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North Bay Small Fish Mercury Monitoring with a Focus on Napa-Sonoma Managed Ponds and Sloughs

version 2

Letitia Grenier and Ben Greenfield - Aquatic Science Center
Darell Slotton and Shaun Ayers - UC Davis

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7770 Pardee Lane
Second floor
Oakland, CA 94621
p: 510-746-7334
f: 510-746-7300

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Introduction

In San Pablo Bay, California, 10,000 acres of historic salt ponds and associated habitats are being restored to tidal marsh or managed as ponds for wildlife (www.napa-sonoma-marsh.org). This complex of wetlands near the Napa River was originally tidal marsh that was converted to ponds for commercial salt production and then was acquired by the California Department of Fish and Game (CDFG). Over time, CDFG has been managing some ponds for wildlife (mainly waterbirds) and restoring some ponds to tidal marsh.

In the larger San Francisco Estuary, methylmercury (MeHg) bioaccumulation in sport fish and other wildlife has been identified as a problem (Davis et al. 2002, Ackerman et al. 2008, Conaway et al. 2008). There is concern that wetland restoration could exacerbate the problem, either through increasing bioaccumulation within the wetland food web or through increased export (biotic or abiotic) to the Bay food web (Davis et al. 2003). There is also growing evidence that the managed ponds (some of which are slated for tidal marsh restoration and some of which will remain as managed ponds) may be as problematic or more problematic than tidal marsh in terms of MeHg bioaccumulation (Slotton et al. 2007, Eagles-Smith and Ackerman 2009, Grenier et al. 2010). Most managed ponds are less connected to the Bay than are most tidal marshes, so the opportunity for MeHg export from ponds to the Bay is less. However, bioaccumulation in wildlife that feed in managed ponds can be significant.

Monitoring MeHg bioaccumulation in tidal marsh and managed ponds in San Pablo Bay is needed to understand how tidal restoration and pond management affect bioaccumulation of MeHg. Such monitoring may help managers determine how to manage and restore wetlands in ways that will minimize the Hg problem. In order to acquire this knowledge, monitoring bioaccumulation of MeHg in the food web using appropriate indicator species, or biosentinels, will be required over the coming years. A data set collected over the time that it takes for restored habitats to evolve and reach their climax state is necessary to understand how restoration and management actions affect MeHg in the long term.

This project provides a baseline of MeHg bioaccumulation in small fish from several sites in the San Pablo Bay watershed, mainly within the Napa-Sonoma marsh-pond complex. A variety of sites of different habitat types and different stages in the restoration trajectory were monitored with the purpose of documenting the current patterns in bioaccumulation of MeHg in small fish across these sites. This monitoring is a regulatory requirement of the San Francisco Bay Regional Water Quality Control Board.

Small fish were chosen as an appropriate biosentinel, because they reflect MeHg bioaccumulation in near-shore subtidal and intertidal habitats, particularly managed ponds, large sloughs, and the Bay margin (Slotton et al. 2007, Eagles-Smith and Ackerman 2009, Greenfield and Jahn 2010). These habitats currently predominate in the Napa-Sonoma marsh complex, because most of the ponds that have been restored to tidal action have not yet become vegetated. The slough and Bay margin habitats also comprise the area where a MeHg export problem from tidal marsh to the Bay could be detected in biosentinels. The fish species and sizes selected for study have small home ranges, are relatively abundant, and are important components of the food web.

The project was designed to address the following monitoring questions:

1. What is the spatial pattern of MeHg bioaccumulation in small fish among different ponds and sloughs? What is the spatial pattern when breeding waterbirds would be at risk in mid-summer?
2. Does restoration to tidal marsh seem to increase or decrease MeHg bioaccumulation?
3. What is the seasonal pattern of MeHg bioaccumulation in ponds and sloughs? Does it provide insight into what factors might relate to higher or lower MeHg bioaccumulation?
4. How does bioaccumulation in these ponds and sloughs compare to other habitat types and locations in the San Francisco Estuary?

Study Design

This study was designed in collaboration with Karen Taylor, CDFG, and Tom Gandesbury, State Coastal Conservancy, to ensure that the sampling locations would address important areas for management concerns. Sampling sites (**Table 1, Figure 1**) were chosen according to the following criteria:

1. Range of managed pond types,
2. Areas restored to tidal action across a range of stages of succession,
3. Upstream control (Kennedy Park) and other ambient locations (Hamilton and Petaluma) away from the Napa-Sonoma marsh complex, and
4. Ponds slated for future restoration to tidal marsh.

Sites and Seasons

The sampling design reflected a balance between achieving a wide spatial coverage and quantifying seasonal variation across a range of important habitat types. Six primary sites were chosen for seasonal monitoring, conducted in December 2009 and March, May, and July 2010. These were:

- Napa River at Kennedy Park, a relative control site, well upstream of the main salt ponds and restoration area.
- Pond 1, a shallow, managed pond with muted tidal influence, unvegetated, formerly the inlet pond from San Pablo Bay and least saline of the historic salt production series.
- Pond 2, another managed, muted tidal pond but differentiated from Pond 1 by its substantially greater depth. Also largely unvegetated.
- Pond 9/10, a fully breached former salt pond with full tidal cycling. A newly restoring site, breached recently (2009).
- Pond 4/5, another fully tidal pond, large and further along in its evolution (breached 2005). Partially vegetated.
- Pond 2A, a fully tidal region that has been undergoing accretion and evolution since 1995, now a heavily vegetated tidal marsh.

The seasonal collections sampled winter (Dec 2009) and summer (Jul 2010) conditions, together with a March collection and one in May when local water bird nesting was underway, with attendant potential Hg risks to their young. Seasonal monitoring at the six primary sites was supplemented by one-time collections at seven additional locations, mostly sampled in July 2010. These were:

- Sonoma Creek at Wingo, a site where a major natural breach was imminent during the winter. A pre-breach collection was made in Dec-09, but the levee held, there was no breach, and so there was no call for a follow-up collection.
- Napa Plant Site/Wash Ponds, another newly breached tidal restoration, similar to Pond 9/10.
- Cullinan Ranch, planned for imminent breaching and tidal restoration, a site currently vegetated with terrestrial plants, so a potentially different set of Hg dynamics post-flooding. A baseline sample was taken from the adjacent Dutchman Slough,
- Pond 6A, a largely closed pond of higher salinity and little or no prior biosentinel Hg data. Shallow with clear water and a benthic mat of filamentous algae.
- Pond 7A, another shallow, basically closed pond with even greater salinity and little or no prior biosentinel Hg data. Different from Pond 6A in being very turbid and with no vegetation. Prominent salt precipitates coat the shoreline.
- Petaluma Marsh, an ambient, comparative location outside the main Napa-Sonoma restoration complex; prior monitoring in other projects.
- Hamilton Air Force Base, another ambient, comparative San Pablo Bay site outside the Napa-Sonoma restoration complex, also of interest for baseline sampling as this area will be undergoing significant tidal wetland restoration.

Biosentinel Fish Species

The small fish species utilized were chosen for a number of relevance factors, including: relatively small home range, importance in local food webs, prevalence and availability for sampling across many or all of the target areas, uptake of significant enough levels of MeHg to allow a differentiation of low vs. moderate vs. high bioaccumulation, and a quick life cycle such that measured concentrations will reflect recent exposure conditions. The primary biosentinel species for this project, Mississippi silverside (*Menidia audens*) fulfills all of these considerations and has been extensively tested and utilized by the UC Davis Hg monitoring team since the mid 1990s. Silversides were sampled at all 6 of the seasonal sites across the project timeline. They were additionally sampled at 4 of the 7 one-time sites, including Sonoma Creek at Wingo, Napa Plant/Wash Ponds, Pond 7A, and Petaluma Marsh. Mississippi silversides are a non-native species in California that have become highly prevalent and even dominant across large areas of the Sacramento/San Joaquin Delta and fringing areas of the San Francisco Bay (Moyle 2002). They are a schooling species that consume a variety of planktonic and benthic invertebrates and larval fish. They accumulate Hg to high levels if there is significant exposure. Earlier work by the authors and Eagles-Smith and Ackerman (2009) indicate that silverside Hg reflects the prior 4-6 weeks of exposure.

Additional biosentinel species that supplemented the Mississippi silverside collections included:

- Rainwater killifish (*Lucania parva*): Taken at 7 of the sites, killifish are a very small, fast growing, highly localized brackish water species that feed on small invertebrates.

- Juvenile striped bass (*Morone saxatilis*): While adult striped bass are noted for their wide ranging migratory habits through fresh, brackish, and even ocean waters, the young-of-the-year tend to reside for the first few months in fairly localized areas. Such young were present at 7 of the locations in July 2010. Predatory throughout life, the juveniles focus on a variety of invertebrates and juvenile fish (Moyle 2002).

- Threespine stickleback (*Gasterosteus aculeatus*): A small, fast growing brackish water species that is generally highly localized due to its limited swimming ability. Consumes small invertebrates. Extensively utilized as a Hg biosentinel by the authors and Eagles-Smith and Ackerman (2009).

- Topsmelt (*Atherinops affinis*): Primarily fully saline, topsmelt extend into brackish water. They have been extensively utilized by the San Francisco Estuary Institute (SFEI) as Hg biosentinels around the greater San Francisco Bay

(Greenfield and Jahn 2010). Available data suggest their home ranges are fairly large. Topsmelt were taken at 6 of the sites in July.

