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Agricultural Wetlands as Potential Hotspots for Mercury Bioaccumulation: Experimental Evidence Using Caged Fish

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Wetlands provide numerous ecosystem services, but also can be sources of methylmercury (MeHg) production and export. Rice agricultural wetlands in particular may be important sites for MeHg bioaccumulation due to their worldwide ubiquity, periodic flooding schedules, and high use by wildlife. We assessed MeHg bioaccumulation within agricultural and perennial wetlands common to California's Central Valley during summer, when the majority of wetland habitats are shallowly flooded rice fields. We introduced caged western mosquitofish (*Gambusia affinis*) within white rice (*Oryza sativa*), wild rice (*Zizania palustris*), and permanent wetlands at water inlets, centers, and outlets. Total mercury (THg) concentrations and body burdens in caged mosquitofish increased rapidly, exceeding baseline values at introduction by 135% to 1197% and 29% to 1566% among sites, respectively, after only 60 days. Mercury bioaccumulation in caged mosquitofish was greater in rice fields than in permanent wetlands, with THg concentrations at wetland outlets increasing by 12.1, 5.8, and 2.9 times over initial concentrations in white rice, wild rice, and permanent wetlands, respectively. In fact, mosquitofish caged at white rice outlets accumulated 721 ng Hg/fish in just 60 days. Mercury in wild mosquitofish and Mississippi silversides (*Menidia audens*) concurrently sampled at wetland outlets also were greater in white rice and wild rice than permanent wetlands. Within wetlands, THg concentrations and body burdens of both caged and wild fish increased from water inlets to outlets in white rice fields, and tended to not vary among sites in permanent wetlands. Fish THg concentrations in agricultural wetlands were high, exceeding 0.2 μg/g ww in 82% of caged fish and 59% of wild fish. Our results indicate that shallowly flooded rice fields are potential hotspots for MeHg bioaccumulation and, due to their global prevalence, suggest that agricultural wetlands may be important contributors to MeHg contamination.

Introduction

Wetland environments provide numerous benefits both ecologically and economically (*1*). However, wetlands often have increased methylmercury (MeHg) production compared to other aquatic habitats, because biogeochemical conditions common within wetlands facilitate methylation of inorganic mercury (Hg; *2*–*4*). Methylmercury is a neurotoxin that biomagnifies through aquatic food chains, and can be toxic to wildlife and humans (*5*). Fluctuating water levels typical of intermittently and shallowly flooded wetlands can enhance the methylation and release of Hg from sediments (*6*). Consequently, wetlands contribute substantially to MeHg bioavailability within downstream environments (*7*–*9*) as well as to in situ bioaccumulation (*10, 11*). The trade-off between wetland benefits and the potential for enhanced MeHg production and associated impairment to wildlife complicate efforts to implement wetland restoration. Currently, the specific wetland habitat types and management practices that might alter MeHg production and bioavailability are unclear.

Rice agriculture is a predominant wetland environment worldwide, comprising about one-fifth of freshwater wetland habitats (*12*) and occupying about 11% (1,956,000 km^2) of the cultivated land area (*13*). Although most rice cultivation occurs within Asia (*13*), California's Central Valley produced about one-fifth of the United States' rice crop over the past decade (*14*). Rice grows in wetlands that are shallowly flooded (<30 cm) during spring and summer. After harvest, rice fields often are drained and then shallowly flooded again during winter to speed rice straw decomposition and provide important waterbird foraging habitat (*15*). This periodic wetting and drying of agricultural fields for rice cultivation may increase MeHg production and its subsequent bioaccumulation, as occurs in other wetland habitats (*6, 10*). Together, their global prevalence, frequency of wetting and drying, and high use by wildlife may make rice fields potential hotspots for MeHg bioaccumulation.

California's Central Valley watershed is highly contaminated with Hg due to historic Hg and gold mining (*16*). Currently, wetlands are estimated to account for 19% of MeHg loadings into the Sacramento-San Joaquin River Delta (*17*), and there is concern that this amount may increase as wetland restoration is implemented throughout the watershed. However, the relative contribution of MeHg production from different wetland habitats is unknown. The Central Valley historically contained 1.6–2.0 million ha of natural wetland habitat (*18*), much of which comprised ephemeral wetlands that were primarily inundated in winter and spring. Over 90% of these wetlands have been lost to agriculture and development with only 121,000 ha remaining (*18, 19*). In contrast, rice agriculture has become a significant wetland habitat in California's Central Valley, with 216,100 ha of white rice (*14*) and 8600 ha of wild rice (D. Klose, California Wild Rice Advisory Board, personal communication) planted in the Central Valley in 2007. Despite the predominance of both Hg and agricultural wetlands, bioaccumulation of MeHg within rice fields has not been quantified relative to adjacent wetlands.

In this study, we assessed Hg bioaccumulation within agricultural and nonagricultural wetlands during the rice growing season. As indicators of Hg bioaccumulation, we introduced western mosquitofish (*Gambusia affinis*) into cages placed at water inlets, centers, and outlets of white rice (*Oryza sativa*), wild rice (*Zizania palustris*), and permanent wetland habitats and exposed fish for 60 days. Concurrent with the removal of the caged mosquitofish, we also sampled naturally occurring wild mosquitofish and Mississippi silversides (*Menidia audens*) at the inlet and outlet of each wetland habitat to compare Hg bioaccumulation patterns between fish that were held in enclosures at discrete locations and those that were free to move throughout and among wetlands. Our main objectives were to examine

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whether Hg bioaccumulation (1) was greater in agricultural wetlands than in permanent wetlands, (2) increased from inlets to outlets with water duration within wetlands, and (3) whether Hg concentrations were influenced by fish length and relative body condition.

Experimental Section

Study Site. We conducted our study in 2007 at Vic Fazio Yolo Wildlife Area (38.33° N, 121.4° W) within Yolo Bypass (23,877 ha floodway; Supporting Information). During summer, the main wetland habitat types were white rice and wild rice fields that were shallowly flooded for rice production, and permanently flooded wetlands.

Caged Fish Study. Western mosquitofish originated from Sacramento-Yolo Mosquito and Vector Control District's aquaculture facility (D. Dokos, Elk Grove, CA). We measured standard length (mm) and fresh wet mass (g), and visually determined sex of each fish. To determine baseline THg concentrations in fish at the time of introduction, we randomly selected 37 female mosquitofish from our stock population and stored them frozen in polyethylene bags at −20 °C until Hg analysis.

We introduced 30 randomly selected female mosquitofish into each cage placed at the inlet, center, and outlet of white rice, wild rice, and permanent wetland habitats on June 28, 2007, shortly after the white rice fields were reflooded after being seeded. Mosquitofish were removed 60 days after introduction. Cages were constructed of 6-mm polypropylene aquaculture mesh (Industrial Netting, Minneapolis, MN) affixed to a polyvinyl chloride pipe frame and measured 122 cm × 61 cm × 61 cm (L × W × H; 454 L). The same cage design was used successfully to examine natural diet and growth rates of juvenile salmon (*20*). We placed fish cages approximately 15 m from the water inlet and outlet within each wetland, and the top of the cages were positioned about 15 cm above the water surface. We also placed 30 female mosquitofish into a second cage at the outlet (located 20 m away) that was removed at the midpoint (29 days) of the 60 day exposure period to assess temporal bioaccumulation patterns. Each wetland habitat type was replicated twice; thus, we introduced a total of 24 fish cages (720 total fish) into six different wetlands. Mosquitofish density was 0.07 fish/L, or 0.11 g of fish/L, which is much lower than most caging experiments assessing contaminant bioaccumulation (review by ref *21*). Upon removal, we remeasured each fish's wet weight (g) and standard length (mm), and stored individuals in polyethylene bags at −20 °C.

Wild Fish Sampling. We also collected male and female wild mosquitofish and silversides at each wetland's inlet and outlet when caged fish were removed using beach seines and dip nets (August 27 to September 19). We measured and stored wild fish similarly to caged fish.

Mercury Determination. We determined whole-body total mercury (THg) concentrations via thermal decomposition and cold-vapor atomic absorption spectroscopy following EPA method 7473 (*22*) and MeHg concentrations at Battelle Marine Sciences Laboratory (Sequim, WA) using cold vapor atomic fluorescence following EPA method 1630 (*23*). We reported THg and MeHg concentrations on a dry weight (dw) basis; moisture content (mean ± SE) was 75.9 ± 0.1% in caged mosquitofish, 73.1 ± 0.2% in wild mosquitofish, and 72.8 ± 0.1% in wild silversides. For comparison with toxicity thresholds developed on a wet weight (ww) basis, we also calculated ww concentrations using individual moisture content values. Additional details and quality assurance measures are in Supporting Information.

Statistical Analysis. We tested whether whole-body THg concentrations ($\log_e$-transformed) of caged mosquitofish exposed for 60 days differed among factors using a mixed-effect analysis of covariance (ANCOVA) while accounting for any effects of fish size or condition (JMP v8.0, SAS Institute, Cary, NC). The global ANCOVA model for THg concentrations included wetland habitat type (white rice, wild rice, and permanent wetland), site (inlet, center, and outlet), fish standard length (length; $\log_e$-transformed), and relative body condition (condition) as fixed effects, wetland replicate as a random effect, and wetland type × site interaction. We determined fish body condition using the Relative Condition Factor (see Supporting Information for details and equations).

Total body burden of THg was calculated for each fish as the product of each individual fish's body mass (dw) and its whole-body THg concentration. The global ANOVA model for THg burden ($\log_e$-transformed) in caged mosquitofish exposed for 60 days was similar to that for THg concentrations, except that this model did not include length or condition as covariates since fish size was incorporated when calculating body burden. Similarly, we tested THg concentrations and burdens in wild mosquitofish and wild silversides using the same model structure as for caged fish, except that we only sampled wild fish from inlets and outlets, and not centers.

