Appendix II: Sampling and Analysis Plan

FINAL

Sampling Plan

Incorporating Wildlife Methylmercury Exposure and Risk Estimates Using Biomagnification Factors into California Lake Monitoring

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Surface Water Ambient Monitoring Program

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I. INTRODUCTION

This document presents a sampling plan for a two-year study that will relate methylmercury exposure in fish from California lakes to exposure and risk in fish-eating birds. Piscivorous birds likely face significant risks from methylmercury exposure in a large number of California lakes. The goal of this study is to assess those risks in a representative sample of lakes and to investigate development of a biomagnification factor to estimate methylmercury exposure in wildlife based on concentrations in lower trophic level prey fish and we will also correlate sport fish mercury concentrations with prey fish and birds.

This work will be performed as part of a two-year field study for the State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). Oversight for this Project is being provided by the SWAMP Roundtable. The Roundtable is comprised of State and Regional Water Board staff and representatives from other agencies and organizations including USEPA, the California Department of Fish and Game (CDFG) the California Office of Environmental Health Hazard Assessment, and the University of California.

The Roundtable has formed a subcommittee, the Bioaccumulation Oversight Group (BOG) that focuses on the Bioaccumulation Monitoring Project. The BOG is comprised of State and Regional Water Board staff and representatives from other agencies and organizations including USEPA, the Department of Fish and Game, the Office of Environmental Health Hazard Assessment, the Southern California Coastal Waters Research Project, and the San Francisco Estuary Institute. The members of the BOG individually and collectively possess extensive experience with bioaccumulation monitoring.

The BOG has also convened a Bioaccumulation Peer Review Panel that is providing programmatic evaluation and review of specific deliverables emanating from the Project, including this Sampling Plan. The members of the Panel are internationally recognized authorities on bioaccumulation monitoring. The BOG was formed and began developing a

strategy for designing and implementing a statewide bioaccumulation monitoring program in September 2006. To date the efforts of the BOG have included a two-year screening survey of bioaccumulation in sport fish of California lakes and reservoirs (in 2007 and 2008), another two-year screening survey of the California coast (in 2009 and 2010), and a one-year screening survey of California rivers and streams (in 2011). This wildlife study (in 2012 and 2013) will begin the next phase of BOG studies to assess the impacts of bioaccumulation on beneficial uses in California water bodies.

II. GENERAL ASPECTS OF THE SWAMP BIOACCUMULATION MONITORING PROJECT

A. Addressing Multiple Beneficial Uses

Bioaccumulation in California water bodies has an adverse impact on both the fishing and aquatic life beneficial uses (Davis et al. 2007). The fishing beneficial use is affected by human exposure to bioaccumulative contaminants through consumption of sport fish. The aquatic life beneficial use is affected by exposure of wildlife to bioaccumulative contaminants, primarily piscivorous species exposed through consumption of small fish. Different indicators are used to monitor these different types of exposure. Monitoring of status and trends in human exposure is accomplished through sampling and analyzing sport fish. On the other hand, monitoring of status and trends in wildlife exposure can be accomplished through sampling and analysis of wildlife prey (small fish, other prey species) or tissues of the species of concern (e.g., bird eggs or other tissues of juvenile or adults of the species at risk).

Over the long-term, a SWAMP bioaccumulation monitoring program is envisioned that assesses progress in reducing impacts on both the fishing and aquatic life beneficial uses for all water bodies in California. In the near-term, however, funds are limited, and there is a need to demonstrate the value of a comprehensive statewide bioaccumulation monitoring program through successful execution of specific components of a comprehensive program. Consequently, the BOG initially focused on sampling that addressed the issue of bioaccumulation in sport fish and impacts on the fishing beneficial use. This approach was intended to provide the information that is the highest priority for the state government and the public. The present study represents a first step in evaluating the impacts of bioaccumulation on the aquatic life beneficial use.