- Yellowfin goby (*Acanthogobius flavimanus*): Known for extensive spawning migrations (Moyle 2002), this bottom-dwelling species is fast growing and presumably relatively localized in its early life stages. Young yellowfin gobies were the dominant species present at the Hamilton air force base site, and so were taken in 4 composites. This species has been utilized by SFEI and UC Davis.

- Arrow goby (*Clevelandia ios*): Another species that has been used to a moderate extent around San Francisco Bay by SFEI. This is a small, bottom-dwelling goby that preys on invertebrates. They were taken at the Hamilton site, as well as at Pond 6A.

Methods

Field Sampling Techniques

Biosentinel fish were collected with a variety of seines and seining techniques. Samples were maintained in water, field sorted and cleaned, individually measured to mm total length, and placed into size-apportioned composite groups. For each collection, target composites of the primary species, Mississippi silverside, included 8 individuals within each of the following size ranges: 45-49 mm, 50-54 mm, 55-59 mm, 60-64 mm, 65-69 mm, and 70-75 mm (48 individuals in total), based on extensive prior work by UC Davis. Composite samples were sealed into labeled, doubled freezer weight bags with water surrounding and air removed. These were field frozen on dry ice and later transferred to laboratory freezers.

Laboratory Techniques

The processing of each composite sample of fish prior to analysis included: thawing of the frozen fish, followed by the determination of wet weight (mg) for each composite. Care was taken to preserve fresh consistency for the initial weighing process, avoiding sample drying or accumulation of excess moisture. Composite fish samples were subsequently dried to constant weight at 55 °C, with this weight recorded for calculation of percentage solids, used for the conversion of dry weight analytical data to corresponding fresh weight concentrations. Following final weighing, dried composite samples were individually ground to a fine powder with a modified coffee grinder for analytical consistency. Samples were analyzed as homogeneous, dry powders.

Whole body Hg was assessed as total Hg. Samples were analyzed for total Hg by standard cold vapor atomic absorption (CVAA) spectrophotometry, using a dedicated Perkin Elmer Flow Injection Hg System (FIMS) with an AS-90 autosampler, following a two stage digestion under pressure at 90 °C in a mixture of concentrated nitric and sulfuric acids with potassium permanganate.

Routine analytical QA/QC included a 67% ratio of QA/QC samples, or 20 for every 30 analytical samples. These were subjected to the same acid digestion, physical and chemical treatment, and detection as analytical samples and included: blanks, aqueous standards, continuing control standards, standard reference materials with certified levels of Hg, laboratory split samples, matrix spike samples, and matrix spike duplicates. Performance was tracked with control charts and sample material was archived in case of the need to re-analyze based on QA/QC samples exceeding control limits. Re-analysis was not necessary for this project, with routine results well within control limits.

Statistical Analysis

All Hg data were log transformed, to achieve normally distributed data, prior to parametric statistical analyses. Analysis of covariance (ANCOVA) was employed to account for the effect of fish total length (TL) on tissue Hg concentrations (Tremblay et al. 1998, Chumchal and Hambright 2009). To evaluate the impact of collection location for individual species, while accounting for length, we used the linear model procedure in R (Version 2.11.1) to perform ANCOVA. In this analysis, the continuous predictor, fish length, is fit in combination with one or more categorical predictors. In ANCOVA, the interaction between length and the categorical predictor variables is also examined; when a significant interaction is present, this indicates differing length versus Hg relationships among the categories (Tremblay et al., 1998). For most species, sufficient data for this analysis were only available during the July, 2010 sampling event. Consequently, statistical differences among sampling locations were evaluated only during July for these species. For Mississippi silverside, data were available for the four sampling periods at all six locations: Kennedy Park, Pond 1, Pond 2, Pond 2a, Ponds 4/5, and Ponds 9/10. Thus, an additional analysis was performed on these stations for Mississippi silverside, including the effects of length, sampling location, and sampling period.

Significance of the effects of length, station, or sampling period was determined based on F-ratio and p value, with the effects included only when significant ($p < 0.05$) in the overall model. These results were corroborated by examination of Akieke's Information Criterion, with bias adjustment for small sample size (i.e., AICc) (Burnham and Anderson 2002). After determination of a significant overall effect, individual site comparisons were performed using the false discovery rate control procedure of Benjamini and Holmes. This procedure controls the frequency of individual occurrences of Type I error, while achieving higher statistical power than procedures that correct familywise error rates (e.g. Bonferroni correction) (Garcia 2004, Verhoeven et al. 2005).

Results and Discussion

Monitoring Question 1: What is the spatial pattern of MeHg bioaccumulation in small fish among different ponds and sloughs? What is the spatial pattern when breeding waterbirds would be at risk in mid-summer?

Hg in small fish varied significantly across study sites for all species examined (Figures 1 and 2, Appendix Figures). The examination of spatial variation focused on July 2010, for which there were available data across all species and the largest number of sites. This timing coincides with the waterbird breeding season. Differences in Hg concentrations among sites were subtle. Pond 7A had the lowest Hg across all sites, and Pond 2 and Pond 6A were also lower than other sites monitored (Figure 2). Ponds 6A and 7A had not previously been monitored; the present study indicates that these ponds posed relatively low risk for Hg exposure, compared to other areas within the study region.

There was no clear spatial pattern in Hg in small fish from groups of ponds that related to habitat type, restoration stage, or pond management. In order to discern such relationships, more ancillary data on physical and biotic parameters and more replication within each habitat and pond management type would be needed. Single-pond site effects, such as the winter spike in small fish Hg in Pond 2, were observed, but the pattern was not repeated in other ponds with similar management,

Monitoring Question 2: Does restoration to tidal marsh seem to increase or decrease MeHg bioaccumulation?

Hg concentrations in the sloughs of restored and fully vegetated Pond 2A were moderate in silverside and striped bass (the only species available at the site). This pond was restored to full tidal exchange in 1995 and is now tidal marsh. The small fish sampled in this study reflect bioaccumulation in the large sloughs that carry tidal waters to and from the marsh. Our study as well as previous small fish data (Slotton et al. 2007) indicate that this site has typical to low-end Hg concentrations in small fish for the region. In South San Francisco Bay, Hg concentrations in biosentinels from perennial managed ponds with limited tidal exchange and from tidal marsh small channels and pannes were lower than concentrations in the seasonally wet Pond A8, suggesting that restoration to tidal conditions may not increase risk to wildlife that feed from these marsh habitats (Grenier et al. 2010). A wider synoptic survey of managed ponds and marshes in South Bay for the same study showed no differences in bioaccumulation of MeHg between ponds and marsh small channels and pannes. Thus for San Francisco Bay, there is no evidence from biosentinels that restoration of managed ponds to tidal marsh is likely to have detrimental effects on MeHg bioaccumulation in the large sub-tidal sloughs within marshes (evidence from this study), small inter-tidal channels, or pannes (Grenier et al. 2010).

Monitoring Question 3: What is the seasonal pattern of MeHg bioaccumulation in ponds and sloughs? Does it provide insight into what factors might relate to higher or lower MeHg bioaccumulation?

Mississippi silverside Hg concentrations varied over the seasons of the year. Interestingly, the pattern of seasonal variation differed among sites and was still present after standardizing fish to 55 mm length (Figure 1, Figure 3). We have observed differences in seasonal variation among locations previously (Slotton et al. 2007), indicating that seasonal patterns are related to site-specific conditions. In the present study, Pond 4/5, and Napa River at Kennedy Park exhibited elevated concentrations in the spring (March or May), compared to December and July. This is similar to observations of elevated Hg in May for threespine stickleback and longjaw mudsucker in a managed pond and an artificially ponded marsh in the South Bay (Eagles-Smith and Ackerman 2009). Hg in silverside from Napa Pond 1 increased over the duration of the study from Dec 2009 through July 2010. In contrast, Hg in silverside from Pond 2, Pond 2A, and Pond 9/10 generally decreased during this time.

For most sites, concentrations varied 1.5 to 2 fold between the lowest and highest seasonal levels. Seasonal variation was most pronounced in Pond 2, with the highest concentration (186 ng/g wet weight in December) being 4.2 times the lowest concentration (44 ng in May). The elevated concentrations in Pond 2 in December were atypical for silverside in the Bay and Delta; concentrations this high have only previously been observed for this species in Alviso Slough, and in the Cosumnes River upstream of the Delta, both subjected to direct inputs of highly elevated, mining-derived exposure (Slotton et al. 2007, Greenfield and Jahn 2010).

Possible mechanisms to explain the seasonal patterns in Pond 2 and the other ponds include differences in ecosystem productivity resulting in changes in biodilution of Hg, seasonal differences in net MeHg production, or direct exposure to an uncharacteristic level of Hg loading. MeHg production will be favored in conditions favoring activity of iron and sulfate reducing bacteria, such as anoxia and high net heterotrophy (Benoit et al. 2003, Tetra Tech 2006). Pond 2 is a managed pond, with muted tidal action, and is notably deeper than the adjacent, comparable Pond 1. As it lacks major inflows or outflows, it seems plausible that water quality in Pond 2 may change dramatically among seasons. Evaluation of other water quality parameters, such as dissolved oxygen, organic carbon, and sulfur speciation in the water and sediments would aid in better understanding the mechanisms behind the seasonal variation. This information could aid in determining what kinds of environmental conditions should be avoided in future pond management and modification activities. Another possibility is that the greater depth of Pond 2 allows MeHg to accumulate relative to the shallower ponds where photodemethylation may maintain net MeHg exposure at lower levels. Finally, we have been advised by DFG that Pond 2 was recently rip-rapped with rock derived from Lake Herman, a known Hg hotspot.

