#### **RMP Small Fish Workplan**

#### Introduction

Mercury (Hg) contamination is one of the highestpriority water quality issues for the Estuary (Johnson and Looker 2004), and the RMP Mercury Strategy indicates that the focus should be on "improving understanding of the production and uptake of methylmercury." Through the Hg TMDL, actions are being initiated to reduce Hg loads to the Bay (SFBRWQCB 2006). On the other hand, it has been hypothesized that large-scale wetland restoration around the Bay margins may cause greater Hg accumulation in the food web (Davis et al. 2003). The greatest health risks due to Hg are faced by humans and wildlife that consume fish (Wiener et al. 2002). Adaptive implementation of the Hg TMDL and adaptive management of habitat restoration will depend heavily on appropriate monitoring of impacts on water quality (Mumley and Looker 2004).

One of the key issues identified by RMP stakeholders is how to predict points of greatest Hg exposure in San Francisco Bay. The RMP Mercury Strategy indicates that, "understanding where and when Hg enters the food web is critical in determining how to reduce food web contamination." The Strategy goes on to indicate that, "spatially and seasonally intensive measurements of methyl mercury bioaccumulation" will be required to answer this question. The interaction of spatial and temporal trends may also pose a management concern because of the potential impact of restoration activities on Hg uptake into the food web. In the RMP Small Fish Pilot Study and other local small fish studies, significant differences in fish Hg exposure have been observed among nearby locations and among different years and seasons (Greenfield et al. 2006, Slotton et al. 2007).

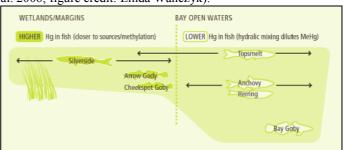
For methyl mercury (MeHg), the causes of spatial variation in concentrations and bioaccumulation remain a topic of intense interest and speculation.

Recent work by USGS has identified spatial differences in shorebird MeHg, with concentration patterns following a general spatial trend: Lower South Bay > South Bay > North Bay > Central Bay (Ackerman *et al.* 2007, Collin Eagles-Smith, pers. comm.). Focused studies are also underway to characterize spatial variation in Hg in wetland and upland biota in the South Bay Salt Ponds region (Grenier et al. 2007). Data collected on Hg in wetland resident fish, brine flies, and songbirds indicate highly variable and site-specific concentrations, which are hypothesized to be affected by gradients in salinity and individual wetland characteristics (Letitia Grenier, SFEI, pers. comm.).

#### Conceptual model

Based on RMP small fish analyses to date, a conceptual model has been developed for spatial patterns of small fish Hg availability and uptake (Greenfield *et al.* 2006). This model explains observed differences in Hg concentrations among species as a function of habitat spatial location (Figure 1).

**Figure 1.** Conceptual model of spatial movement of fish, with respect to Hg sources and habitat (modified from Greenfield et al. 2006; figure credit: Linda Wanczyk).



According to the conceptual model, higher total Hg loading and methylation occurs in the wetlands, sloughs, and Bay margins (Figure 1, left hand side) where Mississippi silverside, arrow and cheekspot goby reside. In the open waters of the Bay (right hand side), MeHg is diluted by tidal mixing with marine waters, resulting in relatively low concentrations for open water species, such as topsmelt and Bay goby.

#### RMP Small Fish Work to Date (2005-2007)

In 2005-7, the small fish sampling effort primarily focused on determining Hg in four fish species that are widely distributed in different habitats of the Bay margins: topsmelt, Mississippi silverside, arrow goby, and cheekspot goby (Figure 1).

A network of ten fixed monitoring stations has been established for trend monitoring of Hg in fish (Table 1). These stations include both areas with planned or ongoing restoration activities and areas with no restoration planned. Six of the fixed stations have been annually sampled since 2005, and three of the fixed stations have been sampled for two of three years (Table 1). Additionally, collaboration with the U.S. Fish and Wildlife Service and California Department of Fish and Game has enabled collection at additional stations on an opportunistic basis. Between 2005 and 2007, 11 midwater stations were sampled opportunistically for pelagic fishes and Bay goby, and 12 additional shoreline stations were sampled to better characterize spatial variation in bioavailable Hg (Table 1).

