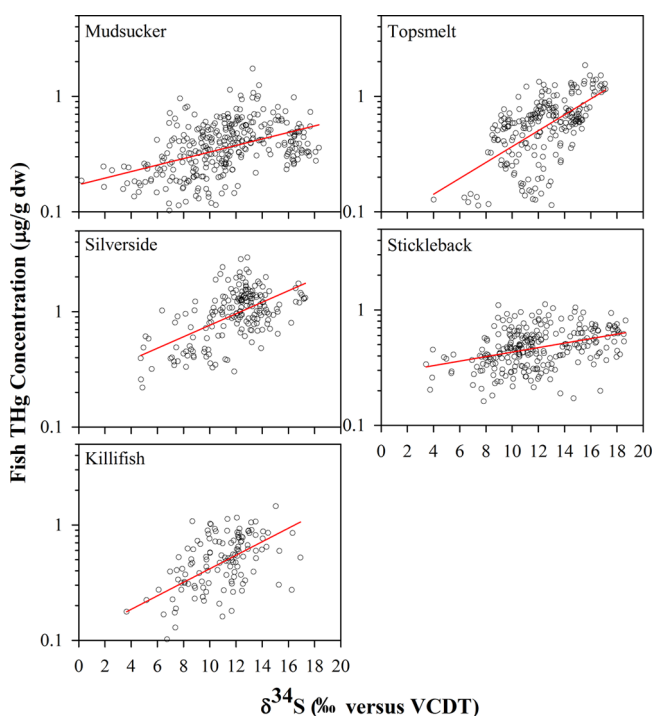

Figure 1. Relationships between sulfur stable isotope ratios (δ^{34}S; ‰ versus Vienna Canyon Diablo Troilite [VCDT]) and model-averaged predictions of size-adjusted total mercury (THg) concentrations in five species of forage fishes from wetland sites throughout the San Francisco Bay Estuary, CA. Predictions account for the effects of carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotopes as fixed effects, and wetland-year as a random effect.