Monitoring Question 4: How does bioaccumulation in these ponds and sloughs compare to other habitat types and locations in the San Francisco Estuary?

The three “control sites” in this study (used to compare Napa Ponds to ambient conditions in the North Bay and tributaries) were Petaluma Marsh at Gambinini, San Pablo Bay at Hamilton Air Force Base, and Napa River at Kennedy Park. In general, Hg concentrations in these three locations were similar to or higher than Hg in the Napa Ponds (with the exception of cool season conditions at Pond 2). This result suggests that potential risk due to Hg exposure for small fish and piscivores at the Napa Ponds complex is generally similar to or less than the risk posed in other parts of San Pablo Bay and the Napa River. Fall monitoring of Mississippi silverside performed Bay wide over several years indicated similar concentrations across most Bay margin locations (including this study), with the exception that concentrations were generally higher in the Lower South Bay, particularly at and adjacent to Alviso Slough (Greenfield and Jahn 2010).

Variation among Species

Interestingly, in this study concentrations were generally quite similar across the species examined (Figure 2), despite large differences in fish size and life history. Of particular interest, Mississippi silverside and topsmelt exhibited similar Hg concentrations, with topsmelt concentrations averaging about 10% - 20% lower than silverside. This pattern is in marked contrast to patterns found in fall monitoring of nearshore locations in San Francisco Bay, where silverside Hg concentrations are generally 2 – 4 times higher than topsmelt (Greenfield and Jahn 2010). This distinction between the two studies may be related to the differences in hydrology, geomorphology, and other habitat features between the Bay versus the more isolated ponds and large sloughs. Fish in San Francisco Bay are free to move Bay-wide, whereas the movements of fish in this study are likely restricted to within the ponds and sloughs due to physical structures (e.g., levees) and the sometimes long distance to the Bay. We have previously hypothesized that topsmelt exhibit greater offshore movement than silverside, resulting in lower Hg exposure (Greenfield and Jahn 2010), within San Francisco Bay. The similar Hg concentrations in the Napa salt ponds for these two species suggest that Hg conditions are similar throughout the managed pond and subtidal slough environment.

Risk to Wildlife

Concentrations of Hg in small fish from this study were generally above the San Francisco Bay TMDL threshold of 0.03 ug/g for forage fish 3 - 5 cm long (SFBRWQCB 2006). However, concentrations were well below a 0.30 ppm threshold at which impaired avian reproductive success has been observed (Albers et al. 2007, Scheuhammer et al. 2007). These findings are generally consistent with forage fish evaluated in other portions of the estuary (Slotton et al. 2007, Greenfield and Jahn 2010). Many piscivorous species feed on the small fish in the Napa-Sonoma pond complex. Piscivorous birds that breed in the area include the Forster's Tern, Caspian Tern, Snowy Egret, Great Egret, Great Blue Heron, and Black-crowned Night-Heron. In winter, piscivorous waterbirds forage in the ponds, including

Double-crested cormorants and various grebes. These birds were exposed to the sharp increase in Hg concentrations of silversides in Pond 2 during winter, but the breeding birds were exposed to much lower concentrations (Figure 1).

Monitoring Recommendations

1. **Clarify and refine key regulatory and management monitoring objectives and design a multi-year monitoring plan to meet them**. The Napa-Sonoma Marsh Restoration Project will be ongoing for many years. Hg likely will continue to be a concern for the foreseeable future. This concern should be translated into a set of clear questions and practical monitoring objectives. At a minimum, Hg monitoring should enable the Regional Water Quality Control Board to assess:
    - I. whether or not the project negatively impacts existing aquatic and wetland habitats, and
    - II. whether or not the restored habitats increase the risk of Hg bioaccumulation within the Napa Sonoma intertidal landscape.

    1a**. The first objective can be met by pre- and post-project comparisons of existing conditions** of intertidal habitat (marsh, channel, mudflat) and shallow subtidal habitat types that are likely to receive water and/or sediment from inside the breached areas or from outside areas that erode due to the project.

    1b. **The second objective can be met by comparing the habitat types within the breached areas to the ambient condition of same types of habitats within the North Bay.** Ambient surveys are also essential to separate the effects of the project from the effects of other possible influences on bioaccumulation (e.g., sea-level rise, changes in sediment and water management in the watershed, etc.).

    1c. **These comparisons should be based on the same biosentinel species for each habitat type that is monitored** (e.g., shallow subtidal, intertidal aquatic, and intertidal marsh).

2. **Use Mississippi silverside as a biosentinel for shallow subtidal (Bay margin), aquatic intertidal (tidal flats and large intertidal sloughs), and managed ponds.** Given that silversides were present in the greatest number of sites and were available year round, they appear to be a particularly appropriate target species for monitoring these habitat types. It should be noted that the silversides likely represent larger areas of habitat than rainwater killifish and threespine stickleback. Silversides are therefore appropriate to indicate differences between ponds and sloughs (as performed in this study) but should not be used to indicate small-scale spatial differences within individual ponds or sloughs. For shallow subtidal and aquatic intertidal habitats, silversides can be

used to address both monitoring objectives. For tidal marsh habitats, the monitoring will need to involve bio-sentinels specific to marsh habitat (see recommendation #3 immediately below).

3. **Bio-sentinel species should be added to the monitoring effort over time to assess new types of habitats that are restored.** The goal of much of the habitat restoration in the Napa-Sonoma tidal landscape is to create intertidal marshes. Despite this fact, the major marsh habitat types (vegetated marsh plain, small intertidal marsh channels, and marsh pannes) have not yet been monitored for Hg bioaccumulation. A scientific rationale should be developed for deciding which of these marsh habitat types to monitor. The monitoring plan should incorporate triggers for when monitoring should begin for the selected intertidal habitat types. For example, vegetated marsh bio-sentinels are monitored in a restored pond when 20% of it has become vegetated or when extant marsh adjacent to a breached pond may be impacted by the restoration. Previous work in South Bay has developed biosentinels for the major habitat types of tidal marsh (Grenier et al. 2010) that should be considered for the Napa-Sonoma marshes.

4. **Determine if seasonal monitoring is necessary.** This study showed that managed ponds can have significantly elevated Hg in small fish over the winter. However, it is not clear that this winter increase in fish Hg burden is a management or regulatory concern. For example, one reason for monitoring small fish is the possible impact of their Hg burden on the breeding success of piscivorous birds. Although piscivorous birds feed in North Bay managed ponds during winter and could therefore gain Hg body burden at that time, the relationship between that burden and subsequent breeding success in summer is unknown. The point of this example is not to discredit seasonal monitoring, but to point out that such monitoring should be carefully justified.

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Table 1. Sampling sites in the Napa-Sonoma Marsh complex and across San Pablo Bay, California.

Site Name	Site Code	County	GPS N	GPS W	Site Description	Restoration and Successional Stage	Tidal Influence
Napa River at Kennedy Park	KENPK	Napa	32.26785	-122.28652	Upstream "control"	River	Full tidal
Pond 1	P1	Solano	38.15428	-122.34816	Shallow, first pond of hydrological series	Little vegetation, to remain a pond (no breach planned)	Muted tidal
Pond 2	P2	Napa	38.16152	-122.33109	Deep	Little vegetation, to remain a pond (no breach planned)	Muted tidal
Pond 2A	P2A	Solano	38.15404	-122.33319	Tidal sloughs in vegetated marsh	Fully vegetated, breached 1995	Full tidal
Pond 4/5	P4/5	Napa	38.16379	-122.31071	Tidal Pond ("bathtub")	Early stages of re-vegetation, breached 2005	Full tidal
Pond 6A	P6A	Napa	38.18125	-122.34060	Essentially closed pond, shallow, clear, submerged vegetation	Benthic algal mats, slated for future breach	Almost no tidal influence
Pond 7A	P7A	Napa	38.19594	-122.34970	Essentially closed pond, shallow, turbid, no vegetation	Slated for future breach	Almost no tidal influence
Pond 9/10	P9/10	Napa	38.20703	-122.29837	Tidal Pond ("bathtub")	Little vegetation, breached 2009	Full tidal

Napa Plant Site Wash Ponds	WASHP	Napa	38.20382	-122.30494	Tidal Pond ("bathtub")	Early stages of re-vegetation, breached 2009	Full tidal
Dutchman Slough	CULLI	Solano	38.13630	-122.31311	Large tidal slough, baseline for Cullinan Ranch restoration	Tule-lined slough, adjacent to area slated for restoration	Full tidal
Petaluma Marsh, Gambinini	GAMB	Sonoma	38.21441	-122.58325	Large tidal slough in tidal marsh	Slough in high marsh, heavily vegetated	Full tidal
Hamilton AFB, Bay slough	HAMIL	Marin	38.04818	-122.49573	Margin of San Pablo Bay, baseline for Hamilton restoration	Adjacent to tidal marsh and area slated for restoration	Full tidal
Sonoma Creek at Wingo	WINGO	Sonoma	38.20968	-122.42778	Large tidal slough, site of potential future levee failure	Tule-lined slough	Full tidal