There were significant interactions between wetland type and site in all models (see Results); we therefore used conditional F-tests (slices) to test the effects of habitat separately by site, and site separately by habitat, while also accounting for other variables in the model. We then used pairwise *t*-tests to examine which habitats and sites differed.

Lastly, we assessed temporal THg bioaccumulation using reference fish at introduction and mosquitofish caged at wetland outlets. For this analysis, we compared THg concentrations and burdens among three time periods: (1) baseline mosquitofish at introduction, (2) mosquitofish removed after 29 days, and (3) mosquitofish removed after the full 60 days of exposure. We used a mixed effect ANCOVA similar to our primary models, but we removed site and added time period (baseline, 29-day exposure, and 60-day exposure) and wetland type × time period as fixed effects. For these temporal analyses, we randomly selected 12 of the 37 reference mosquitofish at introduction to be assigned to each habitat type at time zero (baseline) to avoid pseudoreplication of reference fish.

Unless otherwise noted, we reported model-based mean ± standard error (SE) THg concentrations and burdens based on back-transformed least-squares means ± SEs. We considered results statistically significant when $\alpha \leq 0.05$.

Results

Caged Fish Mercury Bioaccumulation After 60 Days of Exposure. Mosquitofish did not differ in size among cage sites or habitat types upon introduction, but grew most at centers and outlets of agricultural wetlands and grew least at these wetland's inlets or within permanent wetlands (see Supporting Information, Table S2, and Figure S3). Baseline THg concentrations and body burdens in reference mosquitofish at introduction were 0.14 ± 0.01 μg/g dw ($n = 37$; range: 0.08−0.27) and 0.05 ± 0.01 μg/fish dw ($n = 37$; range: 0.01−0.29), respectively. Methylmercury concentrations in a subset of individuals were highly correlated with THg concentrations (linear regression: $n = 9$, $r^2 = 0.98$, $p < 0.0001$; Figure S1), and MeHg accounted for 94.3 ± 4.8% of THg concentrations.

Across all wetland habitat types and sites, THg concentrations in mosquitofish held in cages for 60 days were significantly greater than those at introduction (Figure 1, Table S1). THg burden also was greater than baseline body burdens at all sites, but some sites within permanent wetlands and at white rice inlets were not statistically significant after applying the sequential Bonferroni correction. THg concentrations and burdens in caged mosquitofish increased by 135% to 1197% and 29% to 1566%, respectively (Table S1).

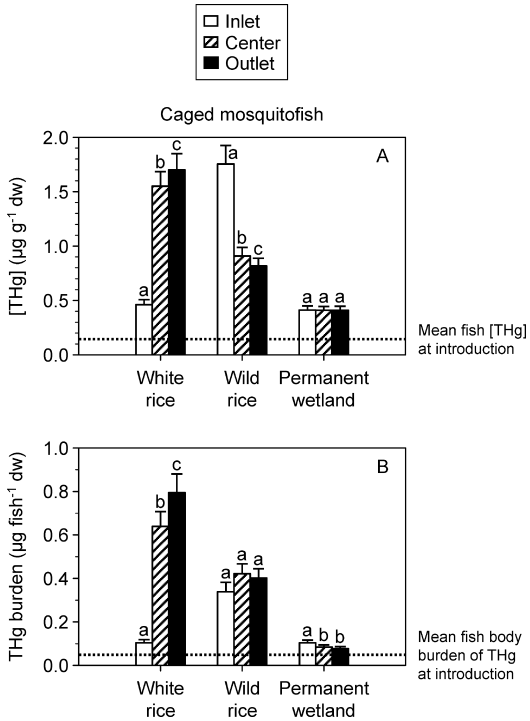

FIGURE 1. (A) Whole-body total mercury (THg) concentrations (μg/g dry weight; dw) and (B) THg burden (μg/fish dw) in western mosquitofish (*Gambusia affinis*) removed from cages after a 60-day exposure period at the inlets (open bars), centers (hatched bars), and outlets (solid bars) of white rice, wild rice, and permanent wetlands during the 2007 rice growing season at the Yolo Bypass Wildlife Area, California. Stippled lines indicate mean THg concentrations and body burdens of reference mosquitofish (n = 37) at the time of introduction into cages. Different lowercase letters above bars indicate that values within a wetland habitat are statistically different (p < 0.05).

Overall, model-based average THg concentrations (μg/g dw) in caged mosquitofish ($n = 304$) at removal were 1.07 ± 0.09, 1.09 ± 0.09, and 0.41 ± 0.04 in white rice, wild rice, and permanent wetlands, respectively, and 0.69 ± 0.04, 0.83 ± 0.04, and 0.83 ± 0.04 at the inlets, centers, and outlets, respectively.

In our global models, we found that THg concentrations in mosquitofish caged for 60 days were positively related to fish length and negatively related to condition, while accounting for wetland habitat type and cage site (Figure S2A). We found significant habitat type × site interactions for both THg concentrations (habitat: $F_{2,3.0} = 43.28$, $p = 0.01$, site: $F_{2,291.9} = 13.02$, $p < 0.0001$, habitat × site: $F_{4,290.9} = 165.66$, $p < 0.0001$, length: $F_{1,292.8} = 38.85$, $p < 0.0001$, condition: $F_{1,292.5} = 35.20$, $p < 0.0001$) and burdens (habitat: $F_{2,3.1} = 70.04$, $p = 0.01$, site: $F_{2,294.0} = 58.83$, $p < 0.0001$, habitat × site: $F_{4,293.9} = 61.89$, $p < 0.0001$). We therefore used conditional F-tests to further interpret whether THg concentrations and burdens differed among habitats and sites.

THg Concentrations in Caged Mosquitofish. THg concentrations in caged mosquitofish differed among wetland habitats at inlets ($F_{2,4.8} = 73.09$, $p = 0.001$), centers ($F_{2,3.5} = 56.51$, $p = 0.01$), and outlets ($F_{2,3.6} = 63.50$, $p = 0.01$; Figure 1A). At inlets, mosquitofish THg concentrations were greater in wild rice than either white rice ($t_{4.8} = 9.82$, $p = 0.001$) or permanent wetlands ($t_{4.8} = 11.00$, $P = 0.001$), but did not differ between white rice and permanent wetlands ($t_{4.8} = 0.85$, $p = 0.43$). At centers and outlets, mosquitofish THg concentrations were greater in white rice than either wild rice (center: $t_{3.5} = 4.40$, $p = 0.02$; outlet: $t_{3.6} = 5.98$, $p = 0.01$) or permanent wetlands (center: $t_{3.5} = 10.61$, $p = 0.001$; outlet: $t_{3.6} = 11.26$, $p = 0.001$), and mosquitofish THg concentrations were greater in wild rice than permanent wetlands (center: $t_{3.5} = 6.32$, $p = 0.01$; outlet: $t_{3.6} = 5.45$, $p = 0.01$).

Mosquitofish THg concentrations also differed among cage sites within white rice ($F_{2,290.7} = 194.89$, $p < 0.0001$) and wild rice ($F_{2,292.0} = 70.87$, $p < 0.0001$), but not permanent wetlands ($F_{2,290.5} = 0.01$, $p = 0.99$; Figure 1A). Within white rice fields, mosquitofish THg concentrations were greater at outlets than inlets ($t_{290.7} = 19.16$, $p < 0.0001$) or centers ($t_{290.7} = 2.25$, $p = 0.03$), and were greater at centers than inlets ($t_{290.7} = 18.35$, $p < 0.0001$). Within wild rice, mosquitofish THg concentrations were greater at inlets than centers ($t_{292.0} = 10.27$, $p < 0.0001$) or outlets ($t_{292.0} = 11.86$, $p < 0.0001$), and greater at centers than outlets ($t_{292.0} = 2.73$, $p = 0.01$).

THg Body Burden in Caged Mosquitofish. THg burdens in caged mosquitofish differed among habitats at inlets ($F_{2,8.0} = 28.34$, $p = 0.001$), centers ($F_{2,4.2} = 91.46$, $p = 0.001$), and outlets ($F_{2,4.2} = 117.33$, $p = 0.001$; Figure 1B). At inlets, THg burdens were greater in wild rice than in white rice ($t_{8.0} = 6.26$, $p = 0.0001$) or permanent wetlands ($t_{8.0} = 6.71$, $p = 0.001$), but body burdens in white rice and permanent wetlands did not differ ($t_{8.0} = 0.01$, $p = 0.99$). At the centers and outlets, THg body burdens were greater in white rice than in either wild rice (center: $t_{4.2} = 2.79$, $p = 0.05$; outlet: $t_{4.2} = 4.46$, $p = 0.01$) or permanent wetlands (center: $t_{4.2} = 12.96$, $p = 0.0001$; outlet: $t_{4.2} = 14.91$, $p = 0.0001$), and body burdens in wild rice were greater than permanent wetlands (center: $t_{4.2} = 10.29$, $p = 0.001$; outlet: $t_{4.2} = 10.66$, $p = 0.001$).