In addition to monitoring of spatial and temporal patterns in Hg, smaller scale projects to address focused management questions are also underway. In 2007, composite topsmelt samples were collected from five sites for analysis of organic pollutants (PCBs, legacy pesticides, and PBDEs), using highly sensitive analytical methods. The objective of this pilot project is to obtain a preliminary Bay-wide estimate of spatial variation in forage fish organic pollutant bioaccumulation.

Results to date (2005-6) indicate the study design is able to detect significant spatial variation. Hg levels in fish from South Bay, Tiburon, and Point Isabel appear to be elevated relative to other sites. Silversides from salt ponds also appear to have elevated levels compared with Bay stations.

Site Name	Region	Site Type	Impact Type	Sampled		
Long-term trend monitoring stations				2005	2006	2007
China Camp Wetland	San Pablo Bay	Control	None	Х	Х	Х
Mouth of Newark Slough	South Bay	Control	None	Х	Х	Х
Hoffman Marsh/Pt. Isabel	Central Bay	Control	None		Х	Х
Benicia State Park	Carquinez Strait	Control	None	X	Х	Х
Candlestick Point	San Pablo Bay	Impact	Wetland creation nearby		Х	X
Eden Landing/Old Alameda Creek	South Bay	Impact	Saltpond breaching	X	Х	Х
Alviso Slough Mouth (Pond A8)	South Bay	Impact	Saltpond breaching	X	Х	Х
Oakland Middle Harbor	Central Bay	Impact	Habitat creation	Х	Х	
Bair Island /Steinberger Slough	South Bay	Impact	Saltpond breaching	Х	Х	Х
Hamilton Army Airfield	San Pablo Bay	Impact	Wetland restoration			X

**Table 1.** Fixed monitoring stations for evaluating long-termtrends in Hg and effects of restoration activities.

## Proposed design for small fish monitoring

The remainder of this document outlines a workplan for monitoring of small fish and ancillary parameters to address key questions identified by the RMP Mercury Strategy. This includes a proposed sampling design for 2008 monitoring. Designs for upcoming years will be developed based on annual evaluation of project progress and findings from this and other related projects. Based on the RMP Mercury Strategy, it is anticipated that this project will continue at a similar level of effort until 2010, with a reevaluation of the need for continued work at that time.

# Questions to be addressed

This workplan is intended to address four questions:

- 1. Where is Hg entering the Bay food web?
- 2. What habitats, conditions, or factors identify hotspots of food web accumulation in Bay margins?
- 3. Are there interannual trends in MeHg bioaccumulation resulting from wetland and margin restoration activities?
- 4. What are the best biomonitoring tools for characterizing hotspots of MeHg bioaccumulation?

Questions 1 and 2 follow the first two questions of the RMP Mercury Strategy. Question 3 addresses the fourth question of the RMP Mercury Strategy, "What effects can be expected from management actions?" It focuses on the short and long-term impacts of the extensive restoration activities planned and underway for the Estuary (Goals Project 1999, Davis et al. 2003). Question 4 addresses the need for continued assessment and development of appropriate biosentinels for this project and future RMP monitoring.

# Approach

This project focuses primarily on monitoring Hg in resident small fish. Resident small fish are widely recommended and used as a Hg biosentinel in the San Francisco Estuary and elsewhere (Snodgrass et al. 2000, Greenfield et al. 2001, Slotton et al. 2002, Wiener et al. 2003, Slotton et al. 2004, Wiener et al. 2007). They have been demonstrated to be readily and conveniently captured in the Bay and associated wetland areas (Greenfield et al. 2006, Grenier et al. 2007, Slotton et al. 2007).

Conceptual benefits of using small fish include that they:

- indicate inter-annual and spatial variation in net MeHg production in aquatic ecosystems,
- accumulate the form of Hg (MeHg) that causes a health risk to biota,
- indicate the net amount of MeHg exposure in their home-range area,

- integrate exposure over a defined period of time (three to six months), making them a cost-effective and informative monitoring tool, and
- indicate spatial patterns over relatively small scales (including near-shore areas)

Naturally occurring fish will be used, rather than caged fish. Caged fish would be costly and present multiple logistical difficulties, including tidal variation, potential for starvation and mortality, and inadequate time for uptake. Other biosentinels, such as benthic invertebrates, are much more difficult to capture consistently at multiple locations and fail to bioaccumulate MeHg as efficiently as small fish.