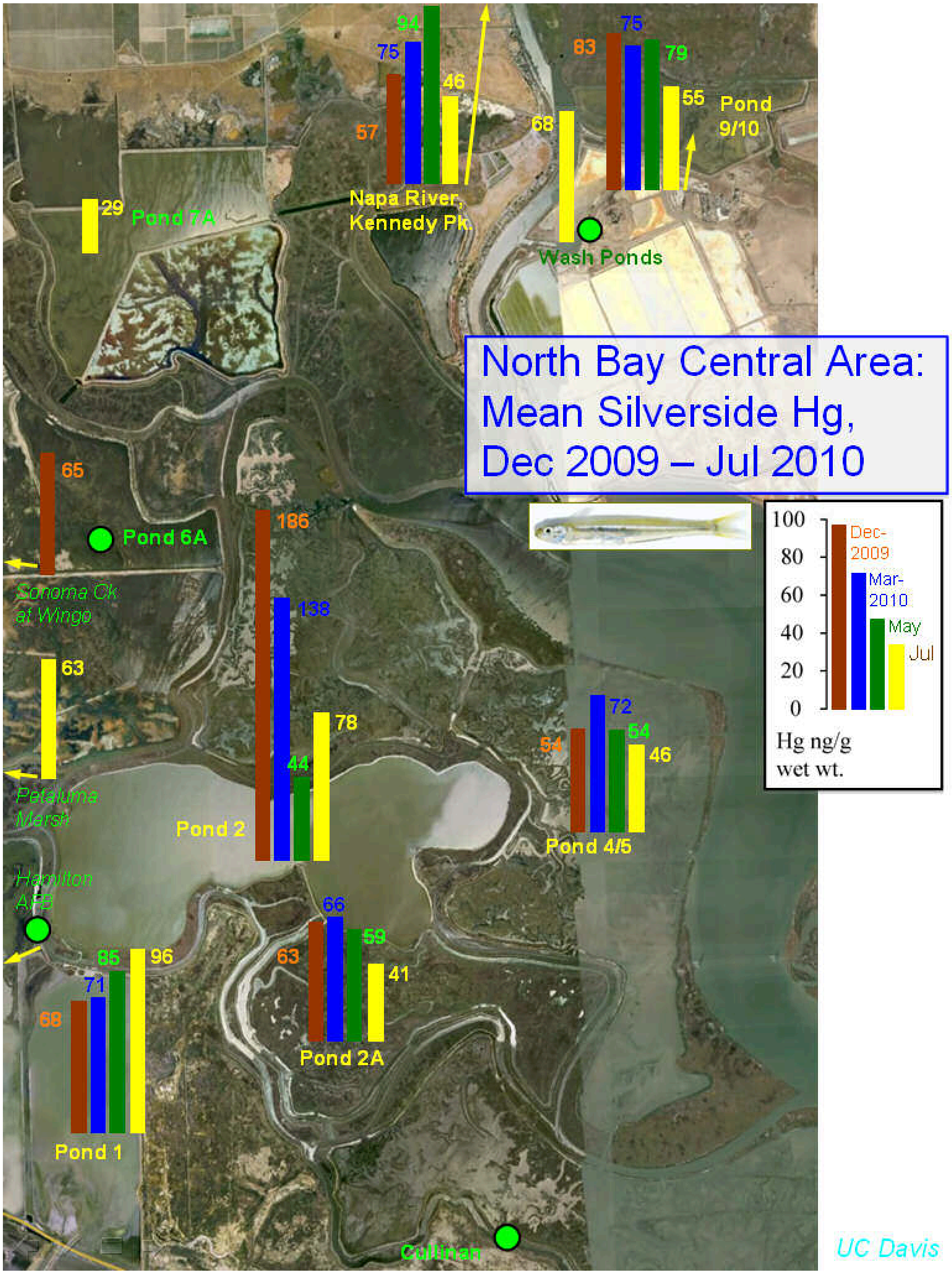

Figure 1. Seasonal and spatial patterns in average Hg in Mississippi silverside collected from 2009-2010 in San Pablo Bay, CA.

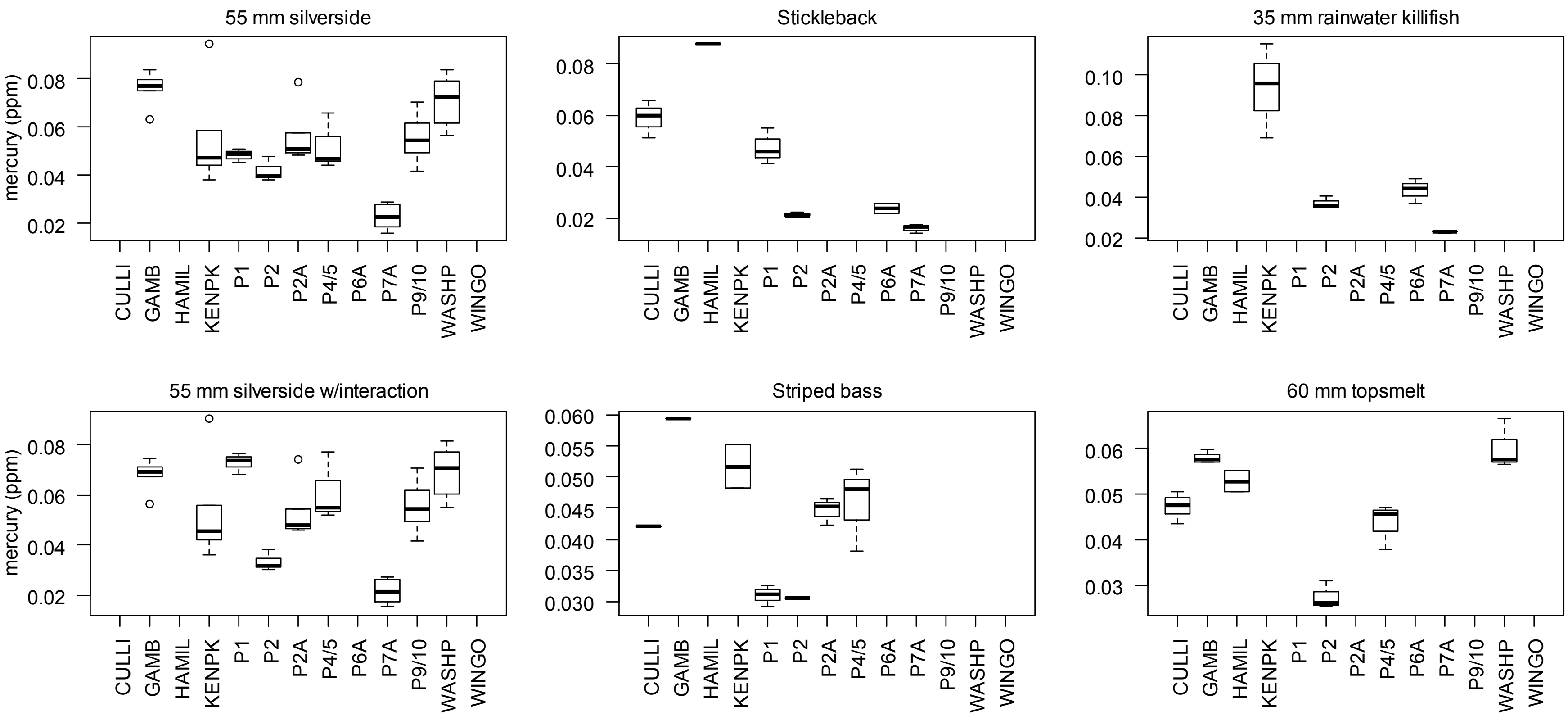

Figure 2. Spatial pattern in Hg for five small fish species monitored in July 2010. All species exhibited significant differences among sites. Results for Mississippi silverside, rainwater killifish, and topsmelt are length-standardized, with silverside results length-standardized using two methods (see Methods section). Hg was not related to total length for threespine stickleback or striped bass. All Hg results are ppm wet weight. See Table 1 for site abbreviations.

