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Sampling and Analysis Plan for a Study of Lakes and Reservoirs with Low Concentrations of Contaminants in Sport Fish

The Bioaccumulation Oversight Group (BOG)

Surface Water Ambient Monitoring Program

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I. INTRODUCTION

This document presents a plan for sampling and analysis of sport fish in a one-year effort to identify California lakes and reservoirs with low concentrations of contaminants in sport fish. This work will be performed as part of 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 Wildlife, the California Office of Environmental Health Hazard Assessment, and the University of California. Interested parties, including members of other agencies, consultants, and other stakeholders are also welcome to participate.

The Roundtable has formed a subcommittee, the Bioaccumulation Oversight Group (BOG), which focuses on bioaccumulation monitoring. 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 Wildlife, the Office of Environmental Health Hazard Assessment, 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 (2007 and 2008), a two-year screening survey of the California coast (2009 and 2010), a one-year survey of California rivers and streams, and a two-year study of mercury accumulation in grebes on California lakes and reservoirs. Final reports on the sport fish surveys are available (Davis et al. 2010; Davis et al. 2012; Davis et al. 2013; http://www.mywaterquality.ca.gov/monitoring_council/bioaccumulation_oversight_group/#mpr).

II. GENERAL ASPECTS OF THE SWAMP BIOACCUMULATION MONITORING PROGRAM

A. Addressing Multiple Monitoring Objectives and Assessment Questions for the Fishing Beneficial Use

The BOG has developed a set of monitoring objectives and assessment questions for a statewide program evaluating the impacts of bioaccumulation (Table 1). This assessment 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 the 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.

This workplan describes an effort to refine the characterization of the status of lakes and reservoirs with regard to impairment due to bioaccumulation. SWAMP surveys to date have focused on identifying water bodies with elevated concentrations of bioaccumulative contaminants so that managers could develop strategies for addressing problem areas. In contrast, this survey will aim to provide information on another facet of status: identification of lakes and reservoirs with relatively low levels of contamination. This information will be useful to managers in their efforts to protect these relatively high quality ecosystems and to replicate these conditions in other water bodies. The information will also be valuable to the fishing public, drawing attention to water bodies where beneficial uses can be enjoyed with reduced exposure to bioaccumulative contaminants.

III. DESIGN OF THE CLEAN LAKES STUDY

A. Management Questions for this Study

Three management questions have been articulated to guide the design of this study: one primary question, and two secondary questions. The primary question is the main driver of the sampling design. The secondary questions will be addressed to the extent possible with the resources available for the study, after assuring that the primary question is appropriately addressed.

Primary Management Question (MQ1)

Which popular lakes in California have relatively low concentrations of contaminants in sport fish?

Answering this question will address the critical need of managers and the public to know which water bodies can be considered relatively clean. With this information, the fishing public can be directed to water bodies where they can enjoy the benefits of fishing and fish consumption and have reduced exposure to contaminants.

The data needed to answer this question are repeated observations of low concentrations of all contaminants of concern in the species with the greatest tendency to accumulate high concentrations. For methylmercury, top predators such as black bass tend to accumulate relatively high concentrations. High lipid, bottom-feeding species such as catfish, carp, and sucker have the greatest tendency to accumulate relatively high concentrations of organic contaminants of concern (PCBs and legacy pesticides). Measuring low concentrations of mercury and organics in both of these types of indicator species provides compelling evidence that a water body has a low overall degree of contamination. Given the variance associated with contaminant concentrations, the evidence becomes even more compelling if the low concentrations are observed on more than one occasion. This higher level of confidence obtained through repeated observation of low concentrations in both types of indicator species is desirable in order to be assured of providing reliable information to the public to guide their decisions on where to fish.

In some water bodies, it is not feasible to obtain both types of indicator species because they are not present in high enough abundance. Lakes at higher elevations with colder water where trout species predominate are a common example. For these lakes, repeated observation of the species most likely to have high concentrations is the best basis that can be obtained for characterizing a lake as one with relatively low concentrations.