Sampling will focus primarily on two fish species: topsmelt and Mississippi silverside (Figure 1). These species are chosen because they are widely distributed throughout the Bay shoreline, relatively easy to capture, and indicate general exposure of the benthopelagic food web (Orsi 1999, Greenfield et al. 2006, Visintainer et al. 2006, Slotton et al. 2007). They are consumed by avian piscivores (Goals Project 2000, Elliott 2005), and therefore indicate spatial differences in potential risk to wildlife. They exhibit statistically significant differences in Hg concentrations among multiple locations in the Estuary (Slotton et al. 2002, Greenfield et al. 2006), and have a limited dispersal range (O'Reilly and Horn 2004), suggesting that they are appropriate indicators of spatial differences in Hg.

The Regional Board has requested that 2008 sampling focus primarily on evaluating spatial patterns in the nearshore zone, but also include assessment of variation over time. Based on this request, Table 2 presents an effort allocation scheme.

With the exception of the seasonal variation component, all sampling will be performed in the fall (September through November), as was conducted in 2005 through 2007. Fall sampling has been the focus of the program for several reasons. First of all, it is anticipated that fall sampling captures as much of the summer increase in growth, consumption, and consequent Hg uptake as possible. Secondly, abundances of the target species are relatively high in the fall (Orsi 1999, Visintainer et al. 2006). Third, fall lies outside the California clapper rail breeding season (April through August), making site access substantially easier. A potential downside to fall sampling is that it may miss short term pulses in bioavailable Hg resulting from winter rainfall and flooding events (Slotton et al. 2007). The seasonal variation survey should aid in evaluating the effect of these events.

**Table 2.** Components of the biosentinel monitoring program, proportion of total effort allocated, and number of stations to be evaluated in 2008.

Component	Proportion of Total Effort	Target Number of Stations
Spatial survey	75%	40
Seasonal variation	10%	2
Interannual variation/restoration effects	10%	8
Comparison of biosentinel tools	5%	5

Four composites of whole fish from each species will be analyzed for total Hg at each location, with at least five fish included in each composite. Composites will be size stratified across the size range that represents the majority of age 0+ nearshore fish, with statistical analyses explicitly considering the effect of fish size (Slotton et al. 2002, Greenfield et al. 2006). To reduce analytical cost, total Hg will be analyzed rather than MeHg, because most Hg assimilated by fish is MeHg (Huckabee et al. 1979).

#### Spatial survey

A key component of the RMP Mercury Strategy is to assess where are the greatest hotspots for Hg entering the Bay food web. The Regional Board has indicated that the highest project priority should be to identify and compare relative impacts of regulated or permitted controllable sources. These include stormwater and municipal discharges (i.e., publicly operated treatment works), as well as restored wetlands. Based on this identified priority, the main focus of this program in 2008 will be to compare among specific site categories to determine whether they exhibit differing evidence of bioavailable Hg. The program will test the hypotheses that small fish Hg concentrations are elevated or more variable at these potential source sites than at control sites. The types of sites that will be compared are:

- Wetlands, emphasizing wetlands likely to exhibit high methylation potential due to bathymetric and hydrological conditions that favor periodic flooding and drying of high marsh areas (Snodgrass et al. 2000, Slotton et al. 2007, Yee et al. 2007).
- Urban stormwater outfall locations (e.g., Figure 2), focusing on urban creek inlets that have extensive storm water discharge points.
- **POTW outfalls** (Figure 3), focusing on sites expected to have high methylation potential due to relatively low flushing rates.

These sites will be compared to control sites (Table 3). Control sites will be adjacent to upland areas, concrete and stone levees, residential locations, or other points having no apparent discharge points or wetlands.