Carbon isotopes were also important predictors of THg concentrations in silversides, but not any other species (SI Table S4). Silverside THg concentrations increased by 38% for each 5‰ increase in δ^{13}C and 74% over the observed range in δ^{13}C. We found moderate support for δ^{15}N as a predictor of THg concentrations in Topsmelt, but δ^{15}N was only weakly important in estimating THg concentrations in the other species (SI Table S4). Although δ^{15}N was included in the top ranked model for Topsmelt, the model-averaged coefficient for
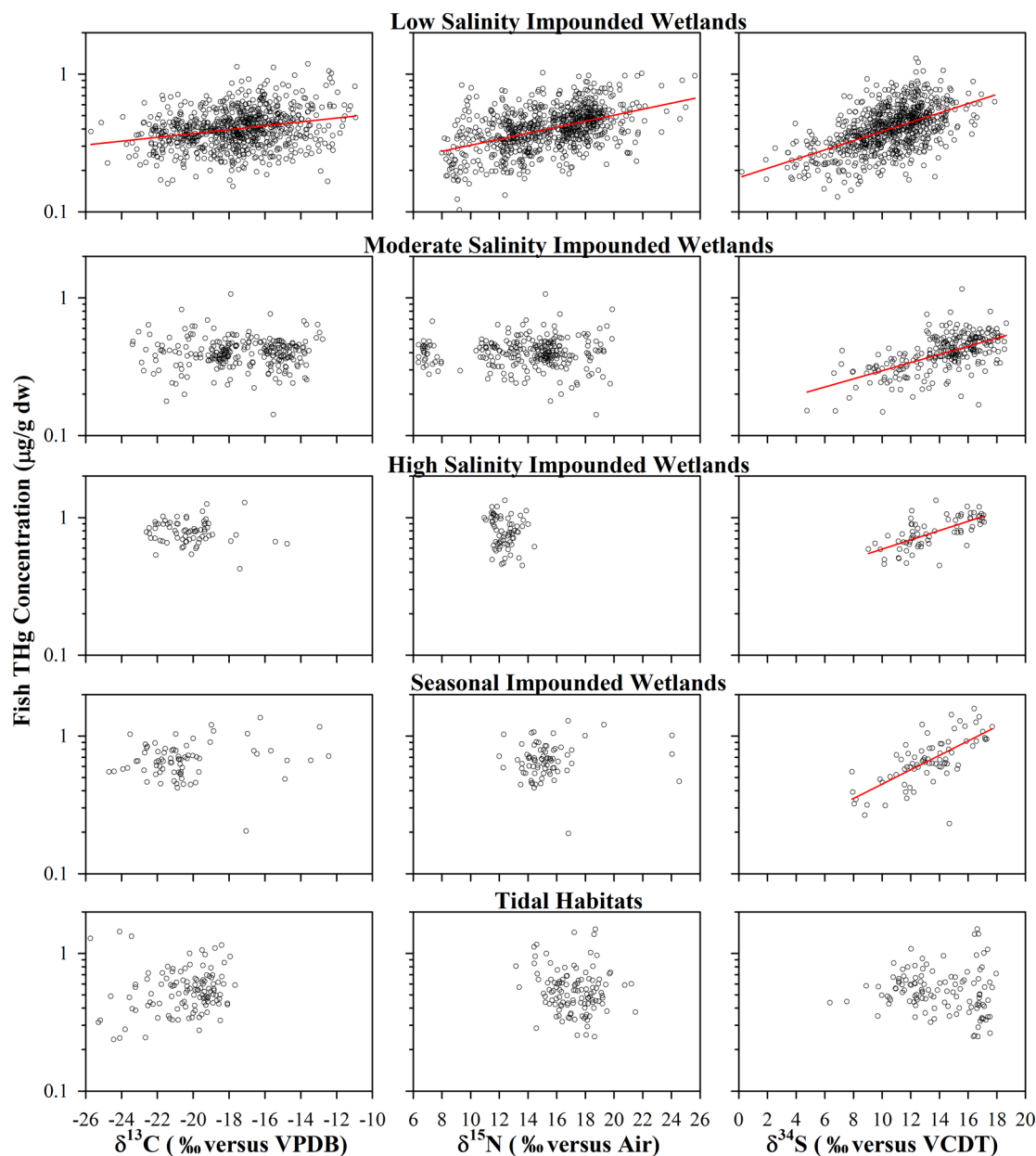
Figure 2. Relationships between carbon (δ^{13}C; ‰ versus Vienna Pee Dee Belemnite (VPDB)), nitrogen (δ^{15}N; ‰ versus Air), or sulfur (δ^{34}S; ‰ versus Vienna Canyon Diablo Troilite (VCDT)) stable isotope ratios and model-averaged predictions of size-adjusted total mercury (THg) concentrations in fish from six habitats in the San Francisco Bay Estuary, CA. Predictions account for δ^{13}C, δ^{15}N, or δ^{34}S as fixed effects, and wetland-year as a random effect.

δ^{15}N overlapped zero (SI Table S4), and thus δ^{15}N was only weakly correlated with fish THg concentrations.

Factors Influencing Fish Mercury Among Habitats. In our second tier analysis, the only plausible ($\Delta AIC_c \leq 2$) model describing variation in fish THg concentrations among habitats included habitat, δ^{13}C, δ^{15}N, δ^{34}S, the habitat $\times$ δ^{15}N and habitat $\times$ δ^{34}S interactions, as well as the random effects of species and wetland-year ($w = 0.90$; SI Table S5). Using evidence ratios, we estimated that this model was 207.6, 2.2 $\times$ 10^3, and 1.5 $\times$ 10^{27} times more likely than the same models without δ^{13}C, δ^{15}N, and δ^{34}S (and the associated habitat $\times$ isotope interactions), respectively. Variable weights (V) strongly supported the importance of habitat, δ^{13}C, δ^{15}N, δ^{34}S, habitat $\times$ δ^{15}N, and habitat $\times$ δ^{34}S (all $V = 1.0$) with little support for the habitat $\times$ δ^{13}C interaction ($V = 0.1$).