Secondary Management Question (MQ2)

Why do some lakes have relatively low concentrations of methylmercury in sport fish?

A statewide control program for methylmercury is being developed by the State Water Resources Control Board: (http://www.waterboards.ca.gov/water_issues/programs/mercury/). Understanding the conditions associated with low concentrations of food web methylmercury is valuable to managers in their efforts to reduce concentrations in waters that are impaired. Simple and inexpensive ancillary measurements may shed valuable light on factors that drive methylmercury accumulation in lake food webs.

Secondary Management Question (MQ3)

Did the 2007-8 survey accurately characterize the status of lakes in which only rainbow trout were collected?

Many of the lakes found to have low concentrations of contaminants in the 2007-8 survey were lakes where only rainbow trout were collected. Rainbow trout generally had low concentrations of methylmercury, with a statewide average of 0.05 ppm. Concentrations of organics in trout were also generally low. To some degree, this was due to lower concentrations of contaminants in these lakes, but other factors also likely played a role. Trout generally occupy a lower trophic position and accumulate lower concentrations of methylmercury and other pollutants than black bass. However, a factor that probably contributed to lower observed concentrations in trout is that, in many lakes, recently planted hatchery fish are part of the catch. A previous study found that hatchery trout consistently had very low concentrations of methylmercury (rainbow trout from four hatcheries all had less than 0.023 ppm – Grenier et al. 2007).

With the level of effort that could be expended in the statewide survey of 2007-8 it is possible that other resident species with a potential to have higher concentrations were missed, such as resident populations of trout or small populations of warmwater predators like black bass or bottom feeders like sucker. With the greater effort planned for the present study, it is anticipated that information will be obtained that will allow for some evaluation of the accuracy of the 2007-8 assessment for lakes where only one species was obtained.

B. Overall Approach

The overall approach to be taken to answer these three questions is to perform a study to revisit a select subset of lakes that were identified as having relatively low concentrations of contaminants in the 2007-8 survey. The same basic design used in the 2007-8 survey will be repeated, as the goal is to obtain confirmation of the earlier results.

C. Coordination

The BOG is seeking to coordinate with other efforts to leverage the funds for this survey and achieve a more thorough evaluation of California lakes with relatively low levels of contamination.

The Colorado River Basin Regional Water Quality Control Board (Region 7) will be conducting a survey of contaminants in sport fish in Region 7 lakes this summer. Region 7 has a relatively large proportion of lakes that meet the criteria for having low concentrations, including 10 of the 14 lakes that will be sampled. Resources for this statewide effort will be pooled with Region 7 resources to allow a more thorough and definitive assessment of the lakes in this region. The data from the Region 7 effort will be processed and reported along with the data from the statewide effort.

The Los Regional Water Quality Control Board (Region 4) is interested in partnering to expand this study in their region. Options for including additional lakes and a more extensive set of ancillary parameters are being explored.

The San Diego Regional Water Quality Control Board (Region 9) is planning a study of cyanotoxins in reservoirs for this summer. One of the lakes to be sampled in that effort (Lake Henshaw) is also a candidate for inclusion in this study. If Lake Henshaw is selected for this study, the work will be coordinated with the cyanotoxin study.

Xx add DFW partnering in sampling Tahoe Keys

D. Selection of Lakes to Be Sampled

The pool of lakes considered for sampling consisted primarily of those included in the earlier SWAMP lakes 2007-2008 survey, with the addition of a few others sampled from 2002-2012 that had their data placed in the California Environmental Data Exchange Network (CEDEN), a centralized repository of data on California's water bodies, including streams, lakes, rivers, and the coastal ocean.

Selection of the lakes to sample in this study was not that straightforward because few lakes meet all of the standards that are under consideration for use California in assessing impairment for the purpose of 303(d) listing. Ideally, it would be good to avoid classifying a lake as having low concentrations and having that same lake appear on the 303(d) list of impaired waters.

303(d) listing determinations are based on the proportion of samples available that exceed the relevant threshold. When more than 10% of the samples exceed the threshold, the water body is classified as impaired.