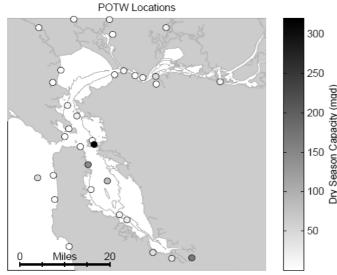
Figure 2. Storm drain at Guadalupe River



component of study.	- <b>I</b>	5
Land Use/Land Cover	N Bay	S Bay
Wetlands	5 sites	5 sites
Urban outfall	5 sites	5 sites
POTW into slough/marsh	5 sites	5 sites
Control (upland, residential, no discharges)	5 sites	5 sites

 Table 3. Proposed sampling design for spatial survey

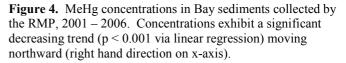
Figure 3. POTW locations in the Estuary. The color scheme indicates dry season capacity (million gallons/day). Data were obtained by Daniel Oros from the Regional Board in 2005.

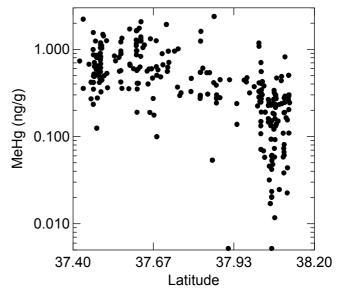


In the Estuary, sediment, bird eggs, and preliminary fish results support a hypothesis that MeHg exhibits spatial trends across the Bay (Figure 4. Greenfield et al. 2006, Ackerman et al. 2007). Therefore, site selection will carefully consider spatial location across the Bay. This will be accomplished by including sites from each category across both northern and southern portions of the Bay (Table 3), and by using GIS to ensure that a spatial gradient exists for all site categories.

The spatial survey will share information gained by the South Bay Mercury Project (SBMP) by establishing some sites adjacent to SBMP sites. The SBMP monitors biosentinel organisms within fringing wetlands and salt ponds. The SBMP has hypothesized several key variables in marsh MeHg bioaccumulation including marsh age, elevation, and salinity (Grenier et al. 2007). In the coordinated

design between the RMP Small Fish Spatial Survey and SBMP, some of the RMP wetland monitoring sites will be established at sloughs draining wetlands monitored by SBMP. This design will help determine whether an elevated Hg signal within a marsh can be tracked in small fish down a major channel to the edge of the Bay. This will aid in addressing Question 1 (Where is Hg entering the Bay food web?).





To address Question 2 (what habitats, conditions, or factors help to identify hotspots of food web accumulation in Bay margins?), there is also a need to identify potential explanatory variables for elevated Hg concentrations in biosentinel fish. This may also aid in determining management options for reducing MeHg. As potential explanatory variables, additional parameters will be collected, including sediment analyses at 20 stations and GIS analyses at all stations. GIS analyses will include multiple spatial parameters that may be associated with Hg loading, loss, and net methylation. Table 4 indicates potential mechanisms for association between spatial parameters and biosentinel fish Hg.

<b>Table 4.</b> Spatial parameters to be developed using GIS for all
sites. These parameters will be statistically compared to Hg
and MeHg in fish and sediments, to help identify potential
drivers of Hg.

Parameter Type	Hypothesized mechanism of		
	influence		
Water residence time	Water dilution and replacement and sediment advective transport may cause net loss of Hg or MeHg, and redox conditions		
Distance to nearest	Loading of Hg and MeHg, as well as		
POTW and nearest	nutrients, fine particulates,		
storm drain discharge	influencing methylation potential		
Number of storm drains feeding into inlet (for urban stormwater outfall sites)	As above.		
Distance to creeks and	As above. Also, movement of fish		
tributaries	upstream to conditions favoring methylation.		
Latitude	Longer residence time in South Bay favoring reduced conditions and consequent methylation.		
Average depth near site	High biotic activity and repeated wetting and drying at shallow sites favoring bacterial methylation activity.		
Abundance of intertidal mudflat near site	As above.		
Nearby Land Cover/Land Uses	Multiple potential mechanisms		

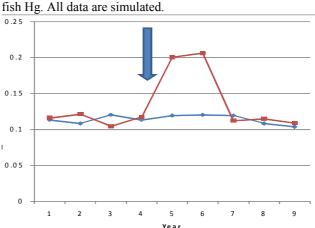
Sediment analyses will evaluate the influence of local sediment chemical conditions, including redox and productivity, on Hg methylation. Sediment parameters will include total and methylmercury, total organic nitrogen, and local redox conditions. This information will be used to assess the extent to which local site characteristics (e.g., wetland vs. POTW vs. control) influence net methylation of Hg in sediments, and consequent bioavailability. This will also evaluate the hypothesis that local site conditions are associated with food web uptake, as measured by the biosentinel organisms.