THg burdens also differed among cage sites within white rice ($F_{2,292.6} = 151.91$, $p < 0.0001$) and permanent wetlands ($F_{2,293.8} = 4.19$, $p = 0.02$), but not wild rice ($F_{2,293.3} = 2.31$, $p = 0.10$; Figure 1B). Within white rice fields, THg burdens were greater at outlets than at inlets ($t_{292.6} = 17.04$, $p < 0.0001$) or centers ($t_{292.6} = 2.80$, $p = 0.01$), and body burdens at centers were greater than inlets ($t_{292.6} = 15.66$, $p < 0.0001$). In contrast, within permanent wetlands, THg burdens were greater at inlets than centers ($t_{293.8} = 1.99$, $p = 0.05$) or outlets ($t_{293.8} = 2.88$, $p = 0.01$), but body burdens at centers and outlets did not differ ($t_{293.8} = 0.91$, $p = 0.36$).

Temporal Mercury Bioaccumulation in Caged Fish. We also examined how quickly Hg was bioaccumulated in caged mosquitofish at wetland outlets, where we removed separate cages after 29 and 60 days of exposure. We found a significant habitat type × time period interaction for both THg concentrations (habitat: $F_{2,3.4} = 18.59$, $p = 0.01$, time period: $F_{2,7.1} = 75.32$, $p < 0.0001$, habitat × time period: $F_{4,11.89} = 10.17$, $p = 0.001$, length: $F_{1,204.5} = 56.93$, $p < 0.0001$, condition: $F_{1,203.8} = 5.64$, $p = 0.02$) and burdens (habitat: $F_{2,4.1} = 35.49$, $p = 0.01$, time period: $F_{2,6.9} = 35.31$, $p = 0.001$, habitat × time period: $F_{4,21.0} = 13.35$, $p < 0.0001$).

Temporal THg Concentrations in Caged Mosquitofish. THg concentrations in caged mosquitofish differed among time periods within white rice ($F_{2,8.0} = 65.09$, $p < 0.0001$), wild rice ($F_{2,7.9} = 29.26$, $p = 0.001$), and permanent wetlands ($F_{2,8.0} = 21.98$, $p = 0.001$; Figure 2A). Within white rice and wild rice fields, THg concentrations were greater after 60 days of exposure than after 29 days (white rice: $t_{8.0} = 8.01$, $p < 0.0001$; wild rice: $t_{7.9} = 4.50$, $p < 0.0001$) and both 29-day and 60-day exposed mosquitofish had greater THg concentrations than reference fish at introduction (29-day white rice: $t_{8.0} = 7.44$, $p = 0.01$; 29-day wild rice: $t_{7.9} = 5.76$, $p = 0.01$; 60-day white rice: $t_{8.0} = 9.54$, $p = 0.001$; 60-day wild rice: $t_{7.9} = 6.95$, $p = 0.01$). Within permanent wetlands, THg concentrations were greater after 60-days of exposure than after 29-days ($t_{8.0} = 5.97$, $p < 0.0001$) and 60-day exposed mosquitofish had greater THg concentrations than reference fish at introduction (29-day: $t_{8.0} = 2.46$, $p = 0.08$; 60-day: $t_{8.0} = 4.00$, $p = 0.02$). Mosquitofish THg bioaccumulation rates ([μg/g dw]/day) were 0.027, 0.015, and 0.005 between introduction and 29 days of exposure, and 0.020, 0.006, and 0.004 between 29

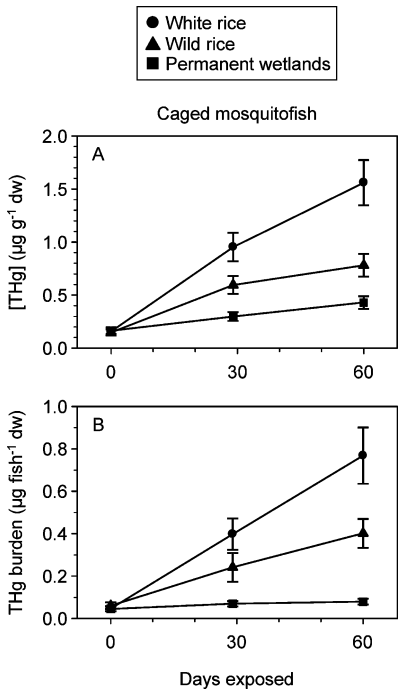

FIGURE 2. (A) Whole-body total mercury (THg) concentrations (μg/g dry weight; dw) and (B) THg burden (μg/fish dw) of western mosquitofish (*Gambusia affinis*) introduced into cages (time zero), removed from cages after 29 days, and removed from cages after 60 days of exposure at the outlets of white rice (circles), wild rice (triangles), and permanent wetlands (squares). The study was conducted during the 2007 rice growing season at the Yolo Bypass Wildlife Area, California.

and 60 days of exposure for white rice, wild rice, and permanent wetlands, respectively. Overall, 57%, 71%, and 50% of THg concentrations at day 60 occurred within the first 29 days in white rice, wild rice, and permanent wetlands, respectively.

Temporal THg Body Burden in Caged Mosquitofish. THg burdens in caged mosquitofish differed among time periods within white rice ($F_{2,9.2} = 46.04$, $p < 0.0001$) and wild rice ($F_{2,9.2} = 23.45$, $P = 0.001$), but not permanent wetlands ($F_{2,9.3} = 1.93$, $p = 0.20$; Figure 2B). Within white rice and wild rice fields, THg burdens were greater after 60 days than after 29 days (white rice: $t_{9.2} = 5.23$, $p < 0.0001$; wild rice: $t_{9.2} = 4.17$, $p < 0.0001$) and both 29-day and 60-day exposed mosquitofish had greater THg burdens than at introduction (29-day white rice: $t_{9.2} = 6.79$, $p = 0.01$; 29-day wild rice: $t_{9.2} = 4.41$, $p = 0.01$; 60-day white rice: $t_{9.2} = 9.10$, $p = 0.001$; 60-day wild rice: $t_{9.2} = 6.19$, $p = 0.01$). Mosquitofish THg bioaccumulation rates ([μg/fish dw]/day) were 0.012, 0.006, and 0.001 between introduction and 29 days of exposure, and 0.012, 0.005, and 0.001 between 29 and 60 days of exposure for white rice, wild rice, and permanent wetlands, respectively. Overall, 49%, 53%, and 71% of THg burdens at day 60 were bioaccumulated within the first 29 days in white rice, wild rice, and permanent wetlands, respectively.

Wild Fish Mercury Bioaccumulation. THg concentrations (μg/g dw) in wild mosquitofish ($n = 140$) were 0.67 ± 0.13, 0.75 ± 0.15, and 0.44 ± 0.08 in white rice, wild rice, and permanent wetlands, respectively, and 0.47 ± 0.06 and 0.79 ± 0.09 at inlets and outlets, respectively. THg concentrations in wild silversides ($n = 135$) were 0.82 ± 0.14, 0.92 ± 0.16, and 0.28 ± 0.05 in white rice, wild rice, and permanent wetlands, respectively, and 0.48 ± 0.05 and 0.74 ± 0.08 at inlets and outlets, respectively.

Similar to our caged fish models, we found significant interactions between habitat type × site for wild mosquitofish (THg concentrations: habitat: $F_{2,2.7} = 2.10$, $p = 0.28$, site: $F_{1,131.8} = 51.95$, $p < 0.0001$, habitat × site: $F_{2,130.6} = 42.71$, $p < 0.0001$, length: $F_{1,126.8} = 1.57$, $p = 0.21$, condition: $F_{1,131.4} = 7.01$, $p = 0.01$; THg burdens: habitat: $F_{2,3.1} = 0.47$, $p = 0.66$, site: $F_{1,134.0} = 26.98$, $p < 0.0001$, habitat × site: $F_{2,133.1} = 6.07$, $p = 0.01$) and wild silversides (THg concentrations: habitat: $F_{2,2.9} = 14.70$, $p = 0.03$, site: $F_{1,126.9} = 49.94$, $p < 0.0001$, habitat × site: $F_{2,126.1} = 24.01$, $p < 0.0001$, length: $F_{1,126.6} = 53.81$, $p < 0.0001$, condition: $F_{1,126.1} = 1.77$, $p = 0.19$; THg burdens: habitat: $F_{2,3.2} = 10.98$, $p = 0.04$, site: $F_{1,122.7} = 7.54$, $p = 0.01$, habitat × site: $F_{2,121.6} = 8.96$, $p = 0.001$). THg concentrations were positively related to fish length for wild silversides, but not for wild mosquitofish, and negatively related to condition for wild mosquitofish, but not wild silversides (Figure S2B,C).

THg Concentrations in Wild Fish. THg concentrations in both wild mosquitofish and silversides differed among wetland habitat types at outlets (mosquitofish: $F_{2,2.9} = 8.90$, $p = 0.05$; silversides: $F_{2,3.5} = 23.92$, $p = 0.01$), but not inlets (mosquitofish: $F_{2,3.6} = 1.13$, $p = 0.42$; silversides: $F_{2,3.3} = 6.68$, $p = 0.07$; Figure 3A,B). At outlets, fish THg concentrations were greater in white rice (mosquitofish: $t_{2.9} = 3.95$, $p = 0.03$; silversides: $t_{3.5} = 6.16$, $p = 0.01$) and wild rice (mosquitofish: $t_{2.9} = 3.22$, $p = 0.05$; silversides: $t_{3.5} = 5.59$, $p = 0.01$) than permanent wetlands, but fish THg concentrations did not differ between wild rice and white rice (mosquitofish: $t_{2.9} = 0.74$, $p = 0.51$; silversides: $t_{3.5} = 0.03$, $p = 0.98$).