The state is in the process of developing a tissue objective for mercury that is anticipated to be 0.2 ppm wet weight (all concentrations mentioned in this document are

presented on a wet weight basis). This threshold will be used for the next round of listing. Advisory Tissue Levels (ATLs) are another set of relevant thresholds developed by OEHHA (Table 2). ATLs factor in the health benefits of fish consumption, and are what OEHHA uses in the development of consumption advice for the public. Because ATLs consider higher rates of consumption (up to three servings per week compared to one serving per week which is the basis for the statewide objective), the lowest ATLs for mercury are lower than the statewide objective.

For organics, it is anticipated that the state will use Fish Contaminant Goals (FCGs) published by the California Office of Environmental Health Hazard Assessment (Table 2). FCGs were developed by OEHHA for potential use by other agencies as cleanup goals. For the organics, the ATLs are higher than the FCGs. For example, the FCG for PCBs of 3.6 ppb is much lower than the lowest ATL (21 ppb).

Through BOG discussion, the 0.2 ppm objective and listing threshold was selected as the criterion for classifying lakes as having relatively low concentrations of mercury. To be confident that a lake truly has fish mercury concentrations below 0.2 ppm, it is desirable to have measured concentrations in species such as black bass that are known to accumulate high concentrations.

For organics, the FCGs are one benchmark to use in assessing the degree of contamination. To be confident that a lake truly has organics concentrations below FCGs, it is desirable to have measured concentrations in species such as catfish, carp, or sucker that are known to accumulate high concentrations.

Only five lakes met these criteria for both mercury and organics, qualifying for Tier 1 of the list of candidate lakes for the study (Tables 3-5, Figure 1).

Given this outcome, slightly less stringent criteria were considered, primarily with consideration to communication of appropriate messages to the fishing public.

Since the FCGs for organics are much lower than the ATLs used to develop advisories, the use of the lowest ATLs for organics was considered. An additional six lakes had concentrations of mercury below the listing criterion and concentrations of organics below the lowest ATLs (Tier 2 in Table 5).

Another way in which the listing criteria are stringent is that they require 90% of the samples measured to be below the threshold. This leads to the fairly common occurrence that a lake has a mean mercury concentration below 0.2 ppm, but gets classified as impaired. The sampling approach employed in the SWAMP survey, which targets a wide range of sizes of black bass to provide a basis for ANCOVA that yields an accurate estimate of a size-standardized mean, has the unintended consequence of tending to trigger impairment listings. Seven lakes had bass with size-standardized mean concentrations below 0.2 ppm and with organics means below the lowest ATLs (Tier 3 in Table 5).

The last tier, Tier 4, is a more numerous category comprised of lakes where both indicator types were not sampled, but concentrations in the fish that were sampled were below the 303(d) listing criteria for mercury and organics.

Other criteria that were considered in selection of lakes for all tiers were having at least a moderate degree of fishing activity and a goal of having some lakes included from each of the regions.

E. Sampling Design At Each Lake

The general goal of this study is to replicate and expand upon the observations of low concentrations observed in some lakes in the 2007-2008 survey. Given this, the sampling design at each location will generally match that of the prior survey (BOG 2007).

1. Sport Fish

a. Sport Fish Species Targeted

Given the focus of the study on the fishing beneficial use, the species to be sampled, as in prior sampling, will be those that are commonly caught and consumed by anglers. Other factors considered include abundance, geographic distribution, and value as indicators for the contaminants of concern. The abundance and geographic distribution of species are factors that facilitate sample collection and assessment of spatial and temporal patterns in contamination. For example, largemouth bass is very common and widely distributed, and these factors contribute to making this an appropriate indicator species even though it is less popular for consumption than some other species.

