MERCURY IN FISH OF THE AMERICAN AND BEAR RIVER WATERSHED RESERVOIRS: TISSUE ANALYSIS AND STRATEGIES FOR MINIMIZING EXPOSURE AT LAKE CLEMENTINE AND ROLLINS RESERVOIR, CALIFORNIA

A Thesis

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by

Alexandria Kathleen Keeble-Toll

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MERCURY IN FISH OF THE AMERICAN AND BEAR RIVER

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LIST OF ACRONYMS

ARD: Acid Rock Drainage

ATLs: Advisory Tissue Levels

BrB: Brown Bullhead

BlkB: Black Bulhhead

BG: Bluegill

BOG: Bioaccumulation Oversight Group

BAL: Brooks Applied Laboratories

BT: Brown Trout

CABY: Cosumnes, American, Bear, Yuba Watershed Region

C: Crappie

CC: Channel Catfish

CEDEN: California Environmental Data Exchange Network

CFCP: Coastal Fish Contamination Program

CVRWQCB: Central Valley Regional Water Quality Control Board

CWA: Clean Water Act

DFW: Department of Fish & Wildlife (formerly Department of Fish & Game)

DOC: Department of Conservation

DWR: Department of Water Resources

EPA: United States Environmental Protection Agency

LMB: Large Mouth Bass

FGC: Fish and Game Commission

FMP: Fish Mercury Project

GS: Green Sunfish

GTLs: Guidance Tissue Levels

Hg: Elemental Mercury

IQR: Interquartile range

MeHg: Methylmercury

NFA: North Fork of the American River

OEHHA: California Office of Environmental Health Hazard Assessment

pg: picogram

RfD: Reference Dose

RT: Rainbow Trout

RS: Redear Sunfish

RWQCB: Regional Water Quality Control Board

SB: Spotted Bass

SLSFCS: Statewide Lakes Sport Fish Contamination Study

SMB: Small Mouth Bass

SVs: Screening Values

SWAMP: Surface Water Ambient Monitoring Program

SWRCB: California State Water Resources Control Board

TL: Total Length

TSF: The Sierra Fund

TSMP: Toxic Substances Monitoring Program

USGS: United States Geological Survey

WC: White Catfish

ABSTRACT

MERCURY IN FISH OF THE AMERICAN AND BEAR RIVER WATERSHED RESERVOIRS: TISSUE ANALYSIS AND STRATEGIES FOR MINIMIZING EXPOSURE AT LAKE CLEMENTINE AND ROLLINS RESERVOIR, CALIFORNIA

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The primary pathway of human exposure to mercury is the consumption of contaminated fish. Identification of patterns of fish tissue mercury levels are a key mechanism for understanding risk drivers and human exposure potential. Site-specific fish tissue data aid the Office of Environmental Health Hazard Assessment (OEHHA) in the development of consumption advisories. This research consists of Year 1 of a three year project to collect fish data from six reservoirs downstream of historic hydraulic mines in the Cosumnes, American, Bear, Yuba watershed region. Angler survey data informed sampling to ensure that commonly caught and consumed species were harvested from Lake Clementine and Rollins Reservoir and was used to evaluate posted fish consumption advice as a mechanism for protecting human health. A total of 72 samples from four species groups were collected in 2015. Geometric mean THg (ppm, wet weight) were highest for black bass at both Lake Clementine (n = 8, THg = 0.40) and Rollins Reservoir (n = 26, THg = 0.54), with a significant positive relationship between fish total length and THg at both water bodies (Lake Clementine: rho = 0.85, $p<0.05$; Rollins Reservoir: rho = 0.85, $p<0.01$). Sunfish data for both reservoirs were lower in THg than black bass (Rollins Reservoir: n = 24, THg= 0.16; Lake Clementine: n = 29; THg = 0.12), with a significant positive relationship between fish total length and THg at Lake Clementine (rho = 0.83, $p<0.01$) but not Rollins Reservoir. These data allow OEHHA to develop site-specific fish consumption advice at both locations and can be used as baseline data to determine if future actions to address inorganic mercury (Hg) sources at legacy gold mines results in reduced human exposure risk at downstream water bodies.

CHAPTER I

INTRODUCTION

Mercury is a contaminant prevalent in aquatic ecosystems across the United States (Herger and Edmond, 2012; Wentz et al., 2014). In a methylated form it has the capacity to enter the food chain, both biomagnifying and bioaccumulating as it moves up through the trophic levels of a fishery (May et al., 2000; Davis et al., 2007; Wiener and Suchanek, 2008; SWRCB, 2010; Wentz et al., 2014). In the State of California, watersheds in regions where historic mercury or gold mining activity occurred are especially impacted by mercury, with the vast majority listed as impaired for mercury under Section 303(d) of the Clean Water Act (Central Valley Regional Water Quality Control Board (CVRWQCB), 1998; State Water Resources Control Board (SWRCB), 2010). Data indicate that fish in mining impacted regions can have very high levels of mercury (>1 ppm) in their tissue, potentially posing a threat to public health (May et al., 2000). Consumption of contaminated fish is the main route of human exposure to mercury (May et al. 2000; Wiener and Suchanek, 2008; Lepak et al., 2009; Carrasco et al., 2011; Engelberth et al., 2013). A strategy used by public health agencies to limit human exposure to mercury-contaminated fish is to provide consumption advice through state-issued advisories.

There are two types of advisories issued by the California Office of Environmental Health Hazard Assessment (OEHHA), site-specific advisories and general advisories. Site-specific fish consumption advisories are issued by OEHHA for water bodies with sufficient fish tissue data ($n \geq 9$ for a species group). General advice has been issued for lakes and reservoirs that lack sufficient data and are based on a large dataset for the entire state (Lim et al., 2013). Mean mercury concentrations in fish vary across California waterbodies. Thus, to develop health-protective general advice, OEHHA used the 90th percentile of fish tissue mercury levels, which has a higher value than the mean, to develop the general advice (Lim et al., 2013). Data that are site-specific may more accurately represent the conditions of individual water bodies because it is representative of local fish species populations and their mercury concentrations. For example, California lakes with high mercury levels in bass have been found primarily in Region 5, where significant historic mining took place (Lim et al., 2013). In addition, site-specific advice may also provide more useful information to anglers because site-specific data collection efforts often rely on angler surveys to help determine species targeted for collection (Gassel and Brodberg, 2005).

Background Literature

Sources of Mercury in Watersheds

The presence of elemental mercury (Hg) and methylmercury (MeHg) in waterways in the U.S. has been traced to a number of factors including atmospheric deposition (from burning of fossil fuels), natural geothermal activity, soil, industrial and domestic waste-water, and the mining of gold and other minerals (Wiener and Suchanek, 2008; Lepak et al., 2009; Shilling et al., 2010; Lim et al., 2013;). At the height of the gold rush, gold mining was the primary use of Hg in the United States (Wiener and Suchanek, 2008). Much of the mercury that was used for gold mining in California was lost to the environment (Churchill, 1999; Alpers et al., 2005a; Wentz et al., 2014). The rate of mercury loss during gold processing has been estimated to be 10 to 30 percent per season and as a result mine sites are often associated with highly contaminated sediments (Alpers et al., 2005). As much as 6,000 metric tons of mercury was lost to the California environment during legacy mining activities (Wentz et al., 2014, p. 33).

In contrast to the Eastern United States, where Hg is associated with coal-based energy generation, in California, past gold and mercury mining activities are "the main source of mercury in the aquatic environment" (Domagalski, 2001; Davis et al., 2007; Lim et al., 2013, p. 7). Within the state of California, numerous abandoned mine sites continue to release mercury and mercury contaminated sediment in the present day (California Department of Conservation (DOC), 2000; Wiener and Suchanek, 2008; Wentz et al., 2014). There are hundreds of mines, mine features (including tunnels, adits, tailing sites, and debris control dams), and sediment deposits in watersheds that have yet to be cleaned up (Davis et al., 2007, p. 45). Many of California lakes, rivers, and reservoirs are impacted by mercury and many water bodies with fish consumption advisories are in mining-impacted systems (Davis et al. 2008; Wiener and Suchanek, 2008; Lim et al., 2013). Research has found that there may be a positive correlation between mercury bioaccumulation in aquatic ecosystems and the intensity of hydraulic mining (Hunerlach et al., 1999).

Due to mercury contributions from mines, the SWRCB cites mine clean up projects including the removal of contaminated sediment from behind reservoir dams as an important and necessary land management action for addressing mercury contamination (Davis et al., 2007). USGS researchers have reached similar conclusions, noting, “The large amounts of mercury mined in ecosystems still contaminate fish decades after mining activity has ceased, and without costly remediation, will likely continue to contaminate fish into the future” (Wentz et al., 2014). Projects at mine sites that SWRCB identifies as successfully removing mercury-laden sediment and tailings, reducing total mercury loads downstream, include the Empire Mine near Grass Valley and the Polar Star Mine near Dutch Flat (Davis et al., 2007).

Transport of Mercury in Watersheds

The downstream transport of legacy mining contaminants impacts California water bodies from summit-to-sea (Davis et al., 2007; Wentz et al., 2014). In Northern California, contamination is “extremely persistent” and substantial loads of mercury are still moving downstream toward the Bay-Delta (Davis et al., 2007, p. 41). California’s first environmental law, the Anti-Debris Act (Sawyer Decision) of 1884, was a response to the impacts of hydraulic debris washing down from Sierra Nevada mines (Alpers et al., 2005a; DOC, 2000). Historical records indicate that, “as early as 1867, tailings from placer mines had accumulated to as much as 70-ft thick in the Bear River drainage and had created major problems with flooding of downstream cities and navigation of the Feather and Sacramento Rivers” (Hunerlach et al., 1999, p. 185). Estimates for the Bear River indicate that 254 million cubic yards of gravel and sediment were added due to hydraulic mine operations, and of this, approximately 139 million cubic yards of hydraulic tailings persist in the lower Bear River (Hunerlach et al., 1999, p. 185). Similar analysis of sediment transport in the North and Middle Forks of the American River found that historically, “approximately 213 million cubic yards of hydraulic gold mining sediment filled the previously bedrock-lined channel" of the North Fork (Tetra Tech, 2007, p. 39).

Over time the sediment stored in Sierra Nevada watersheds moves downstream, often becoming remobilized during major storm or flood events (Hunerlach et al., 1999; Davis et al., 2007). Mercury can be remobilized with the sediment. For example, USGS found total mercury concentrations in bed sediment of up to 26,000 ppm downstream of gold placer operations in the Sierra Nevada (Wentz et al., 2014, p. 50).

Low elevation reservoirs in mine-impacted watersheds can serve as checkpoints, slowing the transport of mercury in watersheds. At Camp Far West Reservoir on the lower Bear River the primary source of Hg is thought to be "transport of contaminated sediments from upstream reaches of the Bear River, especially during high-flow events" (Saiki et al. 2009, p. 2). In spite of reservoir "checkpoints," mercury impacts water bodies in the Central Valley and San Francisco Bay-Delta in addition to higher elevations in the watershed (Davis et al., 2007). Recently it has been recognized that historical mercury inputs "may be responsible for the generation of new methylmercury in downstream reservoirs and the Delta" (Wentz et al., 2014, p. 71).

Deposition of Mercury in Watersheds

Reservoirs play a role in the transport and fate of mercury in Sierra Nevada watersheds. As Saiki et al. (2009) note, "Judging from limited sampling of aquatic biota mostly above and below selected foothill reservoirs, the reservoirs seemingly serve as traps for both sediment-associated inorganic mercury and biologically available mercury" (p. 2). Reservoirs capture and retain sediment and mercury that is being transported downstream; however, particulate-bound mercury may still be transported over reservoir dams during storm events. Though the processes of methylmercury production in ecosystems are not completely understood, the first step is associated with bacteria in anoxic sedimentary environments, especially bacteria associated with the chemical reduction of $SO^2{}_4$- to S^2- (Domagalski, 2001; SWRCB, 2013). In anoxic environments mercury in sediment may be transformed by sulfate or iron reducing bacteria to the more toxic and bioavailable organic form, methylmercury (Lim et al., 2013). The oxygen-poor benthic environments of reservoirs are associated with conditions amenable for the methylation of elemental mercury (SWRCB, 2013). Retention of sediment and methylation potential of mercury have made California reservoirs a target for research and assessment efforts as well as regulatory measures.

The State Water Resources Control Board (SWRCB) and nine Regional Water Quality Control Boards (RWQCB), including the Central Valley Regional Water Quality Control Board (CVRWQCB, Region 5), are in the process of developing a statewide mercury control program for reservoirs (SWRCB, 2013). This control program includes the development of new Total Maximum Daily Loads (TMDL) to limit contaminated discharge. To inform this effort, fish were collected from approximately 350 of California's 1,000+ reservoirs (SWRCB, 2013). Data from more than 90 of these reservoirs were used in statistical analyses conducted by the SWRCB to assess the influence of almost 40 factors on methylmercury concentrations in predatory fish (SWRCB, 2013, p. 4). This analysis found that three factors together are most effective for explaining variability in fish methylmercury concentrations in California reservoirs (SWRCB, 2013, p. 4). These variables include:

1. Aqueous total mercury (reflects the overall magnitude of mercury sources to the reservoir and thus methylmercury potential).
2. Ratio of aqueous methylmercury/[chlorophyll-a] (the magnitude of methylmercury entering the food chain).
3. The magnitude of water level fluctuation (may act upon multiple pathways of mercury cycling). (SWRCB, 2013, p. 4)

Wentz et al. (2014) reached similar conclusions, identifying three key factors that determine the level of mercury contamination in fish nationwide:

1. The amount of inorganic mercury available to an ecosystem.
2. The conversion of inorganic mercury to methylmercury.
3. The bioaccumulation of methylmercury through the food web. (Wentz et al., 2014, p. 1)

Mercury present in reservoirs today has been dispersed and it is possible that fish in these watersheds will have elevated methylmercury levels for years to come (SWRCB, 2013; Wentz et al., 2014). Due to the contamination already present in reservoirs, stakeholders addressing mercury downstream of legacy sources, contend that source control at mine sites may not be as important as “controlling the processes of methylation and bioaccumulation” in reservoirs (SWRCB, 2013, p. 5).

Legacy mines still discharge contaminants and these sources of mercury continue to “be fluvially transported in dissolved and particulate phases,” and long-term management strategies may require addressing both source reduction and reservoir processes (Kuwabara et al, 2003, p. 10). In terms of reservoir processes, understanding whether “some fraction of this sediment-associated mercury can remobilize for transport to the overlying water and subsequently to downstream” will provide insight into how the contaminant should ultimately be managed (Kuwabara et al, 2003, p. 10).

Aquatic Food Webs

Both age and trophic position play an important role in the bioaccumulation of mercury in fish tissue (Davis et al., 2008). Generally, fish that are in high trophic positions have higher levels of mercury than fish at low trophic levels as a result of trophic biomagnification (Herger and Edmond, 2012; Wentz et al., 2014). The largest biomagnification of methylmercury in the aquatic food web occurs at the trophic step between water and algae (SWRCB, 2010; Wentz et al., 2014). USGS studies conducted nationwide during 2002-2009 estimated the increase to be a magnification of approximately 10,000 times (Wentz et al., 2014, p. 38). At each subsequent trophic level transfer (macroinvertebrates; small fish; predatory fish; humans) the biomagnification has been estimated to occur at a rate of 2-5 times (SWRCB, 2010). Increases in mercury from invertebrates to top predators have been estimated to be approximately 100 times (Wentz et al., 2014). The USGS has estimated that as a result of bioaccumulation methylmercury concentrations can increase "1 million times from water to top predator fish" (Wentz et al., 2014, p. 38).

Older high trophic level fish that have spent a lifetime consuming and thus bioaccumulating mercury typically have high levels of mercury as the "levels of mercury in fish depend on what they eat, how long they live and how high they are in the food chain (or their trophic level)" (Herger and Edmond, 2012, p. 4). As a result, smaller, younger fish typically have lower levels of bioaccumulative contaminants including mercury (Scherer et al., 2008). These factors help to explain why upper-trophic-level predators (largemouth bass, *Micropterus salmoides*; smallmouth bass, *M. dolomieui*; and spotted bass, *M. punctatus*), that are both long-lived and predatory, are associated with very high levels of mercury (>1 ppm). This relationship does not always exist, however, and may be impacted by slow growth rates, fish that are "flexible in their foraging," and fish that change their dietary preferences with stages in development (Davis et al., 2008, p. 73). For example, some change in fish tissue mercury level over the lifetime of fish is "due to ontogenetic shifts in diet, especially in predatory species that initially feed on small lower-trophic- level invertebrates as larvae and juveniles, but switch to large higher-trophic-level fish as sub- adults and adults" (Saiki et al., 2009, p. 10).

For purposes of clarity, this research utilizes the following general trophic categories for fish:

- **Upper-Trophic-Level Predators (Predators):** largemouth bass (*Micropterus salmoides)*, smallmouth bass (*M. dolomieui*), spotted bass (*M. punctatus*), and Sacramento pikeminnow (*Ptychocheilus grandis*) (Gassel and Brodberg, 2005).
- **Benthic Omnivores (Bottom Feeders):** channel catfish (*Ictalurus punctatus*), white catfish (*Ameiurus catus*), brown bullhead (*Ameiurus nebulosus*), and black bullhead (*Ameiurus melas*) (Gassel and Brodberg, 2005).
- **Intermediate-Trophic-Level Predators:** bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), and Redear sunfish (*Lepomis microlophus*) (Gassel and Brodberg, 2005).
- **Omnivores:** brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) (Wentz et al., 2014).

Based on these trophic categories, research indicates that the highest fish tissue mercury levels typically occur in upper-trophic-level predators (May et al., 2000; Davis et al., 2008; Shilling et al., 2010; Herger and Edmond, 2012, p. 4). The lowest fish tissue mercury levels typically occur in omnivores like rainbow and brown trout (May et al., 2000). Data collected from California lakes and reservoirs statewide supports this trend of mercury accumulation in trophic levels as Sierra Nevada reservoirs “tended to have the lowest fish methylmercury concentrations, likely because they are dominated by trout, which is lower on the food chain than black bass” (SWRCB, 2013, p. 1). The USGS found that trout in streams nationwide have low mercury concentrations likely because their diet consists of insects and other invertebrates (Wentz et al., 2014, p. 49)

Mercury and Human Health

Fish consumption represents the primary pathway of human exposure to mercury in most populations (May et al. 2000; Lepak et al., 2009; Carrasco et al., 2011; Engelberth et al., 2013; Wiener and Suchanek, 2008). This makes accurate and abundant fish tissue data a key mechanism for assessing exposure risk, in particular in locales where there is known mercury contamination. In the United States mercury contamination is the reason behind the vast majority of fish and wildlife consumption advisories (Wiener and Suchanek, 2008; Wentz et al., 2014). In 2006, the contaminant was responsible for 80%, or 3,080, of all fish consumption advisories posted in the U.S. (Wiener and Suchanek, 2008).

Mercury is a developmental neurotoxin with human health impacts that are numerous and well-documented (Adams and Denton, 2008; OEHHA, 2008; Wiener and Suchanek, 2008; Lepak et al., 2009; Lim et al., 2013; Wentz et al., 2014). Exposure to even low levels of mercury is linked to adverse outcomes that can include damage to the brain, nervous system, kidneys, immune system, and cardiovascular health (Stern and Korn, 2011; Engelberth et al., 2013). Methylmercury exposure is especially dangerous for pregnant women because the compound easily passes through the placenta and the blood-brain barrier (Adams and Denton, 2008). The effects of low-level mercury exposure most frequently cited are neurological impacts on fetuses during the third trimester (Wentz et al., 2014). Children whose nervous systems are still developing are also considered sensitive populations for mercury exposure (OEHHA, 2008; Wiener and Suchanek, 2008; Lepak et al., 2009; Lim et al., 2013). As such, concerns about methylmercury exposure tend to focus on the potential for neurotoxicity during fetal and early childhood development (Wiener and Suchanek, 2008; Lepak et al., 2009).

One public health strategy to reduce mercury exposure is to create fish consumption advisories. Fish consumption advisories delineate parameters for the safe consumption of fish based on species, demographic group, and the recommended maximum number of meals of mercury contaminated fish species that can safely be eaten per week or month. Advisories can be “an important management tool because adverse health consequences can be averted while avoiding potentially large clean-up costs” (Jakus et al., 1998, p. 1019). However, “advisories are considered voluntary recommendations regarding fish consumption and are not subject to regulation” (Scherer et al., 2008, p. 1604). Furthermore, some research suggests that even where advice is posted it may not have the intended impact because “many anglers and fish consumers are not knowledgeable about risk messages, advisory content rationale, or suspected health risks” (Beehler et al, 2003, p. 100).

Due to the fact that the populations with greatest mercury exposure risk are fetuses and children (under 18 years of age), the consumption guidelines are more restrictive for children and women of childbearing age. Data on children was used to provide the scientific basis for the current reference dose (RfD) for methylmercury that is used by the United States Environmental Protection Agency (USEPA) (Wentz et al., 2014). Other at-risk populations include Native Americans, anglers, and ethnic minorities. These groups "often have rates of fish consumption many times higher than that of the general United States population, making them more vulnerable to exposure from this pathway" (Judd et al., 2015, p. 2428). The USEPA recommends assuming a 99th percentile rate of consumption for these groups instead of the 90th percentile rate considered protective of the general population (Shilling et al., 2014). Recently it has been suggested that the inability of California Native American tribes to safely consume fish in historic quantities due to mercury contamination may constitute a violation of the Clean Water Act because these populations are being prevented from enjoying the full designated beneficial use-values of their local water bodies (Shilling et al., 2014). The SWRCB notes that, "the existence of fish consumption advisories issued by OEHHA is one important indicator of the impact of pollutants on the fishing beneficial use of California" (Davis et al., 2007, p. 27).

Within the State of California, OEHHA is the public health agency responsible for the development of fish consumption advisories. OEHHA develops site-specific fish consumption advisories by comparing mean mercury fish tissue concentrations (by species) to the "daily oral exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Gassel and Brodberg, 2005, p. 7). By comparing the daily oral exposure reference dose (RfD) to measured mean mercury concentrations (by fish species) it is possible to determine the number of meals that can be eaten within a specified time frame without exceeding the RfD (Gassel and Brodberg, 2005).

OEHHA has streamlined the development of advisories with the creation of Advisory Tissue Levels (ATLs), which communicate the "maximum numbers of recommended fish servings on a per week basis, that correspond to the chemical levels found in fish" (Lim et al. 2013, p. 24). In this context, serving size refers to one 8-ounce or 227 gram (uncooked or wet weight) serving being consumed by a person weighing 154 pounds (70 kg) (Gassel and Brodberg, 2005). The quantity of fish being consumed should be adjusted up or down based on actual body weight, and the appropriate set of ATLs, dependent on the developmental sensitivity of the population group to mercury exposure (Group 1: Women ages 18-45 and children ages 1 to 17 years; Group 2: women over age 45 years and men) should be consulted. The OEHHA consumption advice is established based on a per-week basis, with "no consumption" recommended when a fish species cannot be eaten at least once a week without deleterious impacts (Lim et al., 2013, p. 39).

Angler Catch and Consumption Patterns

Understanding angler catch and consumption patterns provides key information about the exposure risk associated with specific water bodies. This information can be used to develop appropriate fish consumption advice based on species that are caught and consumed in a given region. Furthermore, surveys can provide insight into angler understanding of risk and reveal important avenues for the dissemination of public health information (Shilling et al., 2010; The Sierra Fund, 2011; Judd et al., 2015). According to Judd et al. (2015), there are two basic survey types used in most fish consumption studies – food frequency questionnaires and 24-hour recall surveys. Food frequency questionnaires are useful for characterizing both daily variation at the individual level and seasonal variation at the population level (Judd et al., 2015). A third type of angler survey that is commonly used is a creel survey where anglers are interviewed in the field as they are fishing and asked to show their catch (Judd et al., 2015). Creel surveys gather information about what species are caught or harvested from a specific waterbody and include information about fishing and consumption patterns (Judd et al., 2015).

In 2009-10, The Sierra Fund collected surveys of 151 anglers at water bodies in the Yuba and Bear watersheds as part of the *Gold Country Angler Survey*. To promote consistency and facilitate the cross-regional comparison of results, the survey used was based on the Sacramento River Angler Survey, which has been administered widely in the Sacramento-San Joaquin Delta. The Sacramento River Angler Survey is a food frequency (FFQ) questionnaire developed by the University of California, UC Davis in collaboration with the California Department of Public Health. The researchers behind the development of this survey contend, "the vast majority of comparable studies using FFQs have reported accurate findings using this approach among a wide range of nationalities and ethnicities" (Shilling et al., 2010, p. 335). The *Gold Country Angler Survey* was administered by volunteers trained in the protocol developed by UC Davis and the Healthy Fish Coalition. The survey used focused on fishing location and frequency, fish species sought or caught, and fish consumption patterns (see Appendix A: Angler Survey Protocol and Survey Forms).

The known human health impacts associated with mercury and the knowledge that waterways in the Sierra's have been contaminated by historic gold mining operations provided the impetus for The Sierra Fund's angler survey research. The main goal of the *Gold Country Angler Survey* (n=151) was to assess mercury exposure based on rates of fish consumption in the Sierra Nevada. The study focused on 12 target reservoirs and rivers, all of which are listed as impaired for mercury per the Clean Water Act Section 303(d). The survey revealed that despite efforts by California's public health agencies to provide advisories, residents in the Sierra Nevada are catching and eating local fish that are high in mercury, and are largely unaware of the danger. Findings indicate that approximately 90% of those surveyed eat the fish that they catch, and of these, 73% reported that they also planned to share it with their family, 50% reported that children in their household had eaten locally caught fish in the last year, and 54% reported that women of childbearing age in their household had eaten locally caught fish in the last year (The Sierra Fund, 2011). The *Gold Country Angler Survey* also found that though 80% of those surveyed had seen some type of health-related warning about mercury in fish, only 2% had any accurate understanding that would allow them to protect their health. For this survey "accurate understanding" was defined as the ability to articulate information on all three components of fish consumption advisories – species, population group (women of childbearing age, children; women >45, men >18), and frequency of consumption.

The *Gold Country Angler Survey* findings substantiate that individuals who lack specific knowledge about the three key points of fish advisories (species, sensitive populations, consumption patterns) are fishing at Sierra Nevada region Clean Water Act 303(d) listed mercury impaired waterways and consuming their catch, as well as feeding the sport fish to their families. Using data on the mercury contamination levels in fish by species that were compiled by the Central Valley Regional Water Control Board (CVRWQCB), individual exposure levels were calculated for the anglers who completed the survey. This analysis showed that the majority of anglers fall below the safe level of methylmercury per OEHHA recommendations of 21 micrograms per gram per day for women over 45 and men (The Sierra Fund, 2011).

The fish data from CVRWQCB that were used to calculate exposure levels for anglers in the *Gold Country Angler Survey* were not specific to the waterways in the assessment, thus there is the potential that exposure levels are underestimated. Depending on species, the data used consisted of average mercury concentrations for fish from multiple water bodies in the Gold County, data from lower in the watershed, and data from the San Francisco Bay/Delta Region. Nonetheless, when CVRWQCB Gold Country fish mercury data were compared to data collected in a similar survey effort that took place in the Bay/Delta (see Shilling et al., 2010), the Gold Country averages were higher, with the exception of bluegill and largemouth bass.

Statement of the Problem

The ability of OEHHA to issue fish consumption advisories for mercury that are protective of human health is contingent upon the availability of adequate fish tissue data for species commonly caught and consumed by anglers and their families (Gassel and Brodberg, 2005). Data for site-specific fish consumption advisories are lacking for water bodies in mining-impacted regions where some species of fish may have high mercury levels as a result of historic mercury use in the watershed (Shilling, 2003; OEHHA, 2009; Lim et al., 2013). Additionally, strategies for conveying fish consumption advice to anglers are needed so that public health information about mercury in local fish reaches and is understood by anglers (Shilling, 2003).

Mercury contamination of soil and water is one of the most prevalent and long-lived impacts of legacy mining in the Sierra Nevada. Research suggests that this contaminant is regional problem due to the number of drainages where historic placer gold mining took place (Hunerlach et al., 1999). Counties in the Gold Country are riddled with abandoned mine sites, and historic mercury use was pervasive at many sites (California Department of Conservation, 2000; Alpers et al., 2005a; Wiener and Suchanek, 2008). Of 130 mines in California listed by the Department of Conservation (2000) as having potentially significant environmental hazards, 19 are in Nevada County, where Rollins Reservoir (Bear River watershed) is located and many more occur in Placer County where Lake Clementine (American River watershed) is located.

It is estimated that 26 million pounds of elemental mercury was imported to the Sierra for use in gold processing, and of this approximately 13 million pounds was lost to the environment (Alpers et al., 2005a; Churchill, 1999). A single typical sluice could result in an annual loss of mercury totaling several hundred pounds (Hunerlach et al., 1999). Historic mercury use in the Sierra Nevada is associated with contamination throughout California watersheds, from the Sierra headwaters to the San Francisco Bay/Delta (California Department of Conservation, 2000; Domagalski, 2001; Alpers et al., 2005a; Davis et al., 2007). Degradation of watershed health in the Sierra Nevada region due to mercury contamination from legacy mining operations is widespread and well documented (May et al., 2000; Alpers et al., 2005a; The Sierra Fund, 2011, 2014). The Bureau of Land Management examined high priority watersheds impacted by legacy mines on public lands and ranked the Bear River watershed fourth in terms of priority and the American River fifth in terms of priority out of 17 ranked California watersheds (Bureau of Land Management, n.d.).

Evidence of mercury in Sierra Nevada aquatic ecosystems is demonstrated in existing data. Research conducted by May et al. (2000) found that mercury levels in the tissue of a number of fish species harvested from Sierra Nevada waterways in proximity to abandoned mine sites routinely exceeded the screening value (SV) of 0.08 ppm delineated by OEHHA (Gassel and Brodberg, 2005). Mercury concentrations in excess of the screening values indicate that additional research to establish mean levels of mercury contamination is needed. Between 1978 and 2003, the Toxic Substances Monitoring Program (TSMP), as part of the State Water Resources Board (SWRB) Surface Water Ambient Monitoring Program (SWAMP), collected fish tissue samples that were also in excess of SVs. Finally, fish tissue samples collected during a more recent study, The Statewide Lakes Sport Fish Contamination Study (conducted by SWAMP in 2008) also in many cases exceeded the 0.08 ppm SV.

Many Sierra Nevada water bodies with known mercury contamination, that are on the Clean Water Act Section 303(d) list for impairment, lack the necessary fish tissue data for California public health agencies to establish consumption advice (OEHHA, 2009). A limited number of site-specific fish consumption advisories issued for bass in the Yuba and Bear watersheds (Combie Reservoir; Camp Far West Reservoir; Lake Englebright) lists it as a “do not eat” species for sensitive populations (Group 1: Women ages 18-45 and children ages 1 to 17 years) (http://oehha.ca.gov/fish/so_cal/). Other lakes and reservoirs in these two mining impacted Sierra Nevada watersheds do not have enough data collected for OEHHA to issue a site-specific advisory (OEHHA, 2009). Angler survey research indicates that these same lakes and reservoirs are popular local fishing locations frequented by anglers who eat their catch and feed it to members of their family (The Sierra Fund, 2011).