THg concentrations in both wild mosquitofish and silversides also differed among sites within white rice (mosquitofish: $F_{1,130.0} = 126.60$, $p < 0.0001$; silversides: $F_{1,124.5} = 76.36$, $p < 0.0001$) and wild rice (mosquitofish: $F_{1,126.8} = 10.83$, $p = 0.001$; silversides: $F_{1,126.4} = 16.29$, $p < 0.0001$), but not permanent wetlands (mosquitofish: $F_{1,129.0} = 2.57$, $p = 0.11$; silversides: $F_{1,124.0} = 0.28$, $p = 0.60$; Figure 3A,B). Within white rice and wild rice fields, fish THg concentrations were greater at outlets than inlets (mosquitofish in white rice: $t_{130.0} = 11.25$, $p < 0.0001$; silversides in white rice: $t_{124.5} = 8.74$, $p < 0.0001$; mosquitofish in wild rice: $t_{126.8} = 3.29$, $p = 0.001$; silversides in wild rice: $t_{126.4} = 4.04$, $p = 0.0001$).

THg Body Burdens in Wild Fish. THg burdens in wild mosquitofish did not differ among habitats at inlets ($F_{2,4.1} = 0.95$, $p = 0.46$) or outlets ($F_{2,3.2} = 1.00$, $p = 0.46$; Figure 3A). However, THg burdens in wild mosquitofish differed among sites within white rice ($F_{1,131.6} = 19.54$, $p < 0.0001$) and wild rice ($F_{1,132.1} = 13.96$, $p = 0.001$), but not permanent wetlands ($F_{1,131.1} = 0.32$, $p = 0.57$; Figure 3A). Within white rice and wild rice fields, body burden was greater at outlets than inlets (white rice: $t_{131.6} = 4.42$, $p < 0.0001$; wild rice: $t_{132.1} = 3.74$, $p = 0.001$).

THg burden in wild silversides differed among habitats at outlets ($F_{2,5.4} = 20.42$, $p = 0.01$), but not inlets ($F_{2,5.7} = 2.50$, $p = 0.17$; Figure 3B). At outlets, burden was greater in white rice ($t_{5.4} = 6.33$, $p = 0.01$) and wild rice ($t_{5.4} = 3.17$, $p = 0.01$) than permanent wetlands, but burdens in wild rice and white rice did not differ ($t_{5.4} = 1.57$, $p = 0.15$). THg burden in wild silversides also differed among sites within white rice ($F_{1,127.3} = 23.64$, $p < 0.0001$), but not wild rice ($F_{1,99.4} = 1.09$, $p = 0.30$) or permanent wetlands ($F_{1,126.3} = 0.91$, $p = 0.34$; Figure 3B). Within white rice fields, THg burden was greater at outlets than inlets ($t_{127.3} = 4.86$, $p < 0.0001$).

Discussion

Within each wetland habitat type and site, THg concentrations and body burdens in mosquitofish removed from cages after 60 days of exposure were substantially greater than baseline values at introduction (Figure 1). Total Hg concentrations and burdens in caged mosquitofish increased by a range of 135% to 1197% and 29% to 1566% among sites, respectively, indicating that Hg was accumulated at all sites despite any potential differences in food availability, prey composition, or fish growth rates (Table S1). Mosquitofish THg concentrations and burdens after 29 days of exposure

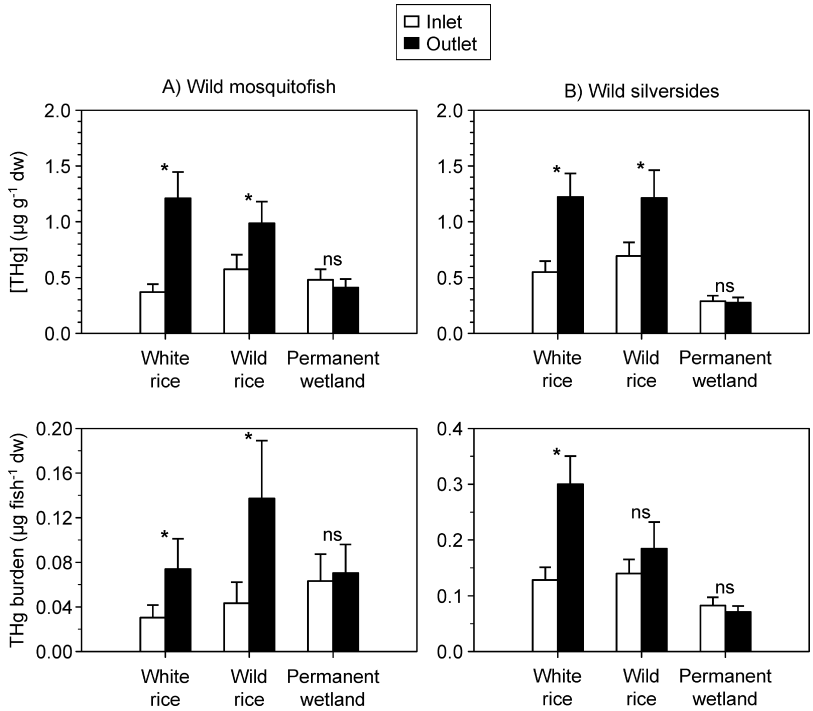

FIGURE 3. Whole-body total mercury (THg) concentrations (μg/g dry weight; dw) and THg burden (μg/fish dw) in (A) wild western mosquitofish (*Gambusia affinis*) and (B) wild Mississippi silversides (*Menidia audens*) caught at the inlets (open bars) and outlets (solid bars) of white rice, wild rice, and permanent wetlands during the 2007 rice growing season at the Yolo Bypass Wildlife Area, California. Asterisks above bars indicate that values within a wetland habitat are statistically different ($p < 0.05$) and "ns" indicates that values are not statistically different.

at rice field outlets also were greater than those at introduction, and concentration-based bioaccumulation rates tended to be slightly higher in the first half of exposure than in the latter half of the 60-day exposure period (Figure 2). Overall, mosquitofish caged at white rice outlets accumulated an average of 721 ng Hg/fish in just 60 days. It is unclear whether THg concentrations in caged mosquitofish would have continued to increase with additional exposure time, but we did find substantial increases between both 0−29 days and 29−60 days of exposure (Figure 2). Recent work in mesocosms from the Experimental Lakes Area found that after a single spike of MeHg into the water column, THg concentrations in small fish increased rapidly for the first 30 days and then reached an asymptote (*24*). In contrast, THg concentrations increased at a more constant rate and did not asymptote in fish enclosed in mesocosms that were spiked repeatedly with lower MeHg doses (*24*). Orihel et al. (*25*) also observed linear increases in MeHg concentrations in juvenile fish across a range of MeHg exposures over 10 weeks. Our results are similar to those studies detecting a linear response of fish Hg concentrations to repeated dosing of the water column, suggesting that MeHg exposure to caged mosquitofish may have been relatively consistent over the 60-day study period.

The greatest increases in THg concentrations and burdens of caged mosquitofish occurred within flooded white rice and wild rice fields, whereas concentrations in permanent wetlands were consistently lower (Figure 1). In particular, THg bioaccumulation in mosquitofish caged at wetland centers and outlets was highest in white rice, followed by wild rice, and then permanent wetlands. After 60 days, THg concentrations increased by 12.1, 5.8, and 2.9 times over baseline values at introduction in white rice, wild rice, and permanent wetlands, respectively. This pattern of bioaccumulation was different at the inlets, primarily due to wild rice where THg concentrations and burdens at inlets were 3−4 times greater than in white rice and permanent wetlands. It is unclear why mosquitofish caged at wild rice inlets had greater THg concentrations, considering the similarities in mosquitofish condition and source water among wetland inlets. Both white rice and wild rice fields had surface water MeHg concentrations lowest at inlets and greatest at outlets (M. Stephenson, Moss Landing Marine Laboratories, unpublished data). Perhaps water duration was longer at inlets of wild rice relative to white rice fields because wild rice was typically flooded deeper (20−30 cm) than white rice (10−15 cm). Congruent with our results for caged fish, we also found that THg concentrations in wild mosquitofish and silversides at outlets were substantially greater in white rice and wild rice than permanent wetlands, whereas THg concentrations in wild fish did not differ among habitats at the inlets (Figure 3).

The disparity in THg bioaccumulation between agricultural wetlands and permanently flooded wetlands may be the result of differences in wetting and drying patterns, resulting in varying redox conditions and MeHg production within the sediments. Analogous to the shallowly flooded, ephemeral nature of rice fields, Snodgrass et al. (*10*) found that Hg concentrations in fish were highest in South Carolina wetlands that were shallower and had larger water level fluctuations. Experimental flooding of wetlands in the Experimental Lakes Area also caused increases in Hg concentrations and body burdens in small fishes held in mesh pens (*11*). Water levels in wetlands we studied were shallowest and fluctuated most in rice fields (<30 cm) compared to permanent wetlands (0.8−1.3 m). Together, these results indicate that intermittent, shallow, and initial flooding of wetlands can enhance the methylation and release of Hg from sediments (*6*).

Mercury bioaccumulation also varied among sites within wetlands (Figures 1 and 3). Total Hg concentrations and burdens of both caged and wild fish increased from inlets to outlets in white rice fields, suggesting that there may be a gradient of bioaccumulation through the wetland as Hg becomes methylated, evapo-concentrated, and transported in the direction of water flow. In permanent wetlands where water was more stagnant, THg concentrations and burdens

showed less spatial variation. Within wild rice fields, the pattern of Hg bioaccumulation was less consistent. Whereas THg concentrations in wild mosquitofish and silversides increased from inlets to outlets, THg concentrations in caged mosquitofish actually declined from inlets to outlets in wild rice fields. However, THg burdens in caged mosquitofish were no different among sites (Figure 1B) indicating that the greater THg concentrations in caged mosquitofish at wild rice inlets (Figure 1A) may have been at least partially due to bioconcentration related to relatively poor fish condition (Figure S3). In fact, THg concentrations also were negatively correlated with the condition of caged and wild mosquitofish, but not wild silversides (Figure S2). This indicates that mosquitofish in relatively poor condition may have had greater THg concentrations due to bioconcentration. For instance, the same body burden of Hg in a fish that loses body mass would result in a greater THg concentration without any additional Hg being necessarily acquired via the diet (e.g., (*26*)).