The interactions between habitat and both δ^{15}N and δ^{34}S indicated that the relationships between fish THg concentrations and the two isotopes differed among habitats, confounding our ability to interpret the main effects alone in our initial model set. Therefore, we conducted separate analyses for each habitat while accounting for variation among species and wetland-year. In low salinity impounded wetlands, the only plausible model for estimating fish THg concentrations included all three isotopes ($w = 0.98$; Table 1). Using evidence ratios, we estimated this model was 40.9, 9.4 $\times$ 10^3, and 4.4 $\times$ 10^{16} times more likely than the similar models without δ^{13}C, δ^{15}N, and δ^{34}S included, respectively. The most parsimonious model explaining fish THg concentrations in each of the moderate salinity, high salinity, and seasonal impounded wetland habitats included only δ^{34}S ($w = 0.55$, 0.51, and 0.44,

respectively; Table 1). In all three of these habitats, other models within $\Delta AIC_c \leq 2$ of the top model consisted of $\delta^{34}S$ with the addition of parameters considered uninformative (Table 1). In contrast to these impounded wetland habitats, the most parsimonious model in tidal habitats was the null model consisting of an intercept with species and wetland-year as random effects. Although all candidate models were within $\Delta AIC_c \leq 2$ of this top model, no model improved upon the null model's explanatory ability, suggesting that these three stable isotopes were not useful predictors of fish THg concentrations in open tidal habitats of the San Francisco Bay Estuary.

In low salinity impounded wetlands, all three stable isotopes were important predictors of fish THg (SI Table S6). Fish THg concentrations in low salinity impounded wetlands increased by 20%, 40%, and 83% for every 5‰ increase in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively. Thus, fish THg concentrations were estimated to increase 60% over the range of observed $\delta^{13}C$, 143% over the range of $\delta^{15}N$, and 291% over the range of $\delta^{34}S$ when the other two isotopes are held at their mean values for each habitat (Figure 2). In contrast, $\delta^{13}C$ and $\delta^{15}N$ were not informative predictors of fish THg concentrations in moderate salinity impounded wetlands, high salinity impounded wetlands, or seasonal wetlands. Rather, variable weights suggested that $\delta^{34}S$ was the only important variable for determining fish THg concentrations in these three habitats (SI Table S6). Each 5‰ increase in $\delta^{34}S$ resulted in an increase in fish THg concentration of 54%, 58%, and 115% in moderate salinity, high salinity, and seasonal impounded wetlands, respectively. Fish THg concentrations increased by 160%, 87%, and 224% over the observed ranges of $\delta^{34}S$ in moderate salinity, high salinity, and seasonal impounded wetlands, respectively (Figure 2). Conversely, variable weights were low for $\delta^{13}C$ and $\delta^{15}N$ in these habitats and the model-averaged coefficients overlapped zero, indicating there was no relationship between fish THg concentrations and any of the three isotopes (Figure 2). None of the three isotopes were strong determinants of fish THg concentrations in tidal habitats (SI Table S6; Figure 2).