Purpose of the Study

The primary objective of this research project was to collect fish tissue data that could be furnished to OEHHA for the purpose of establishing site-specific fish consumption advice, if warranted, for two Sierra Nevada reservoirs that currently lack the necessary data for this determination to be made. OEHHA issues fish consumption advisories for the State of California and to ensure that the data collected were consistent with OEHHA's data requirements, all sampling and analysis protocols were reviewed by OEHHA. The secondary objective of this research project was to assess whether posting fish consumption advice is an effective method for improving angler knowledge on three critical components of local fish consumption: (1) species, (2) population group, and (3) frequency of consumption. This study provides information on mercury levels in local fish and evaluates the posting of fish consumption advisories as an information dissemination strategy to minimize exposure in the Sierra Nevada, California.

Research Questions

Seven research questions were developed for this study in order to quantify the (A) Mercury Exposure Risk at Target Water Bodies; (B) Impact of Age and Trophic Position on Fish Tissue Mercury Concentration; and (C) Strategies for Minimizing Exposure.

(A) Mercury Exposure Risk at Target Water Bodies

Research Question 1. What is the mean mercury concentration (ppm) in fish tissue (n $\geq$ 9) of OEHHA-defined edible size fish by individual water body and species?

Research Question 2. Do the mean fish tissue mercury concentrations (ppm) exceed the EPA threshold of 0.3 ppm by water body and by species?

(B) Impact of Size and Trophic Position on Fish Tissue Mercury Concentration

Research Question 3. Is there a positive relationship between Total Length (TL) and fish tissue mercury concentration (ppm)?

Research Question 4. Do predatory fish have higher fish tissue mercury concentrations (ppm) than non-predatory fish?

(C) Strategies for Minimizing Exposure

Research Question 5. Do most of the surveyed anglers (> 50%) report having heard or seen health warnings about eating fish?

Research Question 6. Do most of the surveyed anglers (> 50%) report seeing fish consumption advice posted at the water body?

Research Question 7. Are most of the surveyed anglers (> 50%) able to articulate information from the posted advice on (a) species group; (b) population group; and (c) frequency of consumption?

Fish Tissue Research in the Sierra Nevada

This research is part of two larger studies being led by The Sierra Fund of Nevada City, California that are funded by the Department of Water Resources (DWR) through the CABY Integrated Regional Watershed Management (IRWM) group. Fish tissue data collection occurred as part of a larger study of mercury concentrations in fish of the Cosumnes, America, Bear, Yuba (CABY) region with the purpose of providing data for site-specific fish consumption advisories for six lakes or reservoirs (Lake Clementine, Rollins Reservoir, Lake Combie, Camp Far West Reservoir, Scotts Flat Reservoir, and Lake Englebright) and three stream or river segments (Bear River, Yuba River, and Deer Creek) in the region. Angler surveys collected and analyzed as part of this research project are part of a larger survey of Sierra Nevada anglers taking place in partnership with the Sierra Native Alliance (of Auburn, California) for the purpose of increasing the overall sample size of the *Gold Country Angler Survey* (2011) to 500 surveys.

Two Sierra Nevada water bodies were selected from the larger target group for analysis in this research, Rollins Reservoir in the Bear River Watershed and Lake Clementine in the American River Watershed. Both water bodies are reservoirs constructed in areas with known historic mining activity (Hunerlach et al., 1999; May et. al., 2000; Alpers et al., 2005a; Tetra Tech, 2007). According to Lim et al's 2013 report, the two water bodies chosen for analysis do not have complete site-specific fish advisories due to a lack of data (Lim et al., 2013).

Site Information

The Bear River Watershed: Rollins Reservoir

Rollins Reservoir is a man-made water body that sits at an elevation of 2,100 feet in Nevada County, CA. It was built by Nevada Irrigation District in 1963 as part of the new public water system in Nevada County. The lake has a surface area of 900 acres, 26 miles of shoreline, and two arms (the Bear River Arm and the Greenhorn Creek Arm). It is one of the Region 5 reservoirs included as part of the Statewide Mercury Control Program for Reservoirs and new reservoir TMDL (SWRCB, 2013).

Existing research indicates that the Bear Watershed is an aquatic ecosystem heavily impacted by legacy mining operations (Slotton et al., 1997; Hunerlach et al. 1999; May et al., 2000; Kuwabara et al., 2003; Saiki et al., 2009). The Bear River is listed as impaired for mercury under Section 303(d) of the Clean Water Act for the 10 miles between Combie Lake and Camp Far West and for an additional 21 miles below Camp Far West Reservoir. Camp Far West Reservoir, Combie Lake, and Rollins Reservoir are also listed as impaired. According to the listing, mercury is present due to resource extraction and all resources extraction sources are abandoned mines (SWRCB, 2010). At Camp Far West Reservoir mercury contaminated inflows from the Bear River are likely responsible for concentrations of mercury in spotted bass, bluegill, and threadfin shad “high enough to warrant concern about toxic effects in fish and consumers of fish” (Saiki et al. 2009, p. 1).

Numerous mines in the Bear Watershed have been identified and assessed, including eight notable mine sites described below in more detail:

1. *Boston Mine:* Boston Mine is located along Greenhorn Creek and is owned by the Bureau of Land Management (BLM) and private land owner(s). It was BLM’s highest priority site in 2005. Remediation at Boston Mine involved the first successful pilot mercury removal/recovery/recycling project undertaken in California (Bureau of Land Management, 2005).

2. *Blue Lead Mine:* Blue Lead Mine is a hydraulic mine owned by United States Forest Service (USFS) and private land owner(s). Historic evidence indicates that 6 million cubic yards of gravel was mined at the site and large volumes of mercury were used (CDOC, 2000, 2007). The site was ranked 88 out of 133 mines inventoried for chemical hazards in California. This mine is currently being proposed for re-opening and permit review was underway in 2015.

3. *Buckeye Mine:* Buckeye Mine is a hydraulic mine owned by USFS and private landowners (CDOC, 2000). According to USGS, more than 6 million cubic yards of gravel were processed as part of Buckeye Hill (Alpers et al., 2005b).

4. *Poore Mine:* Poore Mine is a hydraulic and drift mine adjacent to a popular recreation area and owned by BLM and private land owner(s). The California Department of Conservation (2000) cites mercury as one of the primary problems at the site. Evaluation of Poore Mine is being administered by BLM and a preliminary Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) investigation has been conducted.

5. *Starr Mine:* Starr Mine is owned by BLM and private land owner(s). The California Department of Conservation (2007) identified the problem at the site as Mercury and categorized the site as a Low water quality priority and a Medium human exposure priority. In 2007, it was estimated that the future capital costs for the site would total $1 million (CDOC, 2007).

6. *Numitor Mine:* Numitor Mine is located very near the shores of Rollins Reservoir and has problems associated with acid rock drainage (ARD) and heavy metals

(CDOC, 2000). This mine was ranked #94 out of 133 sites for chemical hazards by the California Department of Conservation in 2000.

7. *Steephollow Mine:* Steephollow Mine is an 18-acre mine owned by USFS that consists primarily of placer tailings (CDOC, 2000). There is potential mercury contamination at the site (CDOC, 2000). This mine ultimately discharges into Rollins Reservoir and the Bear River watershed through the Steephollow drainage.

Sport fish species known to be residing in Rollins Reservoir include rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), spotted bass, bluegill (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*) (CEDEN, data accessed November 19, 2014). Despite the well-documented use of mercury in the vicinity of Rollins Reservoir, the only fish species with sufficient data and OEHHA-issued fish consumption advice at this water body is catfish. Existing fish tissue data for other species is discussed in Chapter II: Methods.

The American Watershed: Lake Clementine

The Lake Clementine impoundment sits at an elevation of 718 feet in Placer and El Dorado counties. It was created in 1939, when the California Debris Commission (CDC) completed the North Fork Dam on the North Fork of the American River. This dam, constructed following the Caminetti Act of 1893, was one of two structures built in California by the CDC as a debris dam to contain sediment and mine waste transported downstream from up-canyon (CDOC, 2000). Passed by Congress, the Caminetti Act allowed mines to continue to operate as long as mine operators built approved debris dams (Hunerlach et al., 1999). The second CDC dam was built in the Yuba River

Watershed, creating Englebright Reservoir, and both dams have subsequently been acquired by the United States Army Corp of Engineers (USACE). Lake Clementine reservoir is 3.5 miles long and characterized by narrow, steep canyon walls. It is shaped like a wide stretch of river and averages 300 yards across in most places. Lake Clementine, created by damming the North Fork of the American River (NFA), has a surface area of 280 acres and a capacity of 14,700 acre-feet. The North Fork of the American River was granted Wild and Scenic Status in 1978.

The American River Watershed is best known for its mining past because the event that prompted the California Gold Rush occurred in 1848 when James Marshall discovered gold on the South Fork of the American River at Coloma. The American River, along with the Bear, Yuba, and Feather Rivers is among the most mining-impacted watersheds in the state (Alpers et al., 2005a). It is estimated that two-thirds of all hydraulic mining in California took place in these watersheds, with most mining in the American River Watershed taking place in the North Fork drainage (Tetra Tech, 2007).

In 2010, the river was listed by the Central Valley Regional Water Quality Control Board (CVRWQCB) as impaired for mercury under the Clean Water Act section 303(d). This determination was based on fish tissue mercury levels in samples collected by the Toxic Substances Monitoring Program below the North Fork Dam. In addition to Lake Clementine, 71 miles of the North Fork of the American River spanning from the North Fork Dam to Folsom Lake, is listed as impaired for mercury due to resource extraction per Section 303(d) of the Clean Water Act.

The American River Watershed has not been as well studied for mine impacts as the Bear River watershed (by USGS, CDOC) and fewer mines have been identified and assessed. Known mines with either chemical or physical hazards in Placer County that may impact Lake Clementine include:

1. *La Trinidad Mine:* La Trinidad mine was located in 1870 and produced an estimated 25,000 ounces of gold (CDOC 2007). The mine is now owned by USFS and was identified in 2007 as having contamination issues due to arsenic (CDOC, 2007). As of 2007 waste-removal had been completed and a total of $258,000 in capital costs incurred with an estimated annual operations and maintenance cost of $10,000 per year.

2. *Gold Run Mine:* The California Department of Conservation (2000) identified the problem at the Gold Run mine site as Mercury with associated hazard, exposure, and risk rankings of 4, 5, 5 (out of 5, a score of 5 being associated with highest risk). The town of Gold Run is California Historic Landmark NO. 405, a location known for its hydraulic mines, which, according to the California Office of Historic Preservation, produced approximately $6.1 in gold between 1865-1878 (California Office of Historic Preservation 2016a).

3. *Iowa Hill:* In 1856 Iowa Hill had an estimated weekly production of $1,000, with approximately $20 million worth of gold produced by 1880 according to the California Office of Historic Preservation. The town of Iowa Hill was repeatedly destroyed by fire and rebuilt (in 1857, 1862, and 1922) and is California Historic Landmark No. 401 (California Office of Historic Preservation 2016a).

4. *Yankee Jim's*: Is California Historic Landmark NO. 398. Hydraulic mining techniques were first utilized in the town of Yankee Jim's in 1853 and by 1857 the town was considered to be the "most important in Placer County" (California Office of Historic Preservation 2016a).

5. *Jenny Lind:* Gold was reputedly discovered at the Jenny Lind Mine in 1852, and, according to the Office of Historic Preservation, the site produced an estimated $1 million in gold (California Office of Historic Preservation. 2016a).

Sport fish species reported to be residing in Lake Clementine include rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), bluegill (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*). Existing data from CEDEN indicates that Sacramento sucker (*Catostomus occidentalis*) exist just below Lake Clementine, in the North Fork of the American River. Fieldwork conducted as part of this research established the presence of green sunfish (*Lepomis cyanellus*), Redear sunfish (*Lepomis microlophus*), Sacramento pikeminnow (*Ptychocheilus grandis*) and common carp (Cyprinus carpio) species in Lake Clementine.

CHAPTER II

METHODS

Fish Tissue Data

Study Design

The Office of Environmental Health Hazard Assessment advocates a two-pronged approach, (1) screening and (2) comprehensive assessment, for research that involves fish tissue sampling with the purpose of developing consumption advisories (Gassel and Brodberg, 2005). This research followed the explicit study design preferred by OEHHA to ensure that the data collected would be accepted and used in the formation of fish consumption advice, if warranted, for Rollins Reservoir and Lake Clementine.

Initial Screening

The sampling process for this research at Rollins Reservoir and Lake Clementine began with a screening study to identify species popular with local anglers, and used existing data available through the California Environmental Data Exchange Network (CEDEN) to determine the associated fish tissue levels of mercury by species and water body. The screening study prioritized 1) those species likely to accumulate mercury, and 2) those species that might be lower in mercury contamination so that during the advisory formation, healthy fish alternatives can be cited. For this research, the objectives of an initial screening study were met via analysis of previously collected data available through CEDEN and published data collected by the United States Geological

Survey. CEDEN fish tissue data included sources from USGS, the Statewide Lakes Sport Fish Contamination Study (SLSFCS), and the Toxic Substances Monitoring Program (TSMP).

Comprehensive Assessment

A comprehensive assessment to characterize all popular sport fish was warranted for Rollins Reservoir and Lake Clementine based on the initial screening (Gassel and Brodberg, 2005). The goal of comprehensive assessment is the collection and analysis of sufficient samples (described below) of all fish species caught and consumed by anglers. This would allow for species inclusive fish consumption advice to be issued.

For the development of a sampling plan for comprehensive assessment at Rollins Reservoir and Lake Clementine existing data were analyzed to determine if samples collected as part of previous research efforts meet OEHHA criteria and could thus be included in the current study. The CEDEN and USGS data used in the initial screening to determine exceedance of SVs (see above) was examined to ensure that parameters required by OEHHA were included in the data. The procedure for this involved importing data for the two reservoirs targeted for research from the CEDEN database and examining fish tissue samples on a per-water body basis (database accessed November 19, 2014). Based on these findings it was possible to identify data gaps and develop individual sampling plans for the number and species of fish samples required (per water body). CEDEN data for Rollins Reservoir and Lake Clementine were examined and data gaps were identified prior to January 2015.

Fish collection began in May 2015, following submission and approval of the Department of Fish and Wildlife (DFW) scientific collecting permit (SCP). Fishing took place from a motorized boat with the assistance of experienced fisherman William Templin, Department of Water Resources (DWR). In addition, fishing was done from kayaks, the shoreline, and docks and piers with assistance from members of the Gold Country Fly Fishers Club. Fish sample handlers were trained in Ultra Clean Hands procedure for collection (USEPA, 1996). Fish were transferred to Brooks Applied Labs (BAL) for processing in a single shipment (see below for field and laboratory methods).

Water Body Selection

Water bodies were selected for inclusion in the larger CABY Fish Tissue study based on geographic location (Sierra Nevada region), popularity with anglers, 303(d) list status under the Clean Water Act for mercury impairment, and lack of and/or incomplete site-specific OEHHA fish advisories. Locations chosen for analysis were verified to be popular fishing destinations with demonstrable local angler activity (The Sierra Fund, 2011). Water bodies selected lacked the required number of fish samples to assess the necessity of edible-species-inclusive site-specific OEHHA fish consumption advisories (Klasing and Brodberg, 2003). See Table 1 for complete information on the water bodies selected for analysis (also see Figure 1).

TABLE 1. RESERVOIRS SELECTED FOR ANALYSIS

Site Identification	Watershed	County	Elevation (feet)	Surface Area (acres)	Shoreline (miles)	Number & Names of Arms
Rollins Reservoir	Bear River	Nevada & Placer	2100	900	26	2 Arms: Greenhorn Creek & Bear River
Lake Clementine	American River (N. Fork)	Placer	718	280	8	N/A

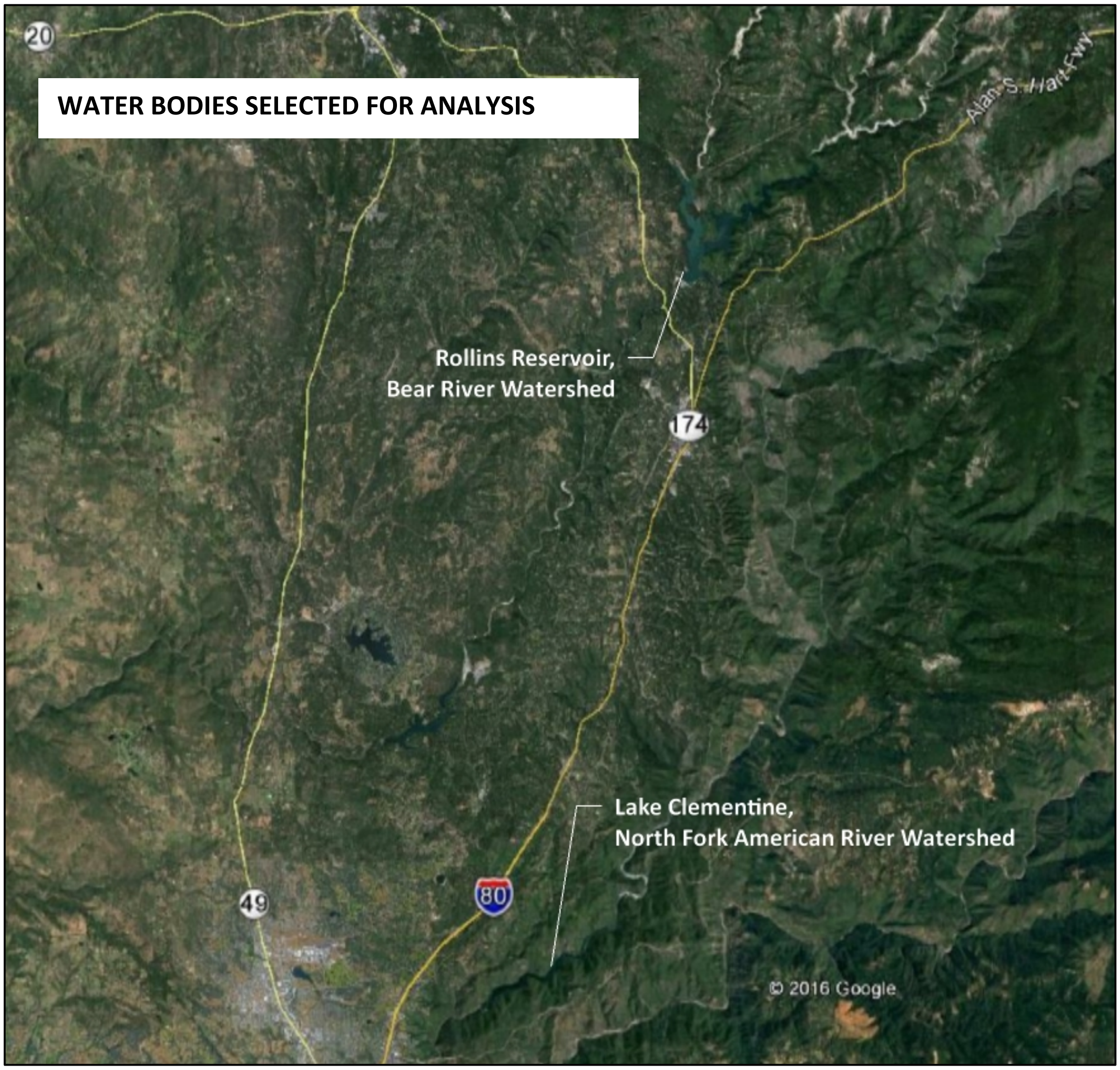

Figure 1. Map of water bodies selected for analysis. Rollins Reservoir (l) and Lake Clementine (r) parallel the Interstate 80 corridor in Nevada and Placer counties. Hydraulic mine debris deposits are visible upstream of both water bodies, evidenced by "white" areas along the Greenhorn Creek (far left) and Bear River watersheds above Rollins Reservoir and the less obvious "white" areas above Lake Clementine in the North Fork of the American (NFA) River watershed.

Species Selection

Fish sampled for this research match those commonly consumed by the anglers who fish each waterway, per EPA recommendations for sampling (U.S. EPA, 2000). Species harvest varies regionally and even locally and depends on a suite of factors including cultural background, socio-economic status, educational attainment level, ethnicity, income, age, and gender (Lepak et al., 2009; Shilling et al., 2010). Consequently, it is crucial to understand the preferences of local anglers. Prior to developing a sampling plan, OEHHA recommends identifying target species from information obtained from local fishers or employees of the Department of Fish and Wildlife (DFW, formerly the Department of Fish and Game), analysis of previously collected data on fish, or angler surveys such as creel surveys (Gassel and Brodberg, 2005). Detailed and comprehensive angler harvest data results in a more efficient use of a research budget by limiting the testing of fish species that are rarely consumed (Lepak et al., 2009).

For this research, angler survey data specific to the region of interest were used to aid in species selection. Responses from 132 anglers surveyed by The Sierra Fund in 2009 and 2010 (see Figure 2) who reported that they consume their catch were cross-referenced with information on fish species known to be present in local waterways to develop a list of potential species.

OEHHA requires fish samples from a minimum of three representative species in order to evaluate a water body to have an inclusive fish consumption advisory (Lim et al., 2013). For OEHHA's California Statewide Advisory for Lakes and Reservoirs Without Site-Specific Advice, data for certain species were combined for analysis as a "species group" based on their taxonomy and evidence suggesting comparable fish tissue mercury levels (Lim et al., 2013). Notable exceptions include Brown Trout and Rainbow Trout which, due to dietary preferences cannot be grouped

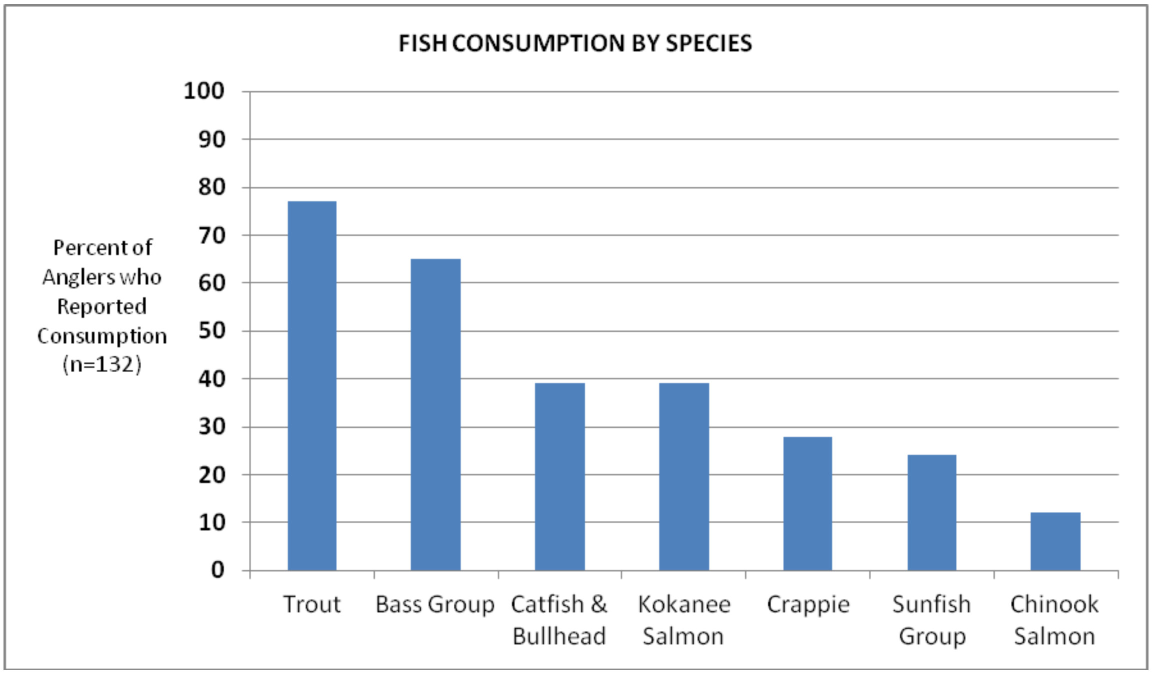

Figure 2. Fish Consumption by species, *Gold Country Angler Survey*. Trout: Rainbow & Brown (n=101), Bass Group: Largemouth Bass, Smallmouth Bass, and Stripped Bass (n=86), Catfish & Bullhead (n=51), Kokanee Salmon (n=51), Crappie (n=37), Sunfish Group (including Bluegill) (n=32), Chinook Salmon (n=16).

together, and Crappie, which is in the same family as Sunfish but have been found to be more contaminated in some studies (Lim et al., 2013). Species groupings utilized by OEHHA for general consumption advisories are as follows (Lim et al., 2013):

- Brown Trout
- Rainbow Trout
- Black Bass Group (largemouth, smallmouth, and spotted bass)
- Catfish and Bullhead Group (brown bullhead, black bullhead, white catfish, and channel catfish)
- Crappie

- Sunfish Group (small sunfish ("panfish"), bluegill, green sunfish, redear sunfish, and pumpkin seed)

OEHHA uses grouping guidelines for site-specific fish consumption advisories as well. Closely related species that are difficult to tell apart and often have similar levels of contamination are evaluated together as a group, including Black Bass (largemouth, smallmouth, and spotted bass), and Sunfish (bluegill, redear, and green sunfish) (OEHHA, 2009).

Based on OEHHA species groupings, the angler survey data (in Figure 2), and additional research to substantiate fish species present in water (including fishing reports, water body owner/operator websites, and social media including Yelp and Facebook) a list of target fish was developed for Rollins Reservoir and Lake Clementine. The list contains (1) popular fish caught and consumed by local anglers, (2) all species considered "edible" by local anglers, (3) at least three representative species, as required by OEHHA, (4) at least one species believed or known to have high levels of mercury contamination, and (5) at least one species believed or known to have low levels of mercury contamination (see Table 2).

TABLE 2. FISH SPECIES SELECTED FOR ANALYSIS

Fish Species or Group	Representative Species Type (per BOG, 2014)	Level of Mercury Contamination (believed or known)
Brown Trout	Predator	High (per BOG, 2014)
Rainbow Trout	Predator	Low (per BOG, 2014)
Bass Group	Predator	High (per BOG & Wentz et al., 2014)
Catfish & Bullhead Group	Bottom Feeder	Potentially Above Threshold (per BOG, 2014)
Crappie	Secondary Target	Higher than Sunfish Group (per OEHHA, 2009)
Sunfish Group	Secondary Target	Lower than Predator

Size

To accurately mimic angler catch patterns for size it is assumed that anglers are fishing legally with a valid California Sport Fishing license. Therefore, fish collected for tissue sampling must meet the legal requirements for minimum and/or maximum sizes per the Department of Fish and Wildlife (DFW). For species lacking an established legal size requirement, fish collected should be of "edible" size (Gassel and Brodberg, 2005). Edible size, as delineated by the SWRCB, is considered to be fish over 150 mm, however, this study prioritized OEHHA's preferred criteria for each targeted species (see Table 3).

Fish size was measured in the field as total length (TL), which OEHHA considers to be the standard measurement. TL refers to the length from the tip of the snout to the tip of the longer lobe of the caudal fin (Lim et al., 2013). For use of existing data that does not include a measurement for total length OEHHA recommends converting fork length (length from the tip of the snout to the end of the middle caudal fin rays) to total length using conversion factors that take into account "the degree of fork in the tail" (Lim et al., 2013, p. 11).

Size Classes

Within the range of legal/edible length, OEHHA recommends sampling a broad range of fish sizes or multiple size classes when adequate resources are available to fund analyses (Gassel and Brodberg, 2005, p. 4). By collecting same-species samples of different sizes it may be possible to determine if variation in contamination level based on fish age or size is a factor, which allows for the formation of more detailed advisories concerning not just species but recommended size fit for consumption. Fish size

TABLE 3. LEGAL AND/OR EDIBLE SIZE CRITERIA BY FISH SPECIES

Representative Species Type	Species Group	Species	Minimum Size (mm) (Per Gassel and Brodberg, 2005, Appendix I)
Predator	Brown Trout	Brown Trout (BT) (*Salmo trutta*)	BT: 200 (min)
Predator	Rainbow Trout	Rainbow Trout (RT) (*Oncorhynchus mykiss*)	RT: 200 (min)
Predator	Bass Group	Largemouth Bass (LMB) (*Micropterus salmoides*) Smallmouth Bass (SMB) (*Micropterus dolomieu*) Spotted Bass (SB) (*Micropterus punctulatus*)	LMB: 305 (min) SMB: 305 (min) SB: 305 (min)
Bottom Feeder	Catfish and Bullhead Group	Brown Bullhead (BrB) (*Ameiurus nebulosus*) Black Bullhead (BlkB) (*Ameiurus melas*) White Catfish (WC) (*Ameiurus catus*) Channel Catfish (CC) (*Ictalurus punctatus*)	BrB: 200 (min) BlkB: 170 (min) WC: 200 (min) CC: 200 (min)
Secondary Target/ Intermediate-Trophic- Level Predator	Black Crappie	Black Crappie (C) (*Pomoxis sp.*)	C: 150 (min)
Secondary Target/ Intermediate-Trophic- Level Predator	Sunfish Group	Bluegill (BG) (*Lepomis macrochirus*) Green Sunfish (GS) (*Lepomis cyanellus*) Redear Sunfish (RS) (*Lepomis microlophus*)	BG: 100 (min) GS: 100 (min) RS: 130 (min))

expressed as total length (mm) was used as proxy for age in this research instead of fish mass (g) because both OEHHA and DFW use fish length for minimum and maximum requirements.

Weight

Legal size for fish harvest is determined by fish length. For purposes of data analysis total weight of fish (in the field) and sample weight (in the lab) were recorded as wet weight measurements. When necessary dry weight measurements recorded in datasets downloaded from CEDEN were converted to wet weight using recorded percent sample moisture and the following conversion: Wet Weight (WW) = Dry Weight (DW) x [1 - Percent Sample Moisture/100] (Lusk et al., 2004). Wet weight measurements are preferred by OEHHA because this is the measurement type used most frequently by anglers when determining serving size. Dry weight is considered useful in contexts where the most common manner of consuming fish is dried or dehydrated, which does not apply to the Sierra Nevada region. Protocol for addressing the weight of fish samples processed as composites is described below.

Number

To establish a comprehensive site-specific fish consumption advisory, OEHHA requires at least nine samples of fish per fish species and samples from three or more representative species (Lim et al., 2013). This applies to lakes and reservoirs 2000 surface acres or less and small to moderate creek or river segments 25 miles or less. For larger lakes or those with multiple arms more samples are required – by arm or from multiple locations where fish are most accessible to fishermen (see Table 4 for recommended number of fish for harvest).