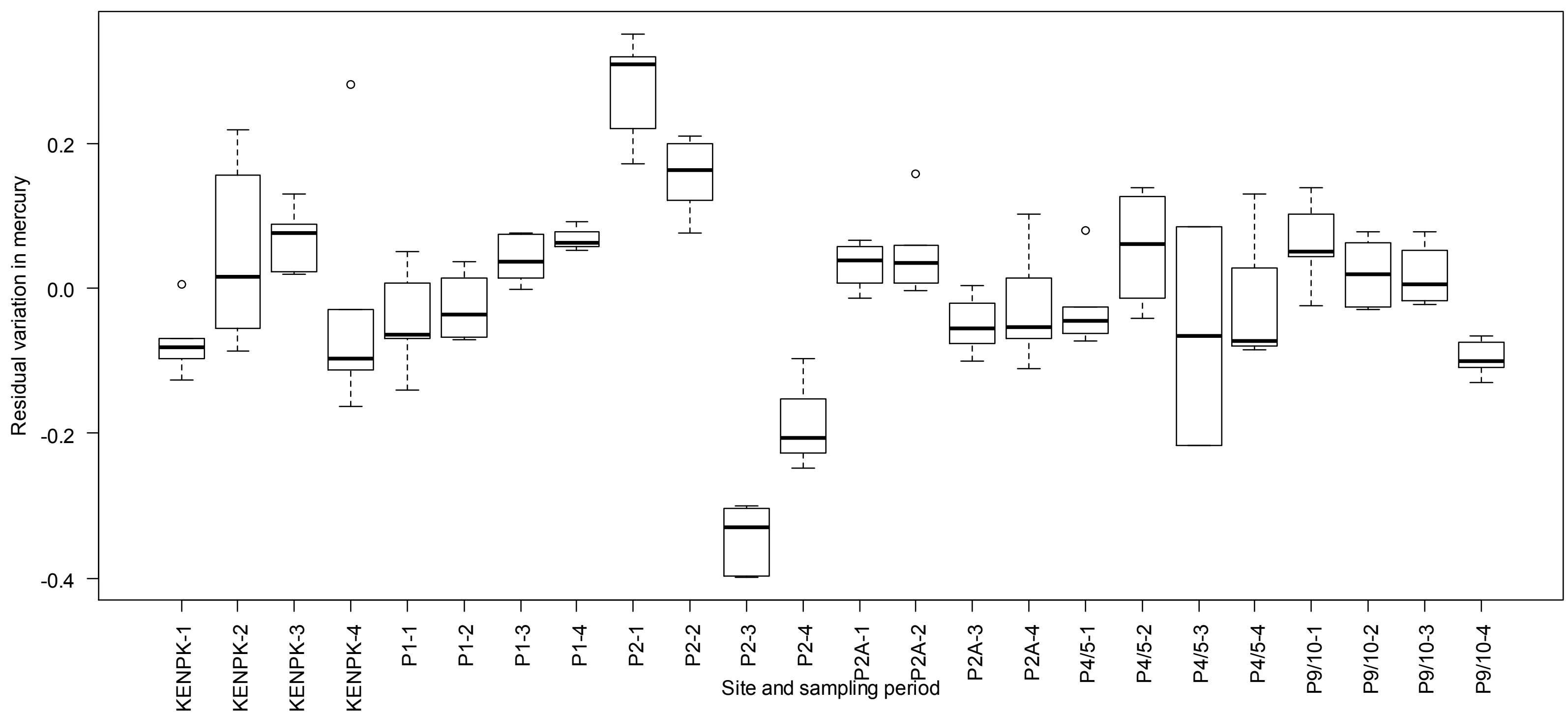

Figure 3. Seasonal variation of Hg in Mississippi silverside was different across the five seasonally sampled sites. Results are the residual variation in Hg after correcting for fish length, and indicate relative differences among sites rather than absolute Hg concentrations. For each site, the site name is followed by a number indicating sampling period (1 = Dec 2009; 2 = March 2010; 3 = May 2010; 4 = July 2010). See Table 1 for site abbreviations.

Appendix 1: Supplemental figures

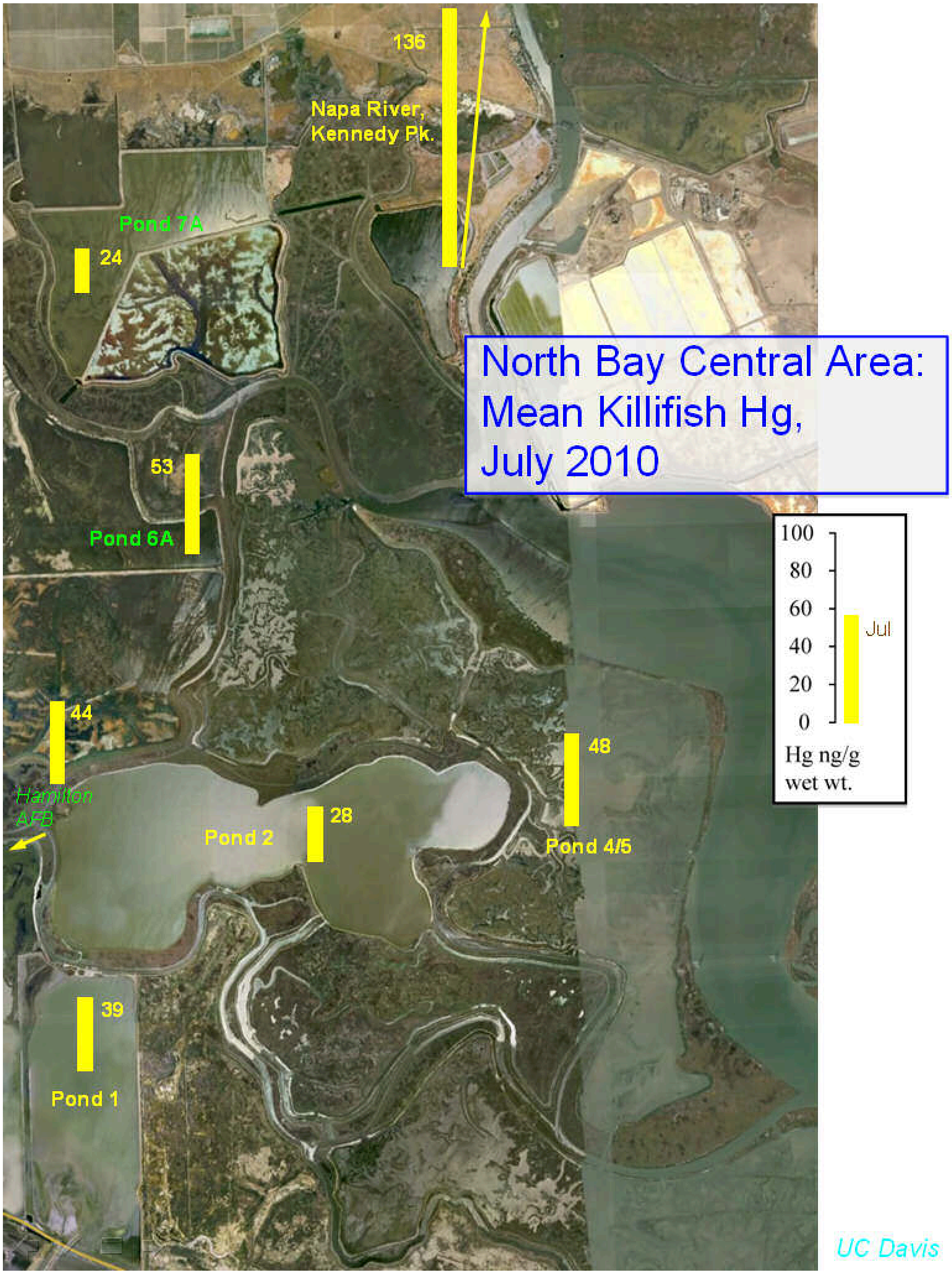

Figure A1. Spatial variation in rainwater killifish Hg, July 2010.

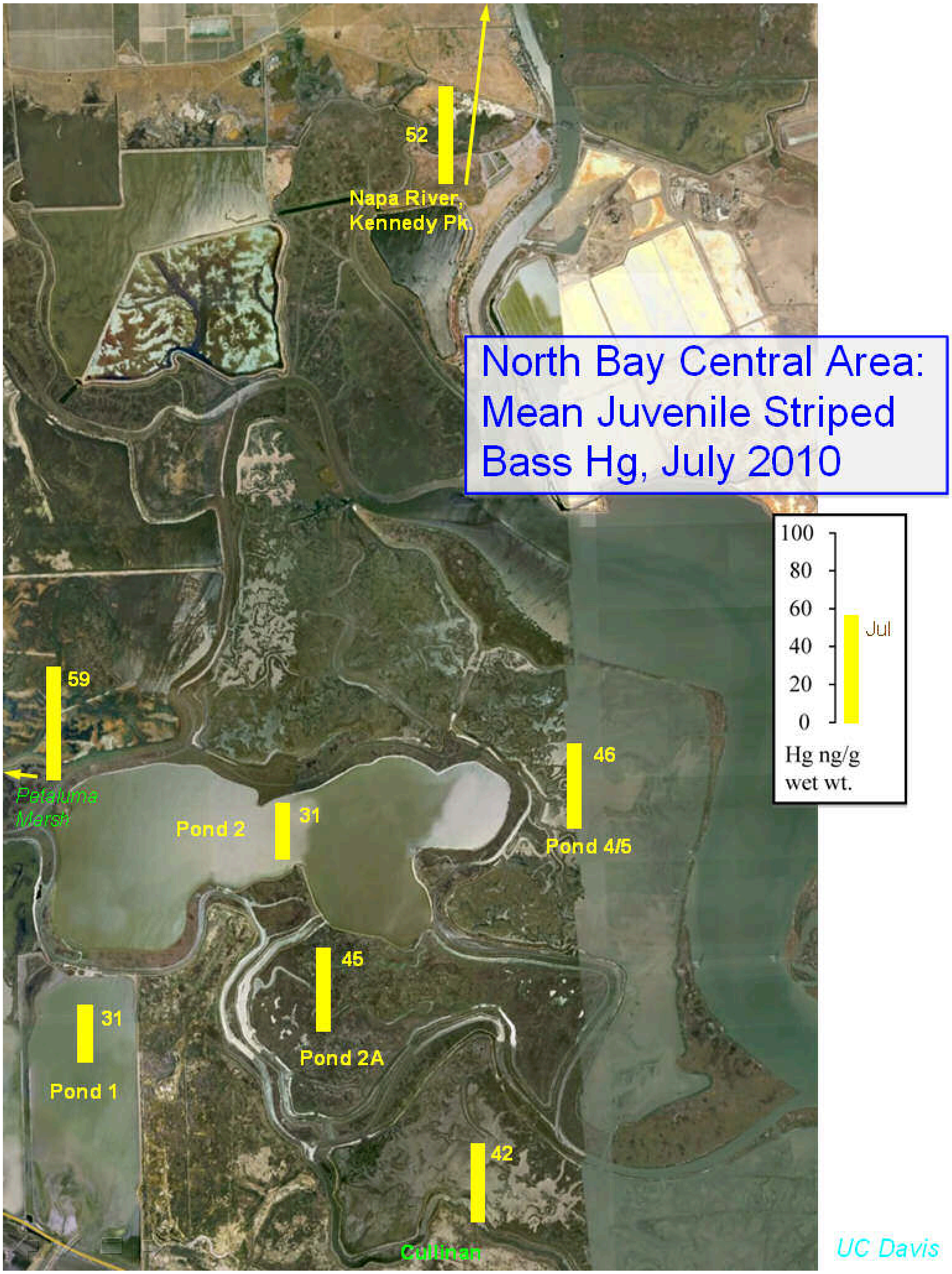

Figure A2. Spatial variation in juvenile striped bass Hg, July 2010.