Factors Influencing Fish Mercury Within Wetland Sites. In our third tier analysis, we examined the relationships between fish THg concentrations and stable isotope ratios within each of 25 wetland sites. We evaluated eight candidate models for each site (SI Table S5), and seven of the eight models ranked as the most parsimonious model in at least one of the 25 sites. The model containing both $\delta^{15}N$ and $\delta^{34}S$ was most commonly ranked as the top model explaining fish THg concentrations (top model in 24% of wetland sites), followed by the model that contained only $\delta^{34}S$ (top model in 20% of wetland sites; SI Table S7). The model containing only $\delta^{15}N$, and the model including all three isotopes, were each the top model in 16% of sites (SI Table S7). Within individual wetlands, $\delta^{15}N$ and $\delta^{34}S$ often were more important predictors of fish THg concentrations (median V = 0.8 and 0.7, respectively) than $\delta^{13}C$ (median V = 0.3). However, variable weights for all three isotopes varied considerably among the 25 sites (SI Table S8). Additionally, THg concentrations were correlated with either $\delta^{15}N$ or $\delta^{34}S$ in more than twice as many sites as $\delta^{13}C$. Model-averaged beta coefficients for $\delta^{13}C$ differed from zero in only four of the 25 wetland sites, with two sites displaying a positive correlation with fish THg and the other two sites displaying negative correlations (Figure 3a). Beta coefficients for $\delta^{15}N$ and $\delta^{34}S$ differed from zero in 10 and 11 of the 25 wetlands, respectively (Figure 3b,c). Fish THg concentrations were estimated to increase by 28−292%
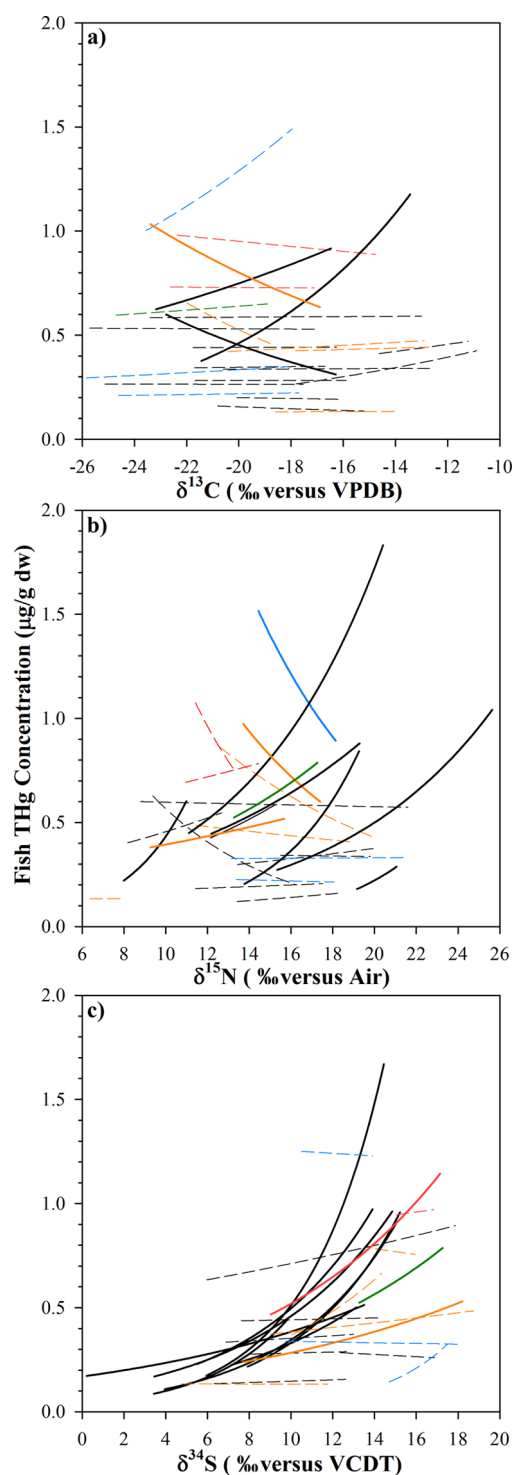

Figure 3. Relationships between total mercury (THg) concentration and stable isotope ratios of (A) carbon ($\delta^{13}C$; ‰ versus Vienna Pee Dee Belemnite [VPDB]); (B) nitrogen ($\delta^{15}N$; ‰ versus Air); and (C) sulfur ($\delta^{34}S$; ‰ versus Vienna Canyon Diablo Troilite [VCDT]), in forage fish from 25 wetland sites in the San Francisco Bay Estuary, CA. Solid lines have model-averaged-coefficients with 85% confidence intervals that do not overlap zero, whereas dashed lines have confidence intervals overlapping zero. Black lines are low salinity impounded wetlands, orange lines are moderate salinity impounded wetlands, red lines are high salinity impounded wetlands, green lines are seasonal wetlands, and blue lines are tidal habitats. Predictions account for $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ as fixed effects, and year (where applicable) as a random effect.

(median = 151%) per 5‰ change in $\delta^{15}N$ (36−317%, median = 142%, over the observed range in each wetland) in the eight wetlands where THg and $\delta^{15}N$ were positively correlated, and declined by 52% and 56% per 5‰ change in $\delta^{15}N$ (39% and 41%, respectively over the observed range in each wetland) in two wetlands where THg and $\delta^{15}N$ were negatively correlated (Figure 3b). Although fish THg concentrations and $\delta^{34}S$ were only correlated in 11 of the 25 wetlands, model-averaged coefficients in these wetlands were consistently positive and THg was estimated to increase 58−506% (median = 193%) for every 5‰ increase in $\delta^{34}S$ (123−860%, median = 325%, over the range of $\delta^{34}S$ observed in each wetland site; Figure 3c) when fixing the other two isotopes at their means in each wetland site.