The sampling plans that were developed for this research project took into account the recommended harvest guidelines established by OEHHA and the existing data available from CEDEN and USGS that met OEHHA criteria for fish samples. Based

TABLE 4. NUMBER OF FISH RECOMMENDED FOR HARVEST BASED ON WATER BODY TYPE

Type of Water Body	Fish Species or Species Group	Maximum Number of Fish per Species or Species Group Proposed for Harvest
Lake or Reservoir, Zero Arms	Brown Trout, Rainbow Trout, Bass Group, Catfish & Bullhead Group, Crappie, Sunfish Group	9
Lake or Reservoir, Two Arms	Brown Trout, Rainbow Trout, Bass Group, Catfish & Bullhead Group, Crappie, Sunfish Group	9-18

Note: Data for table from Lim et al., 2013.

on existing data, individual sampling plans were developed for Rollins Reservoir and Lake Clementine.

Existing Data: Rollins Reservoir

Fish tissue data existed for Rollins Reservoir prior to this research (see Table 5). The Bear River watershed has been the subject of multiple research projects conducted by the United States Geological Survey (USGS). At Rollins Reservoir, USGS collected 28 samples from five species or species groups in 1999 (channel catfish, *Ictularus punctatus,* n=13; black basses, *Micropterus spp*., n= 7; sunfish: blue gill, *Lepomis* macrochirus, n=3; black crappie, *Poxomis nigromaculatus*, n=1; and brown trout, *Salmo trutta*, n=4). Samples were collected from both the Bear River Arm of Rollins Reservoir (18 samples) and from the Greenhorn Creek Arm of Rollins Reservoir (10 samples) Results from these data collection efforts revealed 15 of 28 samples to have mercury concentrations greater than 0.30 ppm (May et al., 2000).

Additional existing data for Rollins Reservoir were collected by The Toxic Substances Monitoring Program (TSMP) (collected in 1984-1985) and the SWAMP

TABLE 5. EXISTING FISH TISSUE DATA FOR ROLLINS RESERVOIR

Sampling Location	Collecting Agency	Fish Species	Number of Samples	Fish Tissue Mercury Concentration (ppm)
Bear River and Greenhorn Creek Arm, Rollins Reservoir	USGS	Channel Catfish	n=13; individual samples	Geometric Mean 0.35
Bear River and Greenhorn Creek Arm, Rollins Reservoir	USGS	Bluegill	n=3; 2 composite samples, 1 individual sample	Range 0.16-0.41
Greenhorn Creek Arm, Rollins Reservoir	USGS	Black Crappie	n=1; composite	0.31
Bear River and Greenhorn Creek Arm, Rollins Reservoir	USGS/TSMP	Largemouth Bass	n=8; 1 sample invalid because TL not recorded	Geometric Mean 0.33
Rollins Reservoir	SLSCS/TSMP	Small Mouth Bass	n=11; 1 sample invalid because TL not recorded	Geometric Mean 0.76
Greenhorn Creek Arm, Rollins Reservoir	USGS	Brown Trout	n=4	Each Sample <0.10

Statewide Lakes Sportfish Contamination Study (SLSC) 2008 (collected in 2007). All data referred to were available from the California Environmental Data Exchange Network (CEDEN), with additional information on USGS collected data available in May et al. (2000). Not all of the existing fish tissue data were suited for the purposes of this research project due to the data parameters specified by OEHHA for the development of fish consumption advice. For example, many of the bass samples were below the legal limit of 305 mm. These samples may be used by OEHHA to supplement data (in excess of 9 samples $\geq$ 305 mm) in the development of fish consumption advice and are also used in this research to help establish the relationship between TL and fish tissue THg concentration.

This research project did not sample any species of the Catfish and Bullhead Group at Rollins Reservoir because OEHHA has already issued site-specific fish consumption advice for this species group. The advisory for Rollins Reservoir is based on data collected by USGS in 1999 totaling 13 channel catfish samples. An additional two samples were collected by TSMP in 1984 and 1985. Total length of the fish sampled is not available for the TSMP samples. Of the 28 samples collected at Rollins Reservoir by USGS in 1999, channel catfish had the highest mercury concentrations (n=13; geometric mean= 0.35 ppm). Non-parametric statistical analysis (Spearman's rank correlation) "indicate non-significant (alpha=0.05) relations between mercury concentration and total length (p=0.94, rho= - 0.02) and between mercury concentration and total mass (p=0.80, rho=0.07)" for catfish at Rollins Reservoir (May et al., 2000, p. 12).

Prior to this research, there were eight existing largemouth bass samples for Rollins Reservoir. Seven samples were collected by USGS from the Bear River Arm and Greenhorn Creek Arm of Rollins Reservoir in September 1999 (9/14/1999-9/15/1999). TSMP collected one additional sample however, for this sample the TL of the fish sampled is not available, so the TSMP sample was not included in this analysis. The largemouth bass samples collected by USGS show a trend in increasing mercury concentration with increasing length and mass. Spearman's rank correlations for these samples indicate a significant (alpha=0.05) relationship between THg and TL (p=0.04, rho=0.79) and between THg and total mass (p=0.01, rho=0.86) (May et al., 2000, p. 12). For this analysis THg in the seven largemouth bass samples ranged from 0.20 to 0.45 ppm (geometric mean THg=0.33 ppm) (May et al., 2000).

Eleven Smallmouth Bass samples existed for Rollins Reservoir prior to this research. Ten samples were collected on 11/27/2007 as part of the Statewide Lakes Sportfish Contamination Study (SLSCS) 2008. One additional sample was collected by TSMP, however, the TL for the TSMP sample is not available and it was therefore not included in this analysis.

USGS collected a limited number of Sunfish Group samples (bluegill, n=3) from Rollins Reservoir in 1999 (from 9/14/99-9/15-99). These data for sunfish consists of seven bluegill analyzed as two composite samples of three fish and one individual sample. The composite samples of bluegill had mercury concentrations of 0.16 and 0.21 ppm and the individual sample had "an anomalously high concentration of 0.41 ppm" (May et al., 2000, p. 12). Three black crappie were also collected from the Greenhorn Creek Arm of Rollins Reservoir in 1999 and analyzed as a single composite sample (n=1; 0.31 ppm) (May et al., 2000).

Four Brown Trout samples were collected by USGS from the Bear River Arm of Rollins Reservoir in 1999. These samples were analyzed as individuals and each "had mercury concentrations less than 0.10 ppm" (May et al., 2000, p. 12).

Existing Data: Lake Clementine

Prior to this research project there were no existing fish tissue data for Lake Clementine. Existing data that were available consisted of 5 composite samples for two species for the North Fork of the American River just below the Lake Clementine impoundment (North Fork Dam) at the Highway 49 bridge (CEDEN data accessed November 19, 2014). These data were collected in September and October of 1981, 1982, and 1988 as part of the Toxic Substances Monitoring Program (TSMP) 1978-2003 project and were compiled on CEDEN as Surface Water Ambient Monitoring Program (SWAMP) Historic Bioaccumulation Data (see Table 6). The TSMP data were used to inform the decision to add the North Fork of the American River to the California 303(d) list and to the TMDL required list for Regional Board 5 (CVRWQCB) in 2010. For this research the data presented in Table 6 were used to establish the need for comprehensive fish tissue research at Lake Clementine. However, because the data in Table 6 were not collected from Lake Clementine they were not used in the development of the individual sampling plan for Lake Clementine or in any fish tissue data analysis for Lake Clementine.

TABLE 6. EXISTING FISH TISSUE DATA FOR NORTH FORK OF THE AMERICAN RIVER

Sampling Location	Collecting Agency	Fish Species	Number of Fish in Composite	Fish Tissue Mercury Concentration (ppm)
NFA, Highway 49 Bridge	SWAMP/TSMP	Sacramento Sucker	5	0.48
NFA, Highway 49 Bridge	SWAMP/TSMP	Sacramento Sucker	6	0.42
NFA, Highway 49 Bridge	SWAMP/TSMP	Small Mouth Bass	13	0.28
NFA, Highway 49 Bridge	SWAMP/TSMP	Small Mouth Bass	5	0.41
NFA, Highway 49 Bridge	SWAMP/TSMP	Small Mouth Bass	6	0.33

Based on the existing data available for Rollins Reservoir and Lake Clementine, individual sampling plans were developed for each water body. Table 7 presents the number of fish, by species, proposed for collection from Rollins Reservoir and Lake Clementine in 2015.

TABLE 7. NUMBER OF FISH, BY SPECIES AND WATER BODY, PROPOSED FOR COLLECTION

Water Body	Type of Water Body	Fish Harvest Brown Trout	Fish Harvest Rainbow Trout	Fish Harvest Bass Group	Fish Harvest Catfish & Bullhead Group	Fish Harvest Crappie	Fish Harvest Sunfish Group	Total Fish Harvest for Water Body
Rollins Reservoir	Lake or Reservoir 2 Arms	14	18	0	0	17	15	64
Lake Clementine	Lake or Reservoir 0 Arms	9	9	9	9	9	9	54
Total Fish Harvest By Species		23	27	9	9	26	24	108

Composite Samples

Individual samples were considered to be preferred indicators for mercury analysis rather than composite samples because they allowed for characterization of individual variation and correlations between size and chemical concentration. However if samples were processed as composites (due to resource availability or size of fish) the following criteria must apply (Gassel and Brodberg, 2005, pp. 3-4):

- Sample must be composed of fish of the same species.
- Sample must be composed of a minimum of 3 fish, 5 preferred, up to 20 small fish.
- A uniform amount of muscle tissue (aliquot) must be sampled from each fish.

- Sample must adhere to the 75% rule: the total length (TL) of the smallest fish in the composite should be at least 75% of the total length (TL) of the largest fish.

For this research, Brooks Applied Labs was instructed to prioritize treating samples as individuals during analysis. However, it was acknowledged that for some smaller species including sunfish, analyzing samples as composites might be necessary. Furthermore, previously collected data (obtained from CEDEN and USGS) containing composite samples was used when it was available, but these samples were excluded from analysis of the relationship between fish length and mercury concentration.

Timing

The Environmental Protection Agency (EPA) advocates sampling fish during the period when they are most commonly harvested by the public (Gassel and Brodberg, 2005). This allows angler catch patterns to be mimicked. Due to the fact that "rates of fish consumption vary seasonally, based primarily on fish availability," it is crucial to collect fish tissue samples in a manner that is representative of actual angler consumption habits (Shilling et al., 2010, p. 341). Additional seasonal considerations include the fact that contaminant levels can fluctuate annually with the lifecycle of fish, and that mercury levels in watersheds may shift in relation to storm events. For example, Ackerman et al. (2015) note that, "seasonal variation in prey fish Hg concentrations can be substantial" (p. 21). This research sought to minimize error associated with seasonal variation in fish and waterways by limiting data collection to a single summer season.

Other Considerations

It is useful to know if the water bodies from which fish samples are being harvested are regularly stocked with sport fish. Fish that do not spend the duration of their lives in a particular location may have different levels of accumulated contaminants relative to resident fish. For example, hatchery trout raised on hatchery feed typically have lower levels of mercury compared fish that forage exclusively from a waterbody food web (Lim et al., 2013). Furthermore, “management actions such as stocking fish and regulating harvest rates can alter the structure of lake food webs and thereby influence Hg concentrations of resident fish” (Lepak et al., 2009, p. 176). Some hatchery trout and all hatchery steelhead found in California water bodies can be identified by the presence of a healed adipose fin clip (adipose fin is absent). This method of characterizing fish as resident/non-resident in combination with consideration of stocking records is considered sufficient by OEHHA. The presence of hatchery fish in sampled water bodies was ascertained by confirming with the owner/operator that the water body is stocked or was stocked in the past. No hatchery fish were harvested during this research effort.

Field Sampling Methods

Procedures used for field sampling in this research were adapted from the SWAMP Bioaccumulation Oversight Group (BOG) 2014 Clean Lakes Study proposal (Sampling and Analysis Plan for a Study of Lakes and Reservoirs with Low Concentrations of Contaminants in Sport Fish). The Clean Lakes Study sampling and analysis protocol is based on the SWAMP Marine Pollution Studies Laboratory-102a, Section 7.4.5. Relevant aspects of this protocol are summarized below.

Documentation & Records

Documentation and record keeping in the field was done using Rite In The Rain professional field notebooks. Site information including date, time, location, weather, and notable circumstances were recorded. Each fish harvest location was documented for longitude and latitude using a standard GPS unit. A Canon digital camera or iPhone was used to photograph individual fish samples. Upon collection, each fish sample was labeled with a coded ID that corresponded to the sample's container and chain of custody documentation.

Fish Harvest

Fish were caught using traditional fishing poles, two per person, with bait, line, and hook that adhered to the current DFW regulations applicable to the given species and water body so as to accurately mimic catch methods utilized by local anglers. Fishing took place from a pier, boat, or shore in a location known or observed to be frequented by anglers. Past and current fishing reports from published news sources were used to corroborate locations and catch methods.

Ultra Clean Hands sampling methods were used for handling fish (USEPA, 1996). Hooked fish were removed from the hook by a trained researcher wearing non-powdered latex gloves sprayed with 409. Immediately following hook removal, individual fish were positively identified for species, labeled, and photographed. Fish were weighed using a 409-cleaned, zeroed, Berkley fish scale and then placed into a clean Ziploc style bag labeled with a sample ID matching that of the field record for the fish. GPS coordinates of catch location were documented following the safe deposit of the sample into the Ziploc bag.

The harvested fish were measured for TL by placing the bagged sample on a clean measuring board covered with a plastic bag. Bagged fish samples were then placed into an ice chest filled with wet ice. The researcher handling the fish removed and disposed of gloves and wiped hands with a hand-sanitizing wipe. The field notebook was checked for appropriate documentation of the harvested sample before fishing efforts continued.

Sample Handling & Custody Requirements

In the field, each fish sample, labeled with a coded ID and bagged in a labeled Ziploc-style bag, was placed in an ice chest filled with wet ice until a given data collection outing was complete. Fish samples were kept cold on ice and frozen within 24 hours of harvest.

Following each data collection effort, documentation for individual harvested fish samples was transcribed from the field notebook into the appropriate chain of custody documents provided by BAL and additional spreadsheets developed by The Sierra Fund. Necessary chain of custody documentation included Sample ID, date, time, sampler's initials, matrix type (fish tissue), number of containers (1 per fish harvested), field preservation (ice only), and analysis requirements (THg, EPA 1631E Appendix). Additional information documented in TSF spreadsheets included water body, water body location (e.g., "arm" if applicable) and GPS location.

Hold protocol for fish samples specified a maximum hold time of 180 days prior to being shipped to the laboratory chosen for analysis. All harvested samples were sent together, frozen and on ice, in a single overnight shipment to Brooks Applied Labs (BAL) (formerly Brooks Rand Laboratories) in Bothell, WA. Samples were accompanied by appropriate chain of custody documentation that was verified by Brooks Applied Laboratories upon receipt of shipment.

Laboratory Analysis

All total mercury analysis of fish tissue samples collected for this research was conducted by Brooks Applied Labs (EPA laboratory code WA00252) of Bothell, WA. BAL is a National Environmental Laboratory Accredited Program (NELAP) certified by the Florida Department of Public Health Services for mercury using the EPA method preferred for analysis by this research. BAL filleted and de-skinned the large fish samples. The fillet tissue of individual samples was homogenized using BAL SOP BR-0106 (Brooks Rand Laboratories, 2013). Any fish sample too small for fillet underwent whole body homogenization. Homogenized samples were tested individually for total mercury using EPA Method 1631E. This analysis assumes that THg in fish is synonymous with methylmercury (MeHg) because the majority of mercury found in fish tissue is in the methylated form (Lim et al., 2013). Previous research has found that "approximately 95-99% of mercury in fish muscle tissue occurs as methylmercury" (Saiki et al., 2009, p. 9). For fish tissue data collected from Camp Far West reservoir in the Bear Watershed "methylmercury averaged 78-93% of total mercury measured in whole fish" (Saiki et al., 2009, p. 9). OEHHA notes that THg analysis is less costly than MeHg analysis, thus most fish studies analyze for total mercury and assume it "to be 100% methylmercury for the purposes of risk assessment" (OEHHA, 2008, p. 18).

Quality Control and Instrument Requirements

As the certified lab contracted for analysis of the fish tissue samples collected for this research, BAL was responsible for sample storage, instrument and equipment testing, and laboratory inspection and maintenance. Methods consistent with BAL's accreditation as an Environmental Laboratory for mercury analysis were utilized at all times. Fish tissue samples were stored at -20 degrees Celsius at BAL. Homogenates were frozen until analysis is performed.

Fish Tissue Preparation

BAL was instructed by The Sierra Fund to remove the skin from individual fish samples during dissection, or to process the samples "skin-off." This method of preparation for laboratory analysis of mercury is preferred by OEHHA (Gassel and Brodberg, 2005). Skin removal is in violation of the guidelines established by the United States EPA, however, it is consistent with the majority of past fish tissue monitoring efforts conducted in the state of California (BOG, 2014). All fish (with limited exceptions) in the Toxic Substances Monitoring Program, the Coastal Fish Contamination Program, and the Fish Mercury Project have been analyzed "skin-off." Furthermore, analysis of axial fillets without skin has been advised by a bi- national workgroup concerned with the monitoring and analysis of mercury in fish (Wiener et al., 2007).

Processing fish with the skin-on results in lower precision because the skin is difficult to homogenize thoroughly, making it challenging to achieve a uniform sample. Additionally, skin-on preparation can actually dilute measured mercury concentration in individual samples because there is typically less mercury in fish skin than muscle tissue (Gassel and Brodberg, 2005). Requiring BAL to process all samples skin-off ideally resulted in more homogeneous samples and a more accurate measure of mercury concentrations, the primary concern of this research (Bioaccumulation Oversight Group, 2014).

In order to avoid potential contamination of the tissue samples, compromised flesh was removed from the fillet prior to homogenization. This included any flesh that had been in direct contact with the fish skin, flesh that had been in contact with instruments that had been in direct contact with the fish skin, and flesh that had been in direct contact with potentially contaminated surfaces such as foil or plastic bag. To ensure clean samples, BAL trimmed the exposed edges of each fillet by 1⁄4 inch with a clean scalpel or fillet knife (BOG, 2014). All fish filleting and cutting was done using stainless steel instruments on a glass or plastic surface.

Tissue Homogenization

The tissue of individual samples was homogenized in the laboratory using BAL SOP BR-0106. Homogenization was done using a Magic Bullet blender. Between every sample the instrument was cleaned using 409 and triple rinsed with deionized water. Homogenization blanks were collected on any new piece of homogenization equipment and whenever a new technician was homogenizing samples.

Mercury Analysis

The homogenized sample for each individual fish was analyzed for mercury using Appendix to EPA Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation. Samples were acid digested with heat and further oxidized with BrCl. Per EPA Method 1631E, samples were analyzed by stannous chloride ($SnCl_2$) reduction, followed by dual gold amalgamation, thermal desorption and atomic fluorescence spectroscopy (CVAFS) using a MERX-T Analyzer. The laboratory performed internal quality assurance-quality control (QA-QC) described in BAL SOP

BR-0106 and EPA Method 1631E. Quality was assured through calibration and testing of the oxidation, purging, and detection systems.

Method Detection Limits and Method Reporting Limits

The method detection limit (MDL) for Hg, Appendix to Method 1631E has been determined to be in the range of 0.24 to 0.48 ng/g when no interferences are present. The laboratory achieved an MDL that was less than or equal to 0.24 ng/g or one-third the regulatory compliance limit, whichever was greater. Brooks Applied Labs detection and reporting limits for Hg using Appendix to Method 1631E are MDL of 0.12 ng/g and method reporting limit (MRL) of 0.40 ng/g.

Tissue Reference Standards

The standard reference material used by BAL is TORT-3 (lobster hepatopancreas), certified by the National Resource Council Canada, which has a certified reference value (CRV) of 292.0 ppt of mercury (wet basis).

Matrix Spikes and Duplicates

Analysis of matrix spikes (MS) and matrix spike duplicates (MSD) were conducted to assess precision and recovery. Per EPA Method 1631E, performance of the Method on a given sample matrix was assessed by the laboratory that spiked, in duplicate, a minimum of 10% (one MS and MSD per 10 samples) from a given sampling site. Concentration of the spike was 1-5 times the background concentration. MS/MSD sets were prepared at the time of sample preparation and, if mercury concentrations were unknown, BAL spiked 1-5 times the minimum level (ML) of 0.5 ng/L or reference historical data for "typical" fish samples.

Percent recovery in each aliquot was calculated according to the following equation:

$$\%R = 100\ (A\text{-}B)/T$$

Where:

%R = % recovery

A = Measured concentration of analyte after spiking

B = Measured concentration of analyte before spiking

T = True concentration of the spike

Acceptable limits for %R for MS and MSD are 70-130%.

System and Method Blanks

Analysis of blanks was conducted to demonstrate acceptable levels of contamination. Four instrument blanks were run prior to calibration and with each analytical batch. Additionally calibration blanks were performed at intervals of every ten samples. Blanks were analyzed through analysis of reagent water using the same amount of reagents used to prepare the calibration standards (USEPA, 2002, p. 19). Individual blanks contained < 10 pg of Hg (the level of the low calibration standard). A minimum of three method blanks were run per analytical batch. Any sample requiring an increased amount of reagent was accompanied by at least one method blank containing an identical amount of reagent. Individual method blanks contained <0.4 ng or < 0.1x sample, whichever was greater.

Percent Moisture

Percent moisture for samples was determined in the laboratory. The preferred method was to determine tissue moisture content by sample weight loss upon freeze-drying and expressing this as a weight percent of the original wet sample. May et al. 2000 used this method for analysis of fish from the South Yuba River, Deer Creek, and Bear River watersheds, noting “Depending on sample size, either the whole sample or a representative aliquot was frozen, then dried under a vacuum until a constant weight was attained” (p. 8). An alternate to the preferred method for determining % moisture was used for this research because time constraints precluded the freeze-drying of fish samples. For this research it was requested that BAL report % moisture, as the inverse of % solids.

Statistical Analysis

Statistical analysis using Microsoft Excel (2004 for Mac, version 11.6.5) was performed on the data. All analyses were performed on wet weight mercury concentrations. Data were grouped by species within each individual water body for initial calculations of mean mercury concentration by species and the relationship between fish length and fish tissue mercury levels. Further analysis compared data by species or species group (e.g., brown trout, rainbow trout, bass group, catfish and bullhead group, crappie, and sunfish group) across water bodies to determine the variability in mercury tissue levels based on species trophic position. OEHHA Advisory Tissue Levels (ATLs) were compared to mean fish tissue mercury concentrations by species and water body to predict the maximum number of servings per week and by population group that would be recommended by OEHHA in the issuance of fish consumption advice.

Non-Parametric Statistics

The data sets associated with this research were small and not normally distributed. This is consistent with past fish tissue research in the watershed (May et al., 2000). Per May et al. (2000), nonparametric statistical methods were used in this research because the data sets for each waterbody and species were small and the data were not normally distributed.

Individual Species and Species Groups

Statistical analysis of fish samples for each water body grouped samples both by individual species (e.g. largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), and spotted bass (*Micropterus punctulatus*)) and by species group (e.g., Black Bass Group). OEHHA typically issues fish consumption advice for species groups, not individual species, and combing species into species groups increased the sample size for each analysis. The grouping of fish by species group for analysis was consistent with previous research in the watershed, but did have limitations. For example, Alpers et al. (2008) grouped species by species group and treated species group samples together (Black Bass: spotted largemouth, and smallmouth; Sunfish: bluegill, green sunfish, and redear sunfish; Trout: rainbow and brown trout). However, they warned that caution should be used in the interpretation of results when the species are grouped this way because of "potentially confounding relations among variables such as age, total length, and growth rate, species, and diet, which can vary from site to site and thereby affect mercury bioaccumulation" (Alpers et al., 2008, p. 22).

Descriptive Statistics

Fish tissue THg (wet weight) and fish TL within each sample water body were examined for each species to determine descriptive statistics (minimum, maximum, geometric mean, standard deviation). Consistent with the data analysis methods used for similar studies, geometric means were calculated for THg concentrations and fish TL (May et al., 2000).

Correlations

The relationship between fish TL and THg was evaluated using Spearman's Rank correlation. This is consistent with the data analysis methods used for similar studies (May et al., 2000).

Addressing Research Questions

(A) Mercury Exposure Risk at Target Water Bodies:

Question 1: What is the mean mercury concentration (ppm) in fish tissue ($n > 9$) of OEHHA-defined edible size fish by individual water body and species? This question was addressed by calculating geometric means for fish species and species groups by water body.

Question 2: Do the mean fish tissue mercury concentrations (ppm) exceed the EPA threshold of 0.3 ppm by water body and by species? This question was addressed by comparing geometric means for fish species and species groups by water body to the EPA threshold.

Comparison of geometric means for fish species and species groups by water body to OEHHA ATLs was conducted to predict the consumption guidelines likely to be issued by OEHHA toxicologists in the development of site-specific fish consumption advice. ATLs are derived from human health toxicity benchmarks and delineate the maximum number of fish servings recommended for consumption for each species on a per week basis (Lim et al., 2013). ATLs for mercury are divided into two categories based on potential sensitivity to developmental neurotoxicity:

Group 1: Women of child-bearing age (18-45) and children ages 1 to 17 years

Group 2: Women over age 45 years and men

For the purpose of this research the endpoint ATLs utilized for comparison were the 440 ppb (0.44 ppm) threshold for Group 1 and the 1,310 ppb (1.3 ppm) threshold for Group 2 (Lim et al., 2013). These thresholds refer to the no consumption ATLs for these groups. For fish tissue mercury levels that fall below these ATLs, appropriate consumption guidelines developed by OEHHA that dictate the number of servings per week considered safe for these population groups (see Table 8).

TABLE 8. OEHHA ADVISORY TISSUE LEVELS FOR MERCURY

Consumption Frequency Categories (number of servings per week)	Population Groups and Advisory Tissue Levels (ppm)	
	Group 1: Sensitive Population (Women ages 18 to 45 years and children ages 1 to 17 years)	Group 2: Women over age 45 years and men
None	>0.44	>1.31
1	>0.15-0.44	>0.44-1.31
2	>0.07-0.15	>0.22-0.44
3	>0.055-0.07	>0.16-0.22
4	>0.044-0.055	>0.13-0.16
5	>0.036-0.044	>0.109-0.13
6	>0.031-0.036	>0.094-0.109

Note: Data for table from Lim et al., 2013, p. 25.

To determine if the statewide advice that currently applies to Rollins Reservoir and Lake Clementine is protective of anglers, the results of mean fish THg were compared to 90th percentile value mean lake mercury concentrations of the Statewide Advisory Dataset (see Table 9).

TABLE 9. OEHHA 90TH PERCENTILE VALUE MEANS

Fish Species	Total Lakes	Total Samples	Mean Mercury Concentration Range (ppm)	90th Percentile Value (ppm)
Bass	142	1666	0.013-1.297	0.699
Sacramento pikeminnow	4	28	No Data	No Data
Catfish and Bullhead	50	145	0.005-0.588	0.346
Crappie	8	23	No Data	No Data
Sunfish	34	189	0.02-0.44	0.26
Brown Trout	23	43	0.035-0.84	0.397
Rainbow Trout	86	228	0.014-0.27	0.1

Note: Data for table from Lim et al., 2013, p. 12, 26.

(B) Impact of Size and Trophic Position on Fish Tissue Mercury Concentration

Question 3: Is there a positive relationship between total length (TL) and fish tissue mercury concentration (ppm)? Spearman's rank correlation was used to evaluate the correlation between mercury concentration and fish total length (TL).

Question 4: Do predatory fish have higher fish tissue mercury concentrations (ppm) than non-predatory fish? Geometric means of species occupying distinct trophic positions at each water body were compared and evaluated.

Angler Survey Data

Study Design

The secondary objective of this research was to examine the effectiveness of posting fish consumption advisories as a means of protecting human health. Angler awareness of health warnings and knowledge of the three critical components of fish advisories (species, population group, and frequency of consumption) was examined both before and after state-issued fish consumption advisories were posted at select water bodies. For this objective angler survey data were collected from target water bodies (Rollins Reservoir and Lake Clementine) before July 1, 2015 and after state-issued fish consumption advice was posted at both water bodies (advice posted July 11, 2016 and September 1, 2015, respectively).

Sampling Plan

A sampling plan was developed to collect 30 angler surveys per water body prior to the posting of state-issue fish consumption advisories and 30 angler surveys per water body after advice was posted (120 surveys total). The actual number of new surveys required to meet project goals was calculated to be 90 because angler survey data collected previously as part of The Sierra Fund's 2010 *Gold Country Angler Survey* was used to satisfy pre-posting sampling requirements at Rollins Reservoir (see Table 10).

TABLE 10. SAMPLING PLAN FOR ANGLER SURVEY DATA COLLECTION

Water Body	Existing Angler Surveys (Prior to April 29, 2015)	Pre-Advisory Posting Surveys Needed (April 29, 2015-July 1, 2015)	Post-Advisory Posting Surveys Needed (September 1, 2015 – November 15, 2015)	Total Target Sample Size (By November 15, 2015)
Rollins Reservoir	33	0	30	63
Lake Clementine	0	30	30	60

Human Subjects Considerations

Appropriate Human Subjects in Research steps were followed for the angler survey component of this research. The CSU Chico application for Human Subjects in Research was filed on April 15, 2015. On April 29, 2015, the Chair of the Campus Institutional Review Board determined the project exempt from full review by the Human Subjects Research Committee (HSRC). Per University policy Informed Consent and Post Survey Disclosures were included as part of the Human Subjects in Research application and a copy of the survey instrument (questionnaire) were furnished with the application. The chair of the HSRC approved the required Human Subjects in Research Post Data Collection (PDC) form in April 2016.

Survey Protocol

The Sacramento River Angler Survey developed by UC Davis and the California Department of Public Health was used to conduct pre-and post-advisory posting surveys at each of the two target water bodies. Surveys were administered according to protocol developed by the Healthy Fish Coalition. Pre-posting surveys were collected April 29, 2015-July 1, 2015. Between July 1, 2015 and September 1, 2015, 2015 OEHHA's *Statewide Health Advisory and Guidelines For Eating Fish From California's Lakes and Reservoirs Without Site-Specific Advice* was posted at the two water bodies. Post-advisory posting surveys were developed by adding an additional set of questions to the original survey to query anglers about whether they saw and understood posted fish consumption advice. Post-posting surveys were collected by Sierra Native Alliance September 6, 2015-February 27, 2016.

The field protocol utilized for survey collection in this research was consistent with CSU, Chico university requirements for Human Subjects in Research and with the Healthy Fish Coalition Protocol for conduction angler surveys. Field Materials consisted of copies of the *Gold Country Angler Survey* or copies of the *Gold Country Angler Survey Post-Posting*, copies of Informed Consent, copies of Post-Survey Disclosures, and copies of educational materials on mercury in fish to be provided upon request at the conclusion of a survey. Educational materials consisted of trifold brochures produced by The Sierra Fund titled *Fish, Mercury and You* and *Get the Mercury Out*.

At the target water bodies (Rollins Reservoir, Nevada/Placer County; Lake Clementine, Placer County) adults with fishing poles were approached and asked to participate in a face-to-face angler survey lasting approximately 20 minutes. Respondents were verified to be over 18 years of age. Oral informed consent was obtained and surveys were conducted per Healthy Fish Coalition Protocol. At survey completion Post-Survey Disclosures of research project objectives were provided and additional educational materials were offered to respondents.