B. Addressing Multiple Monitoring Objectives and Assessment Questions for Aquatic Life Beneficial Uses

The BOG has developed a set of monitoring objectives a statewide program evaluating the impacts of bioaccumulation on the aquatic life beneficial use. This framework is consistent with frameworks developed for other components of SWAMP, and is intended to guide the bioaccumulation monitoring program over the long-term. The four objectives can be summarized as 1) status; 2) trends; 3) sources and pathways; and 4) effectiveness of management actions.

Over the long-term, the primary emphasis of the statewide bioaccumulation monitoring program will be on evaluating status and trends. Bioaccumulation monitoring is a very effective and essential tool for evaluating status, and is most cost-effective tool for evaluating trends for many contaminants. Monitoring status and trends in bioaccumulation will provide some information useful for identifying sources and pathways and for evaluating the effectiveness of management actions at a broader geographic scale. However, other types of monitoring (i.e., water and sediment monitoring) and other programs (regional TMDL programs) are also needed for addressing sources and pathways and effectiveness of management actions.

In the near-term, the primary emphasis of the statewide bioaccumulation monitoring program is on evaluating Objective 1 (status). The reasons for this are:

1. systematic statewide assessment of status has never been performed and is urgently needed;
2. we are starting a new program and establishing a foundation for future assessments of trends;
3. past monitoring has established very few time series that are useful in trend analysis that this program could have built upon.

The study described in this sampling plan will generate information that supports assessment of status. This effort will be the first study of bioaccumulation in California wildlife with a statewide perspective. Information on methylmercury exposure and risk in wildlife will be obtained for a representative group of 24 lakes, providing 1) a direct measure of status in those lakes and 2) an assessment of the degree to which data on exposure in small fish and sport fish can be interpreted as an indication of exposure in wildlife.

C. Addressing Multiple Habitat Types

SWAMP has defined the following categories of water bodies:

- lakes and reservoirs;
- bays and estuaries;
- coastal waters;
- large rivers;
- wadeable streams; and
- wetlands.

Due to their vast number, high fishing pressure, and a relative lack of information on bioaccumulation (Davis et al. 2007), lakes and reservoirs were identified as the first priority for sport fish monitoring. Coastal waters, including bays and estuaries, were selected as the next priority, due to their importance for sport fishing and a relative lack of past monitoring. Rivers and streams were the last in the series of water body types to be covered with a statewide screening study. Wetlands were not covered due to the low fishing pressure in those habitats.

Following the sequence established for the fishing beneficial use, assessment of the impact of bioaccumulation on aquatic life beneficial uses is also beginning with a focus on lakes

and reservoirs. Methylmercury exposure and risk was identified as the greatest concern in this habitat type, and reproduction in piscivorous birds as the taxa and lifestage at greatest risk. The logistics of performing surveys of exposure and risk in wildlife, require much greater effort and time at each water body, and thus do not readily allow for statewide surveys of the same breadth as were performed for sport fish. However, a two-year study covering 24 lakes was considered to be feasible within the scope of available funding and staffing, and is expected to be sufficient to answer some critical general questions with regard to aquatic life beneficial uses. Including other contaminants or habitats is not feasible with existing funding at this time.

Bioaccumulation is likely having negative impacts on aquatic life beneficial uses in all of the habitat types identified by SWAMP, including wetlands, which are among the most important habitats for wildlife. Whether SWAMP will perform surveys in the other habitat types has not yet been determined. The results of this preliminary assessment of methylmercury impacts in lakes and reservoirs will be valuable in informing the decision on the priority of further assessments.

In summary, focusing on one habitat type (lakes and reservoirs), one objective (status), and one category of beneficial use (aquatic life) will allow us to provide reasonable coverage and provide an informative assessment of bioaccumulation in these habitats in a two-year study.

III. STUDY DESIGN

A. Management Questions Addressed by this Study

Management Question 1 (MQ1)
Does methylmercury pose significant risks to aquatic life in a representative sample of California lakes and reservoirs?