■ DISCUSSION

Employing a multiple isotope approach across a gradient of estuarine habitat, we found that sulfur isotope ratios ($\delta^{34}S$) were important predictors of variation in fish THg concentrations, and that the influence of $\delta^{34}S$ was apparent at the landscape scale, among wetland sites, and within nearly half of the wetlands examined. In contrast, we found that the importance of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, which are frequently used as indicators of trophic processes influence on THg concentrations in fish, varied substantially among habitats and among individual wetlands. Further, in many individual wetlands, $\delta^{34}S$ appeared to differentiate consumers more clearly than $\delta^{13}C$, possibly reflecting more variable $\delta^{34}S$ of primary consumers relative to $\delta^{13}C$. Together, our results highlight both the utility of a multiple isotope approach employed across habitats and scales, as well as the important role of sulfur cycling in determining THg concentrations of small fish in wetlands of the San Francisco Bay Estuary.

Sulfur isotope ratios reflect a variety of estuarine processes, including availability of isotopically enriched marine sulfates,[23,24] dietary accumulation of isotopically depleted sedimentary sulfide relative to enriched aqueous sulfate,[19,21,22] and variation in the production or retention of isotopically enriched aqueous sulfates due to differing sulfate reduction rates.[15,33] We observed a wide range (0−19‰) in $\delta^{34}S$ of fish tissue among wetlands. Because of the extremely high concentration (∼28 mM in seawater compared to <100 μM in freshwater) and uniform $\delta^{34}S$ (∼21‰) of the marine sulfate pool relative to other potential sulfate sources, it is unlikely that inputs of marine sulfates alone could explain this large range.[23] Further, it is unlikely that relationships between fish THg concentration and $\delta^{34}S$ would be observed among such a variety of fish species and across sites if variation in $\delta^{34}S$ was due to differences between food webs deriving sulfur predominantly from sulfides (i.e., benthic based) versus sulfates (i.e., pelagic based).[19,21] Thus, the wide range of $\delta^{34}S$ observed among wetlands is likely the result of differential sulfate reduction rates or retention of reduced sulfate, with higher $\delta^{34}S$ values suggestive of higher sulfate reduction rates at particular sites.[15,23,24] Importantly, sulfate reduction is also a key microbial process associated with the production of bioavailable MeHg[18] and increased sulfate reduction rates can increase MeHg concentrations in both water[7,8] and fish.[34−36] Further, sulfate reduction rates, sulfate $\delta^{34}S$, and aqueous MeHg concentrations are positively correlated in some ecosystems.[7,8] Notably, we found that fish THg concentrations were strongly correlated with $\delta^{34}S$ specifically in impounded wetland habitats,

where fish THg concentrations are typically elevated,[26] but not in tidal habitats where THg concentrations are generally lower. The higher importance of $\delta^{34}S$ in impounded wetlands compared to tidal habitats coincides with differences in sulfate reduction and Hg methylation rates in the two habitats, which are typically higher and more variable in impounded habitats.[37]

Sulfur biogeochemistry may also be an important component of Hg cycling in tidal habitats, but may not be detectable using $\delta^{34}S$ because the isotopic enrichment of residual sulfate pools associated with sulfate reduction is unlikely to occur in habitats where the essentially unlimited marine sulfate pool is replenished with tidal action.[21,23] Similarly, tidal flushing may prevent the localized bioaccumulation of MeHg in these tidal habitats, essentially removing any localized effects of MeHg production. For example, repeated impoundment of a coastal marsh in San Francisco Bay resulted in the accumulation of sulfate reduction byproducts, including MeHg, which were not accumulated during periods of tidal connectivity.[38]

Previous studies suggest that foraging habitat (i.e., benthic versus pelagic foraging determined using $\delta^{13}C$) and trophic position (as indicated by $\delta^{15}N$) are important determinants of broad spatial patterns in MeHg bioaccumulation within estuaries, with higher THg concentrations typically associated with more pelagic foraging ecologies and higher trophic position consumers.[11,14,25] However, we found relatively little influence of $\delta^{13}C$ and $\delta^{15}N$ on fish THg concentrations among wetland sites or habitats, despite sampling a variety of species with diverse feeding ecologies. Fishes within low salinity impounded wetlands, where THg concentrations were strongly correlated with $\delta^{13}C$ and $\delta^{15}N$ (Figure 2), were a notable exception to this trend. This suggests that foraging habitat and trophic position play a more important role in influencing fish THg concentrations in low salinity impounded wetlands relative to the other habitats. Such a contrast between low salinity impounded wetlands and the other habitats may stem from the substantial gradient in food web complexity among habitats. In low salinity impounded wetlands, primary producer and invertebrate diversity (i.e., food web complexity) is substantially higher than in the more saline habitats where food webs are dominated by pelagic production and either brine shrimp (*Artemia* spp.) or brine flies (*Ephydra* spp).[39−41] Such low diversity of primary producers and dominance of a single or few prey resources in higher salinity wetlands channels energy through one large pathway with few trophic levels as opposed to a more complex network of trophic interactions associated with the utilization of multiple food resources. As a result of this reduced complexity, the role of trophic variation in determining fish THg concentrations is likely limited in higher salinity habitats (i.e., > 60 ppt in this study). Indeed, the mean variance in $\delta^{13}C$ (6.6) and $\delta^{15}N$ (10.6) within low salinity (40−60 ppt) impounded wetlands was substantially higher than the mean among the other habitats (4.5 and 4.6, respectively). Such variability enables differentiation of macrophyte or algal based benthic food webs from pelagic food webs based on phytoplankton[21,42] and facilitates biomagnification of MeHg.