The goal of this study is to identify lakes and reservoirs with relatively low concentrations of contaminants. Given this goal, the study is focusing on indicator species that tend to accumulate the highest concentrations of the contaminants of concern - if these species have low concentrations, then it is likely that the food web in general has a low degree of contamination. Different contaminants tend to reach their highest concentrations in different species. Methylmercury biomagnifies primarily through its accumulation in muscle tissue, so top predators such as largemouth bass tend to have the highest concentrations. In contrast, the organic contaminants of concern biomagnify, but primarily through accumulation in lipid. Concentrations of organics are therefore are also influenced by the lipid content of the species, with species that are higher in lipid having higher concentrations. Bottom-feeding species such as channel catfish and common carp tend to have the highest lipid concentrations in their muscle tissue, and therefore usually have the highest concentrations of organics.

Consequently, this study will target, where possible, two indicator species at each location: 1) a top predator (e.g., largemouth bass) as a mercury indicator, and 2) a high

lipid, bottom-feeding species (e.g., channel catfish, common carp) as an organics indicator.

Some lakes, particularly high elevation lakes, may only have one abundant high trophic level species (i.e., trout). In the 2007-2008 survey, this one species was often sampled as an indicator of all the target analytes. In contrast, in this study a greater effort (more hours spent fishing per lake) will be made to collect both mercury and organics indicator species.

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. To cope with this, the sampling crew will have a prioritized menu of several potential target species (Table 6). Primary target species will be given the highest priority. If primary targets are not available in sufficient numbers, secondary targets have been identified. Other species will also be observed in the process of electroshocking. 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 additional follow-up studies are needed at any of the sampled lakes.

b. Sport Fish Sampling Locations Within Each Lake

Lakes and reservoirs in California vary tremendously in size, from hundreds of small ponds less than 10 ha to Lake Tahoe at 50,000 ha. As lakes increase in size it becomes necessary to sample more than one location to obtain a representative characterization of the water body. As much as possible, the same sampling locations visited in 2007-2008 will be visited again for this survey.

In sport fish sampling using an electroshocking boat, it is frequently necessary to sample over a linear course of 0.5 – 1 miles to obtain an adequate number of fish. A sampling location in this study can therefore be thought of as a circle with a diameter of 1 mile. For small lakes less than 500 ha in size, one sampling location covers a significant fraction of the surface area of the lake. Therefore, for lakes less than 500 ha, one location will be sampled. For lakes of medium size (500 – 1000 ha), two locations will generally be sampled. For lakes in the large (1000 – 5000 ha) and very large categories (>5000 ha), two to four locations will be sampled. Since the goal of the study is to characterize human exposure, the existing locations have been established near centers of fishing activity.

Decisions regarding the number and placement of any new locations will be made in consultation with Regional Board staff with local knowledge of the lakes. Criteria to be considered in determining the placement of sampling locations will include the existence of discrete centers of fishing activity, known patterns of spatial variation in contamination or other factors influencing bioaccumulation, road or boat ramp access, and possibly other factors.

c. Sport Fish Size Ranges and Compositing for Each Species

Chemical analysis of trace organics is relatively expensive, and the management questions established for the 2007-2008 survey and this study can be addressed with good information on average concentrations. Therefore the compositing strategy employed in the 2007-2008 survey will again be employed for these chemicals (Figures 2 and 3).

Chemical analysis of mercury is much less expensive, and SWAMP partners would like to be able to answer additional questions related to trends over time and differences among lakes. Consequently, the sampling design for the mercury indicator species includes analysis of mercury in individual fish. For the mercury indicator species, an analysis of covariance approach will be employed where possible, in which the size:mercury relationship will be established for each location and an ANCOVA will be performed. The ANCOVA will allow evaluation of differences in slope among the locations and 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 to provide robust regressions, 10 fish spanning a broad range in size are needed (Davis et al. 2003, Melwani et al. 2007).

Specific size ranges to be targeted for each species are listed in Table 7. Black bass (including largemouth, smallmouth, and spotted bass), Sacramento pikeminnow (included in Group 1) and brown trout are the key mercury indicators. These species have a high trophic position and a strong size:mercury relationship. These species will be analyzed for mercury only (unless a bottom-feeding species is not present), and will be analyzed individually. 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). In past sampling, brown trout have been observed to accumulate high concentrations in some lakes, due to the existence in some cases of resident, self-sustaining populations and a switch to piscivory for larger fish. Brown trout will therefore have the same target as black bass - 10 fish analyzed as individuals with the data analyzed through ANCOVA.