Management Question 2 (MQ2)
Can a biomagnification factor approach (for small fish) or correlation approach (for sport fish) be applied on a statewide basis to estimate risks to birds?

Management Question 3 (MQ3)
What are appropriate TMDL monitoring requirements to address methylmercury exposure in wildlife?

To answer these questions, over two consecutive field seasons in 2012 and 2013, we will sample birds and small fish simultaneously at 24 lakes throughout California during the breeding season when birds are particularly vulnerable to potential mercury-induced reproductive impairment. Specifically, the study will have four main components:

1) Sample grebes at 24 California lakes over 2 years to determine mercury levels in a species near the top of the food chain, and compare these data to known effects-thresholds for birds.

2) Simultaneously with grebe sampling, collect small fish (<100 mm) at these same 24 lakes over 2 years to determine if mercury concentrations are above current wildlife diet objectives.
3) Use these data in Objectives 1 and 2 to calculate a bird biomagnification factor, evaluate the biomagnification factor's usefulness for estimating wildlife exposure, and assess whether the biomagnification factor differs by lake type or geographic region.
4) Simultaneously with grebe and small fish sampling, collect sport fish at these same 24 lakes over 2 years to assess correlations of mercury concentrations in sport fish, small fish, and birds.

B. Methods

This project will be led by USGS for the wildlife component and Moss Landing Marine Labs for the small fish and sport fish components. Bird sampling will be conducted immediately before fish sampling, and then bird collection sampling locations will be communicated to fish sampling personnel for subsequent sampling by the fish team within two weeks of bird sampling. It is likely that southern California lakes will be sampled earlier in the summer, and northern California lakes will be sampled later in the summer as grebes nest earlier in southern California sites.

Grebes

We will use western and Clark's grebes as our index of mercury exposure to wildlife in California lakes. We will sample grebe blood (and eggs where possible) from 24 California lakes during April-October of 2012 and 2013. **Figure 1** shows the proposed primary and alternate lake sites which will be investigated further and the final 24 lakes will be chosen after scouting lakes in the field. We will sample up to 12 lakes each year and conduct the field research over a 2-year period in 2012 and 2013 so that we can travel to all 24 lakes and sample grebes and fish during a narrow time window.

Grebes will be captured using boats and a combination of dip nets, net guns, and gill nets. If necessary, we may use shotguns to lethally collect grebes at sites where capture proves too difficult and costly. Grebe eggs also will be sampled when possible, and we will collect 1 egg randomly from each nest (up to 30 nests per lake, but this will depend on colony size and typically only 10 eggs will be collected from most lake sites). For each grebe captured, we will measure wing, culmen, and tarsus lengths, and weight. Each grebe tissue sample will be marked with an individual tag ID. Whole blood will be transferred from the field on wet ice to the lab where it will be stored at -20°C until mercury analysis. For each grebe captured, we will record the latitude/longitude or UTM where it was captured.

After grebes are collected, grebe collection locations will immediately be transferred from USGS to MLML personnel for the capture of small fish and sport fish within 2 weeks of grebe collections.

Small Fish

Small fish (25-100 mm) will be sampled using traps, seines, and dip nets from areas near grebe collections. We will sample 10 individuals each from two different prey fish species from each lake. We will target the following prey fish at all 24 lakes: Mississippi silversides, young of year largemouth bass, young of year bluegill, threadfin shad, shiner, and young of year tui chubs. Efforts will be made to sample the same species across all lakes, and when not possible we will sample fish that overlap in trophic guild. We will keep extra species of fish in the correct size ranges, and then choose which fish to analyze for mercury after all fish are collected each year. Upon collection, each prey fish collected will be tagged with a unique ID. Fish collected will be linked to the latitude/longitude or UTM where it was collected. Several parameters will be measured in the field for each small fish, including total length (longest length from tip of tail fin to tip of nose/mouth), fork length (longest length from fork to tip of nose/mouth), standard length, and weight. The individual prey fish will be placed in uniquely labeled bags and frozen. Small fish will be analyzed as whole fish.