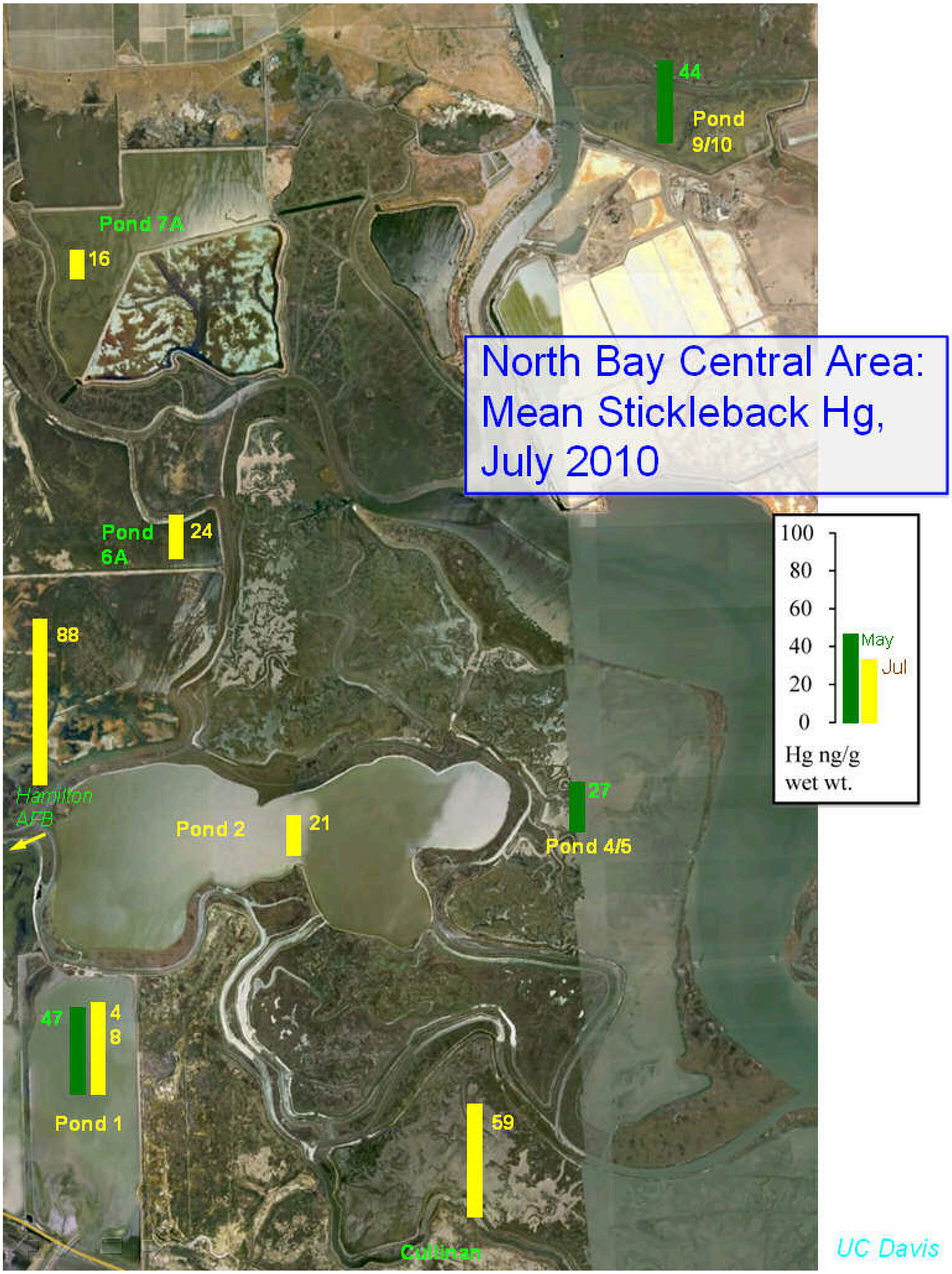

Figure A3. Spatial variation in threespine stickleback Hg, May and July 2010.

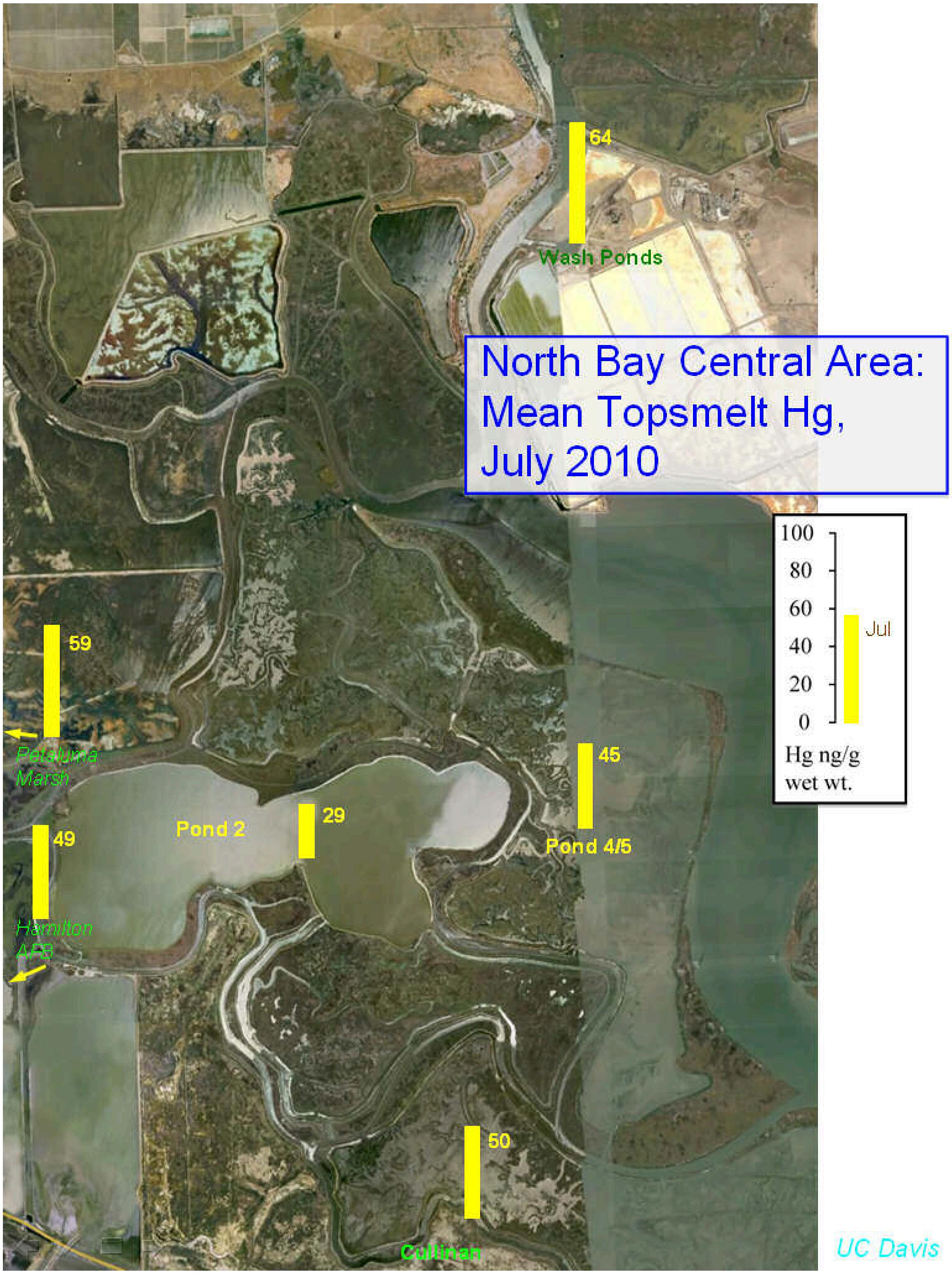

Figure A4. Spatial variation in topsmelt Hg, July 2010.