In many high elevation lakes, trout species predominate, especially rainbow trout. Trout will be sampled again in this study, though a greater effort will be made to obtain resident predators and bottom-feeders in trout lakes. Past sampling of rainbow trout in the Bay-Delta watershed has found low concentrations and a weak size:mercury relationship. Therefore, for rainbow trout the ANCOVA approach will not be used. Mercury will be analyzed in individuals, but a specified size range will be targeted to control for size rather than a wide span to support a regression-based analysis. Trout species will also be analyzed as composites for organics. The size ranges established for trout are based on a combination of sizes prevalent in past sampling (Melwani et al. 2007) and the 75% rule recommended by USEPA (2000) for composite samples.

Catfish, carp, bullhead, and sucker are the primary targets for high lipid bottom-feeders. These species will be analyzed for organics and mercury. Organics are expected to be highest in these species based on past monitoring in the Toxic Substances Monitoring Program and other studies (Davis et al. 2007). Mercury is expected to be highest in the pelagic predators, but concentrations may also be above thresholds for concern in the bottom-feeders, so mercury will be analyzed in these samples as well. Samples for these species will be analyzed as composites. The size ranges established for trout are based on a combination of sizes prevalent in past sampling (Melwani et al. 2007) and the 75% rule recommended by USEPA (2000) for composite samples.

Secondary targets have been identified (Table 7) that will be collected if the primary targets are not available. These species would be processed for potential analysis of mercury and organics. The samples would be analyzed as composites. The size ranges established for trout are based on a combination of sizes prevalent in past sampling (Melwani et al. 2007) and the 75% rule recommended by USEPA (2000) for composite samples.

The sampling crew will be reporting their catch back to the BOG on a weekly basis to make sure that the appropriate samples are collected and to address any unanticipated complications.

d. Sport Fish Compositing and Archiving Strategies

Strategies for compositing and archiving will vary somewhat for lakes of different size. The overall strategy will be described first for small lakes, followed by a discussion of the differences for larger lakes.

Small Lakes

Figure 2 illustrates the approach to be taken for the predator and bottom-feeding species in small lakes (<500 ha). As described above, the predator species will be analyzed for mercury only and as individual fish. All samples of the predator species will be analyzed. Small lakes will be treated as one sampling location, so fish from anywhere in the lake will be counted toward meeting the targets for each size range listed in Table 7. For ANCOVA, one common regression line will be developed to describe the size:mercury relationship for the lake as a whole. Aliquots from these samples will also be archived after they are analyzed in case of any problems or other circumstances calling for reanalysis at a later time.

The bottom-feeding species will be analyzed as composites for organics and mercury, following the same scheme used in the 2007-2008 survey (Figure 2). These composite samples will be analyzed and processed in a stepwise fashion. One representative composite sample will be analyzed first. Another composite sample will also be collected and analyzed in the unanticipated event that the first composite sample has problematic concentrations. Aliquots from all composites will also be archived

whether they are analyzed or not in case of any problems or other circumstances calling for analysis or reanalysis at a later time.

Larger Lakes

For lakes in the medium, large, and very large categories the basic approach will be similar, with a couple of modifications. Figure 3 illustrates the approach using a medium lake as the example. The first difference from the small lake approach is that sampling locations will be treated discretely. For the predator species, this means that 11 fish spanning a wide range of sizes will be targeted for each location to support the development of a size:mercury regression and an estimated mean concentration at standard length for each location. From these location means a lakewide mean will be calculated.

For the bottom-feeder species, discrete composites will be prepared for each location. These composites will be homogenized and archived. Aliquots of homogenate from each location composite will be pooled to form a lakewide composite. The lakewide composite will be analyzed first. If the lakewide composite concentrations of any of the organics are problematic, then all the discrete location composites can be analyzed if that is desired by the Regional Board responsible for that lake. Since the goal of this study is to identify relatively clean lakes, these additional composites will not be automatically analyzed as part of this study. Aliquots from all composites will also be archived, whether they are analyzed or not, in case of any problems or other circumstances calling for analysis or reanalysis at a later time.