Sport Fish

Sport fish (10 individuals per lake) will also be sampled in these same locations as small fish and grebes were formerly sampled, targeting the same individual species among all 24 lakes to the extent possible. One species will be sampled per lake. Fish species are distributed unevenly across the State, with different assemblages in different regions (e.g., high Sierra Nevada, Sierra Nevada foothills, and Central Valley) and a variable distribution within each region (Moyle 2002). To cope with this, the sampling crew will have a prioritized menu of several potential target species (Table 1). If the primary targets are not available in sufficient numbers, secondary targets have been identified. Largemouth bass will be the primary target species where they are present. At higher elevation lakes, resident, self-sustaining trout species will be the primary targets. Other species will also be observed in the process of fish collection. This "bycatch" will not be collected, but the sampling crew will record estimates of the numbers of each species observed. This information may be useful if follow-up studies are needed at any of the sampled locations.

The sampling design includes analysis of mercury in individual sport fish samples. An analysis of covariance approach will be employed, in which the size:mercury relationship will be established for each location and an ANCOVA will be performed that will allow the evaluation of differences in slope among the locations and the comparison of mean concentrations and confidence intervals at a standard length, following the approach of Tremblay (1998). Experience applying this approach in the Central Valley indicates that 10 fish spanning a broad range in size are needed to provide robust regressions (Davis et al. 2003, Melwani et al. 2007).

Specific size ranges to be targeted for each species are listed in Table 2. The numbers and sizes indicated for these species will provide the size range needed to support ANCOVA. In addition, the size range for black bass takes the legal limit for these species (305 mm, or 12 inches) into account. The goal for black bass is to have a size distribution that encompasses the

standard length (350 mm) to be used in statistical comparisons. This length is near the center of the distribution of legal-sized fish encountered in past studies (Davis et al. 2003, Melwani et al. 2007).

Sport fish will be sampled using seines, gill nets, and electroshocking. Upon collection, each sport fish collected will be tagged with a unique ID. Fish collected will be linked to the latitude/longitude or UTM where it was collected. Several parameters will be measured in the field, including total length (longest length from tip of tail fin to tip of nose/mouth), fork length (longest length from fork to tip of nose/mouth), standard length, and weight. Whole fish will be wrapped in aluminum foil and frozen on dry ice for transportation to the laboratory, where they will be stored frozen at -20°C. Fish will be kept frozen wrapped in foil until the time of dissection. Consistent with past SWAMP sport fish monitoring, sport fish will have the skin dissected off, and only the fillet muscle tissue will be used for analysis.

Mercury Analysis

Methylmercury is the form of mercury that biomagnifies and poses risks to wildlife and humans. Methylmercury concentrations will be estimated through measurements of total mercury. Nearly all of the mercury present in fish and in bird blood and eggs is methylmercury, and analysis of tissue for total mercury provides a valid, cost-effective estimate of methylmercury concentration (Wiener et al. 2007).

We will determine mercury concentrations in avian tissues at the USGS Davis and Corvallis Environmental Mercury Labs, and in fish at the Moss Landing Marine Lab following EPA method 7473, "Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" using a Direct Mercury Analyzer. Specifically, using an integrated sequence of sample drying and combustion, coupled with amalgamation and atomic absorption spectroscopy, we will evaluate mercury concentrations in avian and fish tissues in relation to established reference standards. Samples, blanks, and standards will be prepared using clean techniques. ASTM Type II water and analytical grade chemicals will be used for all standard preparations. A continuing calibration verification (CCV) will be performed after every 10 samples. Initial and continuing calibration verification values must be within ±20% of the true value, or the previous 10 samples must be reanalyzed. Three blanks, a standard reference material (such as IAEA-407 or NRCC DORM-3), as well as a method duplicate and a matrix spike pair will be run with each set of samples.

Quality Assurance

This effort will adhere to quality assurance requirements established for the SWAMP. A QAPP specific to this effort is in preparation (Bonnema 2012).