The relatively low variation in $\delta^{13}C$ among primary consumers from higher salinity habitats suggests that $\delta^{13}C$ alone may not adequately differentiate benthic and pelagic food webs in all estuarine habitats or individual wetlands.[43] In these cases, additional biomarkers can be useful for differentiating food web pathways. In the current study, this is demonstrated by the differences in importance of $\delta^{13}C$ and $\delta^{34}S$ within individual wetlands, despite both isotopes presumably

representing differences between benthic and pelagic food web use within wetlands.[13,19,21,22,44] Correlations between fish THg concentrations and $\delta^{13}C$ existed within only 16% of the 25 wetlands, were generally weak, and were both positive and negative in direction (Figure 3). In contrast, fish THg concentrations were correlated with $\delta^{34}S$ in three times as many wetlands (44% of all wetlands) and consistently displayed a positive relationship (Figure 3). As discussed above, variation in $\delta^{34}S$ of fishes from the same wetland is most likely due to variation in reliance on reduced sulfides derived from benthic foraging compared to enriched sulfates derived from pelagic foraging.[19,21] Thus, the positive relationships between fish THg and $\delta^{34}S$ within wetlands suggest that higher THg concentrations in fish were associated with increased reliance on pelagic resources, as has been observed previously in San Francisco Bay forage fish.[26,27] Other studies in wetlands of the San Francisco Bay Estuary also have found that differences in $\delta^{13}C$ of primary producers were much smaller than those in $\delta^{34}S$ (0.3–3.2‰ for $\delta^{13}C$ compared to 10–21‰ for $\delta^{34}S$) and that $\delta^{34}S$ distinguished between benthic and pelagic energy sources more consistently than $\delta^{13}C$ in many wetlands.[45] In fact, for a wide array of estuarine habitats, differences between primary producers were consistently larger for $\delta^{34}S$ than $\delta^{13}C$ and the inclusion of $\delta^{34}S$ significantly improved source attribution compared to $\delta^{13}C$ alone or $\delta^{13}C$ and $\delta^{15}N$ together.[44] Thus, in addition to providing novel insights into the biogeochemical processes controlling MeHg bioavailability at the base of food webs, our results suggests that $\delta^{34}S$ may also be important for accurately characterizing food web processes that lead to bioaccumulation within estuarine food webs.

Our data highlight the value of examining ecological and biogeochemical processes in concert when assessing bioaccumulation of MeHg in estuarine fishes. In particular, the large gradients in $\delta^{34}S$ among estuarine habitats and wetland sites, coupled with the strong relationships between fish THg concentrations and $\delta^{34}S$ at several spatial scales, suggest that variation in sulfur biogeochemistry plays an important role in influencing estuarine fish THg concentrations. Further, our data indicate that the relationship between fish THg concentrations and biogeochemical processes are modulated by ecological variation among estuarine habitats (large scale) and within wetland sites (small scale), underscoring the contribution of local ecological processes in shaping patterns of MeHg bioaccumulation. Thus, our data suggest that management activities aimed at mitigating MeHg risk to wildlife should address both the biogeochemical processes regulating MeHg production and the ecological processes that lead to exposure.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b05325.

Additional methods, map of study sites (Figure S1); species, number, and size of fish sampled (Table S1); model selection tables (Tables S2, S3, S5, S7); and tables of variable weights and model-averaged coefficients (Tables S4, S6, S8) (PDF)

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Notes
The authors declare no competing financial interest.

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