2. Prey Fish xx to be added if agreed upon

3. Sediment xx to be added if agreed upon

4. Water xx to be added if agreed upon

E. Sample Processing and Analysis

1. Sport Fish

Fish will be collected in accordance with MPSL-102a, Section 7.4. Whenever possible an electro-fishing boat will be used, however it may be necessary to employ another method also described in Section 7.4.

The following adaptation to MPSL-102a, Section 7.4.5 (Appendix II) has been made for this study: At the dock, all fish collected will be placed on a measuring board covered with a clean plastic bag; fork and total length will be recorded. Weight will be recorded with a digital spring scale. Small fish will be returned to the lab whole for processing. Large fish will be partially dissected in the field using the following protocol: fish will be placed on a cutting board covered with a clean plastic bag where the head,

tail, and guts are removed using a clean (laboratory detergent, DI) cleaver. The cleaver and cutting board are re-cleaned between fish species, per site if multiple stations are sampled.

Upon collection, each fish collected will be tagged with a unique ID. Each fish collected will be linked to the latitude/longitude 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), and weight. Total length changes with freezing and thawing and is best noted in the field for greatest accuracy and because it is the measure used by fishers and wardens to determine whether a fish is legal size. Determining fork length at the same time simplifies matters, and might help with IDs later to sort out freezer mishaps. For large fish (e.g., salmon, carp, and steelhead which can be greater than 40 lb) there will be times when it is necessary to process fish in the field.

Whole fish or field-processed fish will be wrapped in aluminum foil and placed in a clean labeled zipper-style bag. All samples will be kept cold on ice until frozen in a freezer or on dry ice within 24 hours of collection. Samples will be stored at -20°C at the laboratory until dissection and homogenization. Homogenates will also be frozen until analysis is performed. Frozen tissue samples have a 12 month hold time from the date of collection (USEPA 2000); however, the scientific advisory board has stated that samples kept frozen, with minimal thaw-freeze cycles, for several years have no appreciable degradation of organic contaminants.

All fish will be dissected “skin off” according to MPSL-105, Section 7.1 (Appendix XX); Section 7.2.4 describes homogenization. This is inconsistent with the guidance of USEPA (2000) that recommends that fish with scales have the scales removed and be processed with skin on, and skin is only removed from scaleless fish (e.g., catfish). The BOG is aware of this difference, but favors skin removal. Skin removal has been repeatedly used in past California monitoring. All fish (with limited exceptions) in Toxic Substances Monitoring Program, the Coastal Fish Contamination Program, and the Fish Mercury Project have also been analyzed skin-off. Processing fish with the skin on is very tedious and results in lower precision because the skin is virtually impossible to homogenize thoroughly and achieving a homogenous sample is difficult. Also, skin-on preparation actually dilutes the measured concentration of mercury because there is less mercury in skin than in muscle tissue. The most ubiquitous contaminant in fish in California that leads to most of our advisories is mercury. By doing all preparation skin off we will be getting more homogeneous samples, better precision for all chemicals, and definitely a better measure of mercury concentrations, which are our largest concern. The analysis of axial fillets without skin was also advised by a bi-national workgroup concerning the monitoring and analysis of mercury in fish (Wiener et al. 2007).

Fish are filleted to expose the flesh. It is important to maintain the cleanliness of the tissue for analysis, therefore any flesh that has been in direct contact with the skin, with instruments in contact with skin, or with any potential contaminant surface such as foil or a plastic bag must be eliminated from the analyzed sample. The exposed edges of

the fillet should be trimmed by 1/4 inch with a clean scalpel or fillet knife to remove this contaminated tissue.