Data Analysis

Angler responses to questions contained in the *Gold Country Angler Survey* and the *Gold Country Angler Survey Post-Posting* (Questions 15, 16, and 18) were analyzed in order to understand: (1) if anglers were familiar with health warnings and the issue of contaminated fish; (2) if anglers see and remember posted fish consumption advice at the water bodies; and (3) if anglers have a clear understanding of mercury exposure as result of the health warning posted at the target water body. For data analysis of angler survey responses, Microsoft Excel was used to create data spreadsheets and to calculate percentages.

Addressing Research Questions

(C) Strategies for Minimizing Exposure

Research Question 5: Do most anglers (> 50%) report having heard or seen health warnings about eating fish? This research question was addressed through analysis of responses to Question 15 of the *Gold Country Angler Survey* and the *Gold Country Angler Survey Post-Posting* (see Appendix A: Angler Survey Protocol and Survey Forms): "Have you ever heard or seen any health warnings about eating fish?" (Response categories: Yes; No; Don't Know/Not Sure; Refused)

Percentage of total number of respondents answering "Yes" to the question was calculated. Greater than 50% of "Yes" responses indicates "most" anglers are aware of the issue of contaminated fish.

Research Question 6: Do most anglers (> 50%) report seeing fish consumption advice posted at the water body? This research question was addressed through analysis of responses to Question 18 of the *Gold Country Angler Survey Post-Posting* (see Appendix A: Angler Survey Protocol and Survey Forms): "Have you ever heard or seen any health warnings about eating fish at this reservoir?" (Response categories: Yes; No; Don't Know/Not Sure; Refused)

Surveying using the Gold Country Angler Survey Post-Posting began September 6, 2015, following the posting of OEHHA's Statewide Health Advisory and Guidelines For Eating Fish From California's Lakes and Reservoirs Without Site-Specific Advice in July-August 2015. The percentage of total number of respondents answering “Yes” to Question 18 was calculated. Greater than 50% of “Yes” responses indicates “most” anglers do see posted fish consumption advice at the target water bodies.

Research Question 7: Are most anglers (> 50%) able to articulate information from the posted advice on (a) species group; (b) population group; and (c) frequency of consumption? This research question was addressed through analysis of responses to Question 16 of the *Gold Country Angler Survey* and the *Gold Country Angler Survey Post-Posting* (see Appendix A): “Do you remember what the warning said?” (Response categories: Yes; No; Don’t Know/Not Sure; Refused)

Survey protocol for an answer of “Yes” requires that the exact statement made by the respondent be transcribed. This allows for analysis of respondent understanding of the three critical components of fish consumption advisories (species, population group, and frequency of consumption). During data analysis, exact responses were awarded 1 point per mention of critical component of fish consumption advice; 1 point was also awarded for “some level of awareness” about the content of the health warning. A respondent statement that articulated the health warning by including mention of species, population group, and frequency of consumption received a maximum score of 3 points. A three point score on Question 16 indicates that an angler has a clear understanding of mercury exposure.

CHAPTER III

RESULTS

Initial Screening

The result of the Initial Screening for Rollins Reservoir found that existing data were sufficient to demonstrate that mercury levels in some fish at all trophic levels exceed the screening values (SVs) for mercury concentrations (excess of 0.08 ppm) established by OEHHA. There were no existing fish tissue data for Lake Clementine prior to this research. Data for below Lake Clementine were used in the Initial Screening. For these data mercury levels in two species of fish exceed the screening values (SVs) for mercury concentrations (excess of 0.08 ppm) established by OEHHA.

Fish Tissue Data

A total of 72 samples from four species or species groups of fish were collected from two reservoirs between May 2015 and August 2015 in order to fill gaps in existing data. Samples were held in the freezer at The Sierra Fund office for 130 days post-collection of the first sample on May 2, 2015. On September 8, 2015, all harvested samples were sent together frozen and on ice, in a single overnight shipment to Brooks Applied Labs (BAL) (formerly Brooks Rand Laboratories) in Bothell, WA. Samples were accompanied by chain of custody documentation that was verified by BAL upon receipt of shipment. Samples were confirmed to have arrived intact. BAL analyzed the samples for total mercury and sent The Sierra Fund results within 60 calendar days of receiving tissue samples.

Data Acceptance Criteria

The data were considered acceptable because the criteria for Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Total Mercury on the Automated MERX-T specified by BAL were satisfied. Matrix Spike (MS) and Matrix Spike Duplicates (MSD) were within the specified acceptable limits for recovery (70-130%), ranging from 77%-112%. Analysis results of the forth method blank in BAL batch 151507 was omitted from the batch because it was determined by Brooks Applied Laboratories to be a Grubb's outlier at 0.22 ng/g. In this instance BAL method-blank-corrected the sample results by the average of the three remaining method blank results. All other data were reported without qualification and per BAL all other associated quality control samples results met the acceptance criteria.

Statistical Analysis

For each target water body, fish tissue total mercury levels (THg, wet weight, ppm) and fish total length (TL, mm) were examined by species to determine descriptive statistics (minimum, maximum, geometric mean, standard deviation). Data were then analyzed in relationship to Research Questions 1-4. The results of mean fish tissue mercury levels (Question 1) were compared to the EPA threshold (Question 2) and to OEHHA ATLs to predict the applicable fish consumption advice warranted for the species. The results of mean fish tissue mercury levels were also compared to 90th Percentile Value mean lake mercury concentrations of the statewide advisory dataset to determine if site-specific fish tissue mercury concentrations exceed fish tissue mercury concentrations used for general fish consumption advice. Spearman's rank correlations were used to analyze the relationship between fish TL and fish tissue THg concentration (Question 3). The impact of trophic position on fish tissue THg concentration was also examined (Question 4).

Descriptive Statistics

Rollins Reservoir

Fish tissue data for largemouth bass (*Micropterus salmoides*; n=8), smallmouth bass (*Micropterus dolomieu*; n=11), channel catfish (*Ictalurus punctatus*; n=15), bluegill (*Lepomis macrochirus*; n=3), and brown trout (*Salmo trutta*; n=4) existed for Rollins Reservoir prior to this research. This research collected 31 additional samples of the following species, smallmouth bass (*Micropterus dolomieu*; n=1), spotted bass (*Micropterus punctulatus*; n=8), green sunfish (*Lepomis cyanellus*; n=21), and rainbow trout (*Oncorhynchus mykiss*; n=1).

CEDEN data that did not have information on TL were excluded from the analysis. Samples outside of the OEHHA-required size parameter (TL = $\geq$ 305 mm for black bass) were excluded from comparison to the EPA threshold and OEHHA ATLs but were used in the calculations of mean mercury concentration and were also used to establish the relationship between TL and fish tissue THg concentration. OEHHA had issued a site-specific fish consumption advisory for Catfish and Bullhead at Rollins Reservoir prior to this research. The data used for this advisory development were used in this research to examine the relationship between trophic position and fish tissue mercury concentration.

Black Bass Group at Rollins Reservoir

Prior to this research there were 8 existing largemouth bass samples for Rollins Reservoir. Samples were collected by USGS (September 1999; n=7) and TSMP (excluded from this analysis; n=1). No additional largemouth bass samples were collected as part of this research. Eleven smallmouth bass samples existed prior to this research. Samples were collected for the Statewide Lakes Sportfish Contamination Study (November 2007; n=10) and by TSMP (excluded from this analysis; n=1). One smallmouth bass sample was collected from Rollins Reservoir in July 2015 as part of this research project. Eight spotted bass samples were collected in July and August of 2015 as part of this research project. The earliest sampling date was July 18, 2015 and the last sample was collected on August 5, 2015. See Table 11 and Figure 3.

TABLE 11: BLACK BASS GROUP AT ROLLINS RESERVOIR

Species or Species Group	Number of Fish	Mean THg (ppm)	Standard Deviation (ppm)	Mean TL (mm)	Standard Deviation (mm)
Largemouth Bass	7	0.33	0.09	285	34
Smallmouth Bass	11	0.76	0.24	354	51
Spotted Bass	8	0.52	0.13	270	60
Black Bass Group	26	0.54	0.26	307	62

Of the Black Bass Group, smallmouth bass had the highest mean THg concentration (0.76 ppm), followed by spotted bass (0.52 ppm), and largemouth bass (0.33 ppm). The Black Bass Group at Rollins Reservoir had a mean of 0.54 ppm for THg and a mean of 307 mm for TL (≥305 mm, per OEHHA). Within the Black Bass Group,

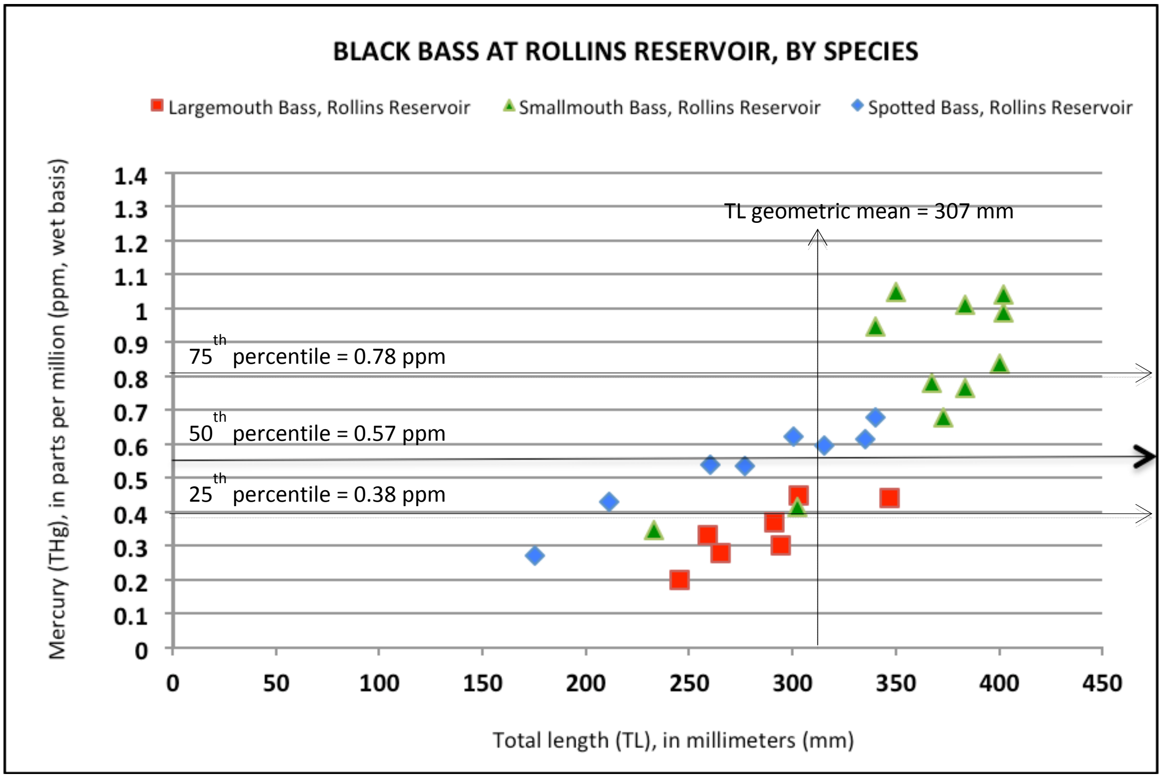

Figure 3. Black Bass at Rollins Reservoir, by species. THg concentrations for Black Bass at Rollins Reservoir in relation to TL. Data were collected by USGS in 1999 (largemouth bass n=8); Statewide Lakes Sportfish Contamination Study in 2007 (n=10); and for this research in 2015 (smallmouth bass n=1; spotted bass n=8) Data collected by USGS and SLSCS were accessed from CEDEN in 2014.

18 samples fell within one standard deviation of the mean THg and 17 samples fell within one standard deviation of the mean TL (n=26). See Figure 4.

Sunfish Group at Rollins Reservoir

A total of 24 samples composed of two species, bluegill (*Lepomis macrochirus*; n=3) and green sunfish (*Lepomis cyanellus*; n=21), were included in this analysis. Bluegill samples consist of two samples analyzed as individuals and one sample analyzed as a composite of three fish (collected by USGS in September 1999). Green sunfish samples were collected as part of this research project between July and August

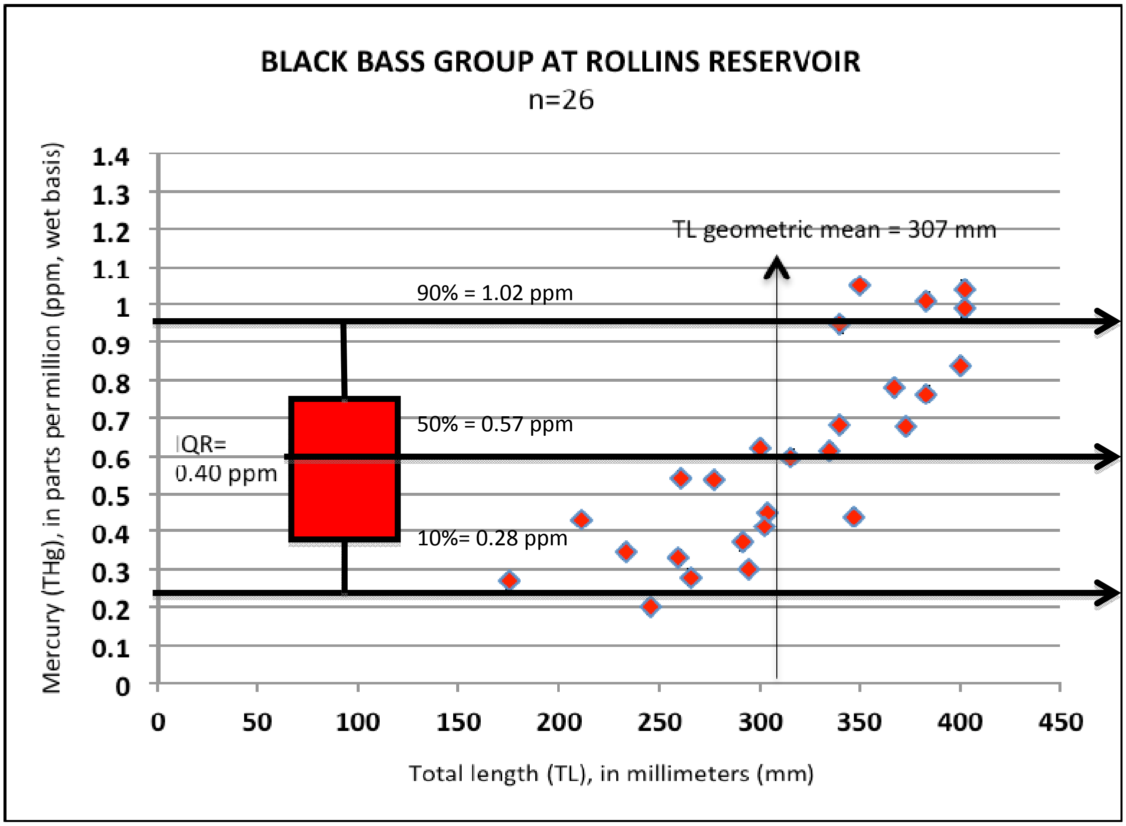

Figure 4. Boxplot of Black Bass group at Rollins Reservoir. Distribution of THg concentrations for Black Bass at Rollins Reservoir (n=26). Inter-quartile range (IQR) shows the spread of the data between the 25th and 75th percentile. Individual sample THg plotted in relation to TL on the right side of the figure.

2015. The earliest sampling date was July 15, 2015 and the last sampling date was August 5, 2015. Of the 24 total samples, four samples fall below the minimum total length specified by OEHHA for the Sunfish Species Group (≥100 mm). See Table 12 and Figure 5.

TABLE 12: SUNFISH GROUP AT ROLLINS RESERVOIR

Species or Species Group	Number of Fish	Mean THg (ppm)	Standard Deviation (ppm)	Mean TL (mm)	Standard Deviation (mm)
Green Sunfish	21	0.15	0.25	115	18
Bluegill	3	0.24	0.13	170	20
Sunfish Group	24	0.16	0.24	120	26

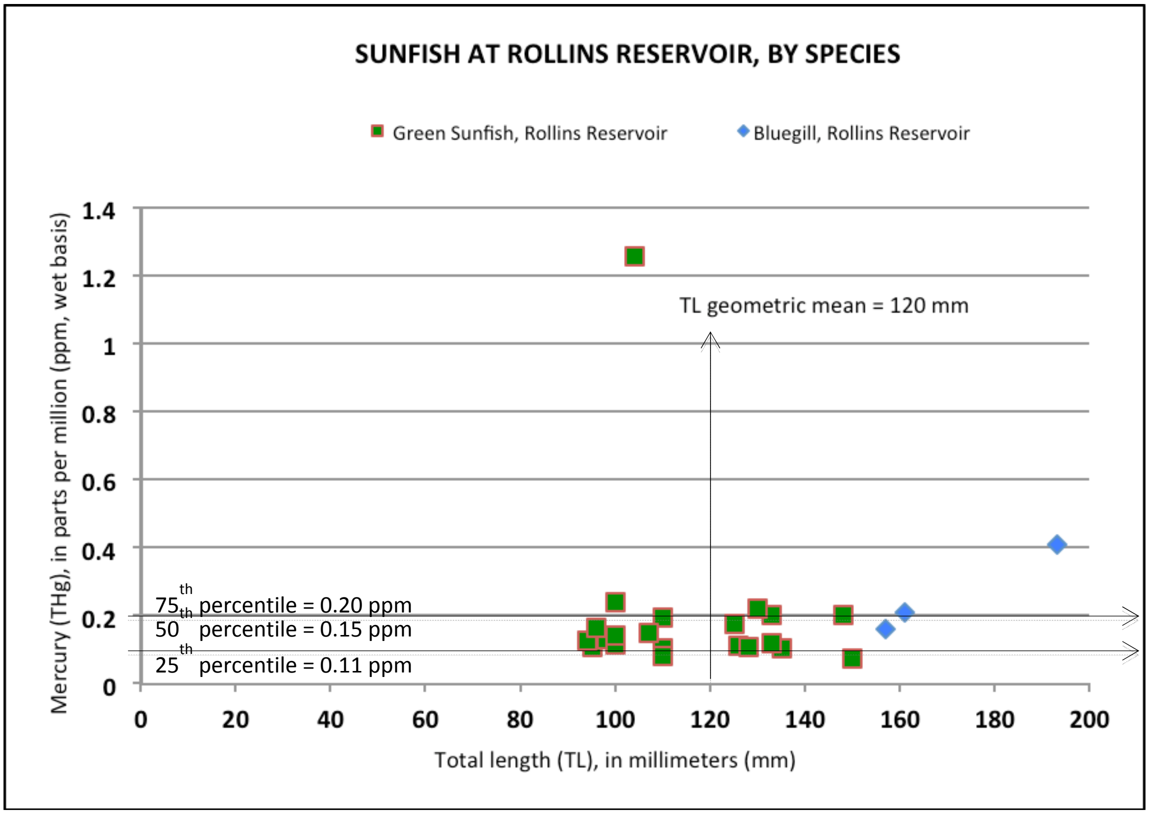

Figure 5. Sunfish at Rollins Reservoir, by species. THg concentrations for sunfish at Rollins Reservoir in relation to TL. Data were collected by USGS in 1999 (bluegill n=3) and for this research in 2015 (green sunfish n=21). Data collected by USGS were accessed from CEDEN in 2014.

The bluegill (n=3) sampled had higher mean for both THg (0.24 ppm) and TL (170 mm) when compared to green gunfish (n=21; THg: 0.15 ppm; TL: 115 mm). The Sunfish Group at Rollins Reservoir had a mean mercury concentration of 0.16 ppm and a mean total length of 120 mm (n=24). Within the Sunfish Group, 22 samples fell within one standard deviation of the mean THg and 19 samples fell within one standard deviation of the mean TL. See Figure 6.

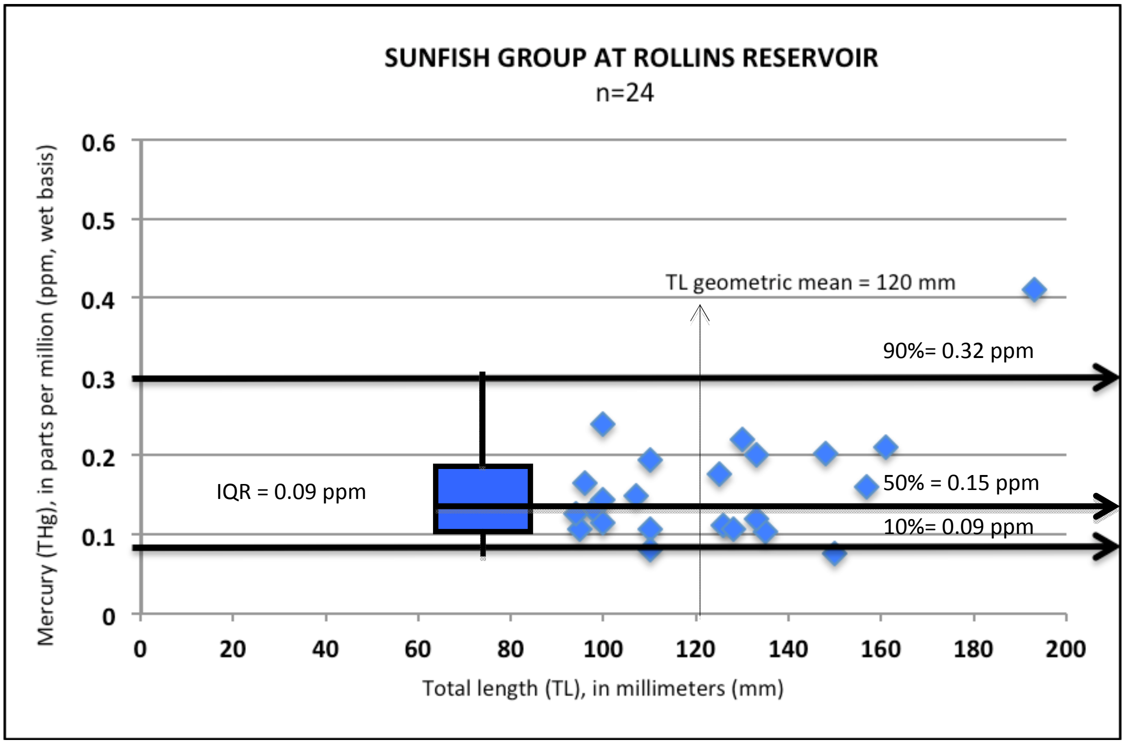

Figure 6. Boxplot of Sunfish group at Rollins Reservoir. Distribution of THg concentrations for Sunfish at Rollins Reservoir (n=24). Inter-quartile range (IQR) shows the spread of the data between the 25th and 75th percentile. Individual sample THg plotted in relation to TL on the right side of the figure, excluding outlier sample with individual THg of 1.3 ppm.

<u>Trout at Rollins Reservoir</u>

Prior to this research brown trout samples were collected from the Bear River Arm of Rollins Reservoir (collected by USGS, September 1999). These samples had a minimum THg concentration of 0.02 ppm, a maximum THg concentration of 0.09 ppm, and a mean (geometric) concentration of 0.05 ppm (n=4). A single rainbow trout sample was collected as part of this research in July 2015 had a THg concentration of 0.02 ppm. See Table 13 and Figure 7.

TABLE 13: TROUT AT ROLLINS RESERVOIR

Species or Species Group	Number of Fish	Mean THg (ppm)	Standard Deviation (ppm)	Mean TL (mm)	Standard Deviation (mm)
Brown Trout	4	0.05	0.03	283	10
Rainbow Trout	1	0.02	N/A	297	N/A

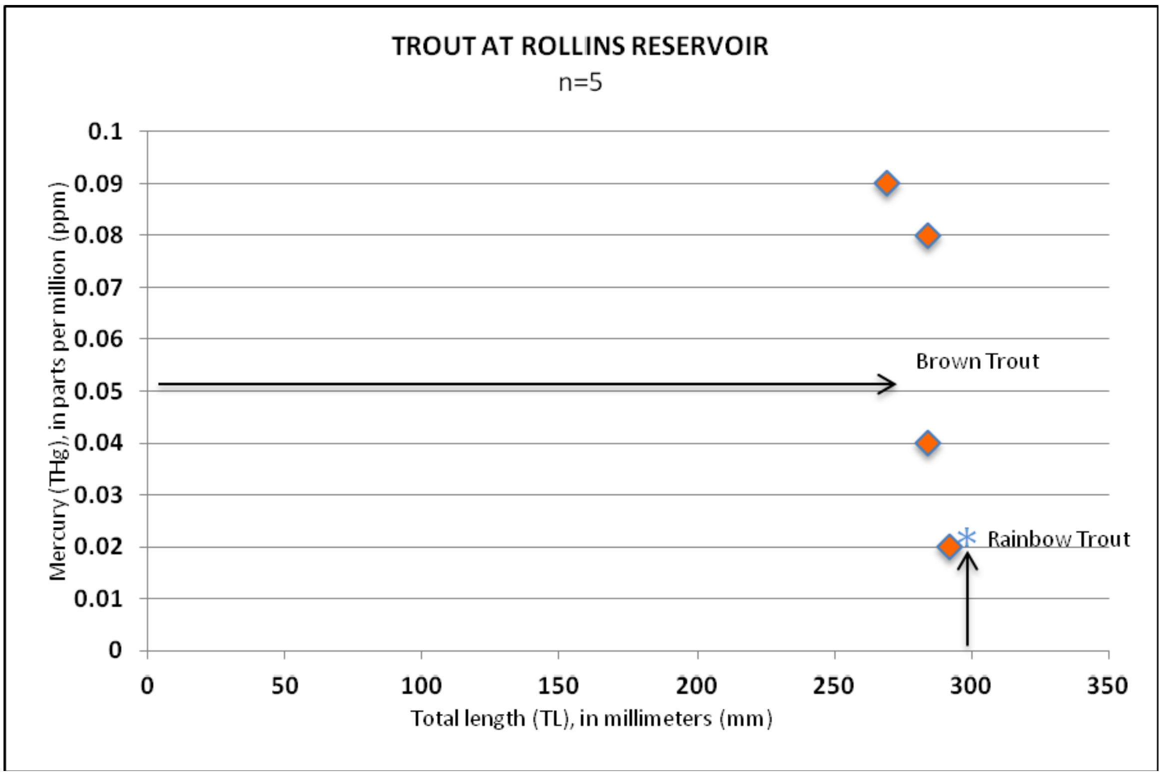

Figure 7. Brown Trout at Rollins Reservoir. THg concentrations for brown trout (n=4) in relation to TL. TL and THg of Rainbow Trout (n=1) denoted by back arrow and blue asterisk on x-axis. Brown Trout data were collected by USGS from Rollins Reservoir in 1999 and accessed from CEDEN in 2014.

Lake Clementine

There were no existing fish tissue data for Lake Clementine prior to this research. This research effort collected 41 fish samples from the following species:

smallmouth bass (*Micropterus dolomieu*), bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), redear sunfish (*Lepomis microlophus*), Sacramento pikeminnow (*Ptychocheilus grandis*), and rainbow trout (*Oncorhynchus mykiss*).

Black Bass Group at Lake Clementine

Eight samples were collected from the Black Bass Group at Lake Clementine. All samples collected were smallmouth bass (*Micropterus dolomieu*). Samples were collected between May and August 2015. The earliest sampling date was May 2, 2015 and the last sampling date was August 16, 2015. The smallmouth bass had a mean THg concentration (n=8) of 0.40 ppm and a mean TL of 308 mm. Five samples were within one standard deviation of the mean THg and five samples were within one standard deviation of the mean TL (n=8). (see Table 14 and Figure 8).

TABLE 14: BLACK BASS AT LAKE CLEMENTINE

Species or Species Group	Number of Fish	Mean THg (ppm)	Standard Deviation (ppm)	Mean TL (mm)	Standard Deviation (mm)
Smallmouth Bass	8	0.40	0.13	308	46

Of Black Bass Group samples collected at Lake Clementine, only five of the eight samples had a TL over the legal limit (≥305 mm) qualifying them for inclusion in the development of fish consumption advice for Lake Clementine. The mean THg concentration in the fish tissue of these five samples was 0.49 ppm (see Figure 8). Per OEHHA, in order for fish consumption advice to be developed a minimum of 9 samples ≥ 305 mm are required for the Black Bass Species Group. Samples in excess of 9 may be of a TL that is below the legal limit for bass. Additional samples from the Black Bass

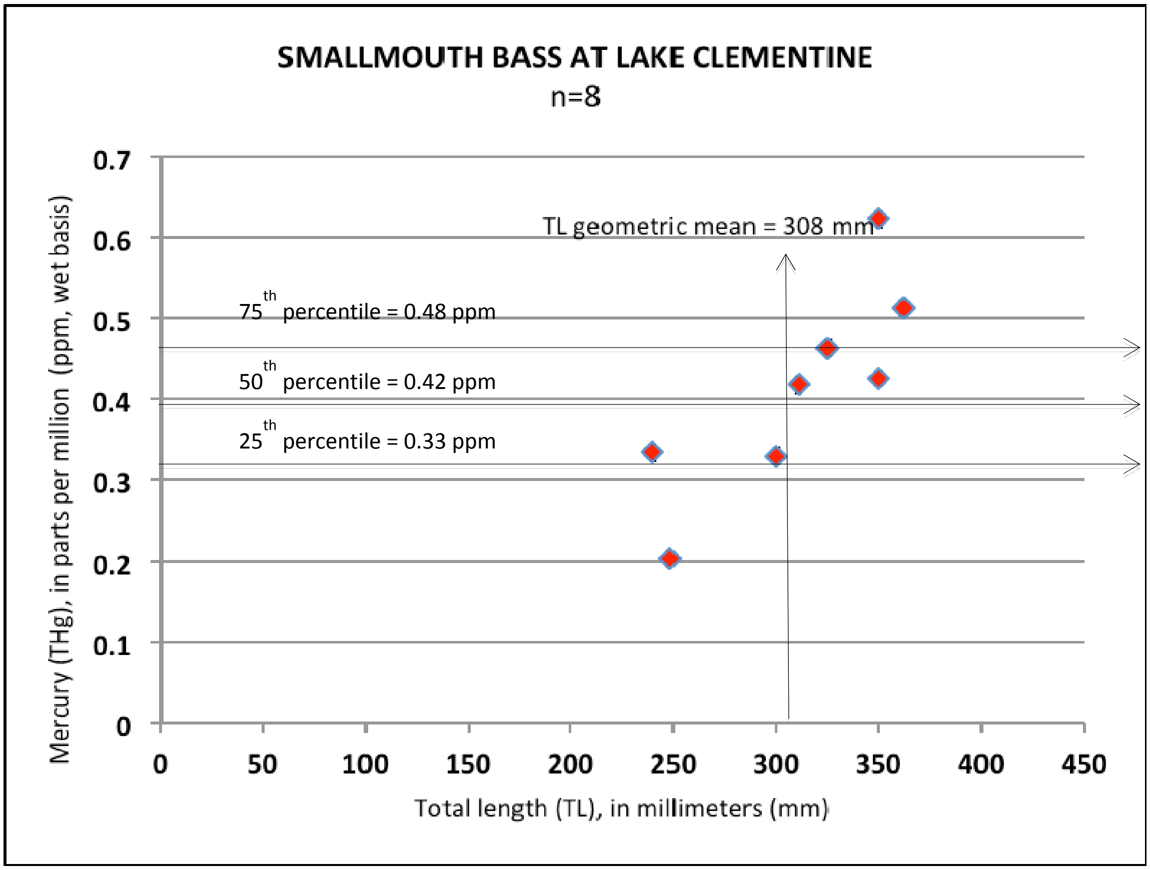

Figure 8. Smallmouth Bass at Lake Clementine. THg concentrations for smallmouth bass in relation to TL. Data were collected for this research in 2015.