In general, THg concentrations in caged and wild fish, as well as aquatic invertebrates (*27*), within agricultural wetlands were high in comparison with other mercury-impaired wetlands and common toxicity levels. For example, mean THg concentrations in wild mosquitofish from agricultural wetlands within Yolo Bypass were more than twice those of mosquitofish (*Gambusia holbrooki*) within the Florida Everglades (*28*). Overall, 82% of caged fish and 59% of wild fish within agricultural wetlands exceeded 0.20 μg/g ww, the fish health risk threshold associated with sublethal end points (*29*). Furthermore, all caged and wild fish exceeded the Central Valley Regional Water Quality Control Board's Total Maximum Daily Load (TMDL) target for Hg concentrations in small fish (0.03 μg/g ww) that is meant to be protective of wildlife (*17*). Although this TMDL target is likely below actual effects to wildlife, such as piscivorous waterbirds, 52% of caged fish and 26% of wild fish exceeded the dietary concentration of 0.30 μg/g ww which is commonly associated with impaired bird reproduction (*30, 31*). Thus, there may be substantial risk of MeHg toxicity to waterbirds and other wildlife that forage within agricultural wetlands. Widespread evaluation of Hg bioaccumulation and concentrations in higher trophic level predators, such as waterbirds, should be conducted in agricultural wetlands to determine wildlife exposure and risk.

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Supporting Information Available

Detailed information on changes in caged mosquitofish mercury concentrations and body size, correlations between fish THg and MeHg concentrations, correlations between THg concentrations and fish length and body condition, and quality assurance methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information for

Agricultural Wetlands as Potential Hotspots for Mercury Bioaccumulation: Experimental Evidence Using Caged Fish

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Total pages (including cover): 12
Number of Tables: 2
Number of Figures: 3

Supporting Information

Experimental Section

Study Area. The Yolo Wildlife Area is approximately 6,475 ha. We studied six wetlands that were <4 km from one another. The flooded area of the wetlands were 31 ha and 78 ha for the white rice fields, 33 ha and 44 ha for the wild rice fields, and 10 ha and 16 ha for the permanent wetlands.

Mercury Determination. We determined whole-body THg concentrations in all fish and whole-body MeHg concentrations in a small subset of fish to confirm that the majority of Hg was in the MeHg form (*1*). We removed the gastrointestinal tracts of caged fish prior to Hg analysis, since a larger proportion of the Hg in the gastrointestinal tract can be inorganic compared to other body tissues (*2*). Fish were dried at 50-60°C for 24-48 h until they reached a constant mass and then homogenized to a fine powder using a mortar and pestle. We analyzed individual fish for THg at the USGS Davis Field Station Mercury Lab on a Milestone DMA-80 Direct Mercury Analyzer (Milestone Inc., Monroe, Connecticut, USA) following EPA method 7473 (*3*). We analyzed MeHg at Battelle Marine Sciences Laboratory (Sequim, Washington, USA) using cold vapor atomic fluorescence following EPA method 1630 (*4*). Quality assurance measures included analysis of two certified reference materials (either dogfish muscle tissue [DORM-2; National Research Council of Canada, Ottawa, Canada], dogfish liver [DOLT-3; National Research Council of Canada, Ottawa, Canada], or lobster hepatopancreas [TORT-2; National Research Council of Canada, Ottawa, Canada]), two system and method blanks, two duplicates, one matrix spike, and one matrix spike duplicate per batch. For THg, recoveries (± standard error [SE]) averaged 99.4 ± 1.8% (n = 60) and 97.9 ± 0.8% (n = 90) for certified reference materials and calibration checks, respectively. Matrix spike recoveries for THg averaged 103.0 ± 0.53% (n = 30), and absolute relative percent difference for all duplicates and matrix spike duplicates averaged 3.4 ± 0.5%. For MeHg, recoveries averaged 91.20 ± 3.8% (n = 3) for certified reference materials. Matrix spike recoveries for MeHg averaged 97.3 ± 1.8% (n = 12), and absolute relative percent difference for all duplicates and matrix spike duplicates averaged 7.8 ± 1.6%.

Statistical Analysis. *Relative Condition Factor*. We estimated the relative body condition of fish using the Relative Condition Factor to account for potential changes in shape as fish grow (*5*), such as often occurs in female mosquitofish. The Relative Condition Factor was calculated as $K_n = W/W'$, where W was mass in g and W' was the predicted length-specific mean mass from a predictive model calculated for that population. To determine W' for the caged mosquitofish population, we used $\log_{10}$-transformed standard length (mm) and $\log_{10}$-transformed fresh wet mass (g) data for the mosquitofish that were introduced into cages as well as the reference mosquitofish analyzed for Hg (caged mosquitofish linear regression: n = 756, r^2 = 0.76, intercept = -4.3379, slope = 2.8584). We also calculated W' for each species of wild fish using all the wild fish captured and analyzed for Hg (wild mosquitofish linear regression: n = 140, r^2 = 0.95, intercept = -5.5443, slope = 3.5573; wild silverside linear regression: n = 135, r^2 = 0.95, intercept = -5.0217, slope = 2.9583).

Statistical Analysis. *Caged Fish Growth After 60-days of Exposure***.** We tested whether the size of mosquitofish removed from cages after 60 days of exposure differed among habitats and sites using a mixed effects analysis of variance (ANOVA). We performed separate ANOVAs for each of three size parameters ($\log_e$-transformed standard length [mm], $\log_e$-transformed fresh wet mass [g], and relative body condition). For each ANOVA, we included wetland habitat type (white rice, wild rice, and permanent wetland) and site (inlet, center, and outlet) as fixed effects, wetland replicate as a random effect, and the wetland type × site interaction. There were significant interactions between wetland type and site in all models; we therefore used conditional F-tests to test the effects of habitat separately by site, and site separately by habitat, while also accounting for the other variables in the models. We then used pair-wise *t*-tests to examine which pairs of habitats and sites differed. We also used two-sample *t*-tests to compare the size of fish at introduction to values 60 days later when fish were removed from cages, and we applied a sequential Bonferroni corrected alpha level to account for the number of tests performed for each variable.

Results

Caged Fish Growth After 60-days of Exposure. Upon introduction into cages, mosquitofish did not differ in standard length or mass among cage sites or habitat types (fish length: habitat: $F_{2,3} = 0.42$, $p = 0.69$; site: $F_{2,532} = 2.81$, $p = 0.06$; fish mass: habitat: $F_{2,3} = 0.53$, $p = 0.64$; site: $F_{2,531} = 0.60$, $p = 0.55$; Table S2). After 60 days of exposure, there were significant habitat type × site interactions for the length (habitat: $F_{2,3.0} = 4.68$, $p = 0.12$; site: $F_{2,294.4} = 34.57$, $p < 0.0001$; habitat × site: $F_{4,294.5} = 22.43$, $p < 0.0001$), mass (habitat: $F_{2,3.0} = 0.53$, $p = 0.64$; site: $F_{2,527} = 0.61$, $p = 0.54$; habitat × site: $F_{4,527} = 2.89$, $p = 0.02$), and relative condition factor (habitat: $F_{2,3.0} = 0.34$, $p = 0.74$; site: $F_{2,294.8} = 16.08$, $p < 0.0001$; habitat × site: $F_{4,294.8} = 4.65$, $p = 0.001$) of mosquitofish removed from cages.

Fish Length. The standard length of mosquitofish removed from cages differed among habitats at the centers ($F_{2,3.7} = 11.55$, $p = 0.03$) and outlets ($F_{2,3.7} = 14.51$, $p = 0.02$), but not at the inlets ($F_{2,6.1} = 1.18$, $p = 0.37$; Figure S3A). At the centers and outlets, fish length was greater in white rice (center: $t_{3.7} = 3.74$, $p = 0.02$; outlet: $t_{3.7} = 4.28$, $p = 0.01$) and wild rice (center: $t_{3.7} = 4.55$, $p = 0.01$; outlet: $t_{3.7} = 4.99$, $p = 0.01$) than in permanent wetlands, but fish length in white rice and wild rice did not differ (center: $t_{3.7} = 0.84$, $p = 0.46$; outlet: $t_{3.7} = 0.69$, $p = 0.53$).

Fish length also differed among cage sites within white rice ($F_{2,293.4} = 19.44$, $p < 0.0001$), wild rice ($F_{2,295.0} = 52.38$, $p < 0.0001$), and permanent wetlands ($F_{2,294.3} = 3.21$, $p = 0.04$; Figure S3A). Within white rice and wild rice fields, fish length was shorter at field inlets than at either the centers (white rice: $t_{293.4} = 5.37$, $p < 0.0001$; wild rice: $t_{295.0} = 8.98$, $p < 0.0001$) or outlets (white rice: $t_{293.4} = 6.18$, $p < 0.0001$; wild rice: $t_{295.0} = 9.93$, $p < 0.0001$), whereas there was no difference in fish length between centers and outlets (white rice: $t_{293.4} = 1.49$, $p = 0.14$; wild rice: $t_{295.0} = 1.22$, $p = 0.22$). Within permanent wetlands, fish length was greater at field inlets than at either the centers ($t_{294.3} = 2.31$, $p = 0.02$) or outlets ($t_{294.3} = 2.23$, $p = 0.03$), whereas there was no difference in fish lengths between centers and outlets ($t_{294.3} = 0.19$, $p = 0.85$).