How a sample is dissected is greatly dependent on the types of analyses being conducted. Tissue from individual fish for mercury analysis only will be dissected from the fillet above the lateral line and analyzed immediately; no homogenization is required. When composites must be created, equal tissue weights are taken from 5 individual fish following the 75% size rule recommended by USEPA (2000) and homogenized with a Büchi B-400 mixer (MPSL-105, Section 7.2.4; Appendix XX) into a Location Composite with a target weight of 200g or greater. Tissue for composites will be taken from the fillet of each fish above the lateral line and from the belly to include areas of higher lipid content. A subsequent lakewide composite will be created from equal portions of each contributing Location Composite within each lake. Figure XX diagrams compositing strategies and target weights for predator and bottom species. Posthomogenization aliquots will be taken from the lakewide composite for mercury, selenium and organics analyses. Aliquots for mercury and selenium will be transferred to pre-cleaned 30ml polypropylene jars (MPSL-101, Section 7.1.5; Appendix XX). Organics aliquots will be transferred to 60ml borosilicate environmentally cleaned jars (example I-Chem class 200).

Mercury will be analyzed according to EPA 7473, “Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry” using a Direct Mercury Analyzer. 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.

Selenium will be digested according to EPA 3052M, “Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices”, modified, and analyzed according to EPA 200.8, “Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry”. 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. Two blanks, a standard reference material (2976 or NRCC DORM-3), as well as a method duplicate and a matrix spike pair will be run with each set of samples.

Organics analyses will be performed by the California Department of Fish and Game Water Pollution Control Lab in Rancho Cordova, CA. Organochlorine pesticides, PCBs, and PBDEs will be analyzed according to WPCL-GC-006 "Analysis of Extractable Synthetic Organic Compounds in Tissues and Sediment (including

Organochlorine Pesticides, Polychlorinated Biphenyls (PCBs) and PBDEs) by GC/ECD or Gas Chromatography with detection and quantitation by tandem mass spectrometry (MSMS)." Microcystins and microcystin metabolites will be analyzed according to WPCL-LC-065, "Determination of Microcystins and Microcystin Metabolites in Water and Tissue by Enhanced LC/MS/MS." 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 ±25% of the true value, or the previous 10 samples must be reanalyzed. One blank, a laboratory control spike (LCS), a CRM (if available), and a method duplicate and a matrix spike pair will be run with each set of samples.

2. Prey Fish xx to be added if agreed upon

3. Sediment xx to be added if agreed upon

4. Water xx to be added if agreed upon

F. Analytes

1. Sport Fish

Table 8 provides a summary of list of analytes for the study. Since the study is focused on assessing the impacts of bioaccumulation on the fishing beneficial use, the list is driven by concerns over human exposure. Contaminants were included if they were considered likely to provide information that is needed to answer the management questions for the study. A detailed list of analytes is provided in Table 9.

Additional discussion of the analytes is provided below.

Ancillary Parameters

Ancillary parameters to be measured in the lab include moisture and lipid (Table 9). Fish sex will also be determined for all samples as it comes at no extra cost and can be valuable in interpreting the data. Each fish collected will be linked to the latitude/longitude where it was collected.

Methylmercury

Methylmercury is the contaminant of greatest concern with respect to bioaccumulation on a statewide basis (Davis et al. 2010). Methylmercury will be measured as total mercury. Nearly all of the mercury present in edible fish muscle is methylmercury, and analysis of fish tissue for total mercury provides a valid, cost-effective estimate of methylmercury concentration (Wiener et al. 2007). Mercury will be

analyzed in all samples because it is possible that samples of each species will exceed thresholds of concern.

PCBs

PCBs are the contaminant of second greatest concern with respect to bioaccumulation on a statewide basis (Davis et al. 2010). PCBs will be analyzed using a congener specific method. A total of 55 congeners will be analyzed (Table 9). PCBs will be analyzed in one composite sample from each location. The species with the greatest expected concentrations (i.e., the organics indicator species where they are present) will be analyzed.

Legacy Pesticides

Legacy pesticides may exceed FCGs or the lowest ATL in some locations. Individual compounds recommended by USEPA (2000) will be analyzed (Table 9). Legacy pesticides will be analyzed in one composite sample from each location. The species with the greatest expected concentrations (i.e., the organics indicator species where they are present) will be analyzed.