Group must be collected from Lake Clementine before site-specific fish consumption advice can be developed. Figure 9 shows the variability in THg concentration for the smallmouth bass samples (n=8) from Lake Clementine.

<u>Sunfish at Lake Clementine</u>

A total of 29 samples were collected as part of this research for the OEHHA Sunfish Species Group at Lake Clementine. Samples were collected from three species of sunfish: bluegill (*Lepomis macrochirus*); green sunfish (*Lepomis cyanellus*); and redear sunfish (*Lepomis microlophus*). Samples were collected between May and August 2015. The earliest sampling date was May 2, 2015 and the last sampling date was August 16,

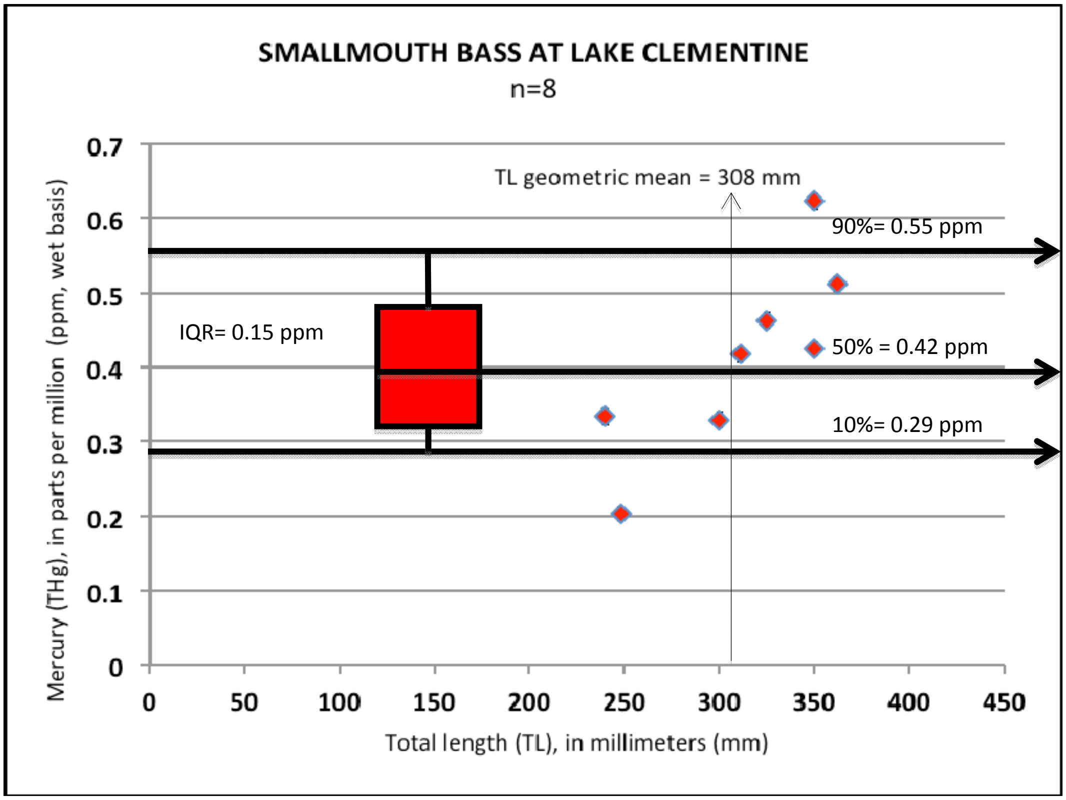

Figure 9. Boxplot of Smallmouth Bass at Lake Clementine. Distribution of THg concentrations for smallmouth bass at Lake Clementine (n=8). Inter-quartile range (IQR) shows the spread of the data between the 25th and 75th percentile. Individual sample THg plotted in relation to TL on the right side of the figure.

2015. The bluegill sampled (n=15) had the highest mean for both THg (0.20 ppm) and TL (187 mm), followed by green sunfish (n=11; THg=0.08 ppm; TL=154 mm). Redear sunfish (n=3) had the lowest mean THg (0.06 ppm) and TL (149 mm) of the three sunfish species sampled. The Sunfish Species Group (n=29) had a mean THg of 0.12 ppm and a mean TL of 170 mm (see Table 15 and Figure 10).

Within the Sunfish Group at Lake Clementine 22 samples fell within one standard deviation of the mean THg and 18 samples fell within one standard deviation of the mean TL. See Figure 11 for the variability in THg concentration for the Sunfish Group at Lake Clementine.

TABLE 15: SUNFISH AT LAKE CLEMENTINE

Species or Species Group	Number of Fish	Mean THg (ppm)	Standard Deviation (ppm)	Mean TL (mm)	Standard Deviation (mm)
Bluegill	15	0.20	0.13	187	19
Green Sunfish	11	0.08	0.04	154	22
Redear Sunfish	3	0.06	0.01	149	17
Sunfish Group	29	0.12	0.12	170	26

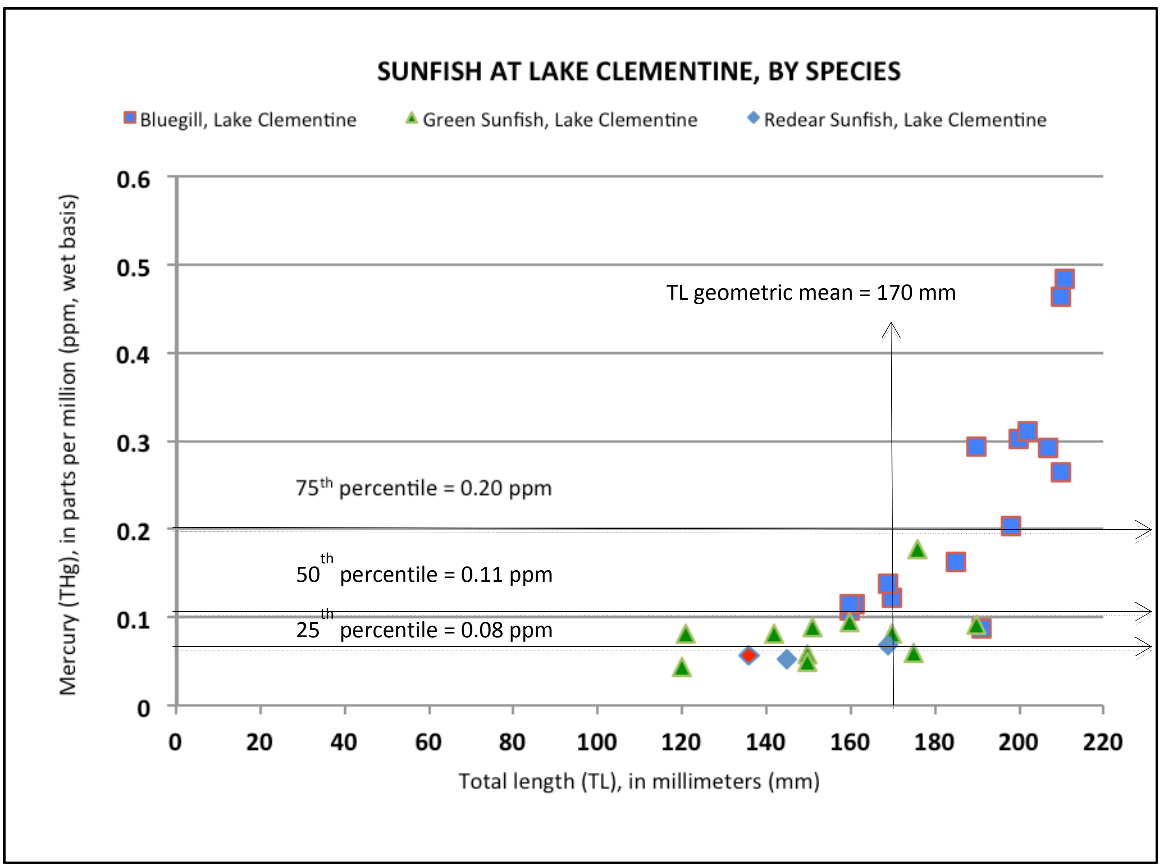

Figure 10. Sunfish at Lake Clementine, by species. THg concentrations for bluegill (n=15), green sunfish (n=11), and redear sunfish (n=3) in relation to total length. Data were collected for this research in 2015.

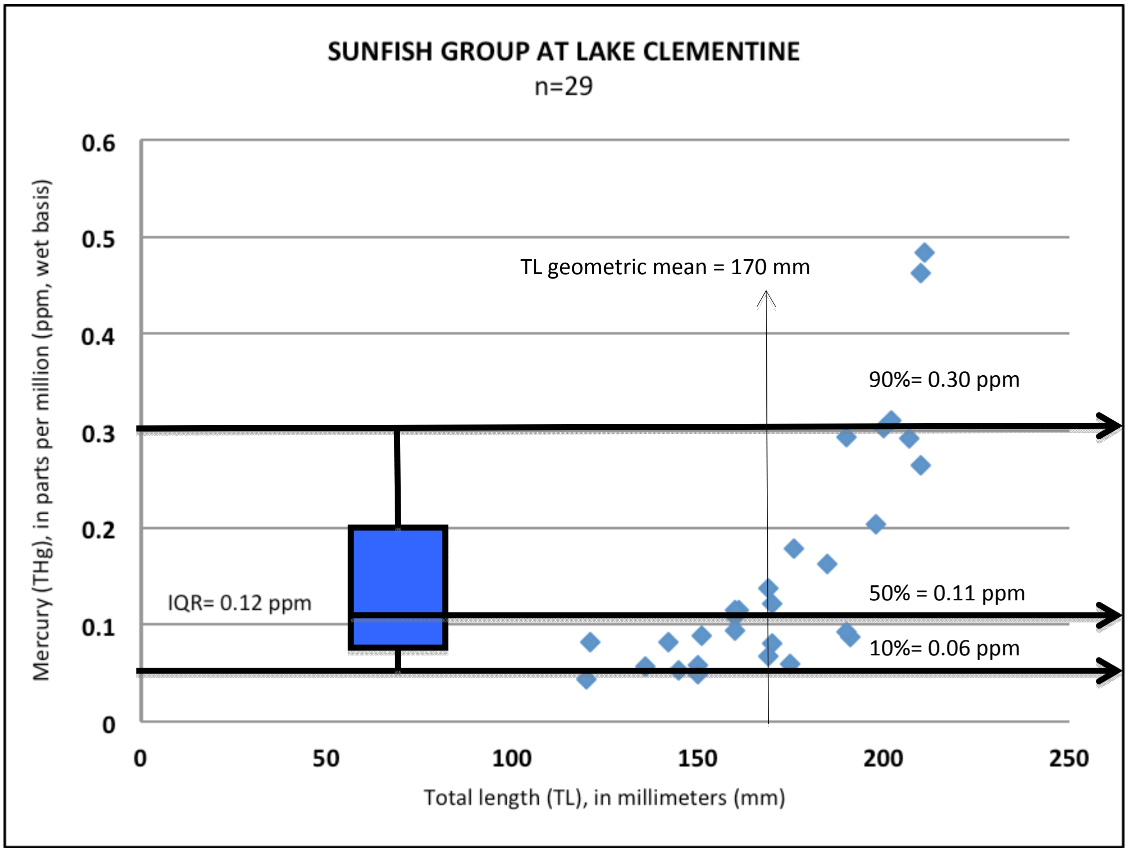

Figure 11. Boxplot of Sunfish group at Lake Clementine. Distribution of THg concentrations for Sunfish Group at Lake Clementine (n=29). Inter-quartile range (IQR) shows the spread of the data between the 25th and 75th percentile. Individual sample THg plotted in relation to TL on the right side of the figure.

<u>Sacramento Pikeminnow at Lake Clementine</u>

Sacramento pikeminnow (*Ptychocheilus grandis*) were added to the list of species proposed for analysis from Lake Clementine based on angler surveys and field experience indicating that Russian and Ukrainian ethnic groups frequent this water body and may have different fish consumption preferences than water bodies surveyed as part of the *Gold Country Angler Survey* (The Sierra Fund, 2011). The goal of developing comprehensive fish consumption advice is to be inclusive of all species being caught and consumed from a given water body to ensure that the advice reflects angler catch and consumption patterns. Limited Sacramento pikeminnow samples (n=3) were caught during the 2015 fishing season due to late addition of this species to the target list for the water body. The mean THg for the pikeminnow samples was 0.46 ppm (mean TL 394 mm). See Table 16 and Figure 12.

TABLE 16. SACRAMENTO PIKEMINNOW AT LAKE CLEMENTINE

Species or Species Group	Number of Fish	Mean THg (ppm)	Standard Deviation (ppm)	Mean Total Length (mm)	Standard Deviation (mm)
Sacramento Pikeminnow	3	0.46	0.40	394	64

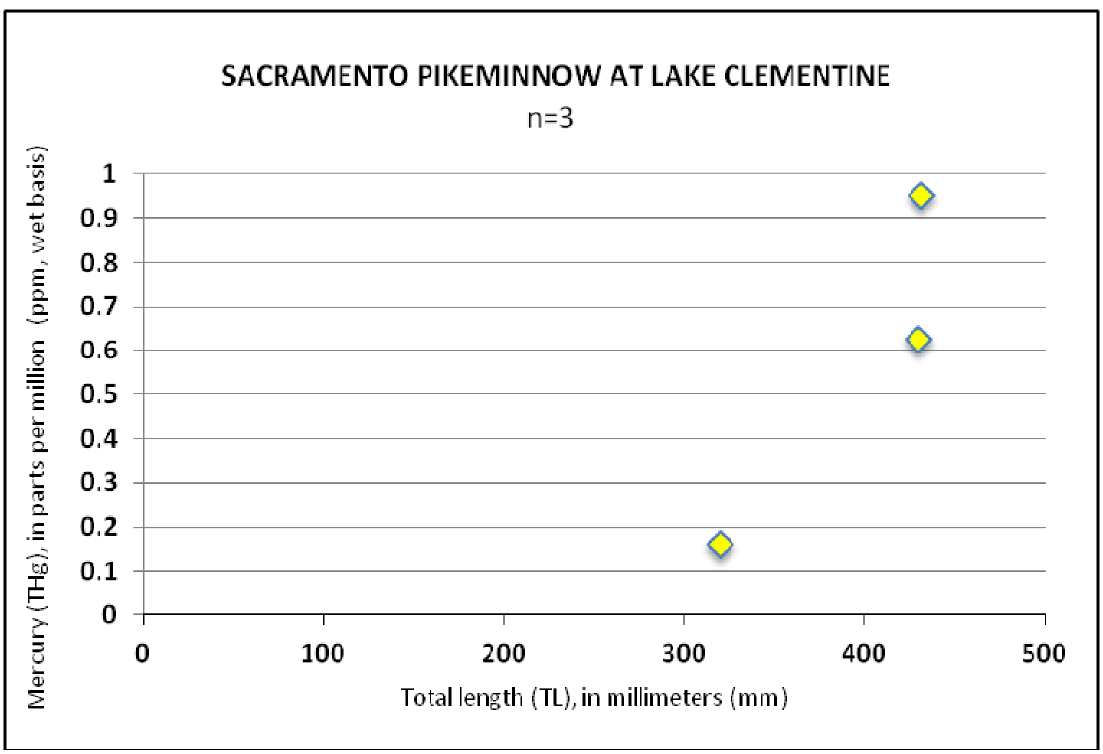

Figure 12. Sacramento Pikeminnow. THg concentrations for Sacramento pikeminnow (n=3) in relation to TL. Data were collected for this research in 2015.

Rainbow Trout at Lake Clementine

A single Rainbow Trout (*Oncorhynchus mykiss*) was sampled from Lake Clementine. This sample had the lowest reported fish tissue THg concentration of any of the samples collected from Lake Clementine (see Table 17).

TABLE 17: RAINBOW TROUT AT LAKE CLEMENTINE

Species or Species Group	Number of Fish	Mean THg (ppm)	Mean TL (mm)
Rainbow Trout	1	0.03	260

Addressing Research Questions: Fish Tissue Data

(A) Mercury Exposure Risk at Target Water Bodies

❑ **Research Question 1.** What is the mean mercury concentration (ppm) in fish tissue (N > 9) of OEHHA-defined edible size fish by individual water body and species?

Results: A sample size of 9 or more fish (≥ OEHHA-defined size criteria) has been achieved for the Sunfish Species Group and Black Bass Species Group at Rollins Reservoir and the Sunfish Species Group at Lake Clementine. This provides OEHHA with the minimum data required to issue site-specific fish consumption advicefor these species groups, at these target water bodies.

Fourteen samples from three species of the Black Bass Group at Rollins Reservoir were over the legal limit specified by the Department of Fish and Wildlife as catchable/edible size for bass (≥305 mm) (largemouth bass, n=2; smallmouth bass, n=9; spotted bass, n=3). The mean THg concentration of these fourteen samples was 0.75 ppm (minimum 0.44 ppm; maximum 1.05 ppm). Inclusion of samples that fall below the legal limit for bass (≥305 mm) and thus below OEHHA's criteria (n > 9; TL ≥305 mm) in analysis of THg results in a lower calculated mean (0.54 ppm; n=26) for the Black Bass Group at Rollins Reservoir.

Twenty samples from two species of the Sunfish Species Group at Rollins Rollins Reservoir were over the OEHHA-determined edible size (≥100 mm) (bluegill, n=3; green sunfish; n=18). The mean THg concentration of these samples was 0.17 ppm. When samples falling below the TL size criteria (green sunfish, n=4) were included in the analysis the mercury concentration for the Sunfish Species Group at Rollins Reservoir was 0.16 ppm (n=24).

For the Black Bass and Sunfish species groups at Rollins Reservoir including samples with TL less than OEHHA size criteria for the species resulted in a lower mean mercury concentration for the species.

At Lake Clementine, 29 samples from three species of the Sunfish Species Group were over the OEHHA-determined edible size (≥100 mm) (bluegill, n=15; green sunfish, n=11; and redear sunfish, n=3). The Sunfish Species Group had a mean THg concentration of 0.12 ppm.

❑ **Research Question 2.** Do the mean fish tissue mercury concentrations (ppm) exceed the EPA threshold of 0.3 ppm by water body and by species?

Results: At Rollins Reservoir the mean fish tissue THg concentrations of all sampled species from the Black Bass Group exceeded the EPA threshold of 0.3 ppm (largemouth bass, n=7, THg=0.33 ppm; smallmouth bass, n=11, THg=0.76 ppm; spotted bass, n=8, THg=0.52 ppm). Of 26 total samples for the Black Bass Species Group at Rollins Reservoir, only two samples fell below the EPA threshold: one largemouth bass sampled by USGS in 1999 (THg=0.20 ppm, TL=245 mm) and one spotted bass sampled as part of this research project in July 2015 (THg=0.27 ppm, TL=175 mm). The mean fish tissue THg concentration for the Sunfish Species Group at Rollins Reservoir (n=24) falls below threshold, however one green sunfish sample was significantly above the EPA threshold (THg: 1.26 ppm, TL: 104 mm).

At Lake Clementine the mean THg concentrations of Sacramento pikeminnow (n=3, THg=0.46 ppm) and smallmouth bass (n=8, THg 0.41 ppm) exceed the EPA threshold of 0.3 ppm. One of the three Sacramento pikeminnow samples falls below the EPA threshold. Of eight smallmouth bass samples collected at Lake Clementine, only one sample falls below the EPA threshold. Four bluegill samples from Lake Clementine exceed the EPA threshold (minimum 0.31 ppm; maximum 0.48 ppm) but the Sunfish Species Group (n=29) falls below the threshold.

Comparison to OEHHA ATLs

For those species groups for which a sample size of 9 or more fish (≥ OEHHA-defined size criteria by species) was achieved (Sunfish Species Group and Black Bass Species Group at Rollins Reservoir and Sunfish Species Group at Lake Clementine) it is possible to predict the recommended frequency of consumption by population group based on the species group mean mercury concentration (see Table 9 for OEHHA ATLs; see Figures 13 and 14 for comparison).

The Black Bass Group at Rollins Reservoir had a mean mercury concentration of 0.54 ppm (n=26). This indicates that Group 1 (women of child-bearing age (ages 18-45) and children ages 1 to 17 years) should not consume any Black Bass from Rollins Reservoir. Group 2 (women over age 45 years and men) can consume one serving per week of Black Bass from Rollins Reservoir (see Figure 13).

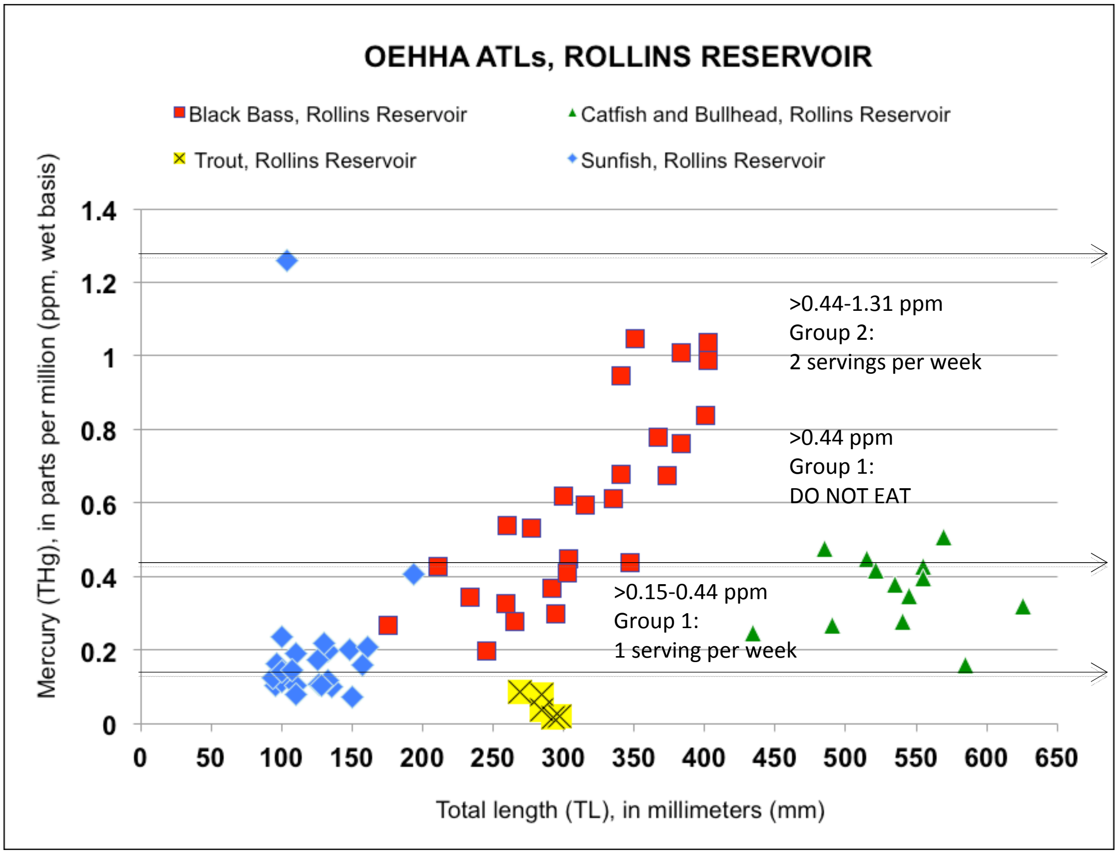

Figure 13. Comparison of THg to OEHHA ATLs, Rollins Reservoir. Mercury concentrations for black bass at Rollins Reservoir in relation to total length. Data were collected by USGS in 1999 (largemouth bass n=8; channel catfish n=13; bluegill n=3; brown trout n=4); Statewide Lakes Sportfish Contamination Study in 2007 (smallmouth bass n=10); and for this research in 2015 (smallmouth bass n=1; spotted bass n=8; green sunfish n=21; rainbow trout n=1). Data collected by USGS and SLSCS were accessed from CEDEN in 2014.

The Sunfish Species Group at Rollins Reservoir had a mean THg concentration of 0.16 ppm (n=24). This indicates that Group 1 (women of child-bearing age (ages 18-45) and children ages 1 to 17 years) can consume one serving per week of Sunfish from Rollins Reservoir. Group 2 (women over age 45 years and men) can consume three servings per week of Sunfish from Rollins Reservoir (see Figure 13).

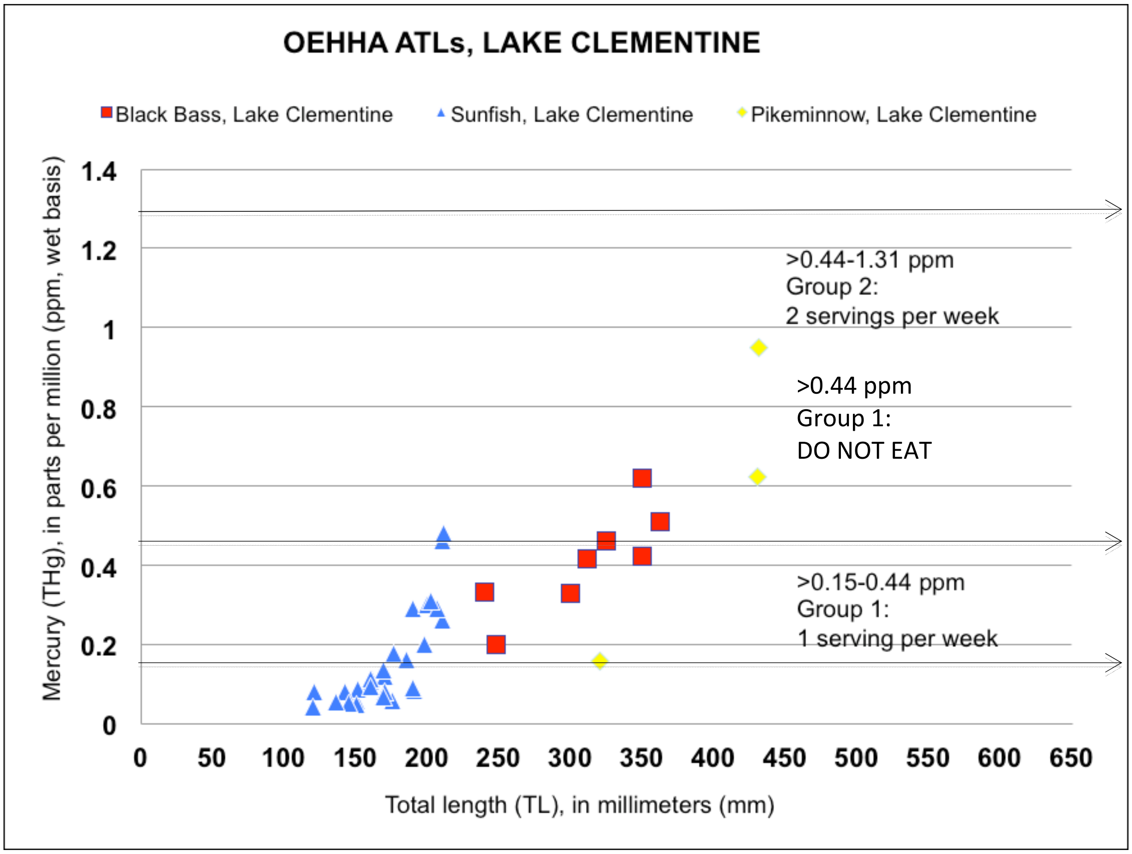

Figure 14. Comparison of THg to OEHHA ATLs, Lake Clementine. Mercury concentrations for Sunfish Group (n=29) (bluegill n=15; green sunfish n=11; and redear sunfish n=3), smallmouth bass (n=8), and Sacramento pikeminnow (n=3) in relation to total length. Data were collected for this research in 2015.

The Sunfish Species Group at Lake Clementine had a mean mercury concentration of 0.12 ppm (n=24). This indicates that Group 1 (women of child-bearing age (ages 18-45) and children ages 1 to 17 years) can consume two servings per week of Sunfish from Lake Clementine. Group 2 (women over age 45 years and men) can consume five servings per week of Sunfish from Lake Clementine (see Figure 14).

Comparison to OEHHA Statewide Advice for Lakes and Reservoirs

The results of mean fish tissue THg concentrations were compared to 90^{th} percentile value mean lake mercury concentrations of the Statewide Advisory Dataset to determine if site-specific fish tissue mercury concentrations exceed fish tissue mercury concentrations used for general fish consumption advice. For Black Bass at both Rollins Reservoir and Lake Clementine, the OEHHA 90^{th} percentile value exceeds the site-specific value for mean mercury concentration. OEHHA has not developed statewide advice for Sacramento pikeminnow so there is no point for comparison. For Catfish and Bullhead, the site-specific mean mercury concentration for catfish (0.35 ppm) exceeds the OEHHA 90^{th} percentile value (0.346 ppm). For Sunfish at both Rollins Reservoir and Lake Clementine, the OEHHA 90^{th} percentile value exceeds the site-specific value for mean mercury concentration. For Brown Trout and Rainbow Trout the OEHHA 90^{th} percentile value is higher than mercury concentrations in the limited number of trout analyzed in this research (see Table 18).

(B) Impact of Size and Trophic Position on Fish Tissue Mercury Concentration

- ☐ **Research Question 3.** Is there a positive relationship between Total Length (TL) and fish tissue mercury concentration (ppm)?

Results: The relationship between total length (mm) and fish tissue mercury concentration (ppm) was analyzed for the Black Bass Group and the Sunfish Species Group at Rollins Reservoir and Lake Clementine.

Rollins Reservoir: At Rollins Reservoir Spearman's rank correlations were calculated for smallmouth bass (n=11) and spotted bass (n=8). Samples for species of the

TABLE 18: COMPARISON OF STATEWIDE ADVICE TO SITE-SPECIFIC MEAN MERCURY CONCENTRATIONS

Fish Species	OEHHA 90th Percentile Value (ppm)	OEHHA Mean THg Range (ppm)	Mean THg (ppm), Lake Clementine	THg Range (ppm) Lake Clementine	Mean THg (ppm), Rollins Reservoir	Mean THg Range (ppm) Rollins Reservoir
Bass	0.699 n=1666	0.013-1.297	0.413 n=8	0.203-0.623	0.539 n=26	0.21-.1.05
Sacramento pikeminnow	No Data	No Data	0.456	0.160-0.950	No Data	No Data
Catfish and Bullhead	0.346 n=145	0.005-0.588	No Data	No Data	0.35 n=13	0.160-0.510
Crappie	No Data	No Data	No Data	No Data	0.31 n=1	No Data
Sunfish	0.26 n=189	0.02-0.44	0.123	0.044-0.484	0.160	0.076-1.26
Brown Trout	0.397 n=43	0.035-0.84	No Data	No Data	0.05	0.02-0.09
Rainbow Trout	0.1 n=228	0.014-0.27	0.031 n=1	Not Applicable	0.021 n=1	Not Applicable

* Significant figures used for this comparison are based on OEHHA's level of reporting precision

Black Bass group were combined with existing largemouth bass samples (n=7) for analysis as a group (n=26). Spearman's rank correlations were also calculated for green sunfish (n=21) and for the Sunfish group (including existing samples for bluegill, n=3; Sunfish Group, n=24).