Fish Mass. The fresh wet mass of mosquitofish removed from cages differed among habitats at the outlets ($F_{2,3.3} = 8.98$, $p = 0.05$), but not the inlets ($F_{2,4.4} = 1.69$, $p = 0.28$) or centers ($F_{2,3.3} = 5.78$, $p = 0.08$; Figure S3B). At the outlets, fish mass was greater in white rice ($t_{3.3} = 3.48$, $p = 0.03$) and wild rice ($t_{3.3} = 3.84$, $p = 0.03$) than in permanent wetlands, but fish mass in white rice and wild rice did not differ ($t_{3.3} = 0.35$, $p = 0.75$).

Fish mass also differed among cage sites within white rice ($F_{2,293.2} = 27.07$, $p < 0.0001$), wild rice ($F_{2,294.5} = 75.40$, $p < 0.0001$), and permanent wetlands ($F_{2,293.7} = 4.23$, $p = 0.02$; Figure S3B). Within white rice and wild rice fields, fish mass was lower at field inlets than at either the center (white rice: $t_{293.2} = 6.88$, $p < 0.0001$; wild rice: $t_{294.5} = 11.14$, $p < 0.0001$) or outlet (white rice: $t_{293.2} = 7.02$, $p < 0.0001$; wild rice: $t_{294.5} = 11.68$, $p < 0.0001$), whereas there was no difference in fish mass between centers and outlets (white rice: $t_{293.2} = 0.50$, $p = 0.62$; wild rice: $t_{294.5} = 0.66$, $p = 0.51$). Within permanent wetlands, fish mass was higher at the inlets than at the outlets ($t_{293.7} = 2.89$, $p = 0.01$), but did not differ between centers and inlets ($t_{293.7} = 1.48$, $p = 0.14$) or centers and outlets ($t_{293.7} = 1.49$, $p = 0.14$).

Fish Relative Body Condition. The relative body condition of mosquitofish removed from cages did not differ among habitats at the inlets ($F_{2,7.4} = 1.98$, $p = 0.20$), centers ($F_{2,4.0} = 0.41$, $p = 0.69$), or outlets ($F_{2,4.0} = 1.80$, $p = 0.28$; Figure S3C). However, fish body condition varied among cage sites within white rice ($F_{2,293.5} = 6.32$, $p = 0.01$), wild rice ($F_{2,294.6} = 12.95$, $p < 0.0001$), and permanent wetlands ($F_{2,294.6} = 4.55$, $p = 0.01$; Figure S3C). Within white rice and wild rice fields, fish body condition was lower at field inlets than at either the centers (white rice: $t_{293.5} = 3.50$, $p = 0.001$; wild rice: $t_{294.6} = 5.01$, $p < 0.0001$) or outlets (white rice: $t_{293.5} = 2.35$, $p = 0.02$; wild rice: $t_{294.6} = 4.30$, $p < 0.0001$), whereas there was no difference in fish body condition between centers and outlets (white rice: $t_{293.5} = 1.63$, $p = 0.10$; wild rice: $t_{294.6} = 1.00$, $p = 0.32$). Within permanent wetlands, fish body condition did not differ between inlets and centers ($t_{294.6} = 1.06$, $p = 0.29$) or inlets and outlets ($t_{294.6} = 1.61$, $p = 0.11$), but body condition at wetland centers was higher than at outlets ($t_{294.6} = 2.99$, $p = 0.01$).

Discussion

Caged Fish Growth After 60-days of Exposure. There was substantial variation in caged mosquitofish growth among wetland habitats and among sites within wetlands, indicating that habitat quality also likely differed. The length and mass of mosquitofish at the outlets were greater in white rice and wild rice than in permanents wetlands. In contrast, the size of mosquitofish removed from cages at the inlets did not differ among habitat types, suggesting that mosquitofish caged near wetlands' water sources experienced more similar environmental conditions and dietary MeHg exposure among habitats than mosquitofish located at wetlands' centers and outlets. Within habitats, the size and body condition of caged mosquitofish were greater at wetland centers and outlets than at the inlets in white rice and wild rice, indicating that conditions for growth improved with water duration within agricultural wetlands. Although no other studies have specifically examined fish growth in these wetland habitat types, two caged fish studies using juvenile chinook salmon (*Oncorhynchus tshawytscha*) in the Yolo Bypass and Cosumnes River, California found that fish growth was greater in ephemeral floodplain habitats than in main river channels during late winter and spring (*6*, *7*). Within the floodplain habitat itself, salmon caged within seasonally inundated wetlands experienced higher growth than those caged in permanent wetlands (*7*). These results are consistent with ours suggesting higher fish growth in ephemerally-flooded, shallow wetland habitats.

We found similar patterns of fish growth and THg bioaccumulation among habitats and within wetlands. Accordingly, in addition to the effects of wetland habitat type and site, fish length and body condition influenced THg concentrations in caged and wild fish (Figure S2). Total Hg concentrations were positively correlated with the length of caged mosquitofish and wild silversides on an individual basis, as often is the case (*8*). Total Hg concentrations were not correlated with length (mean ± SD) in wild mosquitofish, which tended to be smaller (30.9 ± 6.3

mm) on average than those mosquitofish used in the caging experiment at introduction (38.7 ± 4.5 mm) or at removal (42.6 ± 4.9 mm). Total Hg concentrations also were negatively correlated with the relative body condition of caged and wild mosquitofish, but not wild silversides.

Several studies suggest reducing permanent wetland habitats within Central Valley floodplains because they support mainly resident alien fishes, rather than native fishes (*9*, *10*) and fish within ephemeral wetlands typically have higher growth rates, recruitment, and survival than fish in more permanent water bodies (*6*, *7*, *11*). We found that mosquitofish caged in permanent wetlands were smaller upon removal after 60 days than similarly exposed mosquitofish in white rice and wild rice wetlands, indicating that conditions for growth also were less favorable in permanent wetlands compared to more ephemerally flooded habitats. However, caged and wild mosquitofish and wild silversides at the outlets had considerably lower THg concentrations within permanent wetlands than in white rice and wild rice habitats. These results illustrate a trade-off between the quality of agricultural wetland habitats for fish growth and the potential deleterious effects of MeHg bioaccumulation.

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TABLE S1. Total Mercury (THg) Concentrations and Total Body Burden of THg in Western Mosquitofish Removed from Cages After 60 days of Exposure in White Rice, Wild Rice, and Permanent Wetlands

	introduction			removal after 60 days			*t* -test			difference	
	N	mean	SE	*N*	mean	SE	*DF*	*t*	*P*	mean	%
THg concetration (µg g^{-1} dw)											
Permanent wetland 2											
Inlet	37	0.14	0.01	6	0.49	0.08	41	8.41	<.0001*	0.35	246%
Center	37	0.14	0.01	14	0.40	0.02	49	10.62	<.0001*	0.25	176%
Outlet	37	0.14	0.01	20	0.44	0.02	55	13.77	<.0001*	0.29	204%
Permanent wetland 5											
Inlet	37	0.14	0.01	16	0.34	0.02	51	9.08	<.0001*	0.19	135%
Center	37	0.14	0.01	14	0.34	0.02	49	9.16	<.0001*	0.19	136%
Outlet	37	0.14	0.01	15	0.34	0.02	50	9.26	<.0001*	0.20	140%
White rice 31											
Inlet	37	0.14	0.01	6	0.48	0.06	41	8.58	<.0001*	0.34	237%
Center	37	0.14	0.01	24	1.57	0.05	59	33.33	<.0001*	1.42	995%
Outlet	37	0.14	0.01	14	1.64	0.05	49	27.10	<.0001*	1.50	1046%
White rice 64											
Inlet	37	0.14	0.01	6	0.40	0.03	41	7.49	<.0001*	0.26	180%
Center	37	0.14	0.01	26	1.53	0.03	61	35.88	<.0001*	1.39	969%
Outlet	37	0.14	0.01	26	1.86	0.05	61	37.47	<.0001*	1.71	1197%
Wild rice 32											
Inlet	37	0.14	0.01	0	na	na	na	na	na	na	na
Center	37	0.14	0.01	26	0.97	0.02	61	28.56	<.0001*	0.83	579%
Outlet	37	0.14	0.01	21	0.75	0.05	56	19.30	<.0001*	0.60	422%
Wild rice 65											
Inlet	37	0.14	0.01	21	1.79	0.13	56	27.76	<.0001*	1.65	1153%
Center	37	0.14	0.01	24	0.92	0.02	59	27.32	<.0001*	0.78	546%
Outlet	37	0.14	0.01	25	1.02	0.04	60	26.97	<.0001*	0.88	615%
THg burden (µg fish^{-1} dw)											
Permanent wetland 2											
Inlet	37	0.05	0.01	6	0.11	0.02	41	2.52	0.02	0.06	117%
Center	37	0.05	0.01	14	0.08	0.01	49	2.01	0.05	0.03	51%
Outlet	37	0.05	0.01	20	0.07	0.01	55	1.44	0.16	0.01	29%
Permanent wetland 5											
Inlet	37	0.05	0.01	16	0.11	0.01	51	3.52	0.001*	0.05	106%
Center	37	0.05	0.01	14	0.09	0.01	49	2.81	0.01	0.04	78%
Outlet	37	0.05	0.01	15	0.09	0.02	50	2.72	0.01	0.04	80%
White rice 31											
Inlet	37	0.05	0.01	6	0.11	0.01	41	2.42	0.02	0.06	110%
Center	37	0.05	0.01	24	0.63	0.03	59	16.06	<.0001*	0.58	1118%
Outlet	37	0.05	0.01	14	0.71	0.03	49	13.07	<.0001*	0.65	1265%
White rice 64											
Inlet	37	0.05	0.01	6	0.10	0.01	41	2.16	0.04	0.05	93%
Center	37	0.05	0.01	26	0.65	0.03	61	17.05	<.0001*	0.60	1162%
Outlet	37	0.05	0.01	26	0.86	0.03	61	19.04	<.0001*	0.81	1566%
Wild rice 32											
Inlet	37	0.05	0.01	0	na	na	na	na	na	na	na
Center	37	0.05	0.01	26	0.39	0.03	61	12.76	<.0001*	0.34	656%
Outlet	37	0.05	0.01	21	0.32	0.03	56	10.18	<.0001*	0.27	527%
Wild rice 65											
Inlet	37	0.05	0.01	21	0.38	0.03	56	11.67	<.0001*	0.33	640%
Center	37	0.05	0.01	24	0.45	0.03	59	13.62	<.0001*	0.40	779%
Outlet	37	0.05	0.01	25	0.49	0.04	60	13.96	<.0001*	0.44	850%