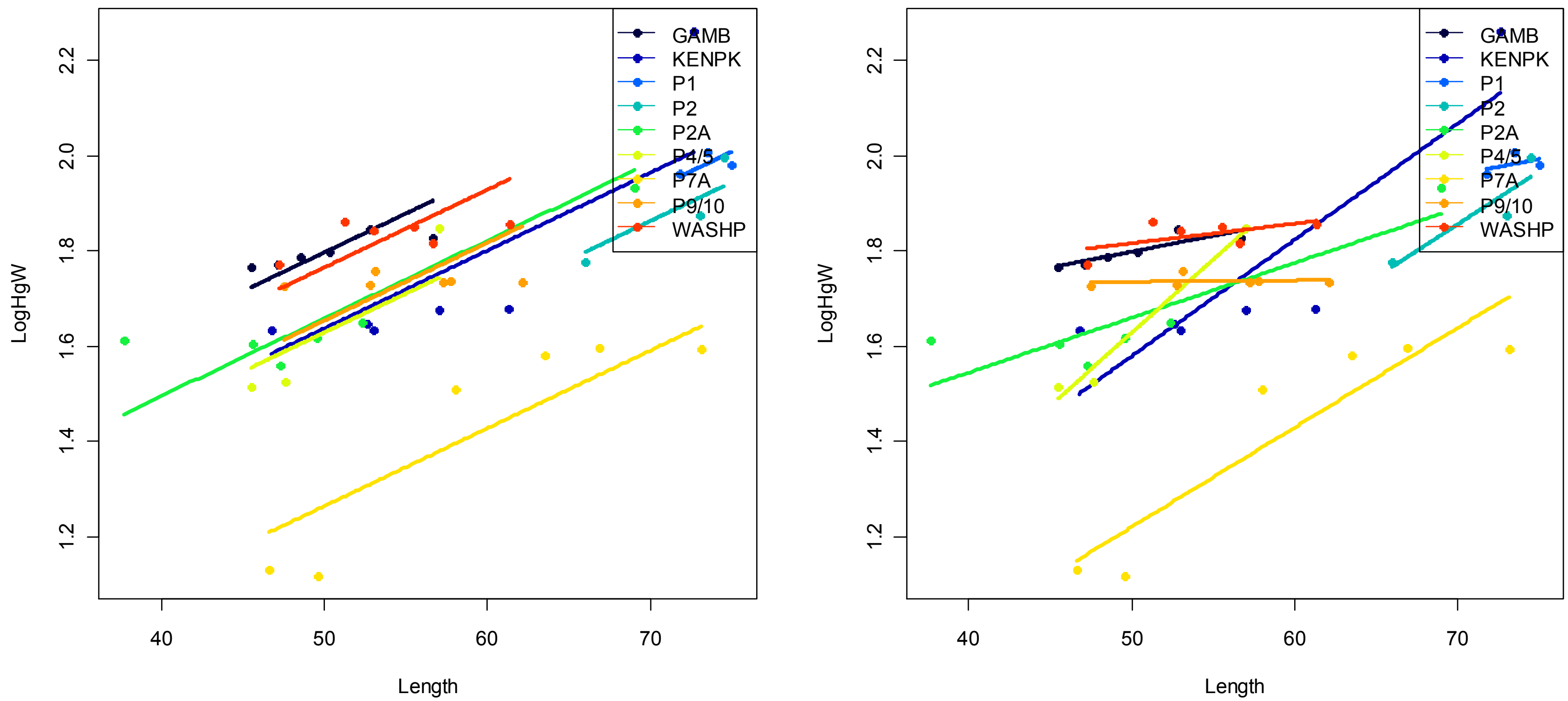

Figure A5. Effect of fish total length and site on Hg in Mississippi silverside, July 2010. The left panel includes a general length effect and site differences. The right panel includes a length effect, site differences, and differences in the length effect among sites. Hg concentrations are presented as the log transformation of the wet weight, whole-body Hg concentration.

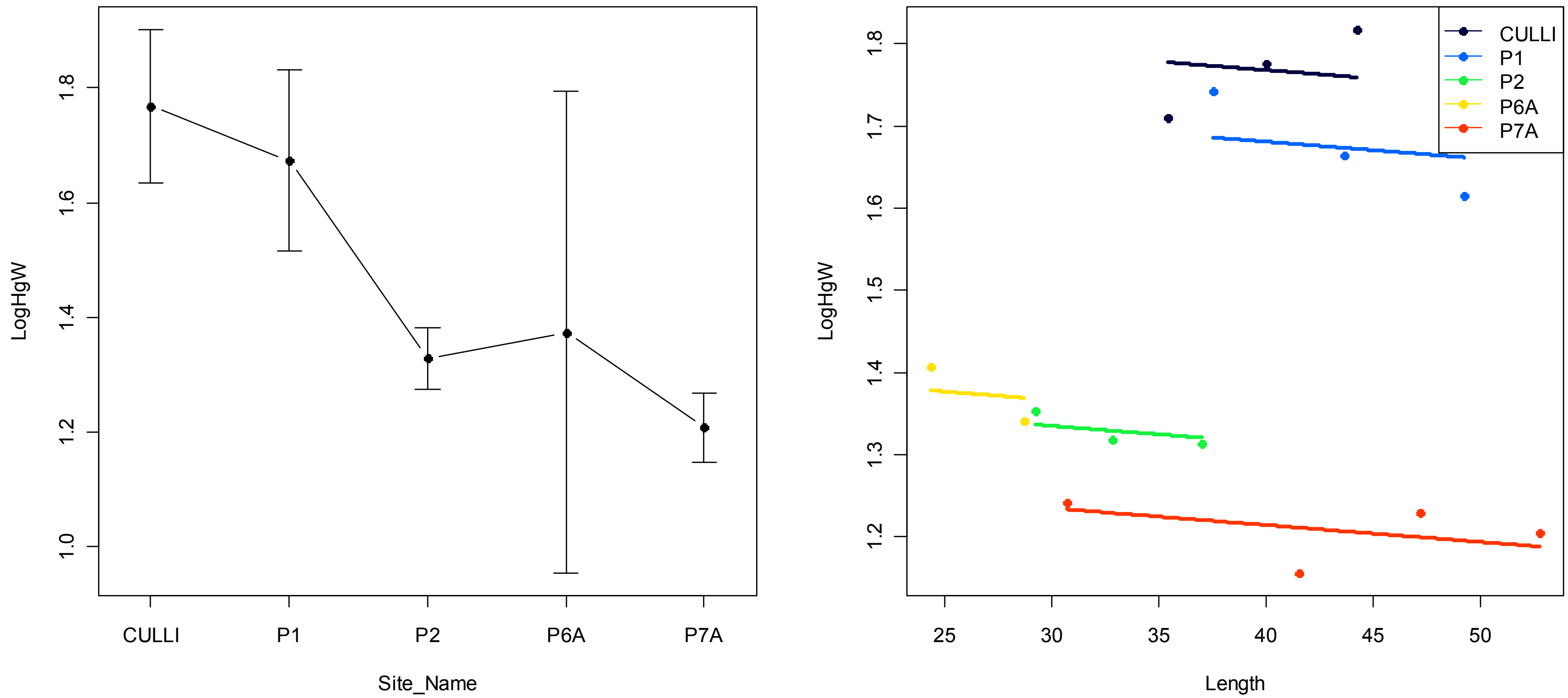

Figure A6. Effect of fish total length and site on Hg in threespine stickleback, July 2010. The left panel includes site differences only. The right panel includes a general length effect and site differences. Hg concentrations are presented as the log transformation of the wet weight, whole-body Hg concentration.

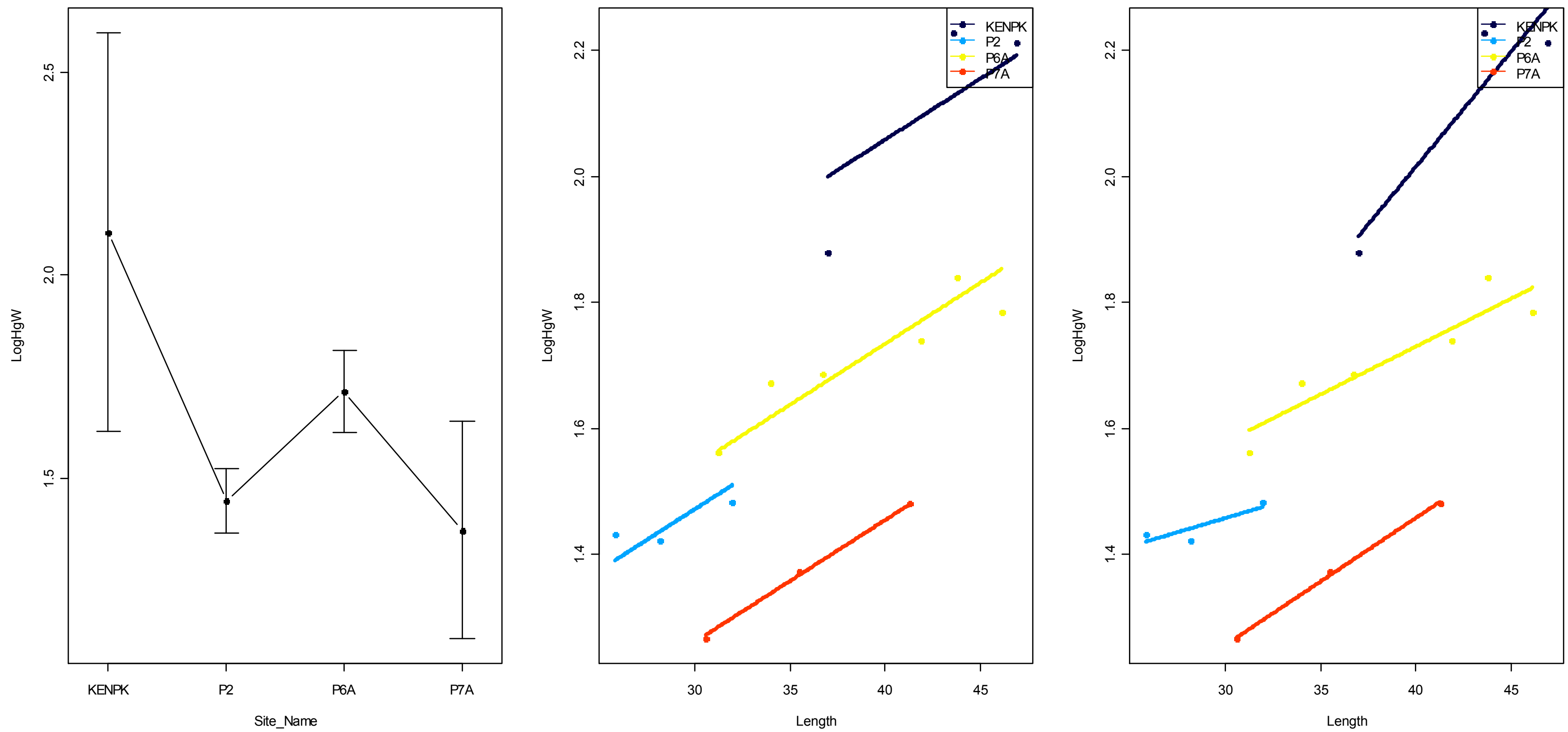

Figure A7. Effect of fish total length and site on Hg in rainwater killifish, July 2010. The left panel includes site differences only. The middle panel includes a general length effect and site differences. The right panel includes a length effect, site differences, and differences in the length effect among sites. Hg concentrations are presented as the log transformation of the wet weight, whole-body Hg concentration.