Black Bass Group: Black Bass Group samples from Rollins Reservoir consist of largemouth bass, smallmouth bass, and spotted bass. Largemouth bass samples (n=7) were collected by USGS in 1999 and analysis indicates a significant (alpha = 0.05) relation between THg and TL (rho = 0.79) (May et al., 2000, p. 12). For spotted bass at Rollins Reservoir the relationship between TL and THg is also significant (n=8, rho= 0.90, p<0.01). There is not a significant relationship between TL and THg for smallmouth bass at Rollins Reservoir (n=11, rho=0.50, p>0.05). See Figure 15.

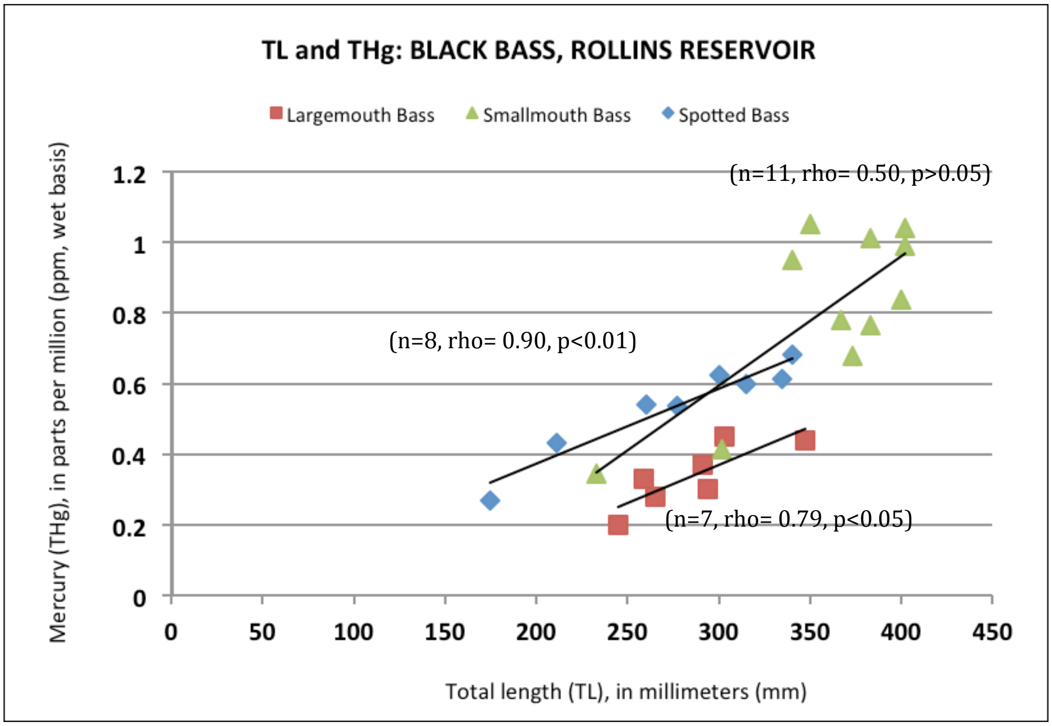

Figure 15. Relationship between TL and THg, Black Bass species, Rollins Reservoir, California. Mercury concentrations for black bass (largemouth bass n=7; smallmouth bass n=11; spotted bass n=8) at Rollins Reservoir in relation to total length.

Spotted bass species have the strongest positive relationship between mercury concentration (ppm) and total length of the three bass species (see Figure 15). When Black Bass Species at Rollins Reservoir were analyzed together as a group the relationship between TL and THg for Black Bass at Rollins Reservoir was significant (n=26, rho= 0.85, p<0.01). See Figure 16.

Sunfish Group: Spearman's rank correlations were calculated for green sunfish (n=21) and for the Sunfish Group (n=24) (including existing samples for bluegill, n=3). The relationship between TL and THg for green sunfish (n=21) at Rollins Reservoir was insignificant (p>0.05). The relationship between TL and THg

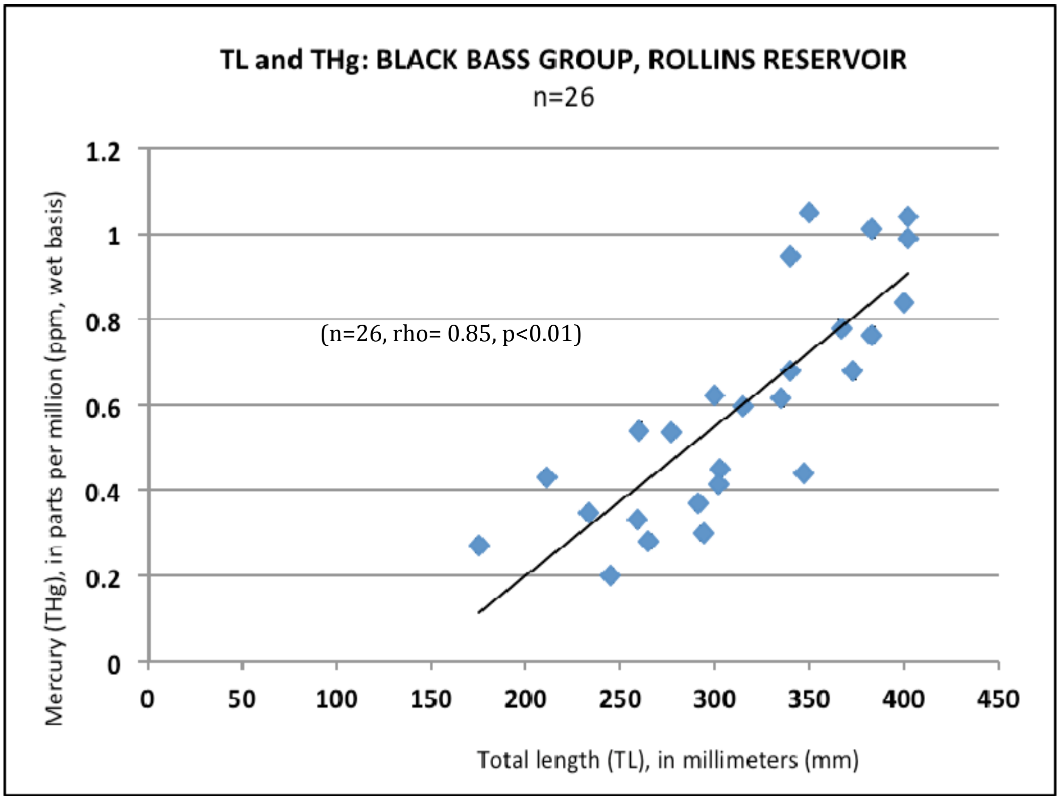

Figure 16. Relationship between TL and THg, Black Bass group, Rollins Reservoir, California. Mercury concentrations for Black Bass Group (n=26) at Rollins Reservoir in relation to total length.

for the Sunfish Group (n=24) at Rollins Reservoir was not significant ($p>0.05$). See Figure 17.

Lake Clementine: At Lake Clementine Spearman's rank correlations were calculated for smallmouth bass (n=8). Spearman's rank correlations also were calculated for bluegill (n=15) and green sunfish (n=11) as separate species. Sunfish data for this water body were combined for analysis as a species group (Sunfish Group, n=29)

Black Bass Group: All Black Bass Group samples from Lake Clementine are smallmouth bass. There is statistically significant relationship between TL and THg for smallmouth bass at Lake Clementine (n=8, rho=0.85, $p<0.05$). See Figure 18.

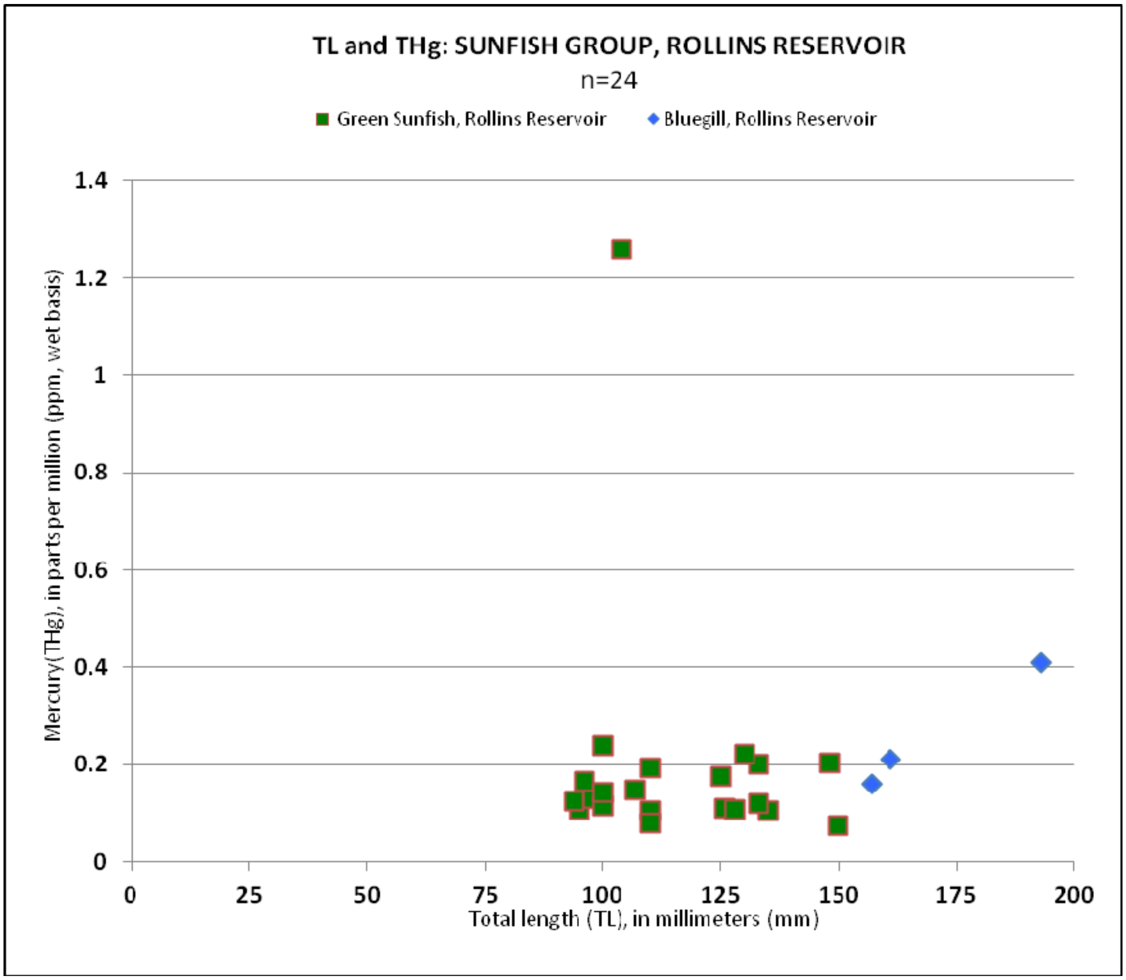

Figure 17. Relationship between TL and THg, Sunfish species, Rollins Reservoir, California. Mercury concentrations for Sunfish Group (n=24) at Rollins Reservoir in relation to total length. Sunfish Group consists of bluegill (n=3) and green sunfish (n=21). The relationship between THg and TL is not significant.

Sunfish Group: Sunfish Group samples for Lake Clementine consist of three species bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), and redear sunfish (*Lepomis microlophus*). The relationship between TL and THg varies by sunfish species (see Figure 19).

Bluegill: The relationship between TL and THg for bluegill at Lake Clementine is significant (n=15, rho=0.81, p<0.01).

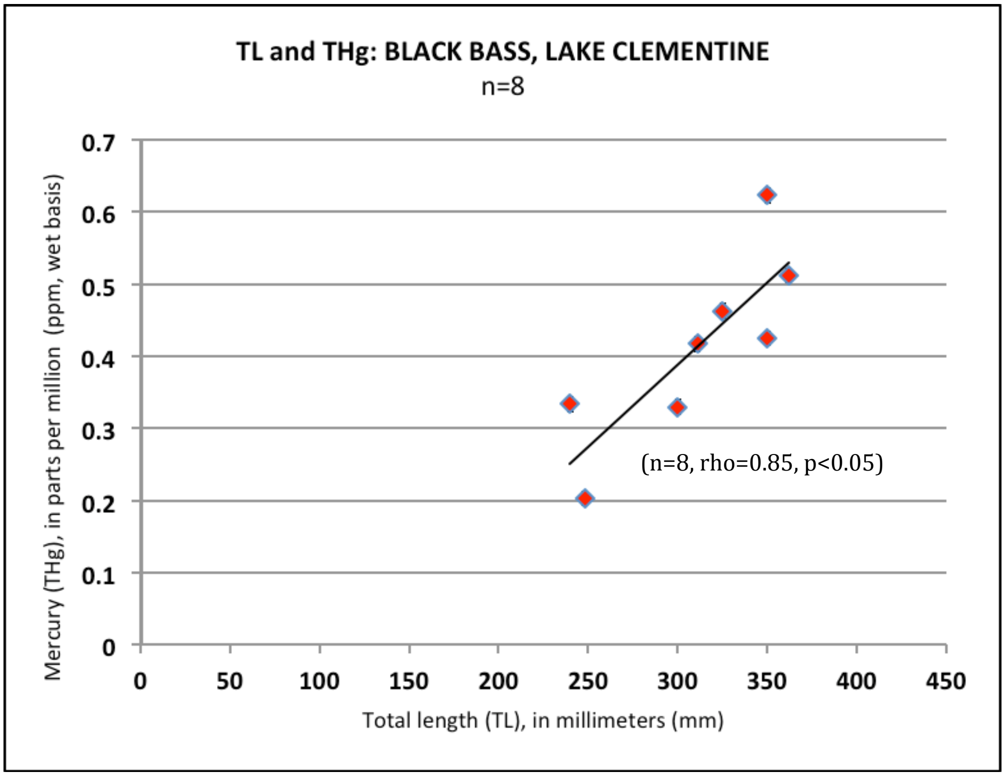

Figure 18. Relationship between TL and THg, Black Bass group, Lake Clementine, California. Mercury concentrations for Black Bass (smallmouth bass) (n=8) at Lake Clementine in relation to total length.

Green Sunfish: The relationship between total length and total mercury for green sunfish at Lake Clementine is not significant (n=11, rho= 0.63, p>0.05). When all three species of sunfish are combined for analysis as a group, there is a statistically significant relationship between TL and THg for sunfish at Lake Clementine (n=29, rho=0.83, p<0.01) See Figure 20.

❑ **Research Question 4.** Do predatory fish have higher fish tissue mercury concentrations (ppm) than non-predatory fish?

Rollins Reservoir: At Rollins Reservoir the impact of trophic level on fish mercury concentration was examined using trophic groups discussed by OEHHA (see Gassel and

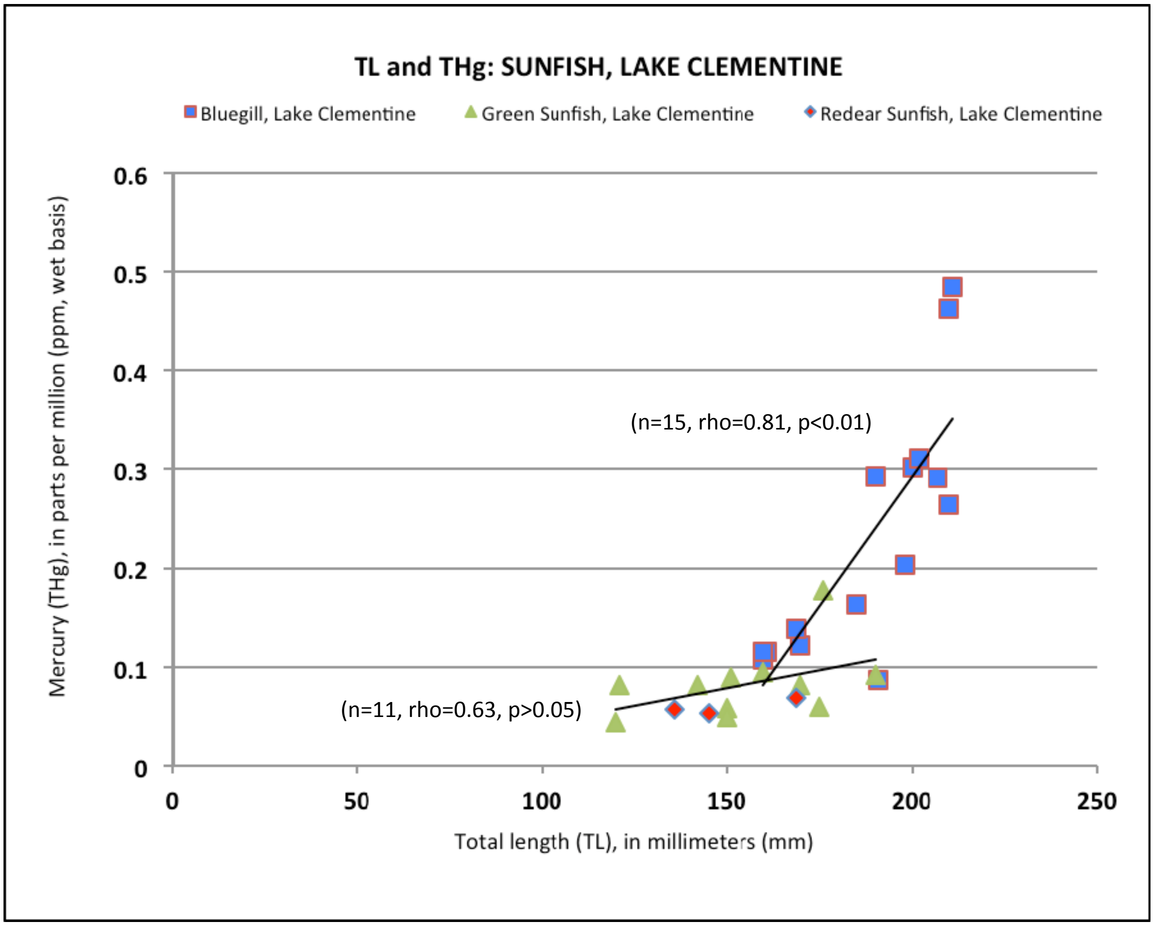

Figure 19. Relationship between TL and THg, Sunfish species, Lake Clementine, California. Mercury concentrations for sunfish species (bluegill, n=15; green sunfish, n=11; redear sunfish, n=3) at Lake Clementine in relation to total length.

Brodberg, 2005). Upper-Trophic-Level Predators were found to have the highest fish tissue mercury concentration (0.54 ppm) followed by Benthic Omnivores (0.35 ppm), Intermediate-Trophic-Level Predators (0.16 ppm), and Omnivores (0.04 ppm). See below and Figure 21.

- **Upper-Trophic-Level Predators (Predators):** The Upper-Trophic-Level Predators sampled from Rollins Reservoir included largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), and spotted bass

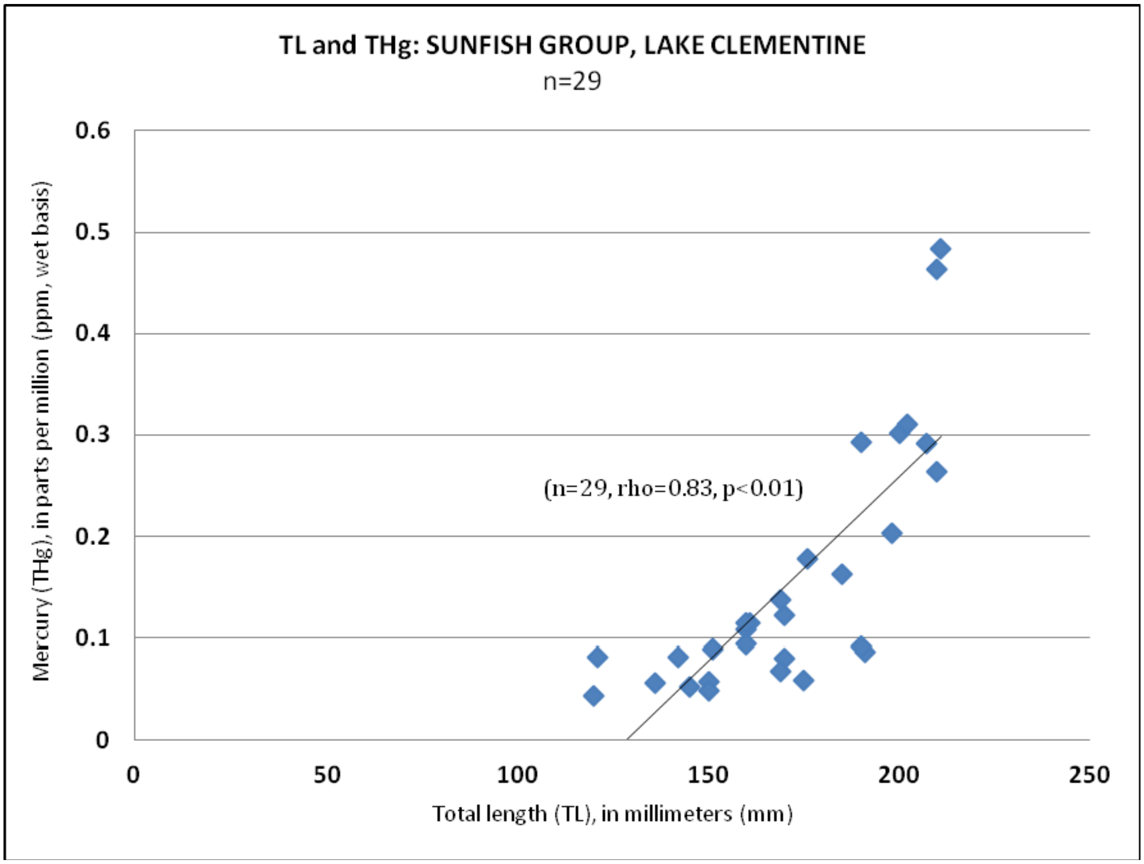

Figure 20. Relationship between TL and THg, Sunfish group, Lake Clementine, California. Mercury concentrations in relation to total length for three sunfish species evaluated as a Sunfish Group (n=29) at Lake Clementine. R^2 value and linear equation is plotted for the Sunfish Group.

(*Micropterus punctulatus*). The geometric mean THg for these samples (Black Bass Group) was 0.54 ppm (n=26).

- **Intermediate-Trophic-Level Predators (Secondary Targets):** The Intermediate-Trophic-Level Predators sampled from Rollins Reservoir included bluegill (*Lepomis macrochirus*) and green sunfish (*Lepomis cyanellus*) The geometric mean THg for these samples (Sunfish Group) was 0.16 ppm (n=24).

- **Benthic Omnivores (Bottom Feeders):** Existing data available from CEDEN was used to characterize Benthic Omnivores at Rollins Reservoir. Channel

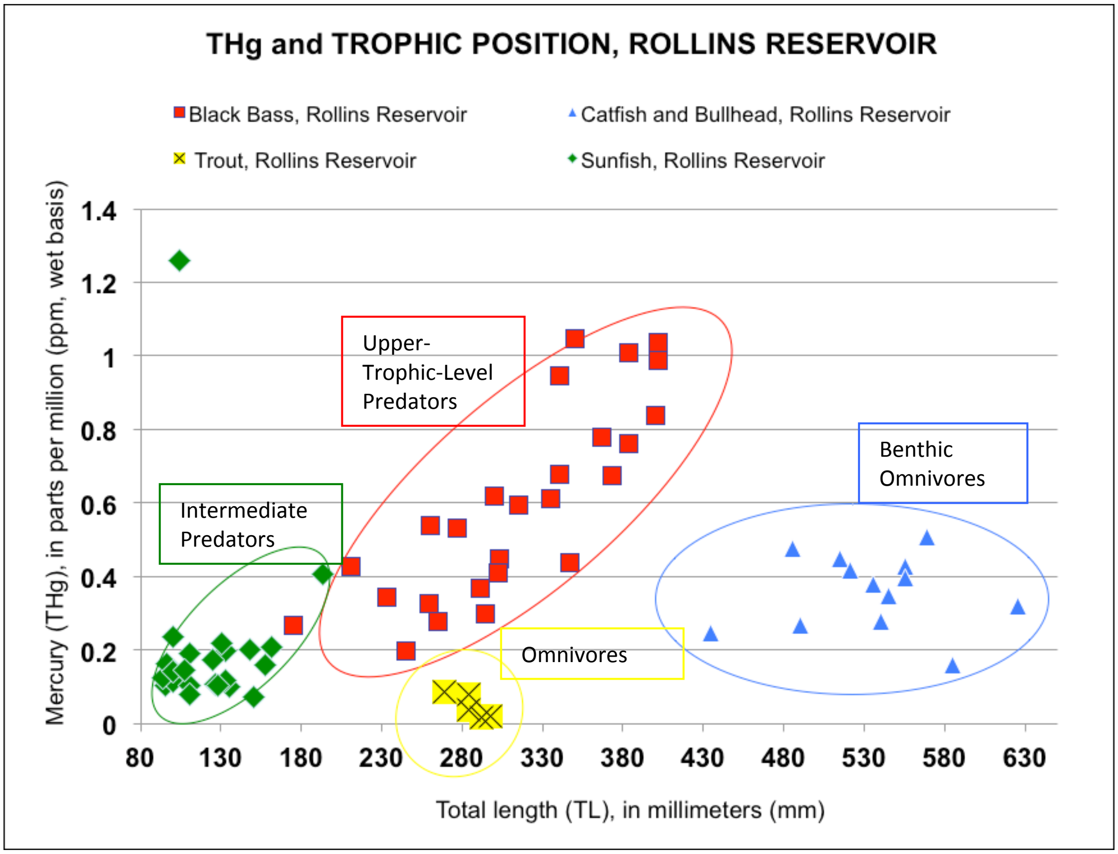

Figure 21. THg and trophic position at Rollins Reservoir, California. Mercury concentrations in relation to TL for Upper-Trophic-Level Predators (Black Bass, n=26), Benthic Omnivores (Catfish and Bullhead, n=13), Intermediate Predators (Sunfish, n=24), and Omnivores (Trout, n=5) at Rollins Reservoir, California.

catfish (*Ictalurus punctatus*) samples collected by USGS in 1999 had a geometric mean THg of 0.35 ppm (n=13) (May et al., 2000).

- **Omnivores:** Data from two species of Omnivores, brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) exist for Rollins Reservoir. Brown trout samples collected by USGS in 1999 had a geometric mean THg of 0.05 ppm (n=4) (May et al., 2000). A single rainbow trout sample was collected as part of this research and was found to have a total mercury concentration of 0.02 ppm. For purposes of comparison by trophic position, the geometric mean THg concentration of the five trout samples available for Rollins Reservoir was taken (brown trout, n=4; rainbow trout, n=1). The geometric mean THg for Trout Species at Rollins Reservoir is 0.04 ppm.

Lake Clementine: At Lake Clementine Upper-Trophic-Level Predators were found to have the highest THg concentrations. The single Omnivore sample had the lowest THg concentration. Intermediate-Trophic-Level Predators (Sunfish Group) had a mean THg concentration falling between the Upper-Trophic-Level Predators and the Omnivore (see below and Figure 22).

- **Upper-Trophic-Level Predators (Predators):** Smallmouth bass (*Micropterus dolomieu*) and Sacramento pikeminnow (*Ptychocheilus grandis*) were sampled from Lake Clementine. Sacramento pikeminnow had the highest mercury concentration (n=3, THg=0.46 ppm) followed by smallmouth bass (n=8, THg=0.39 ppm).

- **Intermediate-Trophic-Level Predators (Secondary Targets):** The Intermediate-Trophic-Level Predators sampled from Lake Clementine included bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), and redear sunfish (*Lepomis microlophus*). The geometric mean THg for these samples (Sunfish Group) is 0.12 ppm (n=29).

- **Benthic Omnivores (Bottom Feeders):** There is no data for Benthic Omnivores at Lake Clementine.

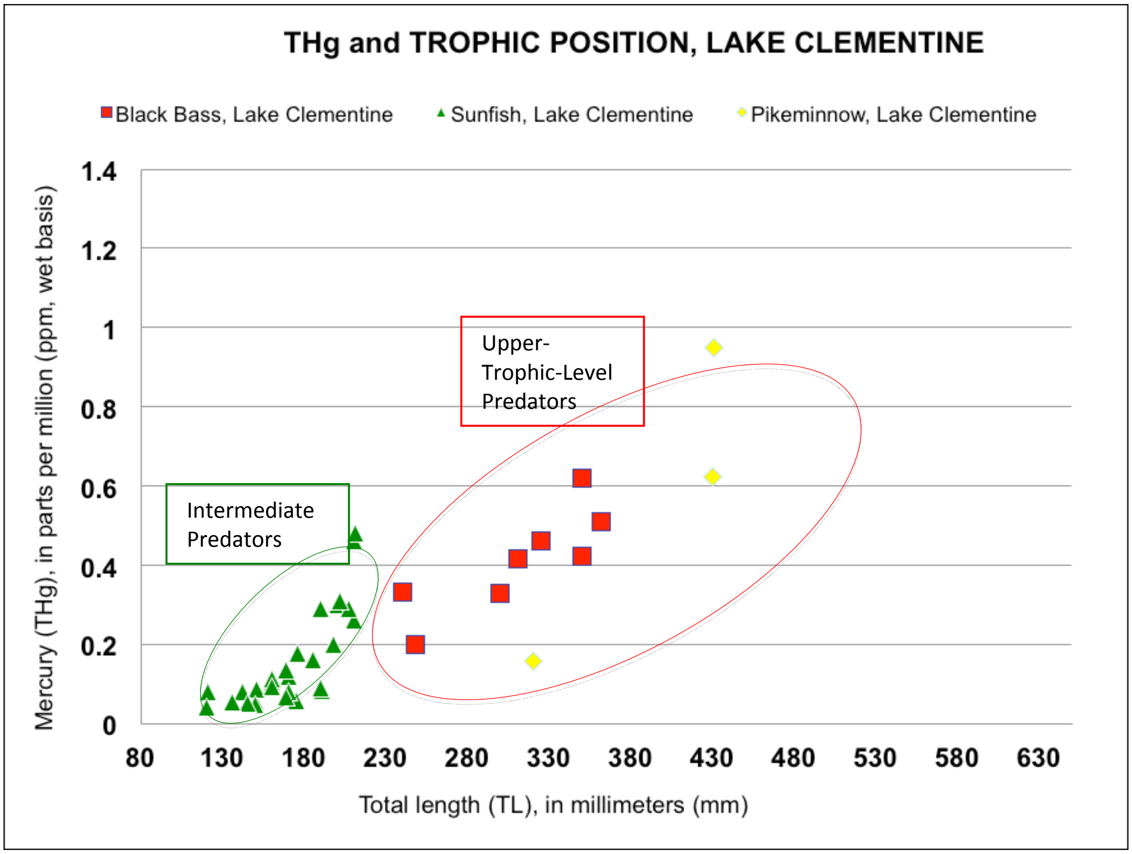

Figure 22. THg and trophic position at Lake Clementine, California. Mercury concentrations in relation to total length for Upper-Trophic-Level Predators (Black Bass, n=8; Sacramento pikeminnow, n=3) and Intermediate Predators (Sunfish, n=29).