Archiving

Grebe Tissues

For the majority of grebe blood samples, we will likely not have any remaining sample mass after we have analyzed blood samples for mercury and performed QAQC. However, any remaining grebe tissue will be stored in short-term archives. Samples in the short-term archive are stored at -20 °C and are intended for use in the identification of short-term time trends (i.e. < 5-10 years), the investigation of yet unidentified chemical contaminants (but note archival jars may not be appropriate for all other contaminant types, and are stored with respect to potential future mercury analysis), and addressing quality assurance issues that may arise during the routine analyses of samples. The short-term archives will be located in a freezer at USGS at either Davis, Dixon, or Corvallis Field Stations. These facilities are not equipped with a backup generator; however, in the event of power failure the facility contingency plans are to keep the freezer closed, providing maintenance of low temperatures for several days.

Fish Tissues

Fish samples will be stored in short-term archives. Samples in the short-term archive are stored at -20 °C and are intended for use in the identification of short-term time trends (i.e. < 5-10 years), the investigation of yet unidentified chemical contaminants, and addressing quality assurance issues that may arise during the routine analyses of samples. The short-term archives will be located in an off-site freezer facility rented by Moss Landing Marine Laboratory. The facility is not equipped with a backup generator; however, in the event of power failure the facility contingency plan is to keep the freezer closed, providing maintenance of low temperatures for several days.

Timeline

Field work for this project will be conducted in the summers (April-October) of 2012 and 2013. Laboratory sample processing and mercury analysis will be conducted in winter and spring of 2012/2013 and 2013/2014. Data analysis and report writing will occur in spring and summer 2014. A draft year one report after the first field season will be delivered in April 2013, with description of any changes for year 2 field sampling. A final report on year one will be delivered in ,March 2014. A final report on the two year study will be delivered in March 2015.

Figure 1. ***Sampling locations for grebes and fish at 24 lakes during 2 field seasons in 2012 and 2013.***

Xx list of likely sampling locations would be good
Xx there is no symbol explaining Ramer Lake on the legend

Proposed lakes for sampling mercury concentrations in western grebes and fish for calculating a biomagnification factor. Red-scale color palette sites are those lakes where grebes are known to have recently bred. Blue-scale color palette sites are those lakes where grebes are known to have bred historically. Green-scale color palette sites are those lakes where grebes occur in the summer but it is not known whether they breed. Darker colored sites indicate a long-term BOG site for sport fish trend monitoring. Stars indicate sites that have been previously sampled by BOG for sport fish. Circles indicate the 24 primary lakes selected, whereas squares indicate alternate lakes that will be used if grebes cannot be sampled at a primary lake after scouting. Other lakes not depicted on this map may also be used for grebe and fish sampling if necessary and further information is found. The relative size of the symbol indicates mercury concentrations in sport fish from BOG sampling during 2007-2011.