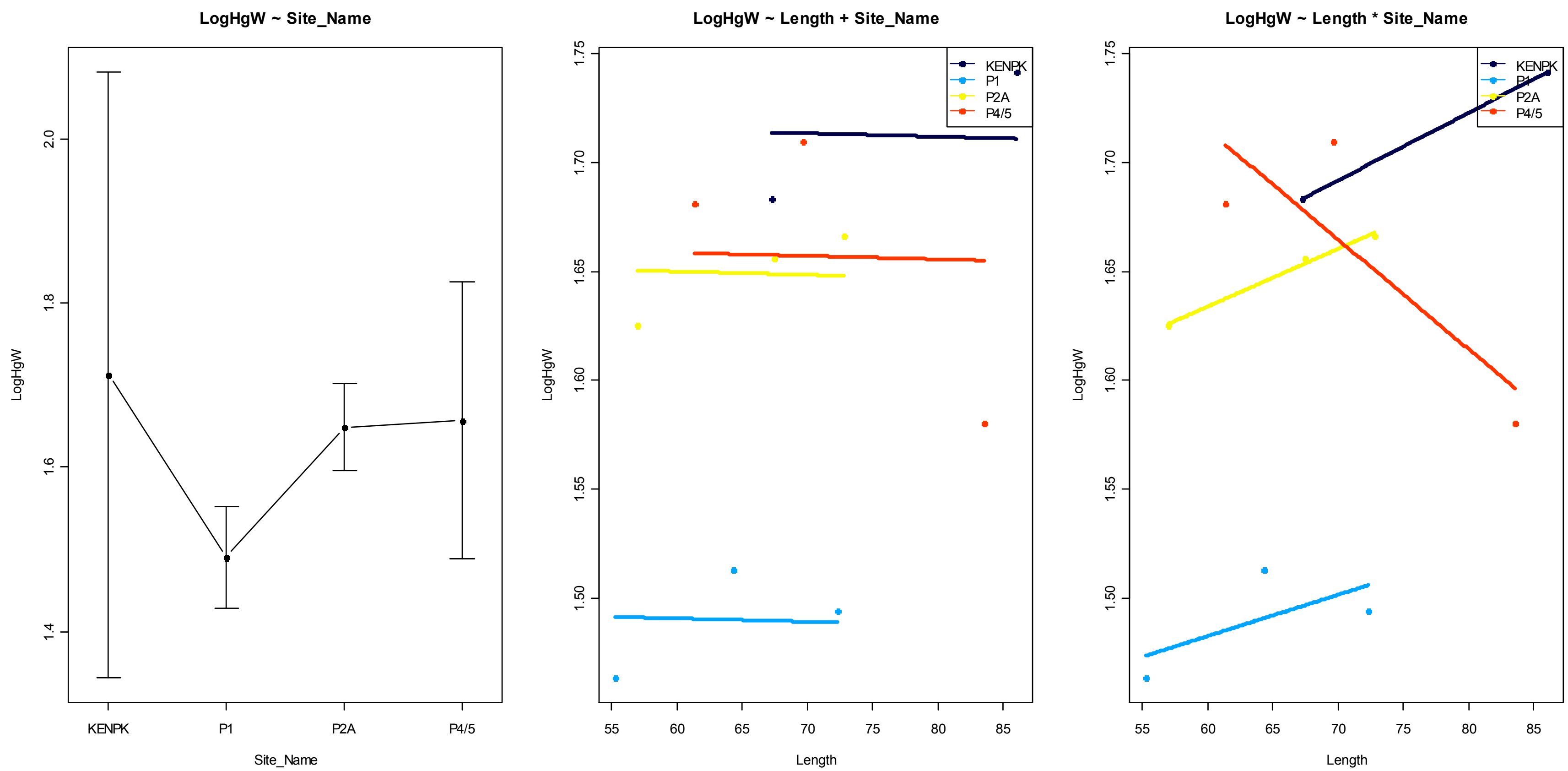

Figure A8. Effect of length and site on Hg in striped bass, July 2010. The left panel includes site differences only. The middle panel includes a general length effect and site differences. The right panel includes a length effect, site differences, and differences in the length effect among sites. Hg concentrations are presented as the log transformation of the wet weight, whole-body Hg concentration.

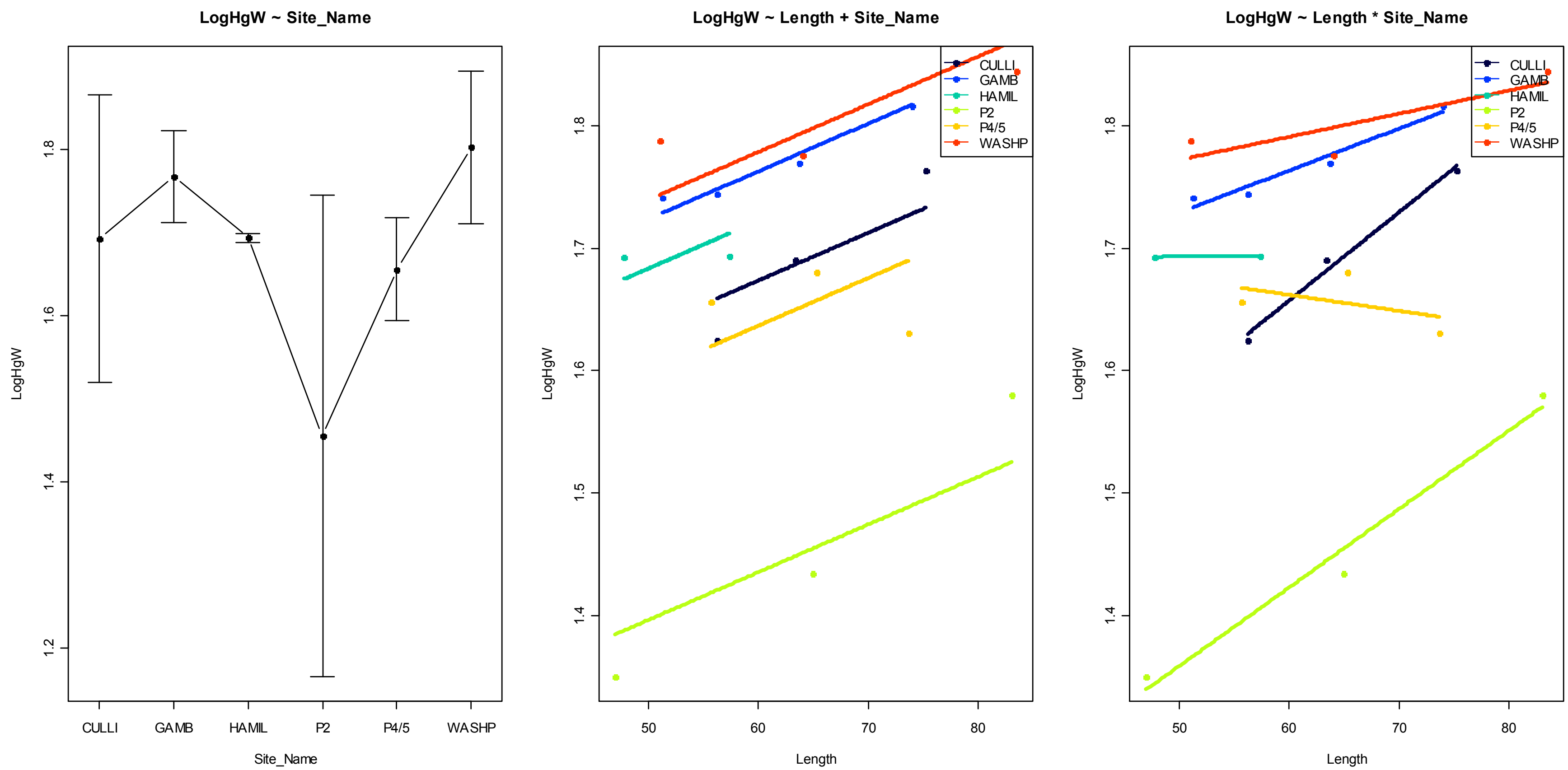

Figure A9. Effect of length and site on Hg in topsmelt, July 2010. The left panel includes site differences only. The middle panel includes a general length effect and site differences. The right panel includes a length effect, site differences, and differences in the length effect among sites. Hg concentrations are presented as the log transformation of the wet weight, whole-body Hg concentration.