- **Omnivores:** A single Rainbow Trout (*Oncorhynchus mykiss*) sample was collected as part of this research and was found to have a THg concentration of 0.031 ppm.

<u>Reservoir Comparison: Trophic Position and THg</u>

The relationship between trophic position and fish THg concentration followed the same general trend at both target water bodies. All sampled Black Bass Species (Upper-Trophic-Level Predator) had fish tissue mercury levels higher than sampled Trout (Omnivores). Trout had the lowest THg levels. Sunfish Species Group

(Intermediate-Trophic-Level Predators) had THg levels falling between Black Bass and Trout (see Figure 23). Sacramento pikeminnow had higher mercury levels than Black Bass at Lake Clementine, but the sample size was extremely small (n=3).

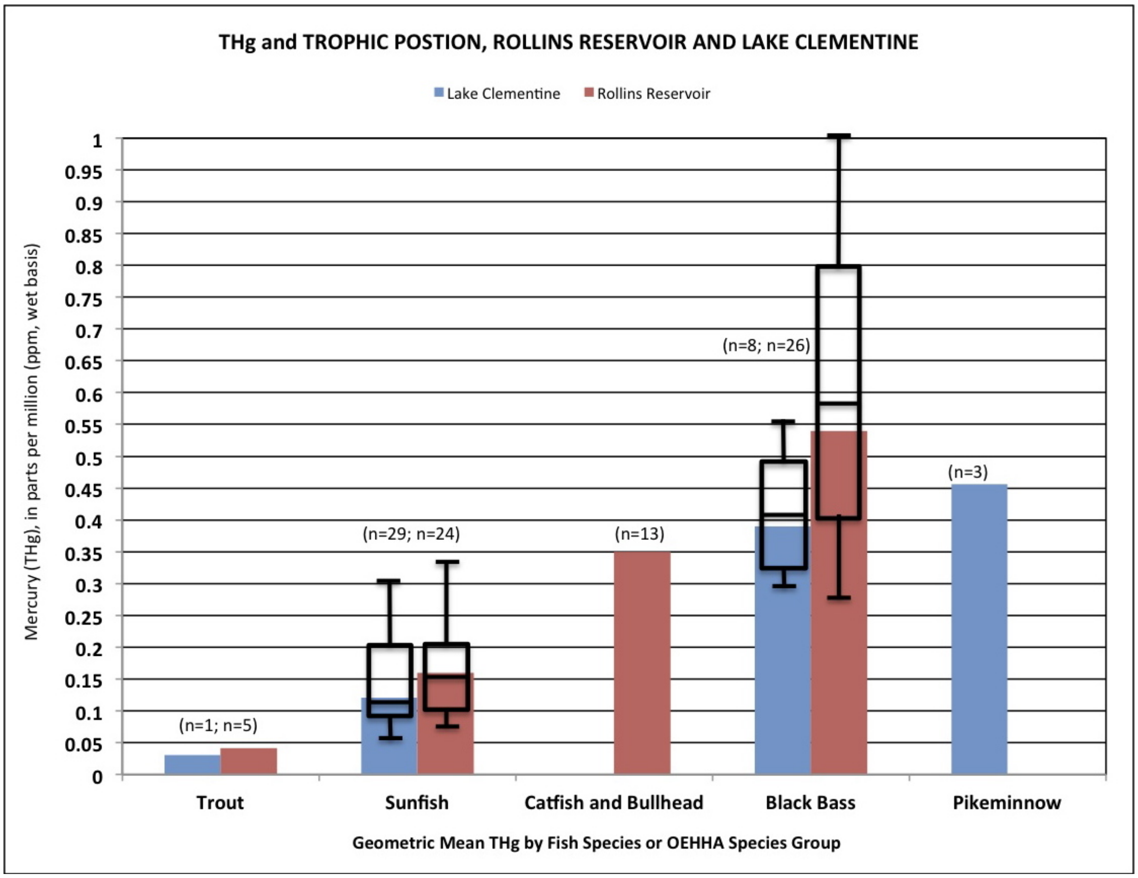

Figure 23. Total mercury and trophic position for edible species, Reservoir Comparison. Mercury concentrations in relation to total length for Upper-Trophic-Level Predators (Black Bass and Sacramento pikeminnow), Benthic Omnivores (Catfish and Bullhead), Intermediate Predators (Sunfish), and Omnivores (Trout). Boxplots for Sunfish and Black Bass at Rollins Reservoir and Lake Clementine show the range of variation in samples that will be provided to OEHHA for the development of site-specific advice.

Addressing Research Questions:
Angler Survey Data

Pre-advisory posting survey data collected as part of the *Gold Country Angler Survey* (The Sierra Fund, 2011) was reanalyzed and used in this analysis (n=151). Rollins Reservoir was a target water body for The Sierra Fund's survey effort, and the largest sample size of surveys was achieved at that location (n=33) (The Sierra Fund, 2011). In addition, pre-advisory posting survey data were collected from Lake Clementine (5/2/2015-8/30/2015; n=17) as part of this research project and used in this analysis. Post-advisory posting data collected at Rollins Reservoir by The Sierra Native Alliance using the *Gold Country Angler Survey Post-Posting* survey and the protocol specified in the methods section of this research was also used (surveys collected 11/6/2015-2/27/2016; n=19). See Table 19 for total samples collected and timeframe collected by water body.

TABLE 19: ANGLER SURVEY DATA FOR ROLLINS RESERVOIR AND LAKE CLEMENTINE

Water Body	Existing Data Number of Surveys (Prior to 4/29/2015)	Pre-Advisory Posting Surveys Collected for this Research (4/29/2015-8/30/2015)	Post-Advisory Posting Surveys Collected by Sierra Native Alliance (11/6/2015-2/27/2016)	Total Sample Size
Rollins Reservoir	33	0	19	52
Lake Clementine	0	17	0	17

<u>(C) Strategies for Minimizing Exposure</u>

❑ **Research Question 5:** Do most anglers (> 50%) report having heard or seen health warnings about eating fish?

Results: This research question was addressed by calculating the percentage of respondents answering "yes" to Question 15 (*Gold Country Angler Survey* and the *Gold Country Angler Survey Post-Posting*): "Have you ever heard or seen any health warnings about eating fish?"

Rollins Reservoir: Findings from the *Gold Country Angler Survey* (n=151), which included Rollins Reservoir (n=33), report that 79% percent of respondents answered "Yes" to Question 15 (The Sierra Fund, 2011). For surveys collected by Sierra Native Alliance at Rollins Reservoir 63% of respondents answered "Yes" to Question 15. These findings indicating that "most" anglers surveyed at Rollins Reservoir are aware of the issue of contaminated fish (>50%). See Figure 24.

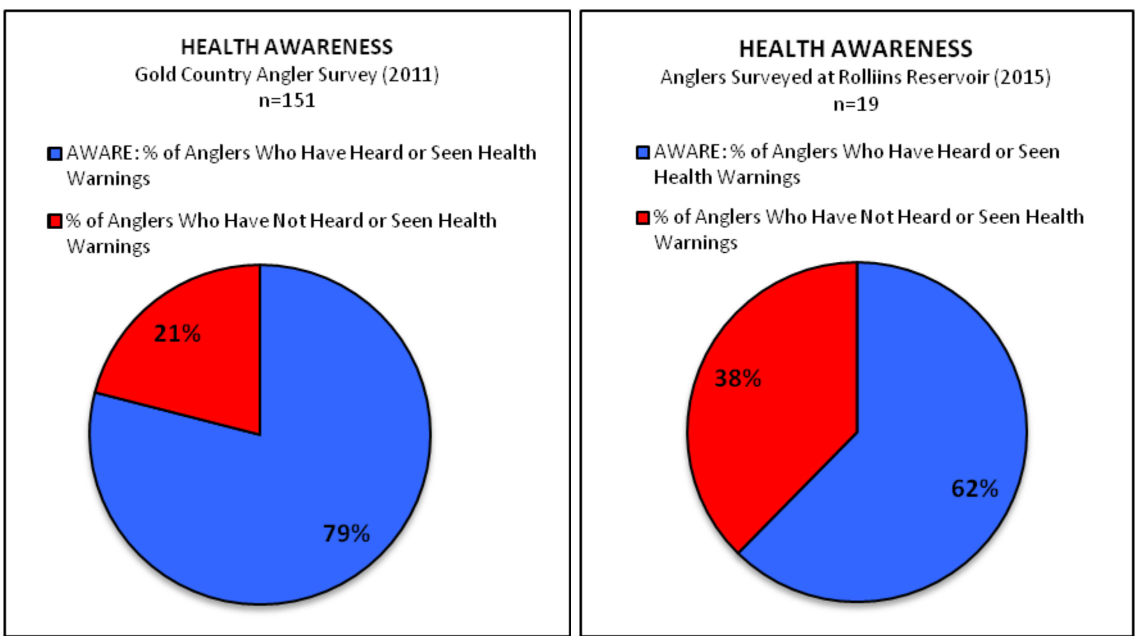

Figure 24. Health awareness, Rollins Reservoir (2009-10 and 2015-16). The percentage of respondents who have seen or heard health warnings about eating fish is shown in blue. Red indicates the percentage of anglers who have not heard or seen a health warning about eating fish. Left pie chart shows percentages from the surveys collected in 2009-10; right pie chart shows surveys collected in 2015-16.

Lake Clementine: For surveys collected as part of this research (n=17) 71% percent of respondents answered "Yes" to Question 15, indicating that "most" anglers surveyed at Lake Clementine are aware of the issue of contaminated fish (>50%). See Figure 25.

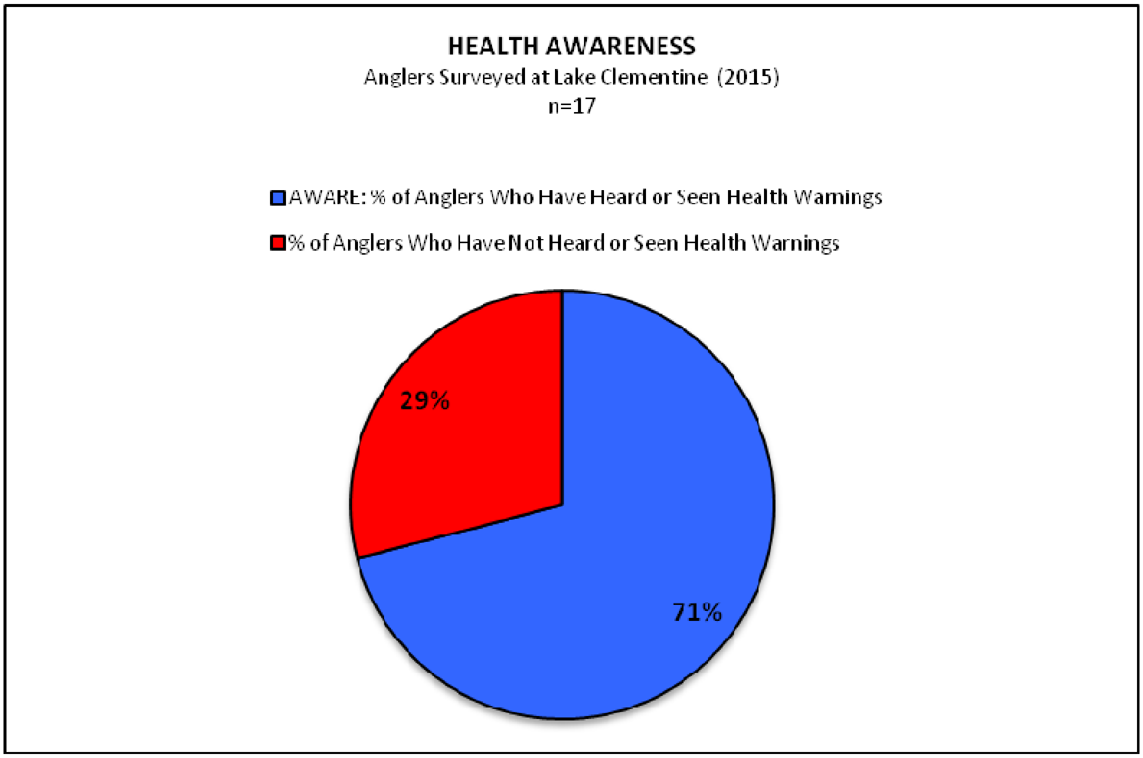

Figure 25. Health awareness, Lake Clementine. The percentage of respondents who have seen or heard health warnings about eating fish is shown in blue. Red indicates the percentage of anglers who have not heard or seen a health warning eating fish. Surveys were collected in 2015.

At both Rollins Reservoir and Lake Clementine most anglers report (> 50%) report having heard or seen health warnings about eating fish.

❑ **Research Question 6:** Do most anglers (> 50%) report seeing fish consumption advice posted at the water body?

Results: This research question was addressed by calculating the percentage of respondents answering "yes" to Question 18 (*Gold Country Angler Survey Post-Posting)*: "Have you ever heard or seen any health warnings about eating fish at this reservoir?"

Gold Country Angler Survey Post-Posting surveys were collected from Rollins Reservoir after 17 fish consumption posters of the general and incomplete site specific advisory was posted at 4 main locations (boat launches and campgrounds) at this water body on July 11, 2015. No survey respondents answered "yes" to Question 18 (n=19). Most anglers surveyed (> 50%) did not report seeing fish consumption advice posted at Rollins Reservoir (see Figure 26).

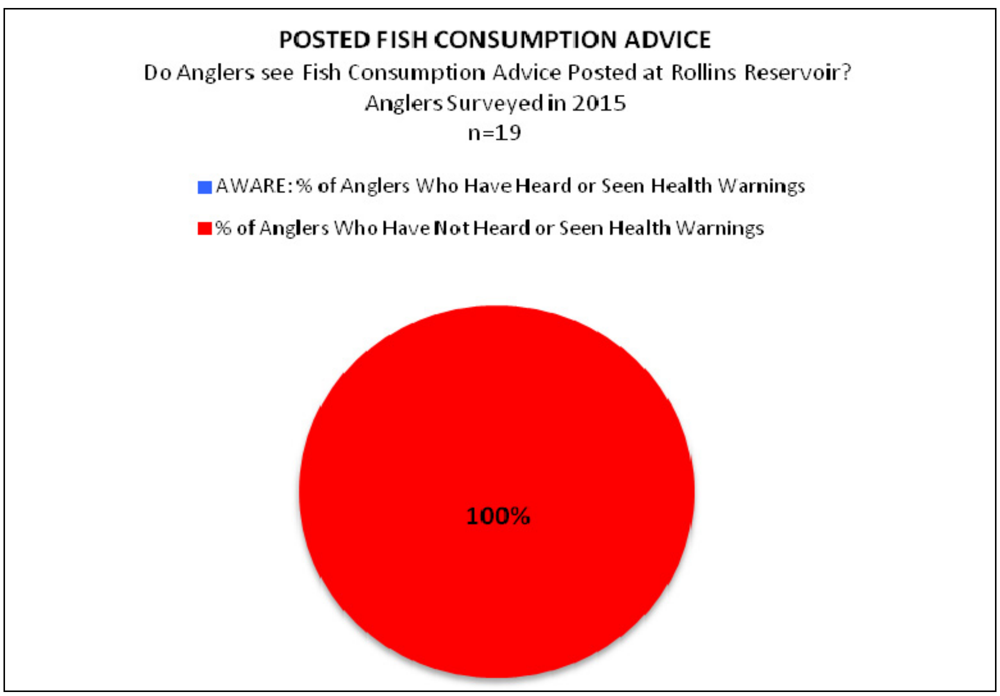

Figure 26. Posted fish consumption advice. The percentage of respondents who have never seen health warnings at the reservoir where they were surveyed (Rollins Reservoir) is shown in red. Of anglers surveyed 100% had not seen fish consumption advisories posted at Rollins Reservoir.

- **Research Question 7:** Are most anglers (> 50%) able to articulate information from the posted advice on (a) species group; (b) population group; and (c) frequency of consumption?

Results: This research question was addressed through analysis of responses to Question 16 of the *Gold Country Angler Survey* and the *Gold Country Angler Survey Post-Posting* (see Appendix A): “Do you remember what the warning said?”

Rollins Reservoir: Findings from the *Gold Country Angler Survey* (n=151), which included Rollins Reservoir (n=33), report that only 2% of respondents who saw a health warning were able to articulate the health warning by including mention of species, population group, and frequency of consumption (receiving a maximum score of 3 points for response to Question 16). Of respondents to the *Gold Country Angler Survey Post-Posting* surveys from Rollins Reservoir (N=19), 38% who saw a health warning articulated information about content on one of three key factors, or had “some awareness” about the content of the health warning. More than half of respondents (53%) to Question 16 articulated information about content on two of three factors, and no respondents included mention of species, population group, and frequency of consumption receiving a maximum score of 3 points (see Figure 27).

Lake Clementine: Of respondents to the *Gold Country Angler Survey* (N=17), 83% who saw a health warning articulated information about content on one of three key factors, or had “some awareness” about the content of the health warning. Only 16% of respondents articulated information about content on two of three factors, and no respondents included mention of species, population group,

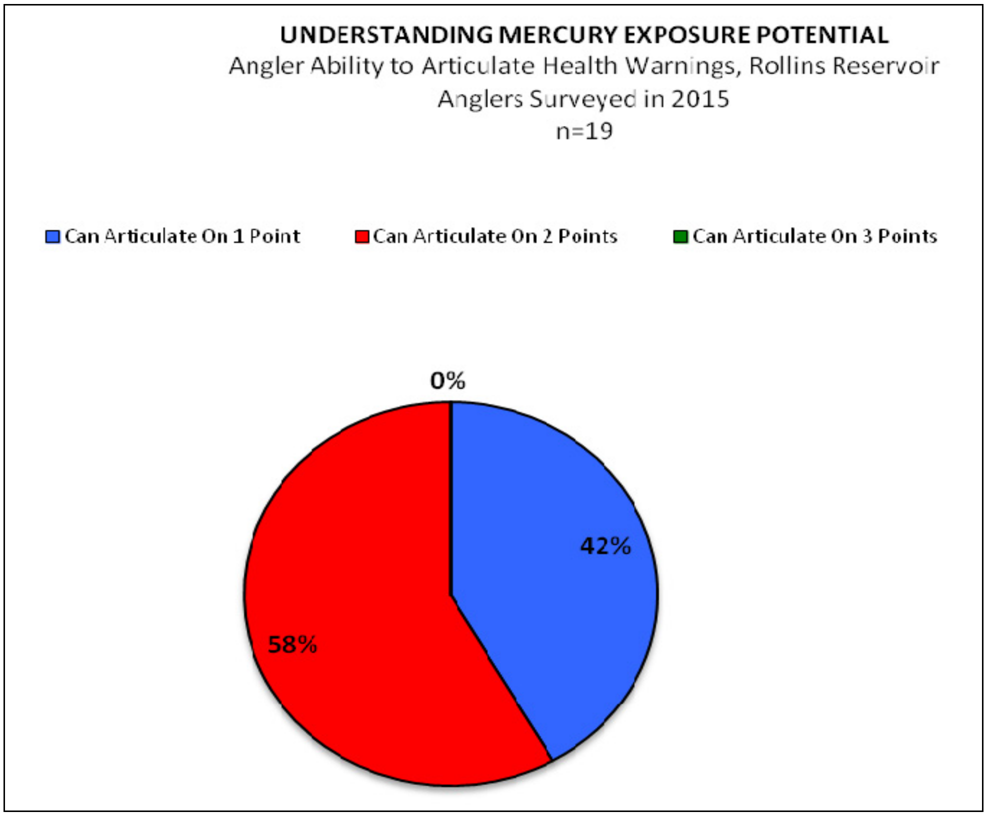

Figure 27. Understanding mercury exposure potential at Rollins Reservoir. The percentage of respondents who have seen health warnings and can articulate on one point, two points, or three points about what the warning said about (1) species of fish, (2) population group, and (3) frequency of consumption.

and frequency of consumption receiving a maximum score of 3 points (see Figure 28).

A three point score on Question 16 indicates that an angler has an "accurate understanding" of mercury exposure as a result of seeing or hearing health warnings. For this survey "accurate understanding" is defined as the ability to articulate information on all three components of fish consumption advisories: (1) species, (2) population group (Group 1: women of childbearing age, children; Group 2: women >45, men >18), and (3) frequency of consumption. Survey

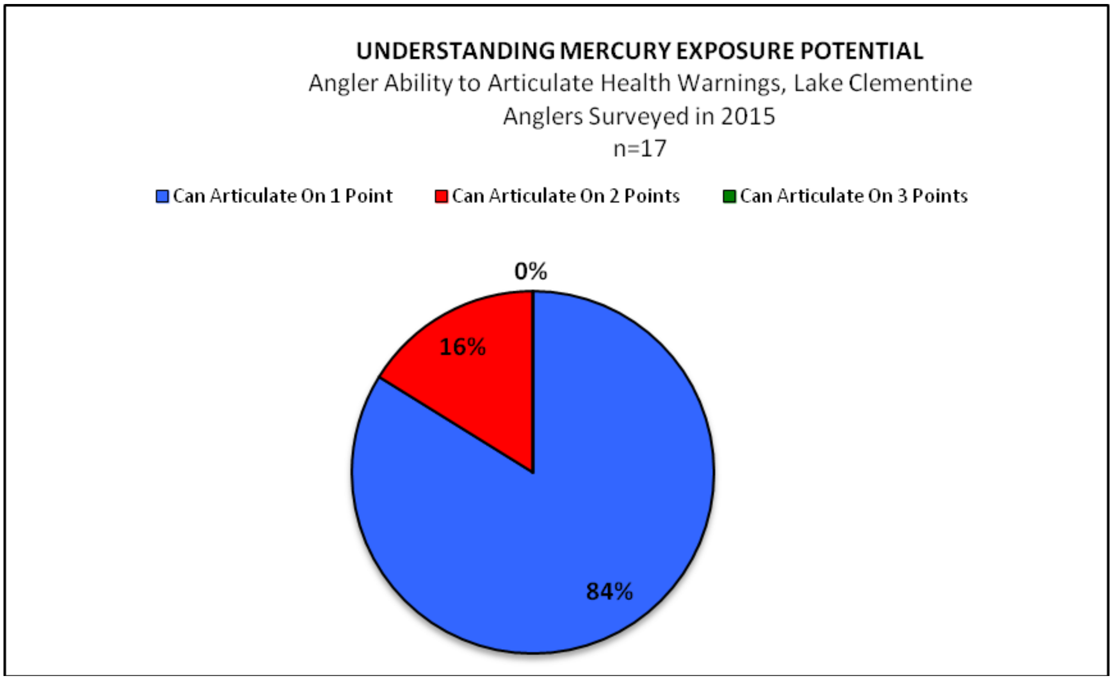

Figure 28. Understanding mercury exposure potential at Lake Clementine. The percentage of respondents who have seen health warnings and can articulate on one point, two points, or three points about what the warning said about (1) species of fish, (2) population group, and (3) frequency of consumption.

respondents in this research did not have accurate understanding (3 point scores = 0%) of fish consumption advisories. Most survey respondents (>50%) who saw or heard health warnings were able to articulate some of the content, but < 50% were able to articulate information from the posted advice on all three components.

Angler Awareness about Mercury

Angler surveys analyzed for this research demonstrate that despite lacking accurate understanding of the three main components of fish consumption advisories, many anglers are aware of health warnings related to mercury. Of 63% of anglers surveyed at Rollins Reservoir (11/6/2015-2/27/2016) that responded “yes” to Question 15 (*heard or seen health warnings about eating fish”*) 38% mentioned mercury in their response to Question 16 (*what the warning said*). At Lake Clementine 70% of anglers surveyed responded “yes” to Question 15 and of these 83% specifically mentioned mercury in their response to Question 16. See Figure 29.

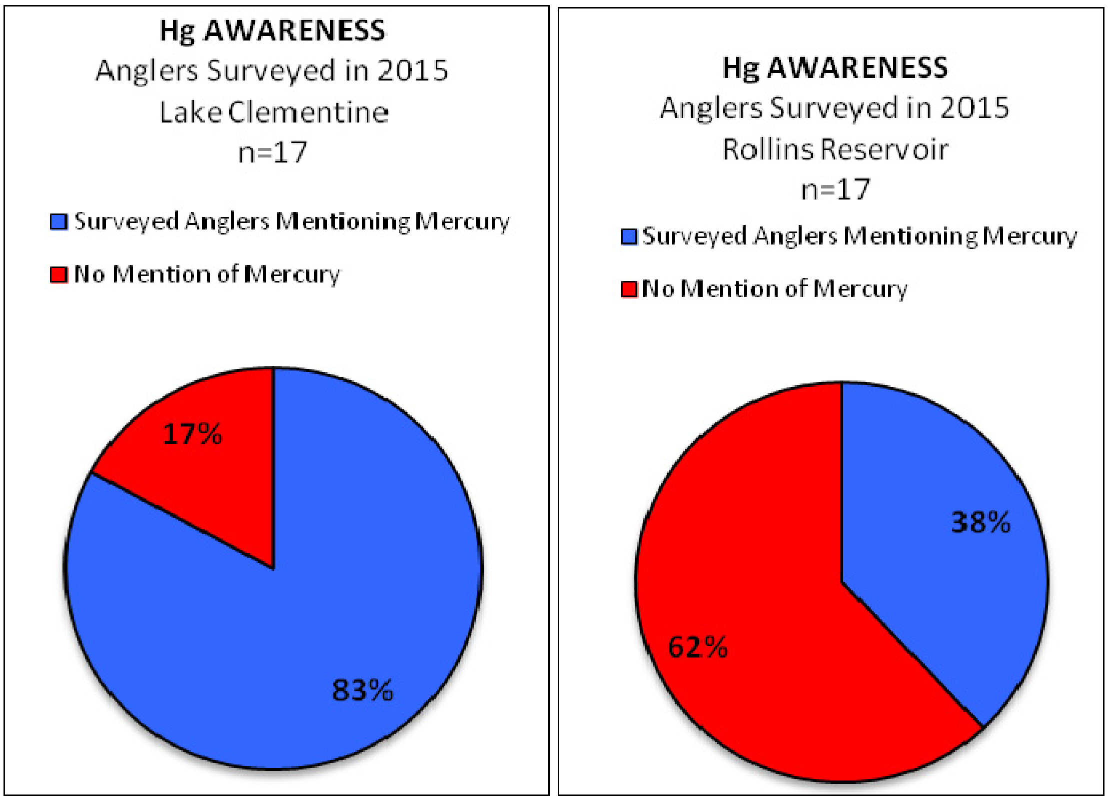

Figure 29**.** Angler awareness of mercury, Lake Clementine (left) and Rollins Reservoir (right). These graphs shows the percentage of respondents who have seen health warnings and mention mercury in their response to question 16.

CHAPTER IV

DISCUSSION

(A) Mercury Exposure Risk at Target Water Bodies

The ability to calculate accurate mercury exposure risk at Rollins Reservoir and Lake Clementine was improved by this research. Total mercury concentrations ranged from a low of 0.21 ppm in rainbow trout (n=1) at Rollins Reservoir to a high of 0.54 ppm (geometric mean) in Black Bass (n=26), also at Rollins Reservoir. An adequate sample size of legal/edible size fish was collected for two species groups at the two target water bodies. Sampling efforts successfully collected a large enough sample size (n > 9) of OEHHA-defined edible size fish for site-specific fish consumption advice to be issued for the Black Bass Group (n=26; geometric mean THg 0.54 ppm) and the Sunfish Group (n=24; geometric mean THg: 0.16 ppm ppm) at Rollins Reservoir and the Sunfish Group at Lake Clementine (n=29; geometric mean THg: 0.12 ppm).

Issuance of advice was particularly warranted for the Black Bass Group at Rollins Reservoir. For this species group 24 out of 26 samples were found to exceed the EPA threshold of 0.3 ppm. This is consistent with previous research in the region, where fish methlymercury concentrations in “largemouth, smallmouth, and spotted bass from five California reservoirs affected by historical hydraulic gold mining were as high as 1.5 ppm” (May et al., 2000; Wentz et al., 2014, p. 51). There is not yet enough data for fish consumption advice to be issued for the Black Bass Group At Lake Clementine, however, the data indicate that advice may be warranted. Seven out of 8 samples collected from Lake Clementine from the Black Bass Group exceed the EPA threshold. The USEPA Fish Tissue Residue Criterion for methylmercury in fish is 0.3 mg/kg (or ppm) for the protection of human health (SWRCB, 2010). Persistent exceedence of the EPA threshhold by black bass in the Sierra Nevada indicates that exposure risk can be minimized if anglers practice catch-and-release with bass species and avoid consumption. For Rollins Reservoir this analysis predicts that the fish consumption advice issued by OEHHA toxicologists states that women of child-bearing age (ages 18-45) and children ages 1 to 17 years (Group 1) should not consume any Black Bass from Rollins Reservoir and that women over age 45 years and men (Group 2) can consume one serving per week of Black Bass from Rollins Reservoir.

Exposure risk due to consumption of fish from the Sunfish Group at both Rollins Reservoir and Lake Clementine is minimal. For Rollins Reservoir this analysis predicts that the fish consumption advice issued by OEHHA toxicologists states that women of child-bearing age (ages 18-45) and children ages 1 to 17 years (Group 1) can consume one serving per week of Sunfish and that Women over age 45 years and men (Group 2) can consume three servings per week of Sunfish. For Lake Clementine this analysis predicts that the fish consumption advice issued by OEHHA toxicologists states that women of child-bearing age (ages 18-45) and children ages 1 to 17 years can consume two servings per week of Sunfish and that women over age 45 years and men can consume five servings per week of Sunfish.

OEHHA's team of staff toxicologists will use the data provided as part of this research to develop site-specific fish consumption advisories, and will make the final decision as to the advice that is issued for Group 1 and Group 2 consumption of black bass and sunfish from Rollins Reservoir and Lake Clementine.

Extremely limited samples available for trout from Rollins Reservoir and Lake Clementine show low values consistent with the recommendations of OEHHA's California Statewide Advisory for Lakes and Reservoirs Without Site-Specific Advice that trout represent a healthy choice for local fish consumption. However, given the small samples size the safety of trout consumption is neither confirmed nor disputed by this research.