a We used two-sample *t* -tests to compare THg concentrations and total Hg burdens of reference mosquitofish at introduction to values of mosquitofish removed from cages 60 days later, and we applied a sequential Bonferroni corrected alpha level to account for the number of tests performed for each variable (Rice 1989).

b "na" indicates no fish were present after 60 days.

* indicates statistical significance after sequential Bonferroni correction was applied.

TABLE S2. Size and Body Condition of Western Mosquitofish Introduced into Cages and Removed After 60 days of Exposure in White Rice, Wild Rice, and Permanent Wetlands

	introduction			removal after 60 days			*t*-test[a]			difference	
	N	mean	SE	*N*	mean	SE	*DF*	*t*	*P*	mean	%
Standard length (mm)											
Permanent wetland 2											
Inlet	30	39.13	0.78	6	38.32	0.42	34	-0.46	0.65	-0.81	-2%
Center	30	35.91	0.40	14	37.08	0.67	42	1.54	0.13	1.17	3%
Outlet	30	38.24	0.38	20	35.80	0.64	48	-3.41	0.001*	-2.44	-6%
Permanent wetland 5											
Inlet	30	40.37	0.93	16	41.43	1.16	44	0.69	0.49	1.06	3%
Center	30	38.02	0.46	14	38.67	0.54	42	0.83	0.41	0.65	2%
Outlet	30	40.85	0.94	16	40.57	1.30	44	-0.20	0.84	-0.28	-1%
White rice 31											
Inlet	30	37.11	0.63	6	38.59	0.96	34	0.95	0.35	1.48	4%
Center	30	35.95	0.61	24	43.51	0.61	52	8.47	<.0001*	7.57	21%
Outlet	30	35.41	0.46	14	44.66	0.67	42	11.01	<.0001*	9.25	26%
White rice 64											
Inlet	30	39.10	0.66	6	38.24	1.11	34	-0.55	0.59	-0.85	-2%
Center	30	39.33	0.79	26	44.26	0.62	54	4.67	<.0001*	4.93	13%
Outlet	30	39.02	0.66	26	45.29	0.54	54	6.83	<.0001*	6.27	16%
Wild rice 32											
Inlet	30	41.14	0.78	0	na	na	na	na	na	na	na
Center	30	40.85	0.61	26	45.06	0.68	54	4.48	<.0001*	4.21	10%
Outlet	30	37.68	0.83	21	45.15	0.81	49	6.04	<.0001*	7.48	20%
Wild rice 65											
Inlet	30	37.37	0.78	21	37.79	0.38	49	0.37	0.71	0.41	1%
Center	30	38.78	0.70	24	45.60	0.78	52	6.35	<.0001*	6.82	18%
Outlet	30	38.51	0.85	25	47.23	0.76	53	7.29	<.0001*	8.72	23%
Wet mass (g)											
Permanent wetland 2											
Inlet	30	1.48	0.10	6	1.26	0.05	34	-0.99	0.33	-0.22	-15%
Center	30	1.40	0.06	14	1.15	0.06	42	-2.71	0.01	-0.25	-18%
Outlet	30	1.69	0.09	20	0.93	0.04	48	-7.77	<.0001*	-0.76	-45%
Permanent wetland 5											
Inlet	30	1.80	0.14	16	1.70	0.14	44	-0.46	0.65	-0.10	-6%
Center	30	1.47	0.07	14	1.48	0.06	42	0.04	0.97	0.00	0%
Outlet	30	1.79	0.13	16	1.55	0.14	43	-1.18	0.24	-0.24	-13%
White rice 31											
Inlet	30	1.40	0.07	6	1.20	0.04	34	-1.31	0.20	-0.20	-14%
Center	30	1.31	0.09	24	2.02	0.08	52	5.24	<.0001*	0.71	54%
Outlet	30	1.25	0.05	14	2.13	0.09	42	7.55	<.0001*	0.87	70%
White rice 64											
Inlet	30	1.64	0.08	6	1.27	0.13	34	-2.10	0.04	-0.37	-23%
Center	30	1.63	0.12	26	2.18	0.08	54	3.48	0.001*	0.55	34%
Outlet	30	1.56	0.09	26	2.19	0.11	54	4.34	<.0001*	0.63	40%
Wild rice 32											
Inlet	30	1.87	0.13	0	na	na	na	na	na	na	na
Center	30	1.80	0.09	26	2.02	0.11	54	1.56	0.13	0.22	12%
Outlet	30	1.54	0.10	21	2.19	0.12	49	3.86	0.0003*	0.65	43%
Wild rice 65											
Inlet	30	1.37	0.10	21	1.11	0.04	49	-2.15	0.04	-0.25	-19%
Center	30	1.56	0.10	24	2.43	0.14	52	5.02	<.0001*	0.86	55%
Outlet	30	1.58	0.10	25	2.38	0.12	53	4.96	<.0001*	0.81	51%
Relative condition factor											
Permanent wetland 2											
Inlet	30	0.91	0.02	6	0.83	0.04	34	-1.80	0.08	-0.08	-9%
Center	30	1.11	0.04	14	0.83	0.04	42	-4.74	<.0001*	-0.28	-25%
Outlet	30	1.16	0.07	20	0.74	0.02	48	-4.88	<.0001*	-0.42	-36%
Permanent wetland 5											
Inlet	30	1.01	0.02	16	0.89	0.02	44	-3.44	0.001*	-0.13	-13%
Center	30	0.99	0.03	14	0.94	0.02	42	-1.12	0.27	-0.06	-6%
Outlet	30	0.97	0.03	16	0.86	0.02	43	-2.32	0.03	-0.11	-11%
White rice 31											
Inlet	30	1.00	0.03	6	0.77	0.05	34	-3.78	0.001*	-0.23	-23%
Center	30	1.03	0.03	24	0.91	0.02	52	-2.89	0.01	-0.12	-11%
Outlet	30	1.05	0.06	14	0.90	0.04	42	-1.71	0.09	-0.15	-14%
White rice 64											
Inlet	30	1.01	0.03	6	0.83	0.04	34	-2.86	0.01	-0.18	-18%
Center	30	0.99	0.02	26	0.94	0.02	54	-1.36	0.18	-0.05	-5%
Outlet	30	0.97	0.02	26	0.89	0.02	54	-2.55	0.01	-0.08	-9%
Wild rice 32											
Inlet	30	1.00	0.03	0	na	na	na	na	na	na	na
Center	30	0.98	0.03	26	0.83	0.02	54	-4.02	0.0002*	-0.15	-16%
Outlet	30	1.05	0.02	21	0.90	0.03	49	-4.35	<.0001*	-0.15	-15%
Wild rice 65											
Inlet	30	0.96	0.02	21	0.76	0.02	49	-6.37	<.0001*	-0.20	-21%
Center	30	0.99	0.02	24	0.97	0.03	52	-0.51	0.62	-0.02	-2%
Outlet	30	1.01	0.02	25	0.86	0.02	53	-5.51	<.0001*	-0.16	-15%

a We used two-sample *t*-tests to compare the size of fish at introduction to values 60 days later when fish were removed from cages, and we applied a sequential Bonferroni corrected alpha level to account for the number of tests performed for each variable (Rice 1989).
b "na" indicates no fish were present after 60 days.
* indicates statistical significance after sequential Bonferroni correction was applied.

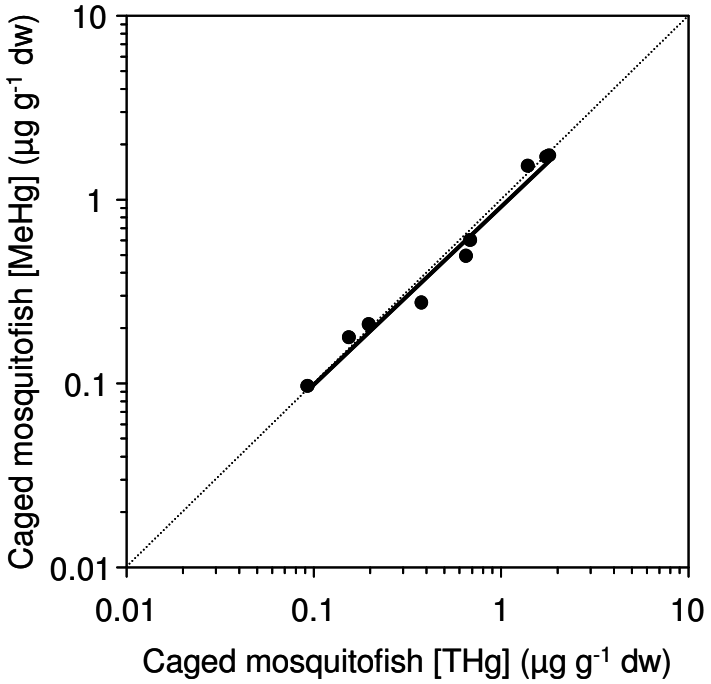

FIGURE S1. Methylmercury (MeHg) concentrations (µg/g dry weight; dw) were highly correlated with total mercury (THg) concentrations (µg/g dw) in western mosquitofish (*Gambusia affinis*) introduced into cages within agricultural wetlands at the Yolo Bypass Wildlife Area, California. The stippled line indicates the 1:1 relationship.