#### Interannual variation and restoration effects

Evaluation of interannual variation and restoration effects (Question 3) will be achieved by continuing

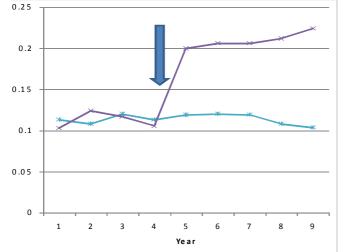
the monitoring design established at the 2005 inception of the small fish Hg program. Trend sites are fixed, rather than randomly located, to allow analysis of trends in bioaccumulation of Hg over time. Prior studies of trends in Bay bivalves and sportfish suggest that continued annual sampling for 10 years will provide sufficient sample size and duration to evaluate effects (Gunther et al. 1999, Greenfield et al. 2005, Melwani et al. 2007). The use of multiple stations is statistically powerful, allowing for the application of models such as Analysis of Covariance to simultaneously test for overall trends across stations (Melwani et al. 2007).

The trend monitoring design includes a similar number of stations with and without planned wetland restorations (Table 1). This should enable assessment of the impacts of the restoration activities, independent of background temporal variation and trends. For example, individual restorations may result in short-term or long-term increases in bioavailable MeHg and consequent uptake by fish (Figures 5 and 6), which would be observed as spikes above the other stations. When the entire data set from trend monitoring stations is examined, a general effect pattern may emerge (Figure 7). In contrast, an absence of effect would indicate limited impacts of restoration on Hg bioaccumulation.

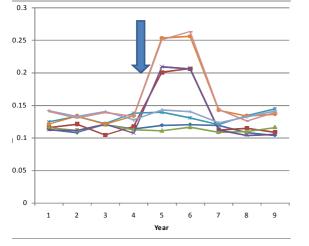


**Figure 5.** Simulated depiction of two trend stations from the small fish monitoring program. The y-axis represents MeHg in small fish. In the station with restoration activity (red line and blue arrow), there is a short-term (2 yr) impact on biosentinel fish Hg. All data are simulated.

**Figure 6.** Simulated depiction of two trend stations from the small fish monitoring program. The y-axis represents MeHg in small fish. In this simulation, restoration activity (blue arrow) results in a long term (>4yr) change in small fish Hg at the restoration station (purple line). All data are simulated.



**Figure 7.** Graphical depiction of multiple station comparison in the small fish monitoring program. The results of 8 stations are depicted, with all stations centered about the impact date. The y-axis represents MeHg in small fish. In this simulation, the restoration activities (blue arrow) result in short-term MeHg increases in all restoration stations (red and purple lines), while the other stations (blue and green lines) are unaffected. All data are simulated.



## Seasonal variation

Assessment of seasonal variation will provide information that is essential for interpreting longerterm trends and identifying management opportunities. The seasonal variation monitoring is intended to complement previous and ongoing work funded by the CalFed program (Slotton et al. 2007, C. Eagles-Smith and Josh Ackerman, USGS, in preparation). Slotton et al. (2007) observed substantial spikes in small fish Hg in late spring, which they attributed to flooding of high methylation areas after large rainfall events. We will determine tissue Hg in one species captured monthly over 2008 at fixed stations. This sampling will test the hypothesis that tissue Hg will be elevated in late winter and early spring, resulting from seasonal rainfall events.