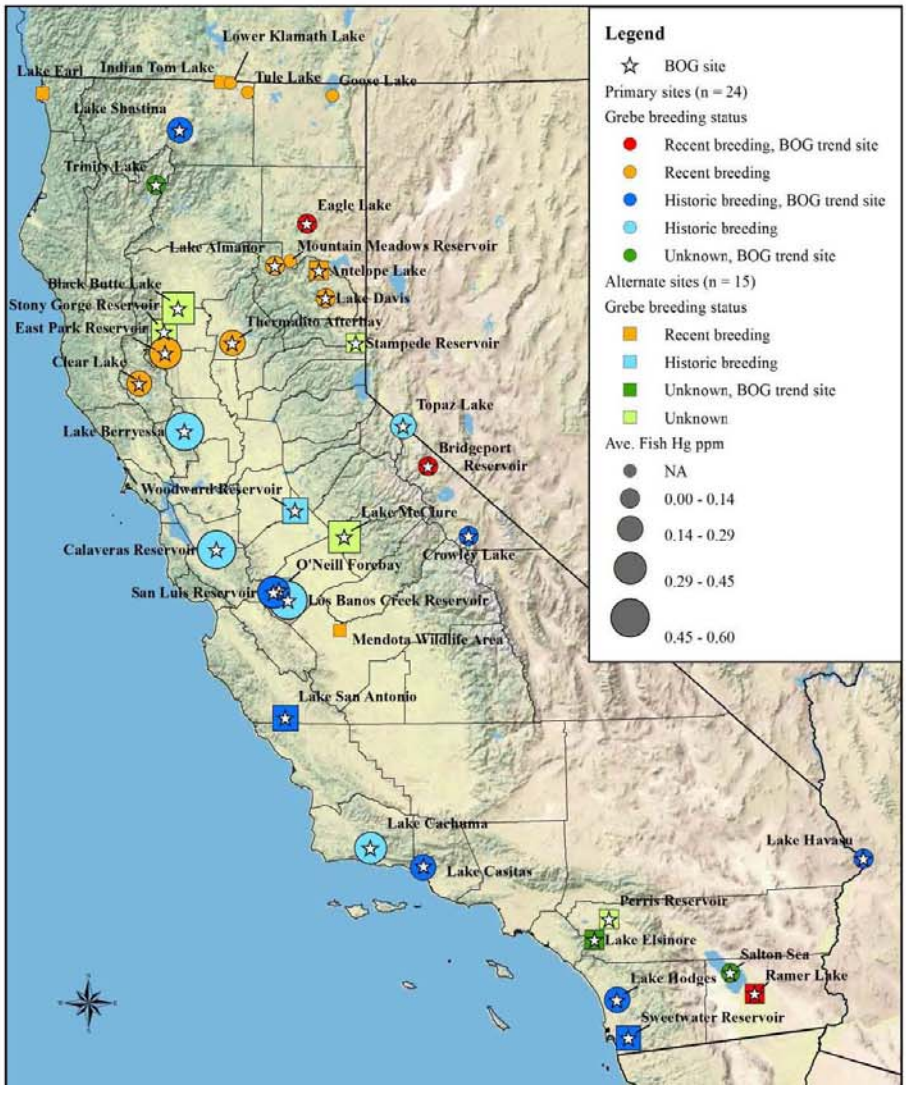

Table 1. Target sport fish species and their characteristics.

	Foraging Type		Trophic Level	Distribution			
Species	Water column	Bottom feeder		Low Elev a-tion	Foothi lls	High Elevati on	Priority for Collecti on
Largemouth bass	X		4	**X**	**X**		**1**
Smallmouth bass	X		4	x	**X**		2
Spotted bass	X		4	x	**X**		2
Sacramento pikeminnow	X		4	x	x		2
Rainbow trout	X		3	x	x	**X**	**2**
Brown trout	X		3/4		x	x	**1**
Eagle Lake trout	X		3			x	2

Trophic levels are the hierarchical strata of a food web characterized by organisms that are the same number of steps removed from the primary producers. The USEPA's 1997 Mercury Study Report to Congress used the following criteria to designate trophic levels based on an organism's feeding habits:

Trophic level 1: Phytoplankton.
Trophic level 2: Zooplankton and benthic invertebrates.
Trophic level 3: Organisms that consume zooplankton, benthic invertebrates, and TL2 organisms.
Trophic level 4: Organisms that consume trophic level 3 organisms.

X widely abundant X less widely abundant "1" primary target for collection "2" secondary target for collection

Table 2. Target sport fish species and size ranges.

	Numbers and Size Ranges (mm)
Black bass (largemouth, smallmouth, spotted)	2X(200-249), 2X(250-304), 5X(305-407), 2X(>407)
Sacramento pikeminnow	3X(200-300), 3X(300-400), 3X(400-500)
Rainbow trout	5X(300-400)
Brown trout	5X(300-400), and keep up to five fish > 400 if present
Eagle Lake trout	5X(300-400), and keep up to five fish > 400 if present