It was not possible to calculate exposure risk for Sacramento pikeminnow due to a limited sample size (n=3; mean THg 0.46ppm). OEHHA did not analyze pikeminnow for the Statewide Advisory dataset so it is not possible to compare OEHHA 90^{th} percentile values to mean THg concentrations of Sacramento pikeminnow data collected as part of this effort. The high (>0.3 ppm) mercury concentration found in the pikeminnow sampled for this research is consistent with limited pikeminnow data available for the region. For example pikeminnow in the lower American River (n=51) had a mean mercury concentration of 0.53 ppm and a mean total length of 317 mm (OEHHA, 2009). Sacramento pikeminnow with high (>0.3 ppm) were also found in the Yuba River (Shilling, 2003). It should be noted that a sample size exception (n<9) "can apply to a few fish species commonly known to build up high levels of mercury, including largemouth bass and Sacramento pikeminnow" (OEHHA, 2009, p. 3). When at least five individuals of pikeminnow have been sampled at a water body OEHHA will consider giving advice for that species (OEHHA, 2009, p. 3). Early in this research effort an additional seven Sacramento pikeminnow were caught and released at Lake Clementine prior to pikeminnow being added to the list of target species and the Department of Fish and Wildlife (DFW) Scientific Collecting Permit (SCP) for this project. Pikeminnow at Lake Clementine were caught with greater frequency than bass species during this research and may be caught by anglers fishing at Lake Clementine with relative frequency. Sampling of additional Sacramento pikeminnow ($n \geq 2$) at Lake Clementine should be prioritized during the larger 2016 sampling effort being undertaken by The Sierra Fund.

Comparison to General Advisory Data

For Black Bass at both Rollins Reservoir and Lake Clementine, the OEHHA 90th percentile value exceeds the site-specific value for mean mercury concentration, indicating that the general advice is protective based on the data analyzed. For Catfish and Bullhead, the site-specific mean mercury concentration for catfish (0.35 ppm) exceeds the OEHHA 90th percentile value (0.346 ppm), indicating that the general advice is not protective, and the OEHHA-issued site-specific advice should be followed. For Sunfish at both Rollins Reservoir and Lake Clementine, the OEHHA 90th percentile value exceeds the site-specific value for mean mercury concentration, indicating that the general advice is protective based on the data analyzed. For brown trout and rainbow trout the OEHHA 90th percentile value is protective, based on the extremely limited number (n=6) of trout analyzed in this research.

(B) Impact of Size and Trophic Position on Fish Tissue Mercury Concentration

<u>Size</u>

The findings of this research show a significant ($p<0.05$) relationship between total length (TL) and fish tissue mercury concentration (ppm) for species of the Black Bass Group and species of the Sunfish Group. This supports research findings that young/small fish have lower fish tissue mercury levels than old/large fish (Scherer et al., 2008 Saiki et al., 2009). Existing studies have found that "mercury levels are usually higher in the muscles of older and larger fish, than in younger specimens, as a consequence of longer exposure time and bioaccumulation" (Carrasco et al., 2011, p. 1645; see also Alpers et al., 2008 and Saiki et al., 2009). Research at Camp Far West in the Bear Watershed follows this trend with study results indicating "mercury concentrations in spotted bass, bluegill, and threadfin shad varied according to fish size, with higher concentrations occurring in larger (older) fish" (Saiki et al., 2009, p. 12).

These findings that indicate a relationship between total length and fish mercury concentration are useful because they can facilitate more detailed communication of exposure information. This may include consumption guidelines that offer advice for size targets to avoid (e.g. *no Brown Trout >16 inches*). However, it is critical to note the importance of ensuring that fish consumption advice is issued based on fish samples that are legal/edible size. As Lepak et al. (2009) note, "if data used to develop consumption advisories include fish shorter than current minimum lengths, the mean Hg concentrations will likely be artificially low relative to the mean Hg concentrations in fish people can legally harvest and consume" (p. 178).

For the Black Bass Group a positive relationship between total length and total mercury concentration in fish tissue was found. There is a significant relationship between TL and THg for largemouth bass and spotted bass at Rollins Reservoir, but not for smallmouth bass. Smallmouth bass were found to have the highest THg levels of the three black bass species sampled at Rollins Reservoir. This is inconsistent with nationwide research on mercury in bass. USGS found that in streams across the United States smallmouth bass, which are more omnivorous than spotted or largemouth bass, had lower mercury concentrations that the two other species of the black bass group (Wentz et al., 2014, p. 49). When black bass species at Rollins Reservoir are analyzed together as a group the relationship between total length and total mercury is significant (n=26; rho= 0.85; p<0.01). For smallmouth bass at Lake Clementine the relationship between total length and total mercury is also significant (n=8; rho=0.85; p<0.05).

For the Sunfish Group a positive relationship between total length and total mercury at Lake Clementine was found but not at Rollins Reservoir. Differences between the water bodies in the sample composition of individual species may have contributed to this finding. At Rollins Reservoir Spearman's rank correlations calculated for green sunfish (n=21) indicated that the realtionship is not statistically signficant. (p>0.05). At Lake Clementine there is a significant relationship between TL and THg for bluegill (n=15). However, like at Rollins Reservoir, there is not a significant relationship between TL and THg for green sunfish (n=11). When all three species of sunfish (bluegill, green sunfish, and redear sunfish, n=3) sampled from Lake Clementine are combined for analysis as a group (n=29), there is statistically significant relationship between TL and

THg (rho=0.83; p<0.01). It is possible that this relationship is being driven by the bluegill samples included in the analysis of the Sunfish Group.

Trophic Position

This research found trophic position to have an impact on fish tissue total mercury. At both target water bodies Black Bass species (Upper-Trophic-Level Predator) had high fish tissue mercury levels while Trout (Omnivores) had low fish tissue mercury levels. Sunfish species (Intermediate-Trophic-Level Predators) had fish tissue mercury levels falling between Black Bass and Trout. This is consistent with existing general fish consumption advice that lists restrictions on bass consumption but states that rainbow trout are typically a healthy choice for local fish, in particular for sensitive populations including women of childbearing age, infants, and children under the age of 18 years.

These research findings are also consistent with existing research that suggests the highest fish tissue mercury levels typically occur in Upper-Trophic-Level Predators (May et al., 2000; Davis et al., 2008; Shilling et al., 2010). This information is useful for informing how species should be prioritized for data collection. For example, targeting omnivores like trout and obtaining a large sample size of that species group could be useful way to confirm that omnivores are healthiest fish choice across the Sierra Nevada. Targeting Intermediate-Trophic-Level species may also be good direction for future research, because species like sunfish are easily caught and thus popular with children. New research by USGS (Ackerman et al., 2015) provides additional evidence that sampling Intermediate-Trophic-Level species may be worthwhile. As part of this research Ackerman et al. developed a Predictive Tool for Managers (modeling tool) that uses “prey fish” (Intermediate-Trophic-Level species including Sunfish that birds and wildlife consume) to predict mercury impact on wildlife (grebes) and sport fish mercury concentrations.

(C) Strategies for Minimizing Exposure

The creation of fish consumption advisories is a public health strategy to reduce mercury exposure. Fish consumption advisories explain parameters for the safe consumption of fish based on species, demographic group, and frequency of consumption. At both of the target water bodies for this research most anglers (> 50%) report having heard or seen health warnings about eating fish. Between 63-79% of respondents answered that "Yes," they had heard or seen health warnings about eating fish. However, the vast majority of those surveyed could not articulate enough information from the health warning to safely consume local fish species. Findings of the *Gold Country Angler Survey* showed that of those surveyed that had seen some type of health-related warning about mercury in fish only 2% had any accurate understanding that would allow them to protect their health (The Sierra Fund, 2011). Not a single angler surveyed in 2015-16 at Rollins Reservoir or Lake Clementine had any accurate understanding that would allow them to protect their health. Between 38%-83% of anglers (depending on water body) who saw a health warning were able to articulate information about one critical component of fish consumption advice. Between 16-53% of respondents articulated information about two critical components of fish consumption advice.

There is evidence to suggest that further analysis should be done to determine any demographic trends in anglers surveyed to see if race/ethnicity may play a role in the inability of anglers to articulate fish consumption advice. For example, Judd et al. (2015) contend “Fish consumption advisories have frequently proven to be an ineffective tool for risk management in tribal communities” (p. 2433). This is because consumption advisories fail to take into account the complex social factors that influence fish consumption including issues of culture, food access, and nutrition (Judd et al., 2015). Findings from Beehler et al. (2003) suggest, “the key to risk reduction is to move beyond simple, written risk communications and provide educational intervention that appreciates and integrates local knowledge and lifestyles” (p. 113). Other evidence suggests that if even most anglers (> 50%) report having heard or seen health warnings about eating fish and are able to articulate information from the posted advice on (a) species group; (b) population group; and (c) frequency of consumption it may not lead to a reduction in exposure. In other words, the medium of communication may not play a role in improving outcomes. In an analysis of angler surveys done in the Central Valley Shilling et al. (2010) found “anglers that were more aware of warnings about fish contamination did not have statistically different rates of fish consumption or corresponding mercury intake than anglers with low awareness” (p. 340).

It was interesting to find that though state-issued fish consumption advice was posted at Rollins Reservoir and Lake Clementine (for the first time ever) during the course of this research project, no anglers that were surveyed reported seeing the posted health warnings. A small sample size (n=19 and time of year (late fall) may have contributed to these findings. It is also possible that angler recognition of posted advice is associated with length of time advice is posted (for example 3 months as with this project versus years). There is some evidence to suggest that once anglers are aware of advisories they respond. Jakus et al. (1998) found that "Anglers who knew of the advisories and who fished primarily to consume their catch were responsive to the advisories, substituting away from reservoirs with advisories and toward reservoirs without advisories" (p. 1023). However, in a region where many water bodies are not posted due to lack of site-specific data and lack of a requirement for landowners to post advice, a substitution response by anglers might not result in reduced exposure potential.

Study Limitations

This study was limited by small sample sizes and non-normally distributed data. Distribution of lake mean concentrations that are skewed is commonly seen with environmental data (Lim et al., 2013, p. 29). Efforts to increase sample size for the fish tissue data analysis by combing species into OEHHA Species Groups, though justified in the context of advisory formation, may have confounded relations among other variables of interest including age, total length, species, and diet (Alpers et al., 2008). Though the data analyzed for this research showed trends in mercury concentrations in aquatic food webs, mercury methylation and bioaccumulation in California reservoirs is not well understood and the individual food webs at both target water bodies warrant more complex analysis.

Obtaining adequate sample sizes from Lake Clementine for both fish tissue data (n>9 per species group) and Angler Surveys (n>30) was especially difficult, perhaps owing to limited accessibility at this water body. Lower Lake Clementine is for boat access only, with a stated limit of 25 boat trailers permitted at one time per Auburn State Recreation Area (ASRA). The water body has a single boat ramp, accessed by a steep and narrow road and the only camping available is at the 15 boat-in only sites. The only shoreline access at the water body is the “swim beach” located at the upstream North Fork American River inflow. Fieldwork conducted at this water body found a high volume of speedboat use during daylight hours, possibility impacting catch outcomes for this research and overall location desirability among local anglers.

This research should be considered a pilot project at Rollins Reservoir and Lake Clementine and results and lessons learned should be used to inform the design and implementation of follow-up research to increase sample size and allow for more accurate analysis of the seven Research Questions associated with this research project.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The ability of the Office of Environmental Health Hazard Assessment (OEHHA) to issue mercury fish consumption advisories that are protective of human health is contingent upon the availability of adequate fish tissue data and an understanding of angler catch and consumption patterns. The primary objective of this research, to provide data for use in the development of site-specific fish consumption advisories, was achieved for at least one species group at each of the two target water bodies. A total of 72 samples from four species or species groups of fish were collected from two reservoirs between May 2015 and August 2015 in order to fill gaps in existing data. As a result it is now possible to provide The Office of Environmental Health Hazard Assessment (OEHHA) with the requisite number of fish tissue samples required for their staff toxicologists to develop and issue fish consumption advice for the Black Bass Group and Sunfish Group at Rollins Reservoir and the Sunfish Group at Lake Clementine.

The secondary objective of this research was to provide insight into the effectiveness of health warnings about fish, including posted state-issued fish consumption advisories, for improving angler knowledge on the issue of mercury in fish. Surveys were collected from Rollins Reservoir and Lake Clementine and responses to four key survey questions were analyzed. This study points to the potential usefulness of Angler Surveys – both for informing sampling plans for fish tissue data collection and for informing best practices for the dissemination of public health information. Together the two objectives of this research project result in data that can be used by public health agencies to provide consumption advice and information about methods of communicating the advice to anglers of the Sierra Nevada. This research contributes to two larger research efforts being undertaken by The Sierra Fund to collect fish tissue data (n≤500) and Angler Surveys (n=500) from nine Sierra Nevada water bodies with known mercury contamination.

Recommendations for Future Research

1. Fill Remaining Data Gaps at Rollins Reservoir and Lake Clementine

- **Rollins Reservoir:** Rollins Reservoir has an advisory for Catfish stating that this species is safe for consumption by Group 1 (women 18-45 and children 1-17) at the rate of 1 serving per week. This project provides data for Sunfish and Black Bass. Nine or more samples from three representative species or species groups are now available, Satisfying OEHHA's sample size criteria for site-specific advisory development. Data for Trout (n=8) are needed from Rollins Reservoir for advice to be inclusive of all edible species (Office of Environmental Health Hazard Assessment 2016e).
- **Lake Clementine:** This project collected adequate Sunfish data (n=29) for Lake Clementine, however additional data are needed for Black Bass (n=4, ≥305 mm), and from one additional representative species (n=9) to satisfy OEHHA's sample size criteria for advisory development. Additional samples should also be collected for Sacramento pikeminnow (n≥3).

2. Identify and Fill Data Gaps for other CABY Region Water Bodies

- **Combie Reservoir:** The advisory for Combie Reservoir was updated in 2009. It includes advice for Black Bass (n=19, THg=0.90 ppm) and Sucker (n=10, Thg=0.53). Black bass and sucker are both listed as Do Not Eat for Group 1 (women 18-45 and children 1-17). Additional data were collected at Combie Reservoir in 2015 as part of The Sierra Fund's CABY Fish Tissue Project. Nine or more samples from three representative species or species groups are now available, satisfying OEHHA's sample size criteria for site-specific advisory development. OEHHA will now have the information needed to establish site-specific advice for Sunfish (n=31, THg=0.20 ppm). Trout data are still needed to establish site-specific advice for Combie Reservoir for this species group (Brown Trout n=7; Rainbow Trout n=7) (Office of Environmental Health Hazard Assessment 2016d).
- **Camp Far West Reservoir:** The advisory for Camp Far West Reservoir was updated in 2009. It includes advice for Sunfish (n=19, THg=0.19 ppm), Black Bass (n=38, THg=0.85 ppm), and Catfish (n=13, THg=0.44). Black Bass and Catfish are both listed as Do Not Eat for Group 1 (women 18-45 and children 1-17). Research as to whether native trout occur at Camp Far West and subsequent collection of trout data are still needed to establish site-specific advice for Camp Far West Reservoir for this species group (Rainbow Trout n=9) (Office of Environmental Health Hazard Assessment 2016a). Office of Environmental Health Hazard Assessment 2016a advice for Lake Englebright was updated in 2009 to include advice for Black Bass (n=56, THg=0.45 ppm), Sunfish (n=31, THg=0.30 ppm), and Rainbow Trout (n=49, THg=0.08 ppm). Black bass is listed as Do Not

Eat for Group 1 (women 18-45 and children 1-17). This water body has site-specific advice for a species known to be high in mercury (Black Bass) a species known to be low in mercury (Rainbow Trout) and an intermediate species (Sunfish). The advisory for Lake Englebright is considered to be complete and no further data collection is necessary at this waterbody (Office of Environmental Health Hazard Assessment 2016c).

- **Scotts Flat Reservoir:** OEHHA previously issued an advisory for a combination of several reservoirs, rivers, and creeks in the Sierra foothills (OEHHA 2009). However, in 2009 this advisory was dropped Scotts Flat Reservoir due to insufficient data. Data collection efforts in 2016-17 should focus on sampling species known to be caught and consumed from this water body ($n \geq 9$ per species or species group).

- **Yuba River, Bear River, and Deer Creek:** OEHHA previously issued an advisory for a combination of several reservoirs, rivers, and creeks in the Sierra foothills (OEHHA 2009). However, in 2009 this advisory was dropped for the South Yuba River, the Bear River below Highway 20, and Deer Creek due to insufficient data. Data collection efforts in 2016-17 should focus on sampling Trout species (Rainbow Trout and Brown Trout) from these three streams/rivers ($n \geq 9$ per species or species group).

3. Increase Angler Survey Dataset (n=500). Increasing the sample size of the angler survey effort will make interpretation of results a more reliable measure of the catch and consumption patterns of Sierra Nevada anglers. Results from The Sierra Fund's larger CABY Fish Tissue Study should be analyzed with Angler Survey data to target sites for

health outreach that are known to have angler activity and high (>0.3 ppm) fish mercury concentrations (Shilling, 2003).

4. Test the Reliability of the Prey Model Developed by Ackerman et al. (2015). The prey model developed by Ackerman et al. (2015) should be tested for reliability in predicting mercury levels in Sierra Nevada Black Bass using known mercury levels in prey fish. Sunfish data collected from Rollins Reservoir as part of this research can be used to predict mercury levels in Black Bass, and this prediction can be checked against the data for Black Bass at Rollins Reservoir.

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APPENDIX A

Angler Survey Protocol

Basic Angler Survey Protocol

The basic angler survey protocol requires that you avoid biasing angler responses by providing information about why the survey is being conducted before the survey is finished. DO tell the angler what organization you are with, if they ask. DO tell the angler that you are HAPPY to provide informational materials and answer their questions at the end of the survey. You can tell the angler that we are trying to get a sense of our local angler population.

1. Adults with fishing poles are the target group for surveys. **Surveyed individuals must be over 18.** If you are not sure, please ask.

2. **Approach anglers in a conversational manner.** Try to memorize the first few questions of the survey so that they can be asked as part of the initial conversation initiation.

3. Start by asking if the angler has ever been surveyed before. The Department of Fish and Wildlife periodically conducts Creel Surveys so it is possible that people may have been surveyed before. **If the answer is NO, proceed.**

4. If the angler agrees to be surveyed THEN get out your clipboard, fill in the answers for the first few questions (that you asked conversationally) and commence the rest of the survey. **If the angler is with a group of fishermen it is ideal to ask if they mind stepping away from the group.** This will prevent the angler from being influenced by others in their group. It will also allow you to survey multiple anglers fishing together (one survey after another) without bias.

5. **For questions that give the option of "don't know" or "refused" please be sure to check the appropriate box.** Don't assume that the person doing post-survey data input will know what was meant by an unchecked box. Surveys must be complete in order to count for data collection purposes. HOWEVER – we also want to know if someone refused to be surveyed OR if someone agreed to be surveyed but changed their mind part way through. Please note this on the survey, if it occurs.

6. At the end of the survey offer to answer any questions or provide informational materials. **Try to answer the angler's questions – however, if you are asked something that you are unsure about, pleaser refer the angler to The Sierra Fund materials or our website**, where interested parties can access the entire 2011 Gold Country Angler Survey report.

7. **Be sure to thank the angler!**

8. As soon as you have completed the survey, answered any questions, and thanked the angler, **quickly go over the survey to double check that it is complete.**

9. **Be sure to fill out Level of Effort forms when you complete a survey outing.** These forms are useful for making subsequent survey outings more successful and are also a critical piece of information for The Sierra Fund.

Have Fun! ALWAYS GO OUT WITH AT LEAST ONE OTHER PERSON (for safety and for enjoyment). Dress appropriately (hat, sunscreen, etc.).

Angler Survey Form

Survey Number:_________

Gold Country Angler Survey: Post-Advisory Posting

Date: __________ **Interviewer name:** ______________ **Time start:** _____:_____ **am** **pm**
end: _____:_____ **am** **pm**

Location of Interview:

- ☐ Lake Wildwood
- ☐ Upper Scotts Flat Lake
- ☐ Lower Scotts Flat Lake
- ☐ Deer Creek- location:________
- ☐ Lake Englebright
- ☐ South Yuba River- location:________
- ☐ Camp Far West Reservoir
- ☐ Lake Combie
- ☐ Rollins Lake
- ☐ Bear River- location:________
- ☐ Other:________

1. Do you have a few minutes to participate in our angler survey?

☐ No [Continue on to 3a]
☐ Yes [Continue on to 2a, skip question 3]

2a. If you don't mind me asking, what is your age: [READ CHOICES. CHECK APPROPRIATE BOX.] 1 ☐ Under 18 (end of survey) 2 ☐ between 18 and 34 3 ☐ between 35 and 45 4 ☐ over 45 5 ☐ Refused	**3a. Please note any known reason that they declined:** ☐ No time ☐ Language barrier ☐ Appeared threatened/uncooperative ☐ Other:____________ ☐ Unknown
2b. Have you ever been interviewed before about fishing or eating fish ☐ Y (fishing ___eating fish ___) By Whom? ☐ N (proceed) (If surveyed before by The Sierra Fund, then do not proceed with the rest of the questions)	**3b. Record observed gender:** ☐ Male ☐ Female
	3c. Record observed ethnicity: ☐ White ☐ Asian/Pacific Islander ☐ Black ☐ Native American ☐ Hispanic ☐ DON'T KNOW ☐ Other ____________

Survey Number:_________

4. What are you trying to catch today?__________________________

5. Are you going to eat the fish you catch today?

☐ Yes
☐ No
☐ Don't know/Not Sure
☐ Refused

If YES	If NO
6. Are you going to feed it to your family? ☐ Yes ☐ No Skip to question 8 below	**7a. What are you going to do with the fish you catch?** ☐ Give it to others to eat ☐ Catch and release it ☐ Other: _________________ ☐ Refused

7b. Do you ever eat fish that you or someone you know catches?

☐ Yes
☐ No [IF NO, SKIP TO **Q10**]
☐ Don't know/Not Sure [SKIP TO **Q10**]
☐ Refused [SKIP TO **Q10**]

Survey Number:_________

8. **About how many times did you go fishing in the last 30 days?**

_____________**[ENTER NUMBER] per** ☐ week
☐ month ☐ Don't know
☐ other_______ ☐ Refused

9a. Do you eat [*NAME OF FISH]* that you or someone you know catches? *Ask about specific fish listed below, as well as any others that are not named.* *Do this question first down the column, then come back and do fish by fish for b-d.*	**9b. How many times did you eat *[NAME OF FISH]* in the LAST 30 DAYS?** *If zero, skip to next row.*	**9c. How much *[NAME OF FISH]* did you eat in one meal?** *SHOW PICTURE OF FISH PIECES. Circle letter and write number of UNCOOKED models per meal.* *Only ask for types eaten in the **last 30 days.*** **A – Small** **C – Medium** **E – Large**	**9d. Where was the *[NAME OF FISH]* caught?** *Only ask for types eaten in the **last 30 days.*** *WRITE RESPONSE AND ENTER CODE* 1= Lake Wildwood 2= Upper Scotts Flat Lake 3= Lower Scotts Flat Lake 4= Deer Creek 5= Englebright Lake 6= South Yuba River 7= Camp Far West Reservoir 8= Lake Combie 9= Rollins Lake 10= Bear River 11= Other Location of survey(write response below)
☐ Catfish/Bullhead		A C E (Circle) ____ # of portion models/meal	
☐ Bass		A C E (Circle) ____ # of portion models/meal	
☐ Sunfish including Bluegill		A C E (Circle) ____ # of portion models/ meal	
☐ Crappie		A C E (Circle) ____ # of portion models/ meal	
☐ Rainbow Trout		A C E (Circle) ____ # of portion models/ meal	
☐ Brown trout		A C E (Circle) ____ # of portion models/ meal	
☐ Kokanee Salmon		A C E (Circle) ____ # of portion models/ meal	
☐ Other__________		A C E (Circle) ____ # of portion models/ meal	
Do you eat *[NAME OF SHELLFISH]* that you or someone you know catches?			
☐ Clams		____ # of clams/meal	
☐ Crawdads/ crayfish		____ # of crayfish/meal	

Survey Number:_________

10. In the last 30 days, have you eaten fish that came from stores, markets, restaurants, or cafeterias? (examples, tuna, fish sticks)

☐ Yes

☐ No [IF NO, SKIP TO **Q12**]

☐ Don't know/ Not Sure [SKIP TO **Q12**]

☐ Refused [SKIP TO **Q12**]

11a. Do you eat *[NAME OF FISH]* from stores, markets, restaurants, or cafeterias? *Ask about specific fish listed below, as well as any others that are not named.* *Do this question first down the column, then come back and do fish by fish for b-d.*	**11b. How many times did you eat *[NAME OF FISH]* in the LAST 30 DAYS?** *If zero, skip to next row.*	**11c. How much *[NAME OF FISH]* did you eat in one meal?** *SHOW PICTURE OF FISH PIECES or models. Circle letter and write number of UNCOOKED models per meal.* *Only ask for types eaten in the **last 30 days.*** **A – Small** **C – Medium** **E – Large**	**11d. Where was the *[NAME OF FISH]* bought?** *Only ask for types eaten in the **last 30 days.*** *WRITE RESPONSE AND ENTER CODE* 1= grocery store 2= restaurant 3= cafeteria 4= Other
☐ Shark		A C E (Circle) ____ # of portion models/meal	
☐ Bass		A C E (Circle) ____ # of portion models/ meal	
☐ Swordfish		A C E (Circle) ____ # of portion models/ meal	
☐ Tile fish		A C E (Circle) ____ # of portion models/ meal	
☐ King Mackerel		A C E (Circle) ____ # of portion models/ meal	
☐ Albacore Tuna		A C E (Circle) ____ # of portion models/ meal	
☐ Canned Tuna		A C E (Circle) ____ # of portion models/ meal	
☐ Salmon		A C E (Circle) ____ # of portion models/ meal	
☐ Trout		A C E (Circle) ____ # of portion models/ meal	
☐ Fish sticks		A C E (Circle) ____ # of portion models/ meal	

Survey Number:_________

☐ Halibut		A C E (Circle) ____ # of portion models/ meal	
☐ Other__________		A C E (Circle) ____ # of portion models/ meal	
Do you eat [*NAME OF SHELLFISH*] from stores, markets, restaurants, or cafeterias?			
☐ Clams		____ # of clams/meal	
☐ Calamari		____ # of calamari/meal	
☐ Shrimp		____ # of shrimp/meal	

Survey Number:_________

HOUSEHOLD & DEMOGRAPHIC INFORMATION

12. In the past year, have any children under 18 in your household eaten fish that you or someone you know catches?

☐ Yes
☐ No
☐ Don't know/ Not Sure
☐ Refused
☐ Not Applicable

13. In the past year, have any women between ages 18 and 45 in your household eaten fish that you or someone you know catches?

☐ Yes
☐ No
☐ Don't know/ Not Sure
☐ Refused
☐ Not Applicable

14. In the past year, have any women expecting a child or who have a baby in your household eaten fish that you or someone you know catches?

☐ Yes
☐ No
☐ Don't know/ Not Sure
☐ Refused
☐ Not Applicable

HEALTH WARNINGS

15. Have you ever heard or seen any health warnings about eating fish?

☐ Yes [continue on to Q16]
☐ No
☐ Don't know/Not sure [SKIP TO Q21]
☐ Refused

Survey Number:_________

16. Do you remember what the warning said?

☐ Yes

☐ No

☐ Don't know/Not sure

☐ Refused

[IF YES, RECORD EXACT RESPONSE]

____

____

17. Do you remember where you saw or heard this warning?
[DO NOT READ RESPONSE CATEGORIES. CHECK ALL THAT APPLY.]

1 ☐ Television 2 ☐ Radio 3 ☐ Sign at fishing location 4 ☐ Friend 5 ☐ Brochure
6 ☐ Market or store 7 ☐ Clinic 8 ☐ Other: ________________
9 ☐ Don't Know/Not Sure 10 ☐ Refused 11 ☐ Internet 12 ☐ News Articles
13 ☐ Fishing Regulation Manual

SITE-SPECIFIC HEALTH WARNINGS

18. Have you ever heard or seen any health warnings about eating fish at this reservoir?

☐ Yes [continue on to Q19]

☐ No [Skip to Q20]

☐ Don't know/Not sure

☐ Refused

19. Do you remember what the warning said?

☐ Yes

☐ No

☐ Don't know/Not sure

☐ Refused

[IF YES, RECORD EXACT RESPONSE]

____

____

Survey Number:_________

BEHAVIOR/ATTITUDE CHANGE

Have the health warnings posted:

20a. Changed how often you eat locally caught fish?

☐ Yes

☐ No

20b. Changed the species you fish for?

☐ Yes

☐ No

20c. Changed who you feed your catch to?

☐ Yes

☐ No

20d. Changed your attitude about eating fish?

☐ Yes

☐ No

[IF YES TO Q20a-d, RECORD EXACT RESPONSE]

____

____

21. Where do you get information about your health, about what is good or bad for you, that you <u>trust, that you really believe</u>?
[DO NOT READ RESPONSE CATEGORIES. CHECK UP TO 3 THAT APPLY.]

1 ☐ Health care provider, clinic or hospital
2 ☐ Internet
3 ☐ Friend, family member
4 ☐ TV
5 ☐ Radio
6 ☐ Books
7 ☐ Newspaper/Magazine
8 ☐ Fishing Regulation Manual
9 ☐ Posted signs
10 ☐ Community center or organization
11 ☐ Church, mosque, or temple
12 ☐ Other (specify): _________________
13 ☐ Don't know/Not sure
14 ☐ Refused

Survey Number:_________

22a. If you don't mind, could you tell me how best to describe your race or ethnicity. [SHOW list. CHECK ALL THAT APPLY]

- ☐ **White**
  - ☐ Russian
  - ☐ Ukrainian
  - ☐ Other __________
- ☐ **Black/African American**
- ☐ **Black/Caribean**
- ☐ **Native American**
- ☐ **Hispanic/Latino**
  - ☐ Mexican
  - ☐ Guatemalan
  - ☐ El Salvadorian
  - ☐ Nicaraguan
  - ☐ Honduran
  - ☐ Caribean (Dominican, Puerto Rican, Cuban, etc.)
  - ☐ Other: _________
- ☐ **Other:** ______________
- ☐ **Unknown**
- ☐ **Refused**
- ☐ **Asian**
  - ☐ Cambodian
  - ☐ Chinese
  - ☐ Hmong
  - ☐ Filipino
  - ☐ Japanese
  - ☐ Khmu
  - ☐ Korean
  - ☐ Lao
  - ☐ Iu-Mien
  - ☐ Vietnamese
  - ☐ Other:_______
- ☐ **Pacific Islander**
  - ☐ Fijian
  - ☐ Hawaiian
  - ☐ Samoan
  - ☐ Tahitian
  - ☐ Tongan
  - ☐ Other:_______

22b. [IF MORE THAN ONE BOX SELECTED] Which race do you most identify with: ______________

22c. [IF REFUSED, RECORD OBSERVED ETHNICITY]: __________________________

23. What zip code do you live in? _______

24. [RECORD GENDER]

☐ male

☐ female

25. [RECORD APPROXIMATE WEIGHT]

☐ under 70 pounds

☐ Between 70 pounds and 150 pounds

☐ over 150 pounds

☐ estimate___________