Because of limited capture success during the winter (Visintainer et al. 2006), it may be difficult to obtain samples of target species during all months. To improve the likelihood of successful collection year round, we will take advantage of an ongoing seasonal sampling program performed by the US Fish and Wildlife Service. This program conducts biweekly beach seine surveys for juvenile salmon in multiple Bay-Delta locations. Their sampling includes nine fixed stations around the perimeter of San Pablo Bay. Previous data indicate that topsmelt are frequently captured at these stations, and the collecting agency has been very helpful in saving and providing samples. Fish will also be collected at an additional station by RMP staff. The proposed site is the Martin Luther King Regional Shoreline, inside San Leandro Bay, and adjacent to the SFEI office. This site is enclosed in an embayment with extensive mudflats exhibiting elevated sediment MeHg. It is also adjacent to a restored wetland and several urban creeks. This combination of conditions is expected to be favorable to strong pulses of bioavailable Hg, with the potential for considerable seasonal variation. Multiple fish will be targeted, with the species most consistently caught year-round used in the final analysis.

#### Comparison of biosentinel tools

In late 2007, a pilot study was established in collaboration with Drs. Elly Best (U.S. Army Corps of Engineers – ERDC) and Holger Hintelmann (Trent University). The study focuses on comparing multiple biosentinels to Diffusive Gradient Thinfilm (DGT) devices as measures of Hg bioavailability. DGTs have been developed as indicators for potential MeHg bioaccumulation at relevant field sites over a range of time periods (up to 28 days, Best et al. 2007). DGTs were deployed at five small fish monitoring sites. Additionally, two clam species were deployed in cages for 28-day bioaccumulation studies, and local bivalve and bulk water samples were collected.

Data from the pilot study will become available for analysis in 2008. The analyses should provide preliminary information on the degree of association between MeHg concentrations in DGT, small fish, caged and local bivalves, and bulk water. This type of information will be useful for evaluating our choice of small fish as the primary Hg biosentinel. It should also aid in determining what monitoring tools best measure bioavailable MeHg in specific management circumstances. Pending results of this comparison, and other research ongoing in the RMP and elsewhere, a new study design will be proposed and developed in 2009.

# *Monitoring mercury in biosentinel organisms – an adaptive program*

The RMP Mercury Strategy includes funding for small fish monitoring at the current funding level in 2008, 2009, and 2010. This workplan presents the study approach for 2008, which will be reviewed by two RMP workgroups (the Contaminant Fate Work Group and the Exposure and Effects Work Group). Pending available funding, our current plan is to draft a new approach for each year that this study is performed. The intent is to adaptively incorporate new information gained to improve the study design on an ongoing basis. At the end of each year, project participants will reevaluate available data, other studies within and outside the RMP, and management recommendations by the Regional Board. We will then draft a new workplan, modify the workplan based on work group and stakeholder review, and perform the new monitoring accordingly.

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# Budget

50	\$77.70	\$3,885
50	\$52.00	\$2,600
		\$6,485
30	\$77.70	\$2,331
60	\$52.00	\$3,120
		\$5,451
120	\$52.00	\$6,240
960		\$58,240
32		\$2,400
		\$2,000
		\$68,880
Samples	Cost	
240	\$75.00	\$18,000
	•	· · / · · ·
40	\$75.00	\$3,000
112	\$75.00	\$8,400
40	\$100.00	\$4,000
40	\$173.00	\$6,920
40	\$20.00	\$800
		\$41,120
24	\$114.00	\$2,736
20	\$85.00	\$1,700
120	\$55.00	\$6,600
		\$11,036
160	\$77.70	\$12,432
120	\$52.00	\$6,240
20	\$100.00	\$2,000
		\$20,672
		\$153,644
R = John R, DY	′ = Don Y	
	30 60 120 960 32 32 32 32 32 32 32 32 32 32 32 32 32	30         \$77.70           30         \$77.70           60         \$52.00           120         \$52.00           960         \$60.67           32         \$75.00           240         \$75.00           40         \$75.00           40         \$75.00           40         \$75.00           40         \$75.00           40         \$75.00           40         \$75.00           240         \$75.00           240         \$75.00           240         \$75.00           240         \$75.00           240         \$75.00           240         \$75.00           40         \$100.00           40         \$100.00           40         \$100.00           40         \$20.00           40         \$100.00           40         \$20.00           40         \$20.00           40         \$20.00           40         \$20.00           40         \$20.00           40         \$20.00           40         \$20.00