Selenium

Past monitoring (Davis et al. 2010) indicates that selenium concentrations are generally not likely to be above thresholds in this study. Selenium analysis will be included for a select few water bodies where selenium may approach thresholds.

Microcystins

Sampling of Lake Henshaw will be conducted in coordination with Region 9, which is conducting a regional study of cyanotoxins. Cyanotoxins will be analyzed in the fish samples collected from Lake Henshaw.

Other Contaminants

Assessment thresholds are essential in this study, and are not available for the other contaminant categories.

2. Prey Fish xx to be added if agreed upon

3. Sediment xx to be added if agreed upon

4. Water xx to be added if agreed upon

F. Quality Assurance

This effort will adhere to quality assurance requirements established for the SWAMP. A QAPP specific to this effort is in preparation (Bonnema 2014).

G. Archiving

1. Sport 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. These samples are intended for the analysis of chemicals which are not expected to degrade in five years of storage at -20 °C. 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.

A number of small volume sub-samples, rather than one or two large volume samples, are prepared for archiving to avoid subjecting the samples to several freeze-thaw cycles. Each sub-sample contains a sufficient amount of material for most chemical analysis, and when needed, can be removed from the freezer and sent to the appropriate laboratory without the need to sub-sample.

For each sampling location, up to five 40-50 g aliquots of each composite analyzed for organics will be archived. This will provide an integrative, representative sample for each location that can be reanalyzed in later years to confirm earlier analyses, look for new chemicals of concern, provide material for application of new analytical methods, provide material for other ecological research, and other purposes. Samples for the short-term archive will be stored in either glass jars with Teflon-lined lids for non-fluorinated organic chemical and trace metal analysis or in polyethylene (PE) or polypropylene (PP) for fluorinated chemical (i.e. PFCs) or trace metals analysis. Four of the five archive jars will be glass with a Teflon lined lid (e.g., I-Chem 200 series glass jars). One separate aliquot will be kept in a polypropylene jar for potential analysis of perfluorinated compounds. These archived samples will be stored at -20°C.

For storage of samples in the short-term archive, glass and plastic containers are pre-cleaned using appropriate acids or solvents by MPSL-DFG or purchased pre-cleaned commercially (e.g. from Fisher or ESS Vial). For containers purchased ‘pre-cleaned’ from ESS Vial or other companies, a minimum of two per shipment will not be opened and kept in storage with the other samples in case container contamination issues arise.

H. Ancillary Data

In addition to the primary and secondary target species, other species will also be observed in the process of sample 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 in any of the sampled locations.

To answer MQ2 (Why do some lakes have relatively low concentrations of methylmercury in sport fish?), additional parameters will be measured. Xx needs to be worked out with Review Panel input. They need to be low-cost additions. Here's a draft list:

- Any data available from reservoir operators
- Sediment
    - Total mercury (if not available already): 3 grabs from the center of the lake
    - TOC
- Depth profile, including
    - Redox
    - Chlorophyll (may not be useful with matching grab samples)
    - Temperature
    - Conductivity
    - pH
    - DO
- Water
    - total, unfiltered mercury - single grab may not be that useful
    - chlorophyll a grab?
- Prey fish

I. Timing

Sampling will be conducted from May 2014 through October 2014. Seasonal variation in body condition and reproductive physiology, as well as limnological characteristics, are recognized as factors that could affect contaminant concentrations. To the extent practical, the seasonal timing of sampling will replicate the timing of the previous round of sampling.

J. Data Assessment

MQ1 will be assessed by comparing results from each location to the thresholds used for 303(d) listing determinations and to ATLs established by OEHHA (Klasing and Brodberg 2008) (Table 2).

MQ2 will be assessed in collaboration with the Water Board staff working on the Reservoir TMDL.

MQ3 will be assessed to the extent possible (depending on how many lakes are successfully sampled in a manner supporting this comparison) through a narrative summary of how the follow-up data compare to the previous results.

K. Products and Timeline

A report on this 2014 sampling will be drafted by June 2015. The final report, incorporating revisions in response to reviewer comments, will be completed and released in September 2015.

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