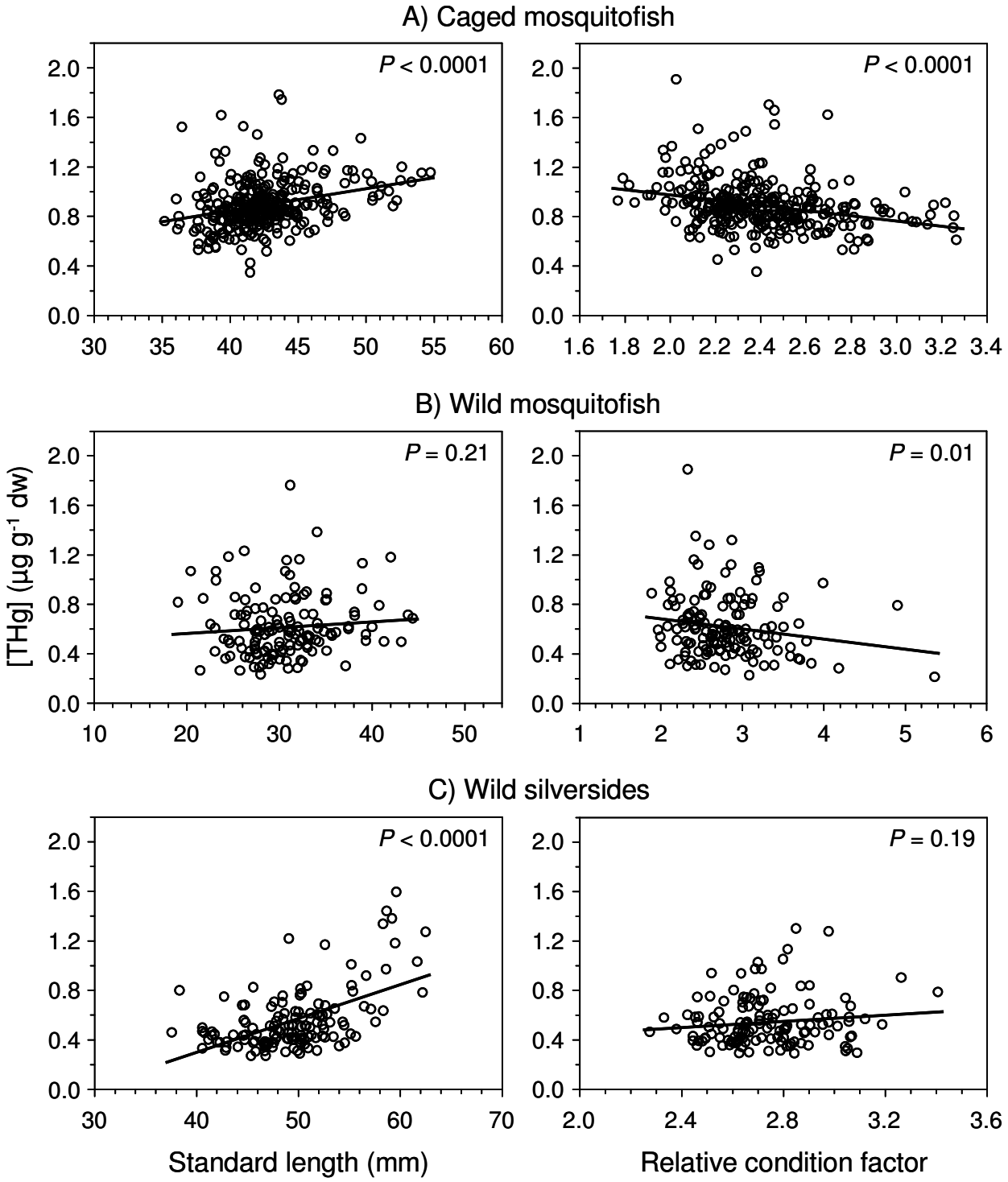

FIGURE S2. Partial leverage plots depicting the relationship between total mercury (THg) concentrations (µg/g dry weight; dw) and standard length (mm) or relative condition factor of (A) caged western mosquitofish (*Gambusia affinis*), (B) wild western mosquitofish, and (C) wild Mississippi silversides (*Menidia audens*) in wetlands at the Yolo Bypass Wildlife Area, California. Partial leverage plots account for the potential effects of wetland habitat type, site within the wetland, habitat × site interaction, standard length, and the relative condition factor as fixed effects, and wetland replicate as a random effect.

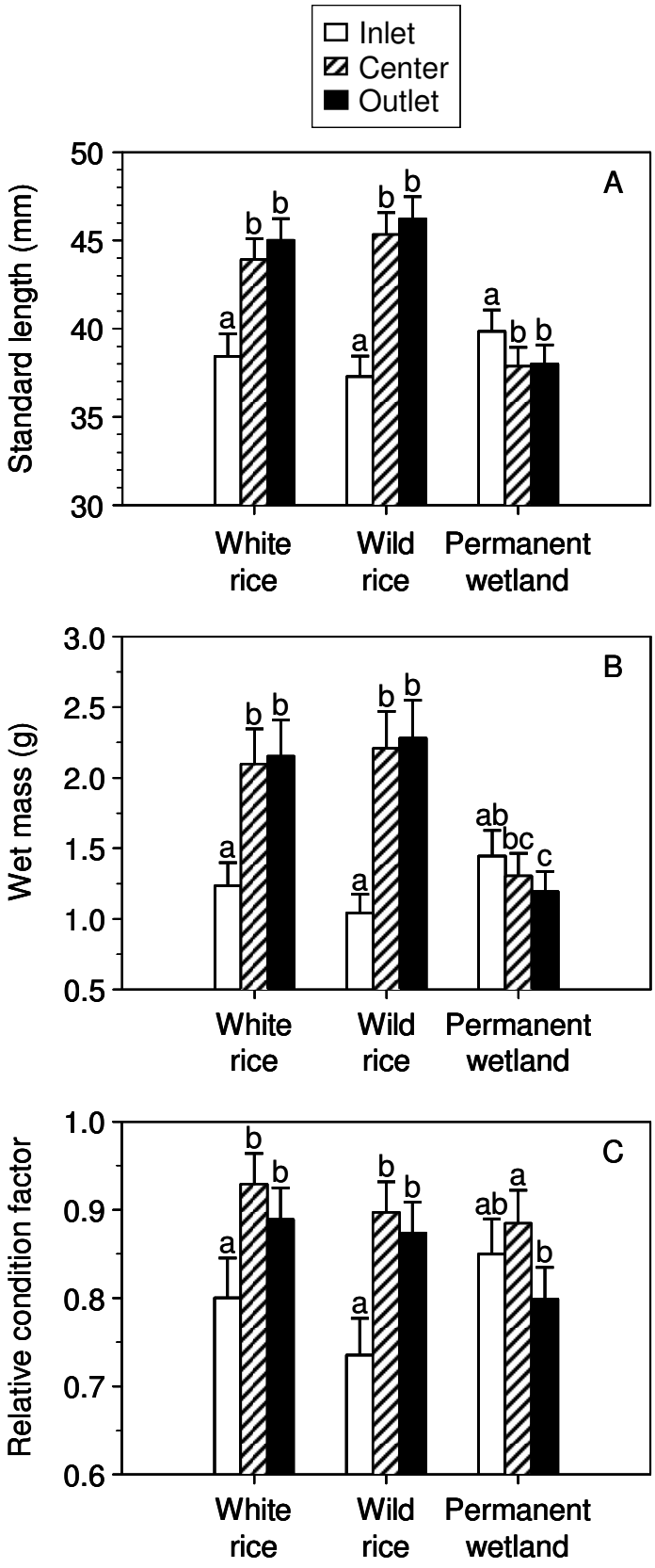
Inlet
Center
Outlet
A
Standard length (mm)
50
45
40
35
30
a
b
b
a
b
b
a
b
b
White rice
Wild rice
Permanent wetland
B
Wet mass (g)
3.0
2.5
2.0
1.5
1.0
0.5
a
b
b
a
b
b
ab
bc
c
White rice
Wild rice
Permanent wetland
C
Relative condition factor
1.0
0.9
0.8
0.7
0.6
a
b
b
a
b
b
ab
a
b
White rice
Wild rice
Permanent wetland

FIGURE S3. (A) Standard length (mm), (B) fresh wet mass (g), and (C) relative condition factor of western mosquitofish (*Gambusia affinis*) removed from cages after a 60-day exposure period at the inlets (open bars), centers (hatched bars), and outlets (solid bars) of white rice, wild rice, and permanent wetlands during the 2007 rice growing season at the Yolo Bypass Wildlife Area, California. Different lowercase letters above bars indicate that values within a wetland habitat are statistically different ($p < 0.05$).